

INFLUENCES OF NATURAL ENEMIES AND RESOURCE AVAILABILITY IN
BIOLOGICAL INVASIONS BY PLANTS

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ABSTRACT

ROBERT WILLIAM HECKMAN: Influences of Natural Enemies and Resource
Availability in Biological Invasions by Plants
(Under the direction of Charles E. Mitchell)

Biological invasions—the establishment and spread of species outside their historical native ranges—has implications for basic ecology as well as conservation and human well-being. As such, identifying the mechanisms that promote invasions is crucial for both applied and basic ecology. While most major invasion hypotheses focus on a single causal mechanism (e.g., nutrient availability, traits of the invasive species), my research examines whether trade-offs between resource allocation to growth of new tissue and defense of tissue against disease and herbivory can explain why some non-native species become invasive in their new range and others do not. Specifically, I tested whether exotic species benefit more from enemy release relative to native competitors in high resource environments. To that end, I conducted a series of field experiments at the level of individual plants and plant communities. This research represents the first thorough test of the assumptions and key predictions of a hypothesis which integrates information about invasive species, invaded communities, and the environment in which invasion occurs to explain invasion success more broadly than previously possible (the Resource-Enemy Release Hypothesis, R-ERH).

I tested this hypothesis in grassland communities and with individuals of several grass species. At the community level, exotics were less damaged than natives, especially in fertilized communities. Moreover, fertilization increased foliar damage on native species. Finally,

fertilization increased exotic dominance only in communities exposed to vertebrate herbivores, and excluding insect herbivores and fungal pathogens reduced exotic dominance regardless of fertilization. At the individual level, species benefitting most from fertilization also benefitted most from exclusion of fungal pathogens and insect herbivores; this relationship was similar for natives and exotics. Within assembled native communities, fertilization increased, and enemy exclusion reduced, exotic dominance. Furthermore, fertilization and enemy exclusion each reduced native colonization of exotic-dominated communities. Together, these results provided partial support for R-ERH. Importantly, they also show that invasions can be driven by multiple independent, not interacting, factors.

To my grandparents, Raymond and Josephine Esch, the best people I know

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CHAPTER I. INTRODUCTION

Species invasions often depend on multiple factors, complicating management efforts. Thus, understanding the factors that allow a small number of non-native species to become dominant, or invasive, in their new ranges is critical for both basic ecology and management efforts. Although numerous hypotheses have been proposed to explain the success of invasive plants, many of these hypotheses have limited applicability because they rely on a single causal factor. In the case of plants, invasion success may be more broadly explained by the Resource-Enemy Release Hypothesis (R-ERH) which integrates multiple factors: the physiological traits of invaders and the resident plant species, the natural enemy (herbivores and pathogens) pressure in the non-native range, and environmental conditions (soil fertility) into a single framework. I tested the primary assumptions and predictions of R-ERH with a series of field experiments at the individual plant and community level. This research constitutes the most thorough examination to date of R-ERH, one of the most integrative hypotheses of plant invasions.

Upon introduction, many non-native species escape from their co-evolved specialist natural enemies (herbivores and pathogens that have evolved to attack one or a few closely related species). The degree to which escape from enemies benefits these exotic species may depend on the physiological traits of the individual. Plants experience a trade-off between rapid growth and defense against enemies (e.g. with thorns or toxic compounds). Plants adapted to fertile soils tend to acquire resources quickly and typically allocate most of their resources (sugars, nutrients) to growth and reproduction rather than defense. Since it is costly to defend rapidly growing tissue, these individuals may be more susceptible to damage from

enemies. These plants subsequently experience a great benefit from escaping their specialist enemies. Conversely, plants that occur in infertile soils typically allocate a large portion of their resources toward defending their tissue. These slow-growing, well-defended individuals may experience less damage from enemies (due to their large defense investment) and receive a much smaller benefit from enemy release. Additionally, increased soil fertility typically increases allocation of resources to growth and reproduction at the expense of defense investment. Thus, nutrient addition to the native community often increases their susceptibility to fungal diseases and insect herbivory. This may reduce the competitive ability of natives relative to non-natives experiencing enemy release, particularly in fertile soil.

CHAPTER SUMMARIES

In collaboration with Justin Wright (Duke University) and Charles Mitchell, I tested both underlying conditions necessary for R-ERH to explain exotic success at the community level, as well as a main community-level prediction of R-ERH. Specifically, we tested three predictions: 1) Native species experience greater enemy damage than exotic species; 2) Increasing nutrient supply increases enemy damage to native species; 3) Increasing nutrient supply increases exotic dominance only in the presence of enemies. We manipulated the supply of soil nutrients and access of vertebrate herbivores, insect herbivores, and fungal pathogens to intact grassland communities containing both native and exotic species. We then measured foliar damage caused by fungal pathogens and insect herbivores and quantified exotic dominance in response to these treatments. We found support for both conditions of R-ERH. Exotics experienced less foliar damage (insect herbivory and total damage) than native species, particularly in communities where soil nutrients were added, confirming the first condition of R-ERH. Moreover, fertilization increased foliar damage on native species, confirming the second condition of R-ERH. We also

found support for the main community-level prediction of R-ERH. Fertilization increased exotic dominance only in communities exposed to vertebrate herbivores. Finally, excluding insect herbivores and fungal pathogens reduced exotic dominance regardless of fertilization, partially supporting the prediction of R-ERH.

In collaboration with Fletcher Halliday and Charles Mitchell, I tested both underlying conditions necessary for R-ERH to explain exotic success at the individual level as well as an individual-level prediction of R-ERH. Specifically, we tested two conditions of R-ERH: (1) native species are more heavily impacted by enemies than exotic species, and (2) species that benefit more from nutrient addition (i.e., quick-growing species) also benefit more from enemy exclusion (i.e., species heavily impacted by enemies). We then tested whether this correlation is weaker for exotics because they benefit less than natives from enemy exclusion. To do this, we manipulated the supply of soil nutrients and access of insect herbivores and fungal pathogens to individuals of 11 grass species growing in pots buried in the field. Overall, we found that species which responded most strongly to fertilization (indicating species adapted to high resource environments) also benefitted most from exclusion of fungal pathogens and insect herbivores (indicating species heavily impacted by natural enemies), confirming the first condition. However, this relationship did not differ between native and exotic species, failing to confirm our second prediction. This suggests that trade-offs exist across species between allocation to growth and defense, but that this trade-off is unaffected by species' provenance (native versus exotic).

In this study, I collaborated with Charles Mitchell to test R-ERH in exotic-dominated communities. We examined whether manipulating soil nutrient supply and enemy access to communities would affect the ability of native species, added as seed, to colonize exotic-

dominated communities. We predicted that enemy exclusion will reduce damage to native colonizers more than resident exotics, increasing colonization of exotic-dominated communities by natives. Furthermore, we also predicted that communities with low nutrient supply from which enemies have not been excluded will be least colonized; communities with high nutrient supply from which enemies have been excluded will be the most colonized. We also tested that native colonizers adapted to high-resource environments will have greater success than those adapted to low-resource environments when nutrient supply is high and enemies are excluded, but not when enemies are present or at low nutrient supply. To test these predictions, we manipulated the supply of soil nutrients, access of insect herbivores and fungal pathogens, and presence of leaf litter to intact, exotic-dominated old field communities. In 2013 and 2014, we added seeds of 11 locally common native species to each plot. Each month, we measured light availability, soil moisture content, and the number of germinating seedlings in each plot. We also measured foliar damage to the resident community (i.e., species already present in plots). In October 2014, we harvested up to 10 seedlings per plot, measured insect herbivory and fungal disease to their leaves, and weighed their above-ground biomass. We used piecewise structural equation modeling to determine the influence of our experimental treatments on factors including light availability, foliar damage, and water availability, which could ultimately influence seedling germination and persistence. We found that litter removal increased seedling success by increasing light availability. Contrary to expectations, enemy exclusion had a net negative effect on seedling success; enemy exclusion reduced foliar damage to seedlings, but this effect was overwhelmed by the benefit of enemy exclusion to the resident community, indirectly reducing seedling success. This suggests that enemies impacted the resident community more than colonizing seedlings. Furthermore, soil nutrient addition reduced seedling success by reducing

light and soil moisture availability. Fertilization also reduced seedling success directly in the SEM, suggesting that fertilization also affected seedling success through another mechanism that we were unable to measure. Also contrary to our prediction, foliar N content, a key predictor of the resource level to which species are adapted, had little influence on seedling establishment. Together, these results do not support R-ERH. Exotic species were not released from enemies relative to natives, but fertilization did benefit exotics by reducing light and water availability to colonizing native species.

In collaboration with Peter Wilfahrt, Fletcher Halliday, and Charles Mitchell, I tested the separate and interactive contributions of nutrient supply, native species richness, and natural enemies to exotic success. To assess these contributions, we planted communities at two levels of native richness (monocultures and five-species polycultures), and factorially manipulated soil nutrient supply and access by aboveground fungal pathogens and insect herbivores. We then allowed natural colonization to proceed for four years and quantified the abundance of all species in each plot annually to calculate exotic dominance of these communities. We found no evidence that nutrient supply, enemy access, and native richness interacted to influence exotic success. Moreover, native richness had no effect on exotic success. In fact, nutrient addition increased, and enemy exclusion decreased, exotic success independently. This suggests that multiple factors may influence invasion simultaneously without interacting.

CHAPTER II. JOINT EFFECTS OF NUTRIENT ADDITION AND ENEMY EXCLUSION ON EXOTIC PLANT SUCCESS

Introduction

Resource availability and natural enemies can each independently influence the success of invasive plants (Huenneke et al. 1990, Shea and Chesson 2002, Agrawal et al. 2005). Moreover, resources and enemies may interact to determine invasion success (the Resource-Enemy Release Hypothesis, R-ERH; Blumenthal 2005, 2006). Many successful invaders grow primarily in high-resource environments (van Kleunen et al. 2010, Leishman et al. 2014), environments in which plants typically allocate fewer resources to defense against enemies (Cebrian and Duarte 1994, Endara and Coley 2011). Consequently, natural enemies may regulate populations of these fast growing, poorly defended plant species (Fine et al. 2004, Lind et al. 2013)—at least in their native ranges. In their introduced ranges, however, plants are commonly released from, or lose, many of their specialist natural enemies from their native range (Maron and Vilà 2001, Keane and Crawley 2002, Mitchell et al. 2006, Heger and Jeschke 2014), and plants adapted to higher-resource environments may be released from more enemies (Blumenthal et al. 2009). Thus, exotic species may benefit from increased resource availability without the higher enemy regulation experienced by the native species with which they compete, thereby allowing exotics to dominate the plant community. This leads to a major prediction of R-ERH: increasing resource availability will increase exotic abundance only in the presence of enemies.

This prediction builds on two conditions being met: (1) exotic species are released from enemies relative to natives, and (2) regulation of natives by enemies increases with resource

availability (Blumenthal 2005, 2006). The first condition, that exotics are released from enemies, has been evaluated in numerous studies, with results differing by study type (Colautti et al. 2004, Torchin and Mitchell 2004, Heger and Jeschke 2014). When considering damage by enemies, there is equal evidence for and against enemy release, while most studies of plant performance have not been consistent with enemy release (Heger and Jeschke 2014). Furthermore, comparisons between species' native and introduced ranges have broadly supported enemy release, while comparisons between native and exotic species have produced mixed results (Colautti et al. 2004, Torchin and Mitchell 2004, Heger and Jeschke 2014).

The second condition, that regulation of native species by enemies increases with resource availability, could occur because greater resource availability should increase the quality of foliar tissue, which is often preferred by herbivores and pathogens (Mattson 1980, Elser et al. 2000, Blumenthal 2006). This may happen on ecological timescales through two non-exclusive mechanisms, resulting because plants face a trade-off between growth and defense against enemies (Coley et al. 1985, Lind et al. 2013). First, greater resource availability may shift the species composition of the plant community toward poorly defended species that are adapted to resource-rich environments where growth is favored over defense against enemies (Coley et al. 1985, Stamp 2003, Endara and Coley 2011). Second, greater resource availability may cause shifts in resource allocation within plant species, favoring phenotypes with more rapid growth and less defense (Throop and Lerda 2004, Veresoglou et al. 2013), without necessarily changing plant community composition.

Testing the main prediction of R-ERH, that increasing resource availability will increase exotic abundance only in the presence of enemies, requires an integrative approach. Specifically, individual performance, population density, or community abundance must be assessed for both

native and exotic plant species, across environments that differ in both resource availability and enemy pressure. Based on these criteria, to our knowledge, only two studies have tested this prediction; neither study supported it (Dawson et al. 2014, Seabloom et al. 2015). Within a plant community, the prediction can be tested by jointly manipulating resource availability and enemy damage, then monitoring changes in the relative abundance by exotic species (Blumenthal 2006). Fulfilling the prediction would require that the reduction in exotic cover in response to experimental manipulation of enemies be greater in high-resource environments than in low-resource environments, or that experimental reduction of enemy damage decreases a positive effect of resource availability on exotic abundance (Blumenthal 2006).

Here, we present what to our knowledge is the first study to experimentally test both the underlying conditions and main predictions of R-ERH. We did this in a grassland by experimentally manipulating soil nutrient supply (nitrogen, phosphorus, potassium, and micronutrients, all in a single fertilization treatment), as well as attack by three major guilds of enemies (vertebrate herbivores, insect herbivores, and fungal pathogens). In this study, we first test both underlying conditions necessary for R-ERH to explain exotic abundance in the plant community, specifically that 1) native species experience greater enemy damage than exotic species, and 2) increasing soil nutrient availability increases enemy damage to native species. We then test a main community-level prediction, that increasing resource availability will increase exotic abundance only in the presence of enemies.

Methods

We performed this study at Widener Farm, an eight hectare old field in Orange County, NC, USA last cultivated in 1996. By the time of the study, the site was dominated by perennial species, including natives (chiefly *Andropogon virginicus* and *Tripsacum dactyloides*) typical of

old fields in the North Carolina Piedmont that have been abandoned for multiple years (Oosting 1942) and exotics (chiefly *Lespedeza cuneata*, *Lonicera japonica*, and *Schedonorus arundinaceus*, together constituting 67% of cover in control plots when the study ended).

This study employed a split-plot experimental design. At the whole-plot level, we factorially manipulated soil nutrient availability and access to the plot by vertebrate herbivores for five growing seasons. At the split-plot level, we manipulated access by both foliar fungal pathogens and insect herbivores for 2.5 growing seasons.

The whole plots and their manipulations were established in April 2008 as part of the Nutrient Network, a globally-replicated experiment in grassland communities, following the network protocols (see Borer et al. 2014a for details); this study involved only a subset of the network's experimental treatments. Soil nutrient availability was manipulated with two treatment levels (fertilized with 10 g N m⁻² yr⁻¹ as slow-release urea, 10 g P m⁻² yr⁻¹ as triple super phosphate, 10 g K m⁻² yr⁻¹ as potassium sulphate, and 100 g m⁻² micronutrients vs. not fertilized). At our site, this fertilization treatment alleviated nutrient limitation and increased aboveground productivity (Fay et al. 2015). To avoid toxicity, micronutrients (Scotts Micromax, Marysville, OH) were applied only once at the start of the experiment. Access by vertebrate herbivores was also manipulated with two treatment levels (fenced to exclude vertebrates vs. not fenced); fences were 2.5 m high to exclude large mammals (primarily deer), with wire mesh surrounding the bottom 1 m to exclude small mammals (primarily voles). An outward facing flange of this wire mesh also extended along the ground ~ 30 cm from the edge of the plot. This 2×2 factorial design yielded four treatment combinations, and each treatment combination was replicated three times, for a total of twelve whole plots. Each whole plot was 25 m².

The split plots and their manipulation were established in August 2010. Access by foliar fungal pathogens and insect herbivores was manipulated with two treatment levels (sprayed with fungicide and insecticide vs. not sprayed). Within each whole plot, four 1 m² subplots were established, and two subplots were randomly assigned to each of the two treatment levels. Establishing four subplots in each of twelve whole plots yielded a total of 48 subplots. We conducted the spraying treatment from August 2010 through October 2012; this involved spraying the non-systemic broad-spectrum biocides on the aboveground portion of all plants every two weeks during the main growing season, from April through October. Neither the fungicide (mancozeb, Dithane[®] DF, Dow AgroSciences, Indianapolis, IN) nor the insecticide (es-fenvalerate, Asana[®] XL, Dupont, Wilmington, DE) had any non-target effects on plant growth under greenhouse conditions (Appendix A1; Appendix A2.1; Appendix A3.1). Each sprayed plot received the equivalent of 3 mm of precipitation per year, or < 0.25% of average annual precipitation. In the field, spraying was highly effective, reducing community damage, community disease, and community herbivory (see below for methods) by at least 80% each relative to unsprayed controls ($P < 0.001$, Appendix A2.2; Appendix A3.2). Like many studies manipulating access to communities by natural enemies (e.g., Mitchell 2003, Allan et al. 2010, Allan and Crawley 2011), we were not able to control for any procedural effects of spraying in the field. Nonetheless, the observations that these biocides did not affect plant growth in the greenhouse, and contributed little water to plots, but substantially reduced damage in the field suggest that the effects of the spraying treatment were attributable to reduced damage.

We visually quantified percent cover of all plant species in each subplot in July and September, 2011 and 2012; because we evaluated cover for all species independently, the sum of cover values for each plot could exceed 100%. We surveyed foliar enemy damage in July and

October 2011 on 20 plant species that occurred at > 5% cover in any plot in the prior cover survey, which comprised an average of 93% of plant cover in each plot. We excluded two species that met this criterion: *Pinus taeda*, the only gymnosperm in the community, and *Vicia* sp., which had senesced prior to our damage surveys. The surveyed species included six native grasses, six native non-leguminous forbs, two native tree and shrub species, one native woody vine, two exotic grasses, one exotic leguminous forb, one exotic shrub, and one exotic woody vine. Of these groups, exotic grasses were, on average, the most abundant (32% cover), while exotic legumes (22%) and native grasses (17%) were the next most abundant groups.

To quantify leaf area damaged, we followed Mitchell et al. (2002) and Mitchell (2003) and haphazardly selected 20 leaves per species varying in age and location from throughout the split plot, but without reference to the leaves' damage level. For each sampled leaf, we recorded the percent of the leaf's area that was damaged, by guild of insect herbivore and fungal morphospecies. Damage was visually quantified by referring to digitized images of known damage severity (James 1971). From these data, we calculated community damage,

$$\text{Community damage} = \sum_{i=1}^n d_{i \text{ subplot}} \left(\frac{c_{i \text{ subplot}}}{c_{t \text{ subplot}}} \right)$$

where $d_{i \text{ subplot}}$ is mean percent of leaf area damaged for the i th species in the subplot, $c_{i \text{ subplot}}$ is percent cover of the i th species and $c_{t \text{ subplot}}$ is the total cover of species within that group (e.g., c_t for exotic damage was total exotic cover) surveyed for damage in the subplot (Mitchell et al. 2002). To determine whether exotic species were less damaged than natives and whether damage to native species increased with fertilization, we calculated community damage separately by plant geographic provenance (native to North Carolina vs. exotic). Due to differences in feeding mode between insect herbivores and fungal pathogens, we examined damage by insects and fungi separately. We combined all insect damage, > 80% of which was chewing damage, to

calculate insect herbivory (hereafter, herbivory). Likewise, we combined all fungal damage to calculate fungal disease (hereafter, disease). To calculate total damage, we summed disease and herbivory (hereafter, foliar damage). These damage estimates all reflected damage within leaves that were present at the time of each survey. Quantifying vertebrate herbivory visually is often unreliable, partially because vertebrates often remove entire leaves or ramets (Oosterheld and McNaughton 2000). Therefore, we did not attempt to quantify it, and inferred impacts of vertebrate herbivory from our fencing treatment, following Oosterheld and McNaughton (2000), Borer et al. (2014b), and Seabloom et al. (2015). Although we cannot be sure that fencing excluded all vertebrate herbivores, fencing effectively reduced both soil-level light availability and species richness at our site (Borer et al. 2014b).

Testing the condition of R-ERH related to enemy release requires accounting for non-independence between natives and exotics within subplots, so we nested the effect of provenance (native vs. exotic) within subplot. Because damage was reduced to very low levels by the spraying treatment, we tested these conditions only in unsprayed subplots.

To test the conditions of R-ERH, we analyzed foliar damage (disease, herbivory) in models including the main effects of fertilization and fencing treatments but not their interaction as whole plot effects. This was because we had *a priori* expectations for the effects of fertilization on damage, but not for fencing. We nonetheless included the main effect of fencing to control for unanticipated effects, e.g. via shifts in vegetation structure. We also included an effect of provenance (native vs. exotic) nested within subplot, and the interaction between fertilization and provenance. Because the relative abundance of native and exotic species differed from 1:1 in all plots, the main effect of fertilization does not indicate the effect of

fertilization on damage across the entire community. Instead, we focused on the fertilization \times provenance effect to test these conditions.

To test the main prediction of R-ERH, we analyzed exotic cover, the ratio of the summed percent cover of exotic plant species to the total percent cover of all plant species in each plot. Because interactive effects of resource availability and enemy exclusion on exotic cover are critical to testing this prediction, this model included interactions between fencing, spraying, fertilization, and year. Fertilization and fencing were whole plot effects and spraying was a subplot effect. Because we measured exotic cover twice in each year (2011 and 2012), we modelled an effect of time at two levels: to account for successional changes between years, we included year as a fixed effect; to account for phenological changes within a year, we included a first-order auto-regressive correlation structure for census date as an ordinal variable using the function `corAR1` in package `nlme`. Our spraying treatment began 2.5 years after our fencing and fertilization treatments, and effects of spraying treatments often take several years to alter community composition, particularly in perennial-dominated systems, limiting statistical power (Allan and Crawley 2011). To increase power to detect effects of spraying, we analyzed the absolute change in exotic cover between 2011 and 2012 (i.e. exotic cover in 2012 minus exotic cover in 2011), in addition to exotic cover. To compare effects of these treatments on exotic cover to effects of these treatments on succession in this old field, we similarly analyzed the absolute change in tree and shrub cover between 2011 and 2012.

We analyzed all data in R version 3.2.2 (R Core Team 2016) with the `nlme` package (Pinheiro et al. 2016) for linear mixed effects models. In order to meet assumptions of homoscedasticity and normality of residuals, we cube-root transformed community damage. When we were unable to fully remove heteroscedasticity via transformation, we also included an

identity variance structure that modelled residual variance separately by treatment level using the `varIdent` function in package `nlme` (Zuur et al. 2009, Pinheiro et al. 2016). In each model, we separately modelled variances of the treatments that—based on visual inspection of the residuals—contained the most heteroscedasticity, then replotted residuals to confirm that heteroscedasticity was eliminated. For all community damage, we allowed variances to differ by fertilization and provenance. For analyses of exotic cover, we allowed variances to differ by fencing and year. Finally, for all models, when significant interactions occurred, we performed Tukey’s HSD using the `lsmeans` package to determine which treatment combinations differed significantly from one another (Lenth 2013).

Results

Are exotic species less damaged than native species?

Exotics experienced less foliar (fungal + insect) damage than natives ($P < 0.001$), particularly in fertilized plots (Fertilization \times Provenance: $P = 0.013$), where exotics had 70% less foliar damage than natives (Tukey HSD: $P < 0.001$, Appendix A3.3a, Fig. 2.1a), supporting the first condition. Exotics tended to experience less disease than natives, although this was not significant ($P = 0.09$, Appendix A3.3b, Fig. 2.1b). Exotics also experienced less herbivory than natives ($P < 0.001$), but only in fertilized plots (Fertilization \times Provenance: $P = 0.004$), where exotics experienced 82% less herbivory (Tukey HSD: $P < 0.001$, Appendix A3.3c, Fig. 2.1c), also supporting the first condition. Overall, these results support the first condition for R-ERH in fertilized plots, but not in unfertilized plots.

Does damage to native species increase with fertility?

Fertilization increased community damage to native species about three-fold (Tukey HSD: $P = 0.015$, Appendix A3.3a, Fig. 2.1a), supporting the second condition. Furthermore,

while fertilization did not influence community disease on natives (Tukey HSD: $P = 0.99$, Appendix A3.3b, Fig. 2.1b), it did increase their community herbivory more than ten-fold (Tukey HSD: $P = 0.006$, Appendix A3.3c, Fig. 2.1c). Overall, these results support the second condition for R-ERH.

Do enemies and nutrients affect exotic abundance?

Fertilization increased exotic cover only in unfenced plots (Fencing \times Fertilization: $P = 0.007$, Appendix A3.4), doing so in 2011 by 58% (Tukey HSD: $P = 0.029$, Fig. 2.2), supporting the prediction that increasing resource availability will increase exotic cover only in the presence of enemies. Also consistent with R-ERH, the sprayed and unsprayed communities began to diverge in exotic cover over time (Spraying \times Year: $P = 0.059$, Fig. 2.3). Specifically, from 2011 to 2012, exotic cover remained constant in unsprayed plots, but decreased by more than 8% in sprayed plots (Spraying, $P = 0.03$, Appendix A3.5, Appendix A2.3a).

Two effects of fertilization on exotic cover were inconsistent with the prediction. First, the effect of spraying on change in exotic cover was not greater in fertilized plots (Fertilization \times Spraying: $P = 0.057$); in fact, spraying reduced the change in exotic cover from 15% to -1% in unfertilized plots (Tukey HSD: $P = 0.026$) but did not influence the change in exotic cover in fertilized plots (Tukey HSD: $P = 0.99$, Fig. 2.4). Second, the effect of fertilization on exotic cover differed by year (Fertilization \times Year: $P < 0.001$, Appendix A3.4, Appendix A2.4); from 2011 to 2012, exotic cover declined by 21% in fertilized plots (Tukey HSD: $P < 0.01$, Appendix A2.4), while exotic cover increased by 13% in control plots (Tukey HSD: $P = 0.04$). These two effects were largely attributable to succession from herbaceous to woody dominance.

Specifically, tree and shrub cover increased from 2011 to 2012 by 17% in fertilized plots only (Fertilization: $P < 0.01$, Appendix A3.6, Appendix A2.3b), corresponding to a 135% increase in

absolute cover of trees and shrubs, all of them native. Furthermore, we observed only one exotic shrub and no exotic tree species in the experiment. These results indicate that there was little potential for exotic cover to increase or be maintained when fertilization accelerated succession to woody dominance.

Overall, these results partially support the prediction of R-ERH for insect herbivory and fungal disease. Because both conditions were met for insect herbivory, but not for fungal disease, the effect of excluding insects and fungi on exotic cover was likely driven by insect herbivores. Moreover, despite being unable to test the conditions for vertebrate herbivory, these results fully support the prediction that fertilization increases exotic cover only in the presence of vertebrate herbivores.

Discussion

Our results provide the first experimental support for the prediction that increasing resource availability will increase exotic abundance only in the presence of enemies. Excluding fungal pathogens and insect herbivores reduced exotic cover over time relative to unsprayed plots, indicating that exotics were released from enemies. However, this reduction occurred only in unfertilized communities, so results for fungal pathogens and insect herbivores only partially support the prediction. Fertilization increased exotic cover in unfenced plots, and did not influence exotic cover when vertebrate herbivores were fenced out, fully supporting the prediction of R-ERH.

Our results support the first condition of R-ERH: exotic species were less damaged than native species. In contrast, previous support for enemy release when comparing native and exotic species within a community is weak (Colautti et al. 2004, Dostál et al. 2013, Heger and Jeschke 2014). This contrast may result from differences in design. To control for phylogenetic

relatedness, most studies have compared exotic species to native species that are confamilial or congeneric (Mitchell et al. 2006, Heger and Jeschke 2014). But, closely related species in the same community often share enemies (Parker and Gilbert 2007, Hill and Kotanen 2009, Parker et al. 2015), which would increase observed damage on exotic species paired with closely related native species, making such comparisons conservative tests of enemy release (Mitchell et al. 2006). Since many successful invaders lack native relatives (Strauss et al. 2006, Parker et al. 2012) and may be subject to introduction bias (Chrobock et al. 2011), it is often ecologically relevant to compare co-occurring exotics to native species that are ecologically similar, regardless of phylogenetic relatedness, as we did here. Our results also support the second condition of R-ERH: damage to the native plant community increased with fertilization. This result is consistent with the growth rate hypothesis, which suggests that fertilization shifted plant species composition by favoring native species that were less defended (Coley et al. 1985, Stamp 2003, Endara and Coley 2011).

Our results may explain conflicting results of previous enemy release studies. Here, we detected greater foliar damage and herbivory on native species than exotics, but disease did not differ between exotic and native species. Many previous studies have focused on a single enemy guild, which may explain why some studies have found exotics to be less damaged than natives, while others have found either the opposite or no difference between natives and exotics (Heger and Jeschke 2014). Similarly, assessing enemy release with a single enemy guild may yield different conclusions than with multiple enemy guilds (Agrawal et al. 2005). Moreover, we detected enemy release only in fertilized plots. Thus, assessing enemy release at a single resource level may yield different conclusions than with multiple resource levels, and could explain non-significant results of some previous studies of enemy release (e.g., Dawson et al. 2014).

To test the conditions of R-ERH that fertilization increases regulation of native species by enemies and that exotic species are released from enemies relative to natives, we followed the common practice of using damage by enemies as a proxy for regulation by enemies (Leger et al. 2007, Han et al. 2008, Dostál et al. 2013). Although enemy damage can be a strong predictor of the magnitude of enemy impact on the performance of a given plant species (Aldea et al. 2006, McElrone et al. 2010), enemy damage may not reliably predict enemy impact across plant species that differ in tolerance of enemy damage, i.e. the relationship between degree of damage and plant performance (Chun et al. 2010). To reliably quantify the impact of natural enemies on native and exotic species, experimental manipulation of enemy damage at the level of the plant community is a strong, but under-utilized approach (e.g., Stricker and Stiling 2012, Suwa and Louda 2012). However, because the community-level approach only quantifies enemy impacts relative to competitors, testing for regulation by enemies at the population level would ideally be done by manipulating enemies in monocultures of each plant species. In most communities, quantifying damage may be the best feasible approach.

Like many studies, enemy guilds differed in the magnitude of their effects on community composition (e.g., Agrawal et al. 2005, Allan et al. 2010, Allan and Crawley 2011). Here, vertebrate herbivores more strongly influenced exotic cover than did fungal disease and insect herbivory. We suggest two non-exclusive explanations. First, vertebrate herbivores may regulate plants in this system more strongly than insect herbivores and fungal pathogens. Although vertebrate herbivores are mostly generalists (Crawley 1983, but see Kartzinel et al. 2015), exotic vertebrate herbivores generally increase exotic abundance (Parker et al. 2006), and native ungulates can increase exotic abundance by preferentially consuming native species (Vavra et al. 2007, Kalisz et al. 2014). Furthermore, exotics may be more tolerant of vertebrate herbivory than

natives (Chun et al. 2010). Second, vertebrate herbivores may have more strongly influenced exotic cover than did insect herbivores and fungal pathogens because we excluded vertebrate herbivores for twice as long as we excluded insect herbivores and fungal pathogens. In a similar study, enemy exclusion took several years to influence community composition (Allan and Crawley 2011). Thus, while we cannot fully separate the effects of enemy identity from length of enemy exclusion, excluding insect herbivores and fungal pathogens longer may have reduced exotic cover to a greater extent than was observed here.

The first study that we know of to test the prediction of R-ERH found no support for R-ERH at the level of the individual plant (Dawson et al. 2014), which is in contrast to our study's support for R-ERH at the level of the plant community. One possible explanation is that processes at the level of the individual plant may not scale up to the plant population, for example because of the potential for compensatory responses among multiple individuals, particularly across the full life-history of the plant (Alexander and Mihail 2000). Thus, experimental manipulation of enemy damage using potted plants may not provide a reliable indication of enemy impacts at the level of plant populations or communities.

We know of only one previous test of our prediction at the community level, which included our site and 33 other Nutrient Network sites worldwide (Seabloom et al. 2015). On average globally, fencing out vertebrate herbivores did not reduce exotic cover, regardless of fertilization treatment. However, sites varied considerably in the effects of vertebrate herbivore exclusion on plant species composition (Borer et al. 2014b, Seabloom et al. 2015), perhaps partly due to variation in herbivore species identity. Such variation could cause herbivores to promote invasion at some sites and not others through at least two mechanisms. First, herbivores can differ strongly in diet preferences (Kartzinel et al. 2015). Second, herbivores vary widely in their

relative impacts on native vs. exotic plants, depending partly on whether the herbivore is native or exotic (Parker et al. 2006) and partly on how tolerant each species is of vertebrate herbivory (Chun et al. 2010). Due to such variation, R-ERH may not explain invasions at all sites, but our experimental confirmation at one site provides a step towards multi-site studies that can test which other factors may modulate the joint effects of resources and enemies.

For any hypothesis, testing not only its predictions but also its conditions can reveal key ecological processes. Like Seabloom et al. (2015), we were unable to quantify damage from vertebrates and thereby, directly test the conditions of R-ERH for vertebrate herbivory. When conditions are not tested, and the prediction is not supported (e.g., Seabloom et al. 2015), then there is little that can be inferred from it about the conditions or the operation of the hypothesis. In contrast, when results fully support a prediction, as for vertebrate herbivory in our study, this suggests that the conditions were met. Testing the conditions for fungal pathogens and insect herbivores allowed us to detect differences in the responses of native and exotic species to fertilization. In fertilized plots, insect herbivory was greater on native than exotic species, but insect herbivory did not differ between native and exotic species in control plots. We also found differences in the responses of different enemy guilds to fertilization; disease did not respond to fertilization, but fertilization increased insect herbivory at the community level, primarily by promoting changes in community composition toward species that exhibited greater damage across the experiment. These mechanistic insights would not have been apparent by simply examining changes in community composition.

The unpredicted decline in exotic cover with fertilization in the experiment's final year appears to be the result of invasion being overwhelmed by succession, which is typically rapid in southeastern US old fields (Wright and Fridley 2010). Prior to our study, annual mowing had

maintained herbaceous dominance; when our study began, mowing ceased, allowing succession to woody species. Tree and shrub cover increased from 2011 to 2012 by 135% in fertilized plots, but remained constant in unfertilized plots. Because fertilization often shifts competition from belowground to aboveground (Borer et al. 2014b), this suggests that herbaceous species were unable to compete for light with larger trees and shrubs. This increase in tree and shrub abundance was entirely due to native species, as the system largely lacked exotic trees and shrubs. The only exotic shrub (*Rosa multiflora*) never occurred in fertilized plots (and in unfertilized plots from 2011 to 2012, it declined in abundance). With no exotic trees or shrubs in fertilized plots, native woody species began to outcompete exotic herbaceous species for light, reducing exotic cover.

In conclusion, our results provide experimental support for R-ERH, one of the most integrative hypotheses for plant invasions, fulfilling both conditions for foliar damage and its primary prediction for vertebrate herbivory: fertilization increased exotic cover only in communities exposed to vertebrate herbivores. Moreover, exotic cover of the plant community responded to multiple guilds of natural enemies, stressing the need to consider the influence of diverse enemies on community processes. Overall, this study demonstrates that enemy release and resource availability can act in concert to facilitate invasion, suggesting that both understanding and managing plant invasions require consideration of the interaction between resource availability and natural enemies.

Fig. 2.1 Effects of fertilizer application on mean A) total damage (disease + herbivory), B) disease, C) herbivory for native and exotic species, calculated using restricted maximum likelihood estimation and back-transformed from a cube-root transformation. Points represent fitted values from the model. Shared letters denote no significant differences between treatments based on Tukey HSD.

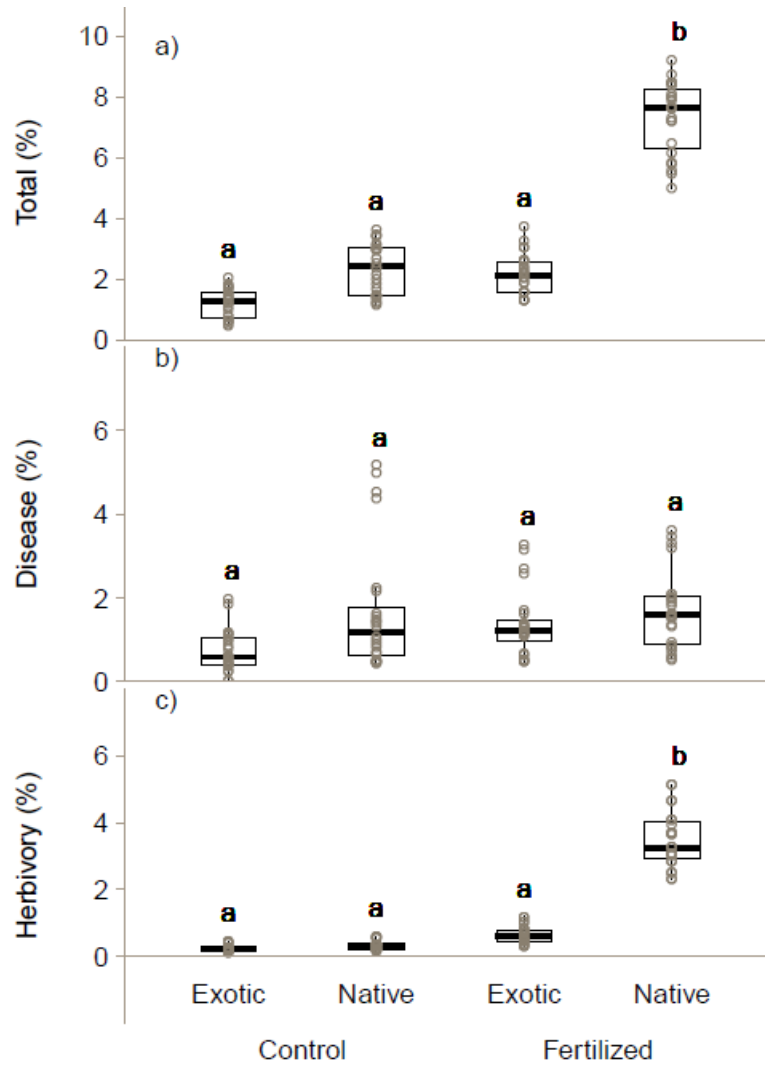


Fig. 2.2 Effects of vertebrate herbivore exclusion (fencing) and fertilizer application on mean exotic cover (the ratio of exotic cover to total cover) in 2011, calculated using restricted maximum likelihood estimation. Points represent fitted values from the model. Shared letters denote no significant differences between treatments based on Tukey HSD.

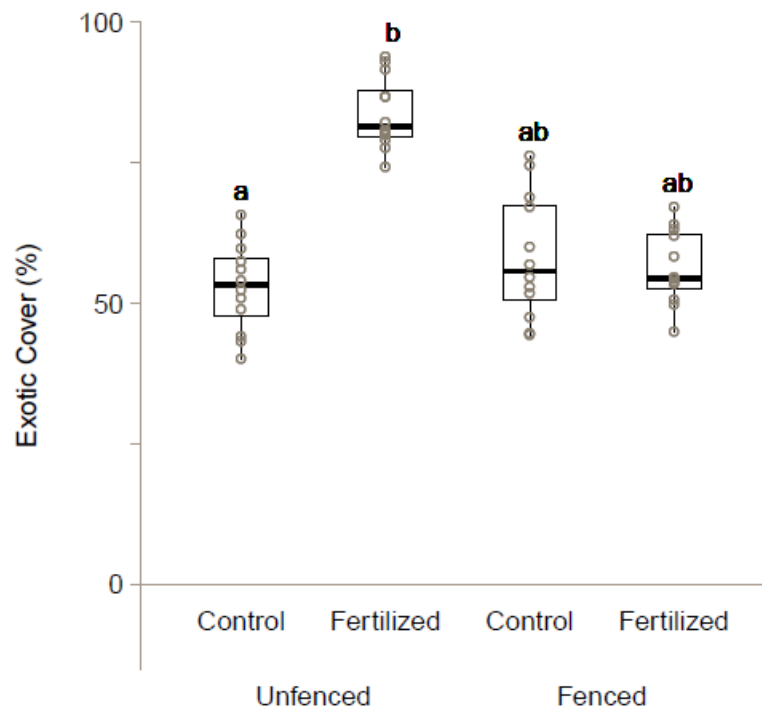


Fig. 2.3 Effects of fungal and insect enemy exclusion (spraying) on mean exotic cover (the ratio of exotic cover to total cover) in 2011 and 2012, calculated using restricted maximum likelihood estimation. Points represent fitted values from the model. Shared letters denote no significant differences between treatments based on Tukey HSD.

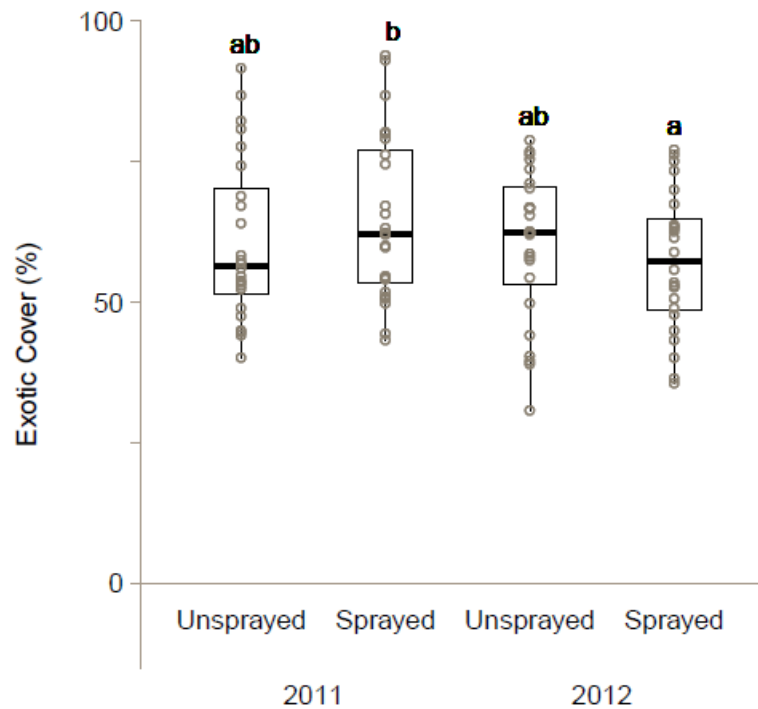
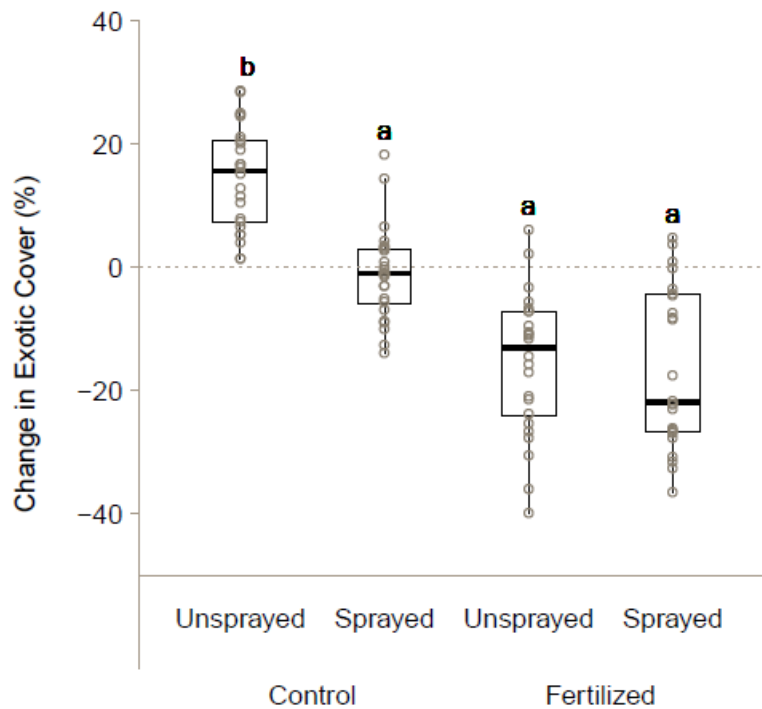


Fig. 2.4 Effects of fungal and insect enemy exclusion (spraying) and fertilization on mean change in exotic cover from 2011 to 2012, calculated using restricted maximum likelihood estimation. Points represent fitted values from the model. Shared letters denote no significant differences between treatments based on Tukey HSD.



CHAPTER III. RESPONSES OF NATIVE AND EXOTIC GRASSES TO DISEASE AND HERBIVORY ACROSS NUTRIENT LEVELS

Introduction

Resource availability and natural enemy pressure (herbivory and disease) may influence exotic success independently (Davis et al. 2000, Keane and Crawley 2002, Shea and Chesson 2002), and may also interact to explain exotic success (Blumenthal 2005, Blumenthal 2006). This interaction may occur because many exotic plants live primarily in high-resource environments (van Kleunen et al. 2010, Leishman et al. 2014), environments where they may benefit considerably from enemy release. In high-resource environments, plants may allocate heavily to growth and less to defense against enemies (Cebrian and Duarte 1994, Endara and Coley 2011), leading to greater impacts of enemies on individuals and populations. Thus, exotic species that have been released from enemy pressure may benefit from increased resource availability without facing the larger enemy impacts that co-occurring native species experience (Blumenthal 2006). Ultimately, this interaction between resource availability and natural enemies might allow exotics to dominate plant communities.

According to the Resource-Enemy Release Hypothesis (R-ERH; Blumenthal 2005, 2006), resource availability and enemy pressure should interact to influence exotic success when two conditions hold: 1) exotic species are released from enemies relative to native species; 2) enemy impacts increase with the resource availability to which plant species are adapted.

Condition 1: Exotic species are released from enemies relative to native species

In a meta-analysis examining tests of the enemy release hypothesis, Heger and Jeschke (2014) found that support for enemy release varied with comparison type. In studies comparing effects of enemies in species' native and introduced ranges, $\frac{3}{4}$ found evidence for enemy release (Heger and Jeschke 2014). In studies comparing effects of enemies between native and co-occurring exotic species, however, nearly half failed to detect enemy release (Heger and Jeschke 2014). These discrepancies may partly result from trade-offs across plant species in allocation of resources to growth and defense against enemies (Blumenthal 2005, Blumenthal 2006).

Condition 2: Enemy impacts increase with the resource availability to which plant species are adapted

The impacts of natural enemies on plants may also vary across resource gradients because plants face a trade-off between resource conservation and resource acquisition (Coley et al. 1985, Stamp 2003). High-resource environments may favor individuals and species with resource-allocation strategies that promote growth through rapid resource acquisition; however, maximizing growth should limit investment in defense against enemies (Coley et al. 1985, Herms and Mattson 1992). Moreover, these poorly-defended species often possess high-quality tissue—especially high foliar nutrient content—which is beneficial to herbivores (Mattson 1980). Thus, herbivores and pathogens should more heavily impact quick-growing, poorly-defended species adapted to high-resource environments. This trade-off in allocation to growth versus defense occurs in many systems (Fine et al. 2004, Endara and Coley 2011, Lind et al. 2013).

A consequence of the trade-off in allocation to growth versus defense can occur within populations and communities: increasing resource availability can increase enemy damage (Mitchell et al. 2003, Dostál et al. 2013). Such an increase in damage can occur by two non-

exclusive mechanisms. First, allocation to growth and defense may primarily vary *between* rather than *within* species (i.e., defense investment within species is constant). According to this mechanism, changes in enemy pressure or resource availability alter competitive interactions between plant species (Blumenthal 2006, Heckman et al. 2016). Increasing resource availability could therefore promote larger enemy impacts on plant communities by replacing well-defended, slow-growing species with poorly-defended, quick-growing species (Lind et al. 2013). Second, allocation to growth and defense may vary considerably within species depending on the environment (i.e., defense investment within species is plastic). According to this mechanism, individuals allocate to growth and defense based on environmental conditions, such as resource availability. Increasing resource availability could promote larger enemy impacts on plant communities by stimulating greater allocation to growth (and less to defense) in individuals, which also enhances their nutrient content, making them more favorable to herbivores (Throop and Lerda 2004, Dostál et al. 2013, Veresoglou et al. 2013, but see Dawson et al. 2014). Because plant species may differ in their tolerance of enemy damage, the same amount of damage could impact species differently (Mitchell et al. 2006, Chun et al. 2010, Agrawal 2011). Thus, in addition to quantifying foliar damage, it is important to consider the effects of enemies on plant growth, survival, or reproduction.

If both conditions—that exotic species are released from enemies relative to natives, and that enemy impacts increase with the resource availability to which plant species are adapted—hold, the trade-off in allocation to growth and defense should be stronger for natives than for exotics (Blumenthal 2005, Blumenthal 2006). To our knowledge, this prediction has never been fully tested.

The goal of this study was to determine whether the impacts of natural enemies are correlated with plant resource-allocation strategy, and whether this correlation differs between native and exotic species. Specifically, we tested two conditions of R-ERH: (1) native species are more heavily impacted by enemies than exotic species, and (2) species that benefit more from nutrient addition (i.e., quick-growing species) also benefit more from enemy exclusion (i.e., species heavily impacted by enemies). Furthermore, we tested whether this correlation is weaker for exotics because they benefit less than natives from enemy exclusion. We then tested a possible explanation for variation in enemy impacts across species: resistance to enemies depends on resource supply to plants and foliar nutrient content, an indicator of plant quality for natural enemies. To do this, we manipulated the supply of soil nutrients and access by aboveground fungal pathogens and insect herbivores to six native and five exotic grass species common in North Carolina old fields.

Methods

We performed this study in an old field in Duke Forest (Durham, NC) on individual plants of eleven co-occurring grass species that differed in provenance (native to North Carolina vs. exotic). Our species pool comprised six native and five exotic grasses. We factorially manipulated soil nutrient supply and enemy access to individual plants at the whole-plot level and the identity of individual species at the subplot level, within a randomized complete block design. This yielded a study that comprised 220 plants ($11 \text{ species} \times 5 \text{ replicate blocks} \times 2 \text{ nutrient supply levels} \times 2 \text{ enemy access levels}$).

In this study, we used 11 perennial grass species common in old fields in the North Carolina Piedmont, which were present at our research site. We chose only species that occurred locally to increase the likelihood that natural enemies would be present to exploit each species,

and to focus on species that are likely to compete. Thus, instead of using phylogenetically-paired native and exotic species (e.g., Agrawal et al. 2005, Dawson et al. 2014), we chose to focus on co-occurring species within a single family (Poaceae) in order to maximize comparisons of ecological relevance. Three of the five exotic species and one of the six native species used the C₃ photosynthetic pathway. One species utilized a photosynthetic pathway intermediate between C₃ and C₄ (*Steinchisma hians*), while the other six species used C₄ photosynthesis. We collected seed for nine of these species locally, and purchased seed for two species from commercial seed sources (Table 3.1).

To improve germination rates, we stratified all seeds in moist sand at 4°C for at least one month prior to sowing. Next, in the greenhouse at the University of North Carolina at Chapel Hill, we sowed up to 15 seeds of each species into one of twenty 0.94 L pots (Deepots, Stuewe and Sons, Corvallis, OR). At the two-leaf stage, we thinned each pot to a single individual. We grew these plants in the greenhouse for four weeks, then moved them to a semi-shaded outdoor area for an additional week to acclimate the plants to higher light intensity and temperature. After hardening for a week, on 23 June 2014, we moved all plants to the field. Each pot was buried (~ 20 cm) so that the soil level in the pot was flush with the ground, and spaced far enough apart (~ 15 cm) to avoid most contact between individual plants. The entire experimental area was surrounded by a fence to exclude vertebrate herbivores. We watered plants when necessary.

We grew all plants in a 50:50 mixture of Fafard 3B soil (Sun Gro Horticulture, Agawam, MA) and sterilized sand. The high sand content of the soil mix allowed finer control of nutrient supply. To avoid limitation by micronutrients, all plants received the equivalent of 100 g m⁻² of micronutrients as Scott's Micromax (Marysville, OH). Each plant in the high-nutrient treatment

received the equivalent of 10 g m⁻² each of N, P, and K each plant in the low-nutrient treatment received the equivalent of 2 g N, P, and K m⁻². To create these two levels of nutrient supply, we added slow-release forms of phosphorus, as triple super phosphate, and potassium, as potassium sulphate, directly to the soil mix. Because slow-release nitrogen is more volatile than potassium or phosphorus, and an aqueous solution is readily available, beginning on 26 June 2014 and ending on 21 August 2014, we applied nitrogen to pots in five biweekly applications of aqueous ammonium nitrate (NH₄⁺NO₃⁻).

Once the plants were in the field, we began the enemy exclusion treatment. We applied a contact foliar fungicide (Mancozeb, Dithane DF) and insecticide (es-fenvalerate, Asana XL) bi-weekly to all aboveground tissue on each plant. A previous study found no significant effect of either biocide on plant growth under greenhouse conditions (Heckman et al. 2016).

We collected three types of data on these plants: foliar damage, foliar functional traits, and above-ground biomass. We harvested the youngest fully emerged leaf on each plant in September 2014 for functional trait analysis. After removing each leaf, we placed it flat on a moist paper towel in a plastic bag for up to eight hours, until we could scan the leaf and measure its area. We then dried each leaf at 60°C for at least 72 hours before weighing the leaf. We calculated leaf mass per area (LMA) as the ratio of fresh leaf area to dried leaf mass following Pérez-Harguindeguy et al. (2013). LMA is a measure of leaf construction costs that is often ecologically relevant to plants. After calculating LMA, we ground leaves to a fine powder in a ball grinder. Samples were analyzed at the Environmental Chemistry lab at the University of Georgia for foliar nitrogen and carbon content by mass. Foliar N content (by mass) is highly correlated by photosynthetic capacity and other traits that indicate the resource allocation

strategy of a plant (Wright et al. 2004, Reich 2014). Furthermore, foliar N content indicates plant tissue quality for herbivore nutrition (Mattson 1980).

We surveyed each plant for damage by natural enemies three times—on 5 August, 29 August, and 19 September 2014. During each survey, we assessed foliar damage on five leaves from each plant. To sample across leaf age classes, we haphazardly selected a focal tiller (ramet) on each plant, assessed the oldest leaf, then assessed each progressively younger leaf, up to five leaves total. If that tiller had fewer than five leaves, we repeated with additional tillers until five leaves were assessed. On each leaf, we visually estimated the percent of leaf area damaged by fungal pathogens, insect herbivores, and mollusk herbivores by visually comparing damage on leaves to reference images of leaves of known damage severity (James 1971, Mitchell et al. 2002, Mitchell et al. 2003).

We harvested all aboveground biomass in October 2014. Because our goal was to determine enemy impacts on plant performance, we maximized the time that plants were exposed to enemies in the field. As a consequence, the roots of some individuals of several species grew out of the bottom of the pot and could not be recovered. Therefore, we use aboveground biomass to indicate plant performance.

To test the prediction that species benefitting most from fertilization would benefit most from enemy exclusion, and that the exotics benefitting most from fertilization would benefit less than natives from enemy exclusion, we calculated the change in aboveground biomass of each species in response to fertilization and enemy exclusion (hereafter, response to fertilization and response to enemy exclusion). To calculate the response to fertilization:

$$\frac{(\text{High nutrients, sprayed, species } i, \text{ block } j - \text{Low nutrients, sprayed, species } i, \text{ block } j)}{\text{Low nutrients, sprayed, species } i, \text{ block } j} \times 100$$

To calculate the response to enemy exclusion:

$$\frac{(\text{Sprayed species } i, \text{ block } j, \text{ nutrients } k - \text{Unsprayed species } i, \text{ block } j, \text{ nutrients } k)}{(\text{Unsprayed species } i, \text{ block } j, \text{ nutrients } k)} \times 100$$

where species_i = 1 of the 11 grass species in the study, block_j = 1 of 5 replicates of each resource × enemy exclusion treatment combination, and nutrients_k = 1 of 2 nutrient supply treatments.

Thus, for each species × block combination, there was one response to fertilization and two responses to enemy exclusion (high and low nutrients). Because we could not attribute mortality directly to either of our treatments, response to nutrient and enemy treatments were only calculated for blocks in which both plants used in the calculation survived until the end of the study. We then calculated the mean response of each species (i.e., averaged across five blocks) to fertilization, and to enemy exclusion at high and low nutrient supply.

In order to analyze the relationship between response to fertilization and response to enemy exclusion, we followed Lind et al. (2013) and performed standardized major axis regression (SMA regression) using the ‘sma’ function in the smatr package in R (Warton et al. 2012). Standardized major axis regression is used to calculate the slope of a relationship between two variables where no clear causal relationship exists; unlike linear regression, SMA regression assumes that both variables are measured with error (Warton et al. 2006). The ‘sma’ function can be used to analyze the relationship between two variables across levels of a single grouping variable. For response to enemy exclusion, we used this single grouping variable to account for both nutrient supply treatment and the provenance of each species, by creating a grouping variable with four levels (e.g., exotic-low nutrients, native-low nutrients). The relationship between response to fertilization and response to enemy exclusion did not differ between these four groups, so we dropped nutrient supply from the model. We report the results of this reduced

model, which examined whether the relationship between species' response to fertilization and response to enemy exclusion is modulated by the species' geographic provenance.

To assess potential phylogenetic autocorrelation, we created a phylogeny using Phylomatic v. 3 (Webb and Donoghue 2005), which included our 11 target species and *Juncus tenuis* (from a different but closely related family) as an outgroup species. Using the phylotools package in R (Revell 2012), we created a correlation matrix between these 11 species, which we used to test for phylogenetic signal in response to fertilization, response to enemy exclusion, and foliar N content. Using Blomberg's K in the 'phylosig' function in picante (Kembel et al. 2010), we found no phylogenetic signal for any of these three variables. Thus, we present results for analyses without phylogenetic structure.

To assess potential explanations for the relationship between response to fertilization and response to enemy exclusion, we quantified the effects of nutrient supply, provenance, and foliar N content on foliar fungal disease, insect herbivory, and mollusk herbivory to unsprayed plants (at the plant-level across the three surveys) using the 'lmer' function in lme4 (Bates et al. 2012) and lmerTest (Kuznetsova et al. 2016). Because incidence of fungal disease, insect herbivory, and mollusk herbivory were each considerably < 100 % (70%, 60%, and 20% of plants, respectively), analyzing damage severity by damage type was heavily zero-inflated, leading to violations of the homoscedasticity and normality assumptions. To account for this, we combined all damage types (fungal disease, insect herbivory, and mollusk herbivory) into a single analysis of total foliar damage. We quantified the severity of each damage type separately on each plant by averaging the percent leaf area damaged across all leaves within each of the three surveys. We then calculated the area under the disease progress stairs (AUDPS) for each plant across the three surveys using the package agricolae (de Mendiburu 2016) in R version 3.2.2 (R Foundation

for Statistical Computing, Vienna). Like the area under the disease progress curve (AUDPC), AUDPS integrates the seasonal development of disease or herbivory progress into a single value, but improves estimates of the first and last observations (Simko and Piepho 2012). Because AUDPS and AUDPC integrate foliar damage over time, they estimate the disease and herbivory experienced by each plant over the season (Madden et al. 2007). We cubed-root transformed total damage to improve normality of residuals and reduce heteroscedasticity. Nutrient supply and provenance were whole-plot categorical predictors and foliar N was a subplot continuous predictor; the model also included crossed random effects of species identity, block, and whole-plot (to account for the nested nature of individual plants within the nutrient supply treatment).

Results

Enemy exclusion reduced foliar fungal disease by 85% ($P < 0.001$, Appendix B2.1a, Appendix B1.1), but did not significantly reduce foliar insect herbivory ($P = 0.19$, Appendix B2.1b, Appendix B1.1). This probably occurred because insect herbivory was low when enemies had access to plants; consequently, spraying could not further reduce herbivory. Because median fungal disease was considerably higher than median insect or mollusk herbivory, enemy exclusion reduced total damage (fungal disease + insect and mollusk herbivory) by $> 80\%$ ($P < 0.001$, Appendix B2.1c).

Total foliar damage (disease + herbivory) did not differ between native and exotic species ($P = 0.63$, Appendix B2.2, Fig.3.1), contrary to the first condition of R-ERH that exotic species are released from enemies relative to natives. Moreover, nutrient addition increased total foliar damage by $> 80\%$, but this effect was only marginally significant ($P = 0.079$, Appendix B2.2, Fig. 3.1). Additionally, the relationship between foliar N content and total foliar damage depended on nutrient supply ($P = 0.011$, Appendix B2.2, Fig. 3.2): the increase in total damage

with foliar N content was significantly higher in high nutrient pots than in low nutrient pots. This suggests that despite the strong positive relationship at the species-level between response to fertilization and response to enemy exclusion, nutrient supply may not strongly influence how individuals allocate resources to growth versus resistance to disease and herbivory.

Across all species (native and exotic), there was a strong positive relationship between the response to enemy exclusion and the response to fertilization (slope = 1.05, $R^2 = 0.25$, $P = 0.017$, Fig. 3.3). This supports one condition of R-ERH, that enemy impacts increase with the resource availability to which species are adapted. But, because the relationship between the response to fertilization and the response to enemy exclusion did not differ by provenance ($P = 0.45$), this fails to support the prediction that enemy impacts increase to a greater degree with the resource availability to which plant species are adapted in natives than exotics. Thus, these results do not fully support R-ERH.

Discussion

We found limited support for the conditions of R-ERH. Contrary to R-ERH, total foliar damage did not differ between native and exotic species. This suggests that exotics were not released from enemies relative to natives. Consistent with R-ERH, there was a positive relationship between enemy impacts and the response of species to fertilization, indicating that species which are adapted to high resource environments are also heavily impacted by natural enemies. This result also supports the growth rate hypothesis of plant defense evolution (Coley et al. 1985, Stamp 2003, Endara and Coley 2011). However, this relationship was similar for native and exotic species. Thus, these results support only one condition necessary for R-ERH (Blumenthal 2006). This is partially contrary to one study, which found that exotic plants adapted to high-resource environments lose more pathogens from their native ranges than exotic

plants adapted to low-resource environments (Blumenthal et al. 2009). Together, this study supports others showing that exotic species may not always be released from natural enemies, but that trade-offs between allocation to growth and defense may be more general (e.g., Lind et al. 2013).

Several factors could explain why native and exotic species responded similarly to enemy exclusion in this study. First, severity of total foliar damage from natural enemies did not differ by species' provenance. This result suggests two non-exclusive mechanisms: that exotic species were not able to escape their natural enemies; and that native and exotic species did not differ in resistance to enemies. However, assessing damage may not be the most effective way to compare enemy impacts among plant species. This is because a second possibility exists: plant species may differ in their tolerance of damage (Mitchell et al. 2006, Chun et al. 2010, Agrawal 2011), and different enemy guilds may impact plant performance differently (Aldea et al. 2006, Allan and Crawley 2011). For instance, the same quantity of visible damage may reduce the performance of each species by a different amount. In fact, some attributes of successful invasive species, like rapid growth rates (van Kleunen et al. 2010, Leishman et al. 2014), may also promote increased tolerance of damage (Agrawal 2011). Thus, differences in tolerance of damage between species could compensate for any differences in foliar damage seen in this study.

Studies comparing native and exotic species often select native-exotic pairs that are closely-related phylogenetically (e.g., Agrawal et al. 2005, Dostál et al. 2013, Dawson et al. 2014), and are thus likely to share enemies, making them conservative tests of enemy release (Mitchell et al. 2006). Furthermore, experimental phylogenetic pairs may not compete within natural communities. On the other hand, comparisons between phylogenetically-distant species

that interact within communities may be more ecologically relevant (e.g., Vasquez and Meyer 2011, Parker et al. 2015), but these comparisons may be confounded by the species' distinct evolutionary histories and ecological traits. A compromise that is potentially informative could involve selecting multiple species from a single phylogenetic group that occur in the same habitat and are likely to interact (e.g., Pearson et al. 2011). The grass family (Poaceae) is widespread, species-rich, and its members are important components of many ecosystems worldwide, making grasses as ideal system in which to test enemy release. However, this may also explain why exotics were not released from enemies. Because the species in this study were related and in close physical proximity, enemies may have been able to exploit both native and exotic species. Another possible explanation for these results is that grasses typically possess greater concentrations of quantitative defenses like silica, than other taxonomic groups (Moles et al. 2011). Because grasses primarily exhibit quantitative defenses against enemies (Adler et al. 2004), they may be more susceptible to evolutionarily-naïve pathogens and herbivores than taxonomic groups that exhibit primarily qualitative defenses (Joshi and Vrieling 2005). Consequently, release from enemies may be weaker in grasses than in some other groups.

Upon introduction to a new range, exotic species are exposed to new natural enemies, most of which are not specialized to exploit these new potential hosts. Generalist natural enemies, which are already present in the introduced range, may also equally favor native and exotic species, or prefer exotics (Parker and Hay 2005, Reinhart et al. 2010, Halbritter et al. 2012), limiting the benefit of escaping natural enemies from their native range. Furthermore, escape from natural enemies may be transient. Over time, pathogens and herbivores can adapt to exploit introduced species, limiting enemy release (Stricker et al. 2016), especially on widespread species that were introduced long ago (Mitchell et al. 2010). This may help explain

why exotics were not released from enemies in our study; three of the five exotic grasses are widespread throughout North America, while the other two exotic species are widespread throughout the southeastern United States. Moreover, each of these exotic species was present in North America by 1875 (Hitchcock 1971, Grant 1978, Mitchell et al. 2010).

Although enemy exclusion experiments provide valuable information about enemy impacts that are more difficult to determine from studies that measure enemy damage (e.g., Mitchell et al. 2006, Allan et al. 2010, Allan and Crawley 2011, La Pierre et al. 2015, Seabloom et al. 2015), enemy exclusion has drawbacks. Because enemies are being removed rather than added, these studies depend on ambient enemy pressure; however, enemy pressure can vary considerably within a year, and can also vary between years based on many factors, including the weather (Agrawal et al. 2005). This may be more acute when comparing across species (Mitchell et al. 2006). In this study, enemy exclusion did not reduce the severity of insect herbivory, perhaps because enemy pressure was too low to be influenced by pesticide application. If enemy pressure had been higher, it might have been possible to see larger differences in enemy impacts between exotic and native species. Nonetheless, enemy exclusion experiments are often relevant for explaining the influence of natural enemies on community-level outcomes (Keane and Crawley 2002, Shea and Chesson 2002, Mitchell et al. 2006). For instance, ambient enemy pressure is likely to influence an individual's ability to compete and coexist with neighbors (Chesson and Kuang 2008). Adding enemies may lead to artificial conclusions: herbivores avoid some exotic species in the field, but will consume these species in feeding assays. Thus, enemy addition studies demonstrate the range of ecological possibilities, but fail to convey the likely conditions that plants experience in the field.

In other studies, fertilization has increased damage by natural enemies (Veresoglou et al. 2013, Heckman et al. 2016). This may occur because plants face a fundamental trade-off between growth and defense against enemies (Coley et al. 1985, Stamp 2003, Endara and Coley 2011). In high resource environments, where tissue is relatively cheap to produce, there should be a strong evolutionary benefit to rapidly acquiring new tissue rather than defending pre-existing tissue (Coley et al. 1985, Stamp 2003). Because individuals in high resource environments exhibit high quality (highly nutritious) tissue that is poorly defended against enemies (Mattson 1980), they should support large enemy populations. This may lead to populations that are strongly regulated by natural enemies. Supporting this idea, here foliar damage, an indicator of enemy pressure, increased with foliar N content in high nutrient pots, but not in low nutrient pots. Moreover, fertilization increased total foliar damage only marginally significantly. This increase was large—80%, on average, but because nutrient supply was a whole plot treatment, we had little power to detect significant differences. This marginally significant effect may have also resulted from integrating multiple types of damage (fungal disease, insect herbivory, mollusk herbivory), rather than any individual functional group of enemies responding to increasing nutrient supply.

Species using the C₃ photosynthetic pathway typically possess higher foliar N content than species using the C₄ pathway (Barbehenn et al. 2004). Because three of five exotic grasses and only one of six native grasses in this study use the C₃ pathway, exotic species should, on average, possess greater foliar N content than C₄ species (Scheirs et al. 2001). If enemy damage is primarily driven by foliar N content, then C₃ species should be impacted more by enemies than C₄ species regardless of provenance. Thus, exotics may have been released from enemies relative

to natives with the same photosynthetic pathway, but this effect would have been difficult to detect due to the confounding effects of provenance and photosynthetic pathway in this study.

Importantly, the predictions of R-ERH, that exotic species will benefit most from increasing resource availability can occur *between* species and *within* species (Heckman et al. 2016). In other words, fertilization promotes increasing dominance by species adapted to high resource environments, but fertilization may also promote more resource-acquisitive phenotypes within a species. Thus, comparing species that differ in their inherent resource allocation strategies across resource gradients can help to determine the importance of each mechanism. Because the response to enemy exclusion did not differ with nutrient supply, and the severity of total damage increased only marginally significantly with increasing nutrient supply, our results suggests that *interspecific* changes in community composition, or increasing dominance by species adapted to high-resource environments often drives the community's response to fertilization (Hahn and Maron 2016).

Overall, our results provide partial support for R-ERH. Species adapted to high-resource environments were impacted most strongly by herbivores and pathogens. Contrary to R-ERH, we found little evidence that exotics were released from enemies relative to natives. Together, this suggests that a trade-off between allocation to growth and defense exists across species, and that this trade-off is general across exotic and native species.

Table 3.1. Grass species (family Poaceae) used this study, provenance in North Carolina (native, exotic), photosynthetic pathway (C3, C4, C3/C4 intermediate), and the source of seed. Ernst = Ernst Conservation Seeds, (Meadville, PA), Everwilde = Everwilde Farms (New Auburn, WI), Local = seeds collected at Widener Farm, Durham, NC.

Species	Provenance	Pathway	Source
<i>Andropogon virginicus</i> L.	Native	C4	Local
<i>Anthoxanthum odoratum</i> L.	Exotic	C3	Local
<i>Dactylis glomerata</i> L.	Exotic	C3	Local
<i>Danthonia spicata</i> (L.) P. Beauv. ex Roem. & Schult.	Native	C3	Everwilde
<i>Paspalum dilatatum</i> Poir.	Exotic	C4	Local
<i>Schedonorus arundinaceus</i> (Schreb.) Dumort.	Exotic	C4	Local
<i>Setaria parviflora</i> (Poir.) Kerguélen	Native	C4	Local
<i>Sorghastrum nutans</i> (L.) Nash	Native	C4	Local
<i>Sorghum halepense</i> (L.) Pers.	Exotic	C4	Local
<i>Steinchisma hians</i> (Elliot) Nash	Native	C3/C4	Local
<i>Tridens flavus</i> (L.) Hitchc.	Native	C4	Ernst

Fig. 3.1 Effects of nutrient supply and provenance on severity of total foliar damage (fungal disease, insect herbivory, mollusk herbivory), calculated using restricted maximum likelihood estimation and back-transformed from a cubed-root transformation. Points represent fitted values from the model, error bars represent 95% confidence intervals.

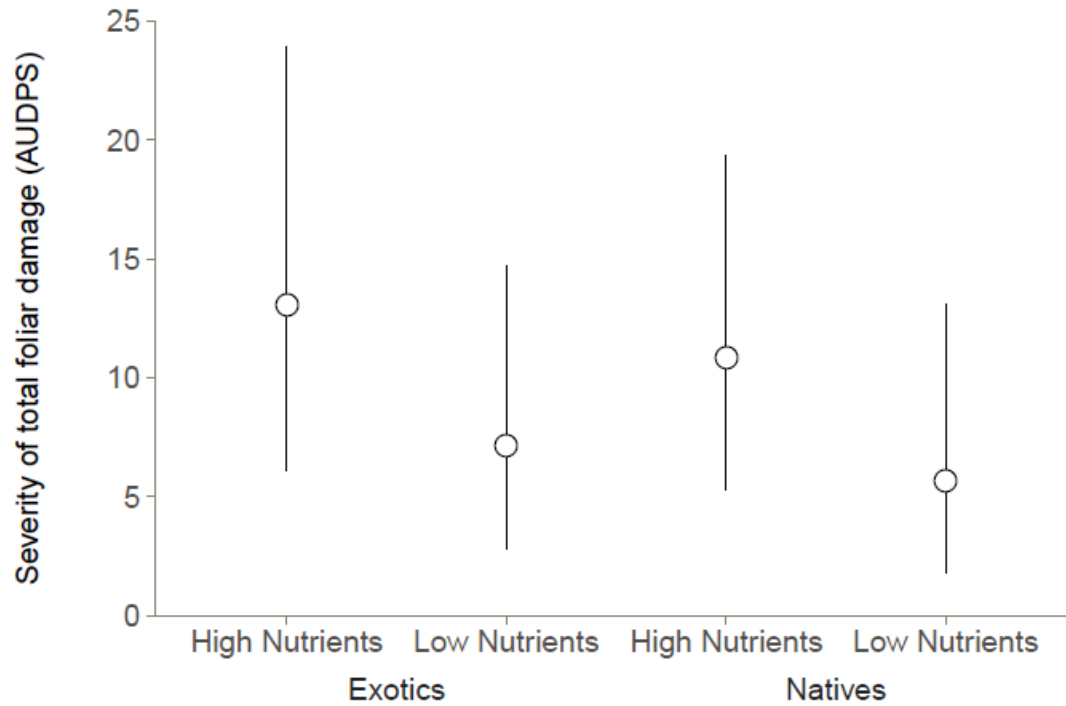


Fig. 3.2 Influences of standardized foliar N content on severity of total foliar damage (fungal disease, insect herbivory, mollusk herbivory), by nutrient supply. Individual points represent raw values. Model-fitted trend lines were back-transformed from a cubed-root transformation.

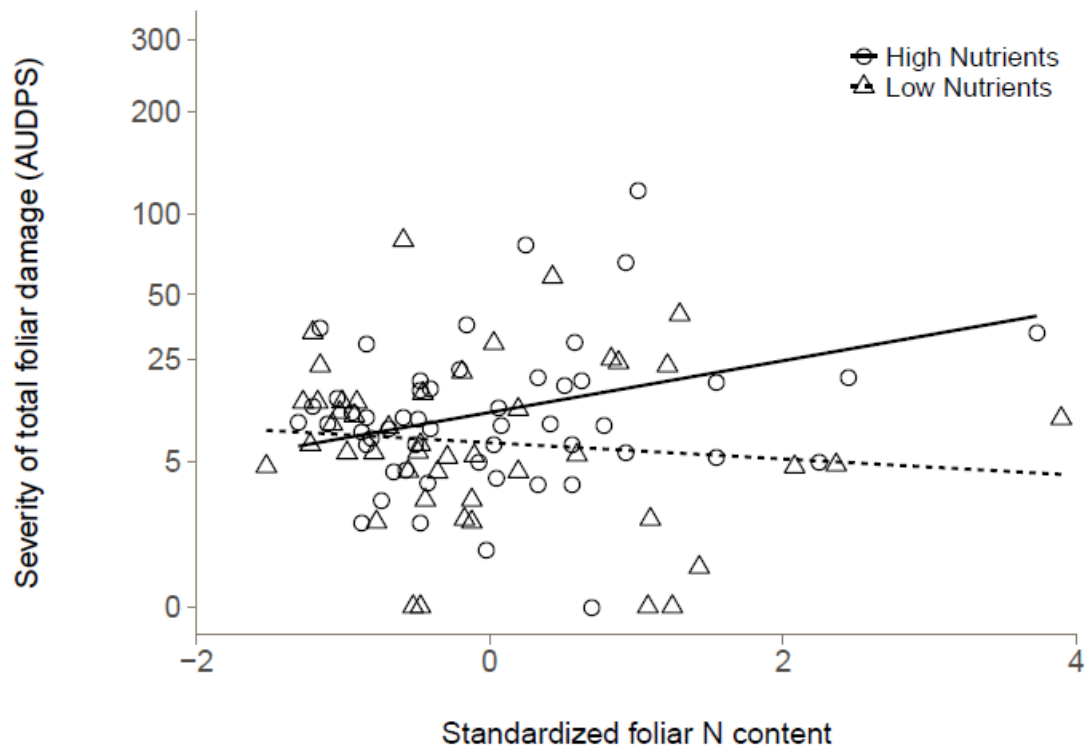
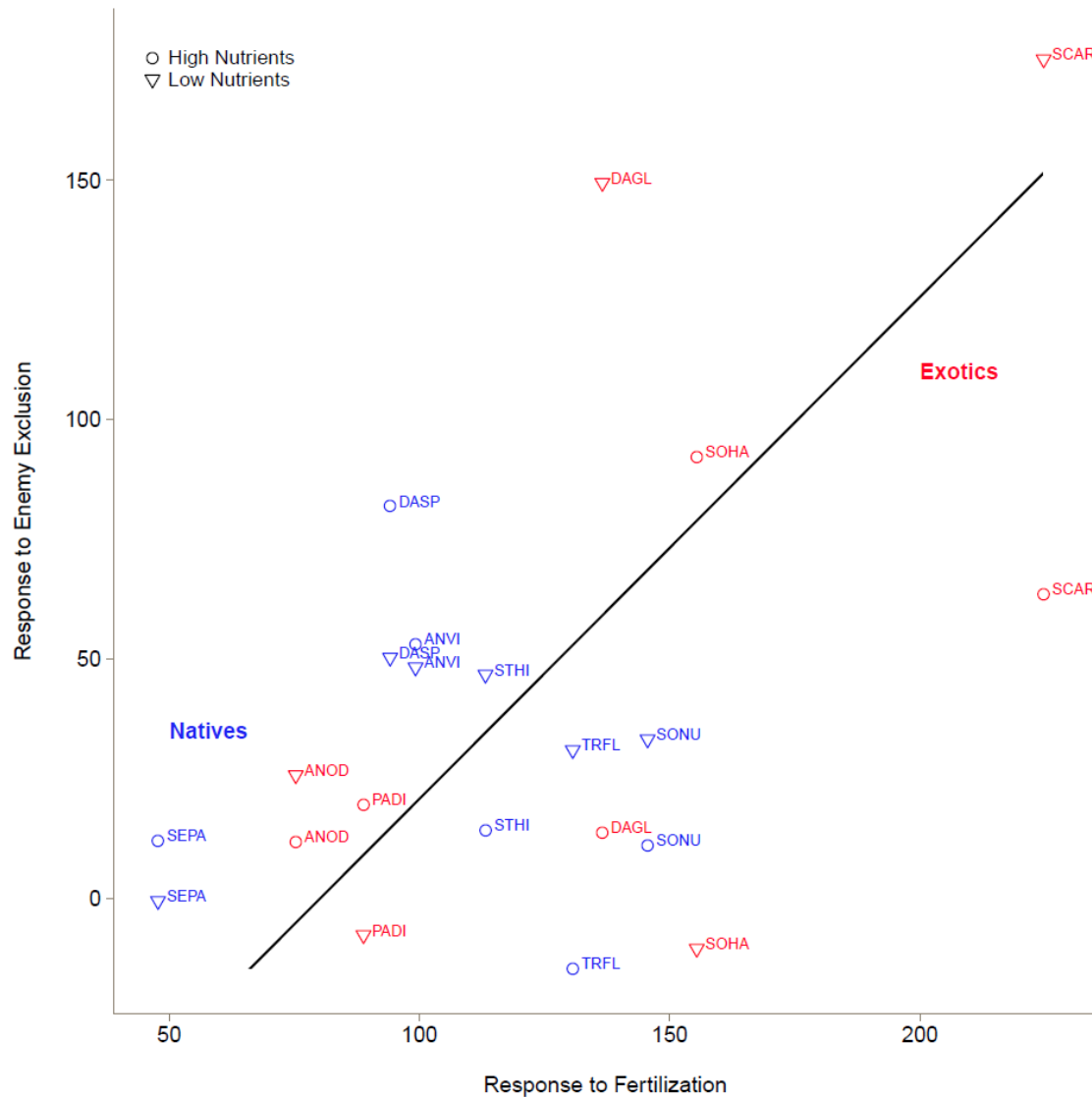


Fig. 3.3 Relationship between response to fertilization and response to enemy exclusion by provenance (native vs. exotic species). Response to fertilization is the percent change in biomass from low to high nutrient supply in sprayed (enemy-exclusion) pots, averaged across all five blocks for each species. Response to enemy exclusion is the percent change in biomass from unsprayed to sprayed pots, calculated separately for high-nutrient and low-nutrient pots, and averaged across all five blocks for each species. Red points represent exotic species and blue points represent native species. Circles represent response to enemy exclusion at high resource supply; triangles represent response to enemy exclusion at low resource supply.



CHAPTER IV. THE ROLES OF NATURAL ENEMIES, LIGHT, AND NUTRIENTS IN COLONIZATION OF EXOTIC-DOMINATED OLD-FIELD COMMUNITIES BY NATIVE SPECIES

Introduction

In many habitats, establishment of new populations within communities is influenced by a combination of biotic and abiotic factors. These factors may also contribute to community assembly. Within plant communities, seedling establishment can be influenced by nutrient availability and enemy pressure, two factors that might be particularly important because they can alter community dynamics in several ways. While increased nutrient availability can have positive effects on establishment, by reducing nutrient limitation, it can also indirectly reduce the availability of other resources like light and water (Rose et al. 2012, Borer et al. 2014b), which are required by establishing seedlings. Moreover, several factors, including species traits and nutrient availability can influence plant-enemy interactions, increasing disease and herbivory to individuals (Throop and Lerdaun 2004, Veresoglou et al. 2013) and communities (Heckman et al. 2016). Because there are multiple potential mechanisms, ultimately, the effects of nutrients and enemies on seedling establishment depend on the strength of each mechanism.

Nutrient addition can influence seedling establishment in numerous ways. When the resident community is unable to fully sequester available nutrients (i.e., nutrient supply is greater than nutrient uptake), the surplus nutrients can facilitate seedling establishment (Davis et al. 2000). Alternatively, when the resident community is able to sequester most of the added nutrients (i.e., nutrient supply is equal to nutrient uptake), nutrient addition may reduce seedling

establishment and survival (Renne et al. 2006). The latter scenario is likely to occur in communities like perennial grasslands, which are dominated by large, long-lived individuals.

In perennial-dominated grasslands, nutrient addition frequently increases aboveground plant productivity (Fay et al. 2015), which can affect the availability of other resources. For instance, increased productivity can increase transpiration, reducing water availability (Rose et al. 2012). Increased productivity can also reduce light availability (Borer et al. 2014b), by producing large amounts of standing vegetation and leaf litter (Goldberg and Werner 1983, Foster and Gross 1998, Wilsey and Polley 2003). This may promote priority effects that can benefit larger, established residents and limit establishment of seedlings, which are considerably smaller (Craine and Dybzinski 2013).

Nutrient supply and leaf litter may also influence plant-enemy interactions. Fertilization can increase plant tissue quality, leading to higher rates of herbivory and disease (Mattson 1980, Veresoglou et al. 2013). Leaf litter can also alter interactions between the plant community and their natural enemies: litter may promote fungal disease by increasing the prevalence of overwintering fungal spores that can attack new vegetation (García-Guzmán and Benítez-Malvido 2003, Roy et al. 2014). Leaf litter also provides habitat for insect herbivores (Karban et al. 2012). Thus, if more litter accumulates following fertilization, this may increase disease and herbivory. Alternatively, more leaf litter may reduce disease and herbivory by obscuring growing vegetation from foraging insects and dispersing fungal spores.

Effects of nutrients and enemies on seedling establishment may depend on the provenance (native or exotic) of colonizing species and the resident community. Because many habitats are already dominated by exotic species, it may be advantageous to examine natural or experimental colonization of exotic-dominated communities by native species. For instance,

Davis and Pelsor (2001) found that colonization is greatest following resource addition by adding native ‘invaders’ to exotic-dominated communities. Further, because resource addition can influence plant-enemy interactions, colonization and establishment may depend on the pressure that enemies exert. In high-resource environments, enemy impact is often higher than in low-resource environments (Mattson 1980, Wardle et al. 1998, Heckman et al. 2016).

Additionally, the provenance of resident and colonizing species may influence establishment. If exotic species are released from enemies, enemies should reduce the fitness of native individuals without influencing exotics (Keane and Crawley 2002). Thus, in exotic-dominated communities colonization by natives should be reduced when enemies are present, and this effect of enemies should be largest in high-resource environments (Blumenthal 2005, Blumenthal 2006).

Alternatively, if exotics are not released from enemies, enemies may benefit natives by suppressing exotic residents.

Species’ responses to nutrient availability may be associated with broad trait syndromes (Craine 2009, Reich 2014). For instance, species that grow well in high-resource environments typically possess resource-acquisitive traits—high growth rate, photosynthetic rate, and foliar nutrient content (Wright et al. 2004, Valladares and Niinemets 2008, Reich 2014, Díaz et al. 2016), but due to physiological trade-offs, these species allocate minimally to defense against enemies (Coley et al. 1985, Stamp 2003). These tradeoffs may lead to predictable changes in community composition: species that benefit from high-resource environments may be most strongly impacted by enemies, and are likely to benefit most from enemy exclusion (Lind et al. 2013). One way to assess the consequences of this trade-off between growth and defense against enemies involves adding propagules of native species that differ in resource-allocation strategies to exotic-dominated communities varying in nutrient supply and enemy access.

In this study, we tested whether multiple factors interact to influence community structure. To do this, we experimentally manipulated soil nutrient supply (nitrogen, phosphorus, and potassium), attack by insect herbivores and fungal pathogens, and the presence of leaf litter in exotic-dominated grassland communities to which we added seeds of 11 native species. Because soil nutrient addition, litter removal, and enemy exclusion can influence native establishment by multiple mechanisms, we measured light and water availability, as well as disease and herbivory in these communities. We predicted that 1) invaded communities with high nutrient supply from which enemies have not been excluded will be least colonized by natives, and 2) native colonizers with quick-return phenotypes will have greater success than those with slow-return phenotypes when nutrient supply is high and enemies are excluded, but not when enemies are present or at low nutrient supply. To determine the mechanisms that most influenced seedling establishment, we used structural equation modelling to analyze the effect of nutrient supply, enemy exclusion, and litter removal on these environmental variables (light and water availability, and disease and herbivory) and, ultimately, native seedling establishment in exotic-dominated communities.

Methods

We performed this study in an old field in the North Carolina Piedmont (Orange Co., NC) that was abandoned in 1996. Since 1996, annual mowing has maintained herbaceous dominance by perennial species, including three dominant exotic species—*Lespedeza cuneata*, *Lonicera japonica*, and *Schedonorus arundinaceus*, which in another study at this site together constituted two-thirds of community cover (Heckman et al. 2016)—as well as several native species typical of North Carolina old fields (Oosting 1942).

This study used a split-plot experimental design. We factorially manipulated soil nutrient supply and access by fungal pathogens and insect herbivores to communities for three years at the whole-plot level. At the subplot level, we manipulated the presence of leaf litter for two years.

In May 2012, we established 40 2×2 m plots with 1 m aisles between plots and began the nutrient supply and enemy access treatments within one month. We manipulated soil nutrient supply with two levels (fertilized with $10 \text{ g m}^{-2} \text{ yr}^{-1}$ N, P, and K vs. not fertilized). This level of fertilization successfully eliminated nutrient limitation to communities of similar composition at our site (Fay et al. 2015). We manipulated fungal pathogen and insect herbivore access to plots at two levels (sprayed with fungicide and insecticide vs. not sprayed). For this purpose, we sprayed plots every two – three weeks throughout the main growing season (April – October) from May 2012 through October 2014 by spraying all aboveground biomass within a plot with non-systemic, broad-spectrum biocides. Neither the fungicide (macozeb, Dithane® DF, Dow AgroSciences, Indianapolis, IN) nor the insecticide (es-fenvalerate, Asana® XL, Dupont, Wilmington, DE) affected plant growth under greenhouse conditions.

This site was dominated by perennial grasses that can produce thick litter layers (~ 5 – 15 cm)—unmanipulated communities reduced light availability at ground-level by ~90% before the main growing season began. To increase light availability, and to reduce overwinter fungal inoculum and habitat for herbivorous insects, in February 2013 we implemented a litter removal treatment at the subplot level. Specifically, we raked all standing dead biomass from one of two randomly-selected 0.5 m^2 subplot within each whole plot twice over the experiment, first in February 2013 and again in March 2014. These two adjacent 0.5 m^2 subplots were in the center

of each 4 m² whole plot. These subplots reduced edge effects and the area over which we surveyed seedling performance (e.g., germination and survival, biomass, foliar damage).

Each year immediately after we implemented the litter removal treatment, we added seeds of 10 native species to each plot at a rate of 1 g seed m⁻² species⁻¹ year⁻¹. This seed addition rate accounts for the general increase in colonization ability with increasing seed size (Tilman 1994, Turnbull et al. 1999). Adding the same number of seeds of each species would likely have biased our results toward greater establishment of large-seeded species (Turnbull et al. 1999). However, we added a greater mass (4 g seed m⁻² year⁻¹) of seeds of one species, *Tripsacum dactyloides*, because its seeds were considerably larger than any other species. Due to their large size, 1 g seed m⁻² would have been insufficient to account for possibly limited germination.

We then followed seedling germination throughout the 2013 and 2014 growing seasons. In 2013, we counted the total number of established seedlings in each subplot once, in September. In 2014, we counted and identified to species all established seedlings in each subplot monthly from June through September. At the end of the experiment in October 2014, we harvested and weighed up to 10 individual seedlings from each subplot. We also quantified foliar disease and herbivory on the harvested seedlings (see below for details).

In 2014, we measured light availability at ground level monthly from March through October using an Accupar LP80 Ceptometer (Decagon Devices, Pullman, WA). Following Borer et al. (2014b), we measured photosynthetically active radiation above, and at two points below, the canopy of each subplot within two hours of solar noon. Light availability is the ratio of light availability at ground level to light availability above the canopy $\times 100$. To simplify our models, we integrated light availability over the entire growing season (March – September), using the ‘auc’ function in the R package MESS (Ekstrøm 2016). Thus, light availability represented the

average proportion of light ($\mu\text{mol PAR m}^{-2} \text{ s}^{-1} \text{ day}^{-1}$) available at ground level at solar noon over the duration of the study.

We also measured soil moisture availability at 10 cm using a time-domain reflectometer (HydroSense Soil Water Content Measurement System, Campbell Scientific Australia) once per month (May – September, 2014). Unlike light availability, there was no strong directional trend in soil moisture availability over the growing season. Thus, we calculated the average soil moisture availability within a subplot as the arithmetic mean of the five soil moisture measurements.

We visually quantified percent cover of each plant species in each subplot in August each year from 2012 through 2014 using a modified Daubenmire method (Daubenmire 1959, Borer et al. 2014a). Because we evaluated cover for each species independently, the sum of cover values for each plot could exceed 100%. To quantify foliar damage to the resident community, we surveyed damage on those most abundant species necessary to account for at least 80% of the total cover of each subplot. For instance, in a plot with three species (A, B, C), where cover_A = 90, cover_B = 20, cover_C = 10, species A and B make up 92% of total cover of the subplot. Thus, we would only survey species A and B. In total, we surveyed 23 species—12 grasses, six non-leguminous forbs, three woody vines, one tree, and one legume.

Following Heckman et al. (2016), Mitchell et al. (2002) and Mitchell (2003), we haphazardly selected 20 leaves per target species varying in age and location from throughout the subplot, but without reference to the leaves' damage level. For each sampled leaf, we recorded the percent of the leaf's area that was damaged (by guilds of insect herbivore and fungal morphospecies) by referring to digitized images of known damage severity (James 1971). From these data, we calculated community damage,

$$\text{Community damage} = \sum_{i=1}^n d_{i \text{ subplot}} \left(\frac{c_{i \text{ subplot}}}{c_{t \text{ subplot}}} \right)$$

where $d_{i \text{ subplot}}$ is mean percent of leaf area damaged for the i th species in the subplot, $c_{i \text{ subplot}}$ is percent cover of the i th species and $c_{t \text{ subplot}}$ is the total cover of species surveyed for damage in the subplot (Mitchell et al. 2002). To calculate community damage, we summed disease and herbivory. To quantify foliar disease and herbivory on established seedlings, we calculated the mean damage on up to five leaves of each harvested seedling. Seedling damage was the mean damage to all seedlings within the subplot.

To quantify the resource-allocation strategies of the native species added as seeds, we grew individuals of each species in the greenhouse at the University of North Carolina at Chapel Hill. To improve germination rates, we stratified all seeds in moist sand at 4°C for at least one month prior to sowing. We then sowed up to 20 seeds of each species into one of twenty 0.94 L pots (Deepots, Stuewe and Sons, Corvallis, OR). At the two-leaf stage, we thinned each pot to a single individual. Pots were randomly divided into two groups: high nutrient supply, receiving the equivalent of 10 g NPK m⁻², and low nutrient supply, receiving the equivalent of 2 g NPK m⁻². To avoid limitation by micronutrients, both levels of nutrient supply received 100 g m⁻² micronutrients (Scott's Micromax). To create these two levels of nutrient supply, we added slow-release forms of P, K and micronutrients to our soil mix, which was a 50:50 mixture of Fafard 3B soil (Sun Gro Horticulture, Agawam, MA) and sterilized sand. The high sand content of the soil mix allowed finer control of nutrient supply. Because slow-release nitrogen is more volatile than potassium or phosphorus, and an aqueous solution is readily available, we applied nitrogen to pots in five biweekly applications of aqueous ammonium nitrate (NH₄⁺NO₃⁻).

After eight weeks growing in the greenhouse, we collected the youngest fully-emerged leaf on each plant for functional trait analysis. We removed each leaf, dried it at 60°C for at least

72 hours, ground it to a fine powder, and analyzed foliar %N and %C using micro-Dumas combustion analysis (Environmental Chemistry Lab, University of Georgia). Foliar N content (by mass) is highly correlated by photosynthetic capacity (Heckman, unpublished data) and other traits indicative of resource-allocation strategies (Wright et al. 2004, Cronin et al. 2010b, Reich 2014). Because three species had poor germination and growth in the greenhouse (*Asclepias syriaca*, *Symphyotrichum pilosum*, and *Tripsacum dactyloides*), we were unable to assess their physiological phenotypes.

We examined seedling performance in three ways. First, we determined the effects of our experimental treatments on total seedling establishment in each subplot. To do this, we performed confirmatory path analysis (Shipley 2009) using the piecewiseSEM package (Lefcheck 2016) in R. We used the piecewiseSEM package instead of likelihood-based structural equation modelling for two reasons: the split-plot experimental design require mixed-effect models, and seedling establishment, a count response, is most appropriately analyzed in a generalized linear model with Poisson errors. We first performed a full path analysis that including all hypothesized paths from our experimental treatments to our ultimate response (total seedling establishment) via environmental variables (Fig. 4.1). To improve the interpretability of path analysis, we subsequently performed a reduced path analysis (Fig. 4.2) that included only significant paths; this reduced path analysis is presented in the Results. To create this reduced path analysis, we simplified each component model independently using the procedure of Zuur et al. (2009), which entails systematically dropping non-significant terms until all terms in the model are significant.

For both sets of analyses, we hypothesized that nutrient addition would influence seedling establishment directly (i.e., direct effects of fertilization on seedlings) or indirectly (i.e.,

via influences on light and soil moisture availability, or damage to the resident community). We hypothesized that enemy exclusion and litter removal would only influence seedling establishment indirectly, via the same pathways. For each of these two path analyses, we ran four mixed-model analyses, each of which included a random effect of whole plot to account for the split plot nature of our experimental design. Three of the analyses used linear mixed-models with nutrient supply, litter removal, enemy exclusion, and their interactions as categorical predictors with the ‘lme’ function in the nlme package (Pinheiro et al. 2016). For these three models, the response variables light availability, soil moisture availability, and community damage (fungal disease + insect herbivory) were standardized (mean = 0; standard deviation = 1). To determine the effects of experimental treatments and standardized environmental variables on total seedling establishment, we performed a fourth model: a generalized linear mixed-model with Poisson errors, a log link, and a random effect of whole plot using the ‘glmer’ function in the lme4 package (Bates et al. 2015).

We also examined the effect of experimental treatments on the per capita biomass of seedlings that we harvested in October 2014. Because many plots had no seedlings established in October, a model examining per capita seedling biomass across all plots would have been severely zero-inflated. For this reason we examined the response with a pair of analyses. First, we performed a generalized linear mixed-model with binomial errors, a logit link, and a random effect of whole plot using the ‘glmer’ function in lme4 to test whether the effect of nutrient supply, enemy exclusion, and litter removal (but not interactions between these factors) influenced the probability that any seedlings had established in a subplot. Second, we examined the effect of litter removal, enemy exclusion, and their interaction on log-transformed per capita seedling biomass in plots containing seedlings, using the ‘lme’ function with whole plot as a

random effect. Because seedlings only established in one fertilized plot, we examined per capita seedling biomass only in unfertilized plots.

Similarly, we examined the effect of litter removal, enemy exclusion, and their interaction on logit-transformed seedling damage. We did this using the ‘lme’ function with whole plot as a random effect.

Finally, we examined the influence of experimental treatments, environmental variables, and the resource-allocation strategies of seedling species on seedling establishment by species using Bayesian inference using the ‘stan_glmmer’ function in rstanarm (Gabry and Goodrich 2016). Based on the results of the path analyses detailed above, we examined these effects in two separate models, one that included standardized environmental variables (light, soil moisture, community damage) and standardized seedling foliar N content (under greenhouse conditions) as continuous predictors, nutrient supply as a categorical predictor, interactions between nutrient supply and foliar N content. The second analysis contained nutrient supply, litter removal, and enemy exclusion as categorical treatment effects, standardized foliar N content as a continuous predictor, and all interactions between these predictors. Each model also included crossed random effects of species identity and whole plot. These models only included the eight species for which we were able to collect foliar N content.

Results

Nutrient addition reduced seedling establishment via three paths: directly, indirectly by reducing light availability, and indirectly by reducing soil moisture availability. Nutrient addition directly reduced seedling establishment, on average, by 3.49 individuals subplot⁻¹ ($P < 0.001$; Appendix C1.1, Fig. 4.2, Fig. 4.3a). Nutrient addition also reduced soil moisture availability, on average, by 1.12 sd ($P < 0.001$, Appendix C1.1, Fig. 4.2, Fig. 4.4a); reducing soil moisture

availability by 1 sd reduced seedling establishment, on average, by 1.16 individuals subplot⁻¹. Additionally, nutrient addition marginally significantly reduced light availability ($P = 0.083$; Appendix C1.1), but this relationship depended on litter removal: nutrient addition reduced light availability by 0.49 sd more in raked subplots, presumably because in unraked plots, light availability was too low to be further reduced ($P = 0.01$, Appendix C1.1, Fig. 4.2, Fig. 4.4b). Reducing light availability by 1 sd reduced seedling establishment by 1.30 individuals subplot⁻¹, on average ($P < 0.001$, Appendix C1.1, Fig. 4.2, Fig. 4.3c). Together, these results indicate that the direct negative effect of nutrient addition on seedling establishment was larger than the indirect effect of nutrient addition mediated through light or the indirect effect of nutrient addition mediated through soil moisture.

Litter removal influenced seedling establishment via two indirect paths: by increasing light availability and by increasing community damage. Removing litter increased light availability by 1.75 sd ($P < 0.001$, Appendix C1.1, Fig. 4.2, Fig. 4.4b); increasing light availability by 1 sd increased seedling establishment by 4.23 individuals subplot⁻¹ ($P < 0.001$, Appendix C1.1, Fig. 4.2, Fig. 4.3c). Removing litter also increased community damage by 0.43 sd ($P = 0.012$, Appendix C1.1, Fig. 4.2, Fig. 4.4c); increasing community damage by 1 sd increased seedling establishment by 3.71 individuals subplot⁻¹ ($P < 0.001$, Appendix C1.1, Fig. 4.2, Fig. 4.3d). Overall, these results suggest that litter removal primarily increased seedling establishment by increasing light availability; increasing damage to the resident community also enhanced seedling establishment to a lesser degree.

Enemy exclusion influenced seedling establishment by one indirect path—by reducing damage to the resident plant community (community damage). Enemy exclusion reduced community damage by 0.88 sd ($P < 0.001$, Appendix C1.1, Fig. 4.2, Fig. 4.4d); reducing

community damage by 1 sd reduced seedling establishment by 0.95 individuals subplot⁻¹ ($P < 0.001$, Appendix C1.1, Fig. 4.2, Fig. 4.3d). Enemy exclusion also reduced damage to seedlings ($P = 0.027$, Appendix C1.2, Fig. 4.5a). This indicates that the benefit of enemy exclusion to colonizing seedlings was outweighed by the benefit of enemy exclusion to the resident community, allowing the resident community to more strongly suppress seedling establishment. As a result, the net effect of enemy exclusion was to reduce seedling establishment. This result is contrary to our prediction that enemy exclusion would primarily benefit native seedlings. Instead, enemy exclusion primarily benefitted the exotic-dominated resident community, suggesting that release of exotic species from enemies was not a major factor limiting colonization by native seedlings.

Nutrient addition reduced the probability that any seedlings would survive until the end of the study ($P < 0.001$, Appendix C1.3, Fig. 4.5b); this probability was not influenced by either litter removal ($P = 0.14$) or enemy exclusion ($P = 0.10$). Because seedlings only survived in one fertilized subplot, we examined per capita seedling biomass only in unfertilized plots. In the 31 of 40 unfertilized subplots with seedlings surviving until the end of the study, per capita seedling biomass did not differ with enemy exclusion treatment ($P = 0.79$), litter removal treatment ($P = 0.23$), or their interaction ($P = 0.78$, Appendix C1.4, Fig. 4.5c). This suggests that although litter removal and enemy exclusion influenced seedling establishment, these experimental treatments did not influence the growth of seedlings that survived until the end of the study.

When examining variation in establishment among species based on a key functional trait, the results largely agree with the SEM, and indicate only a limited role for functional traits. Seedling establishment increased with increasing light and soil moisture availability, and with increasing damage to the resident community (Fig. 4.6a). Likewise, seedling establishment

declined considerably with nutrient addition (Fig. 4.6a). Unlike the SEM, litter removal and enemy exclusion treatments interacted to influence seedling establishment; neither litter removal nor enemy exclusion influenced seedling establishment alone, but seedling establishment was lower where enemies were excluded and litter was intact (Fig. 4.6b, Fig. 4.7a). Finally, the effect of the litter removal treatment on seedling establishment depended on species' functional traits. Specifically, species with higher foliar N content (an indicator of resource-acquisitive strategies) established more successfully in plots with intact litter (Fig. 4.6b, Fig. 4.7b). This indicates that quick-return species were better able to cope with low light availability.

Discussion

In this study, nutrient supply, enemy exclusion, and litter removal each influenced seedling establishment. As predicted, nutrient addition reduced seedling establishment. This effect of nutrient addition occurred through three paths: directly, indirectly by reducing light availability, and indirectly by reducing water availability. These indirect effects were probably driven by increased productivity in fertilized plots, which increased the amount of light intercepted by the community and increased transpiration. Light availability was also influenced heavily by the presence of leaf litter. Removing litter increased seedling establishment by increasing light availability. Moreover, litter removal and enemy exclusion each reduced community damage, which increased seedling establishment. This positive effect of enemy exclusion on seedling establishment was contrary to our prediction, indicating that enemies had a stronger influence on the resident community than on establishing seedlings. Also contrary to our prediction, foliar N content, a key functional trait, had little influence on seedling establishment. These results demonstrate that multiple mechanisms influence native seedling establishment in exotic-dominated communities.

Nutrient addition reduced seedling establishment in this study. This effect occurred by several mechanisms. Nutrient addition reduced light availability (Borer et al. 2014b), but only in raked communities. This interaction probably occurred because intact leaf litter already reduced light availability substantially, preventing nutrient addition from further reducing light availability. Moreover, nutrient addition reduced soil water availability, probably because the greater primary production of fertilized plots promoted greater transpiration (Rose et al. 2012).

Nutrient addition also reduced seedling establishment in the SEM directly. Because a direct negative effect of nutrient addition on seedlings is implausible, this result suggests that we were unable to account for all important environmental variables or because of discrepancies in the spatial and temporal scales of our measures of light and soil water availability from the spatial and temporal scales most relevant for establishing seedlings. For instance, seedlings may have been influenced by soil water availability in the top soil layers (Padilla and Pugnaire 2007), rather than the deeper soil layers that we measured. Moreover, seedlings may have only been affected by soil moisture during the driest periods of the season (Harrington 1991, Padilla and Pugnaire 2007). Because we measured soil moisture monthly, we could have missed important periods of acute drought stress. Although light availability declined over the growing season in a more predictable manner than soil water availability, which varies throughout the growing season based on temperature, evapotranspiration, and precipitation, there may have been a critical period in seedling development when light was most important (Hutchings and Booth 1996). Because we measured light availability at ground-level (i.e., below the litter layer), we may have also underestimated the amount of light that larger seedlings (i.e., those that succeeded in growing above the litter layer) received.

Beyond measurement error, other environmental factors may have influenced the effect of fertilization on seedling establishment. Fertilization often promotes rapidly-growing species (Davis et al. 2000), which may have exacerbated priority effects in this system (Fukami 2015). When we fertilized plots in May, *Schedonorus arundinaceus*, the dominant species in fertilized communities, had already been growing rapidly for several weeks. During this period, larger, established *S. arundinaceus* individuals could have outcompeted small seedlings (Schwinning and Weiner 1998, Craine and Dybzinski 2013). Using slow-release fertilizers may have also benefitted residents with large, established root systems. Over several weeks, the resident vegetation probably would have been able to utilize enough of the available nutrients to slow seedling establishment (Renne et al. 2006).

Contrary to expectation, nutrient addition did not influence community damage (Mattson 1980, Dostál et al. 2013, Veresoglou et al. 2013, Heckman et al. 2016). This may have occurred because the communities were dominated by exotic species, which may be released from their natural enemies at this site (Heckman et al. 2016). If exotics were released from enemies, there would be little reason to expect that fertilization would increase community damage (Keane and Crawley 2002, Blumenthal 2006). However, it appears unlikely that exotics were released from enemies because enemy exclusion reduced community damage significantly. Reducing community damage also reduced seedling establishment, suggesting that enemies had large enough impacts on the resident community to influence competition with establishing seedlings. One explanation for the apparent lack of enemy release is that the most abundant species, *S. arundinaceus*, which is abundant locally and continentally, has acquired new enemies since being introduced to North America (Mitchell et al. 2010, Stricker et al. 2016). In fact, *S. arundinaceus* may be more heavily impacted by enemies than other common grasses at this site

(Heckman, Halliday, and Mitchell in prep). Alternatively, enemies may impact populations of resident species more than establishing seedlings (Heckman et al. in review). Because populations of resident species were denser than the establishing seedlings, enemies that regulate plant populations in a density-dependent manner would have larger impacts on resident species than on establishing seedlings (Gilbert 2002). However, as the populations of these establishing species grow, they may become more heavily impacted by enemies, losing any advantage over the competing residents. Thus, enemies may promote population growth when species are rare, but limit population growth later (Chesson and Kuang 2008, MacDougall et al. 2009, Mordecai 2011). Moreover, these communities were dominated primarily by a single species, *S. arundinaceus*, which increased in abundance following fertilization. Fertilization typically increases herbivory and disease to communities by shifting community composition toward species adapted to high-resource environments, which tend to be less defended against enemies (Coley et al. 1985, Hahn and Maron 2016). Thus, if community composition does not change substantially, fertilization may have a small effect on herbivory and disease.

Leaf litter also had strong negative effects on seedling establishment. Contrary to our prediction, leaf litter also reduced community damage (Karban et al. 2012, Roy et al. 2014). This may have happened because intact leaf litter can intercept dispersing fungal spores or prevent insect herbivores from finding more palatable, less defended tissue. Leaf litter also reduced light availability, as predicted (Borer et al. 2014b). In unraked plots, where light availability was low, species with high foliar N content established most successfully. Species possessing nitrogen-rich leaves often exhibit high photosynthetic rates and shade intolerance (Valladares and Niinemets 2008, Reich 2014). This supports research showing that shade-intolerant species are able to photosynthesize rapidly, even in low-light environments (Valladares and Niinemets

2008). Species using the C₃ photosynthetic pathway—here, non-leguminous forbs—often possess higher foliar N content than species using the C₄ photosynthetic pathway—here, grasses (Knapp 1993). Thus, the effect of seedling foliar N on establishment may be partly attributable to differences between grasses and forbs within this study. Furthermore, because enemy impacts on shade intolerant species should be largest in low-light environments (Myers and Kitajima 2007), this also suggests that establishing seedlings were not heavily impacted by natural enemies.

Over longer time frames, fertilization may reduce community richness by increasing the abundance of species that are better competitors for other resources, especially light (Hautier et al. 2009, Isbell et al. 2013, Borer et al. 2014b). Furthermore, fertilization promotes increased productivity, and subsequently, increased litter production, both of which can reduce the availability of light needed by establishing seedlings (Carson and Peterson 1990, Foster and Gross 1998). Thus, disturbances that remove vegetation and standing litter may be required to promote establishment in productive grasslands (Jutila and Grace 2002).

In conclusion, these results show that nutrients and enemies influence seedling establishment through multiple independent mechanisms. Increasing nutrient supply can influence seedling establishment by reducing the availability of other potentially-limiting resources. Moreover, as predicted, reducing light availability substantially reduced seedling establishment, suggesting that larger resident plants were able to preempt light that is necessary for seedlings to establish. Contrary to our prediction, enemies benefitted native seedlings in these exotic-dominated communities. This suggests that exotic species were not released from enemies relative to natives. Alternatively, enemies might regulate populations in a density-dependent manner, explaining why native seedlings, which occurred at very low densities, would be less impacted by enemies than resident populations, regardless of whether the exotic species had

escaped some (but not all) of their natural enemies. Together, these results show that lower nutrient supply and ambient enemy pressure can enhance native colonization of exotic-dominated communities, an important step in many restoration efforts.

Fig. 4.1 Hypothesized relationship between experimental treatments (nutrient addition, litter removal, and enemy exclusion) and seedling establishment, mediated by three environmental variables: light and water availability and community damage (fungal disease + insect herbivory). Dotted lines indicate negative hypothesized relationships and solid lines indicate positive hypothesized relationships.

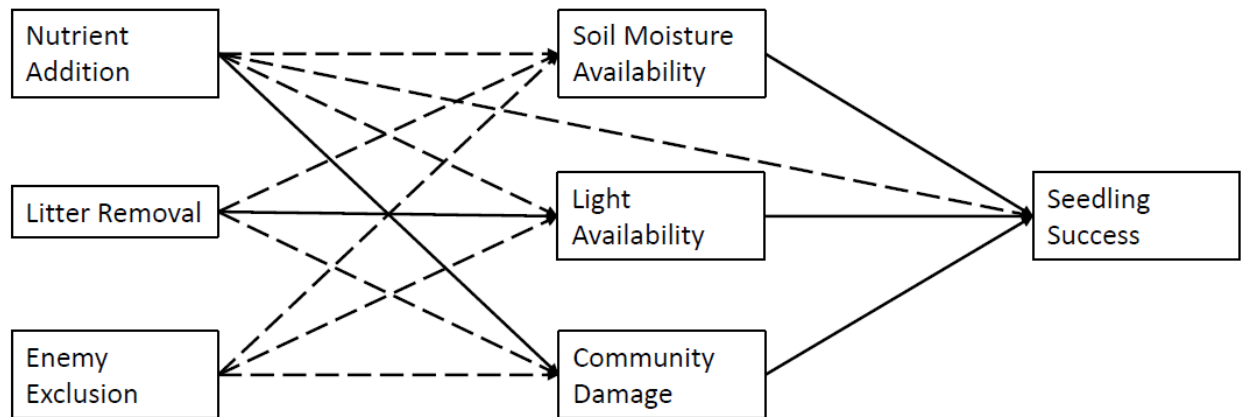


Fig. 4.2 Path diagram of the best model explaining the effects of experimental nutrient addition, enemy exclusion, and litter removal on environmental variables (soil moisture availability, light availability, and fungal disease and insect herbivory to the resident community) that influence the total number of surviving seedlings in each plot (seedling establishment). Dotted lines indicate negative effects, solid lines indicate positive effects. Direct effects of nutrient addition on seedling establishment appeared to be stronger than indirect effects of nutrient addition on seedling success (via changes in light and soil moisture availability). Litter removal increased seedling success via two indirect pathways: community damage and light availability, with the light availability pathway being stronger. Enemy exclusion had an indirect negative effect on seedling success by reducing community damage. Furthermore, the effect of enemy exclusion was larger than the effect of litter removal on community damage. Thus, enemy exclusion contributed more than litter removal to the effect of community damage on seedling success. For each response variable, R^2_M indicates the marginal R^2 and R^2_C indicates the conditional R^2 .

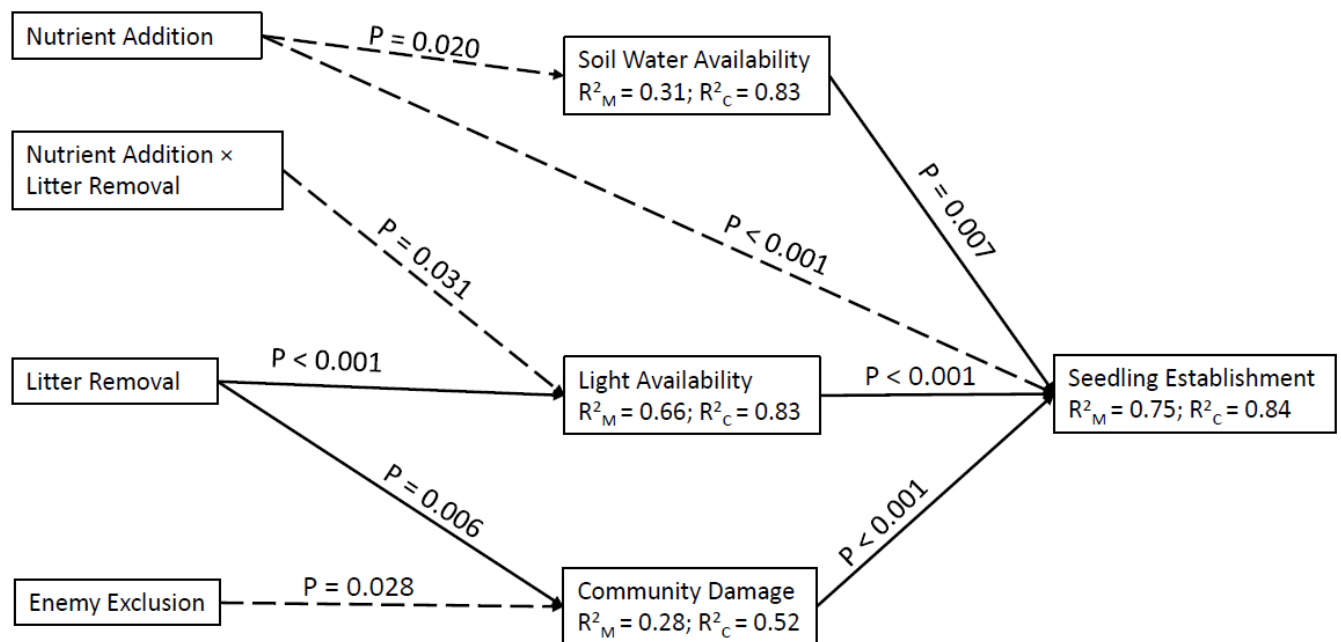


Fig. 4.3 Effects of A) nutrient supply, B) standardized soil water availability, C) standardized light availability, and D) standardized fungal disease and insect herbivory to the resident community (community damage) on seedling establishment (seedlings subplot⁻¹). Individual points show raw values. Box plots show the distribution of fitted values; the middle line represents the median, the edges of the box represent the first and third quartiles, and whiskers extend up to 1.5× the interquartile range. The x-axis above panels B, C, and D shows raw values of environmental predictors.

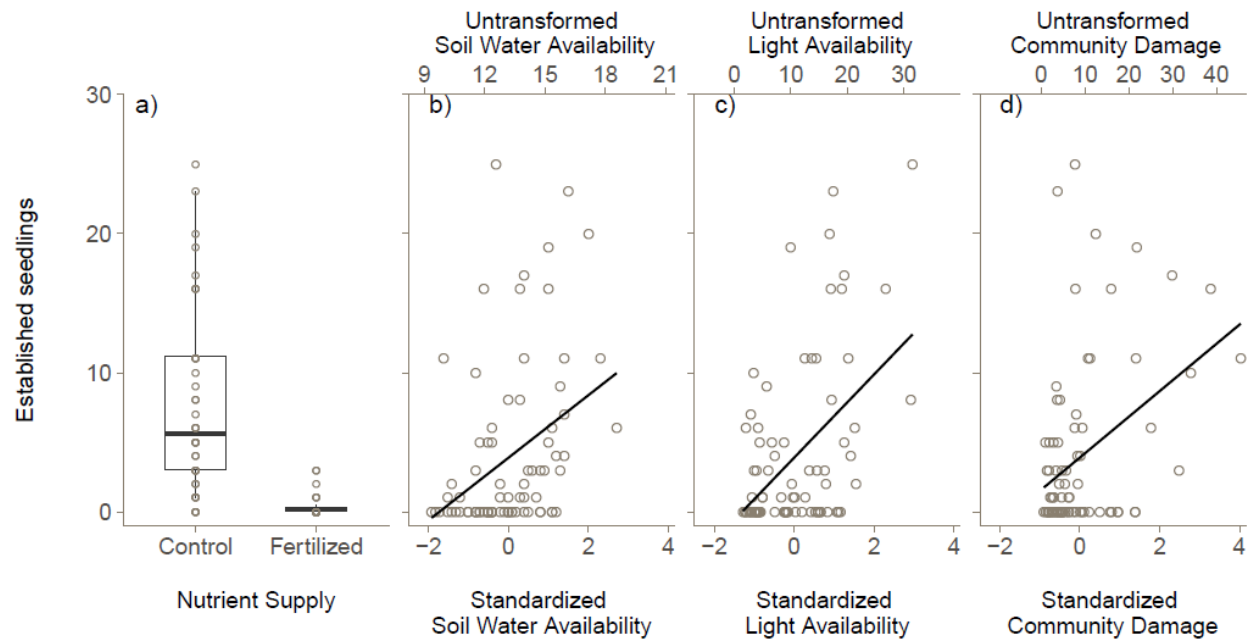


Fig. 4.4 Effects of A) nutrient supply on standardized soil water availability, B) nutrient supply and litter removal on standardized light availability, C) litter removal on standardized fungal disease and insect herbivory to the resident community (community damage), and D) enemy exclusion on standardized fungal disease and insect herbivory to the resident community (community damage). Individual points show raw values. Box plots show the distribution of fitted values; the middle line represents the median, the edges of the box represent the first and third quartiles, and whiskers extend up to $1.5\times$ the interquartile range. The y-axis to the right of each panel shows raw values of the response.

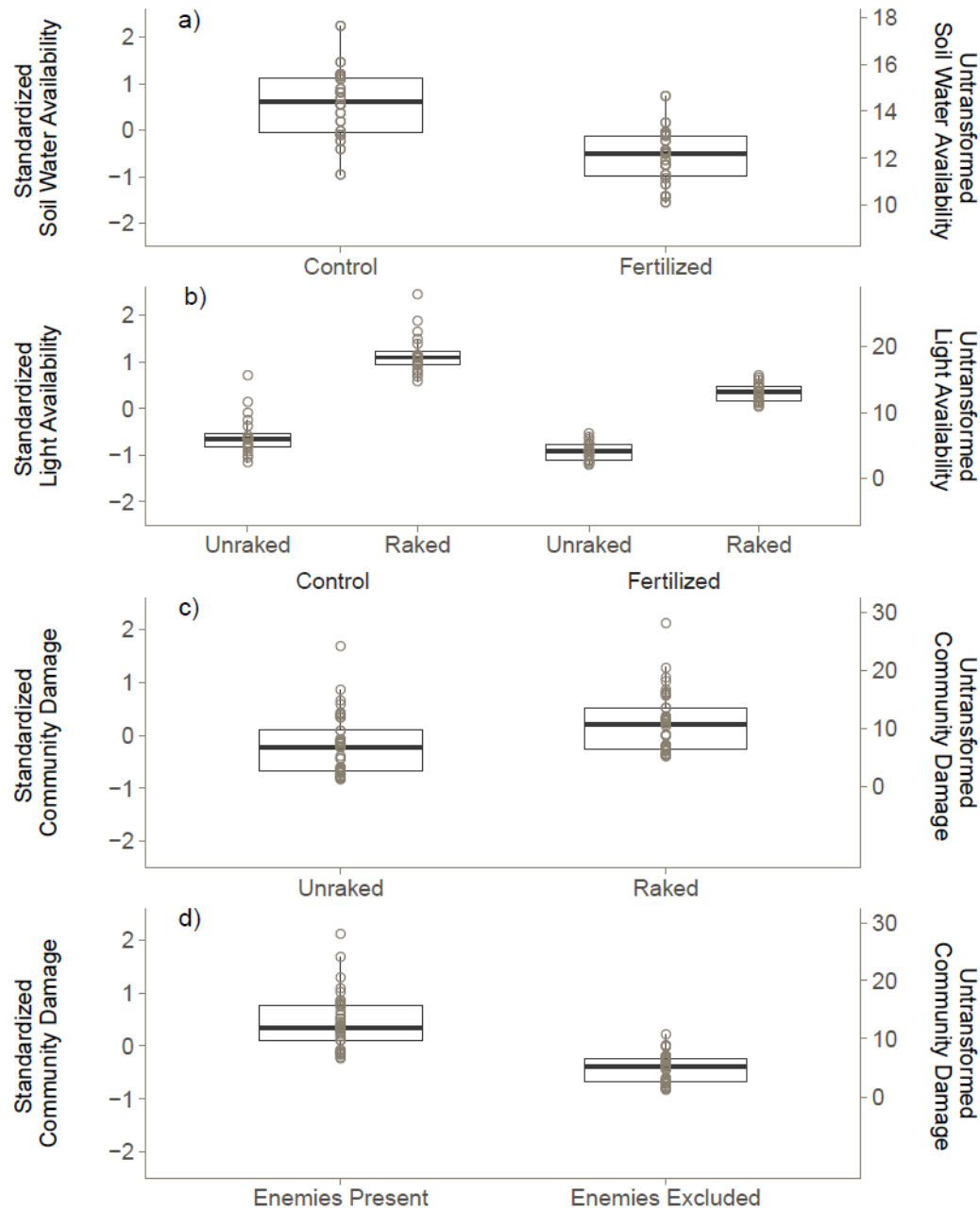


Fig. 4.5 A) Effects of enemy exclusion on fungal disease and insect herbivory on up to ten surviving seedlings per plot in unfertilized plots, B) effects of nutrient supply on the probability that any seedlings in a subplot survived until October 2014, and C) effects of litter removal and enemy exclusion on per capita seedling mass (g seedling⁻¹) in plots with at least one seedling surviving until October 2014. Because seedlings survived until October in only one fertilized plot, panels A) and C) only include unfertilized plots. Individual points show fitted values calculated using restricted maximum likelihood estimation. Box plots show the distribution of fitted values; the middle line represents the median, the edges of the box represent the first and third quartiles, and whiskers extend up to 1.5× the interquartile range.

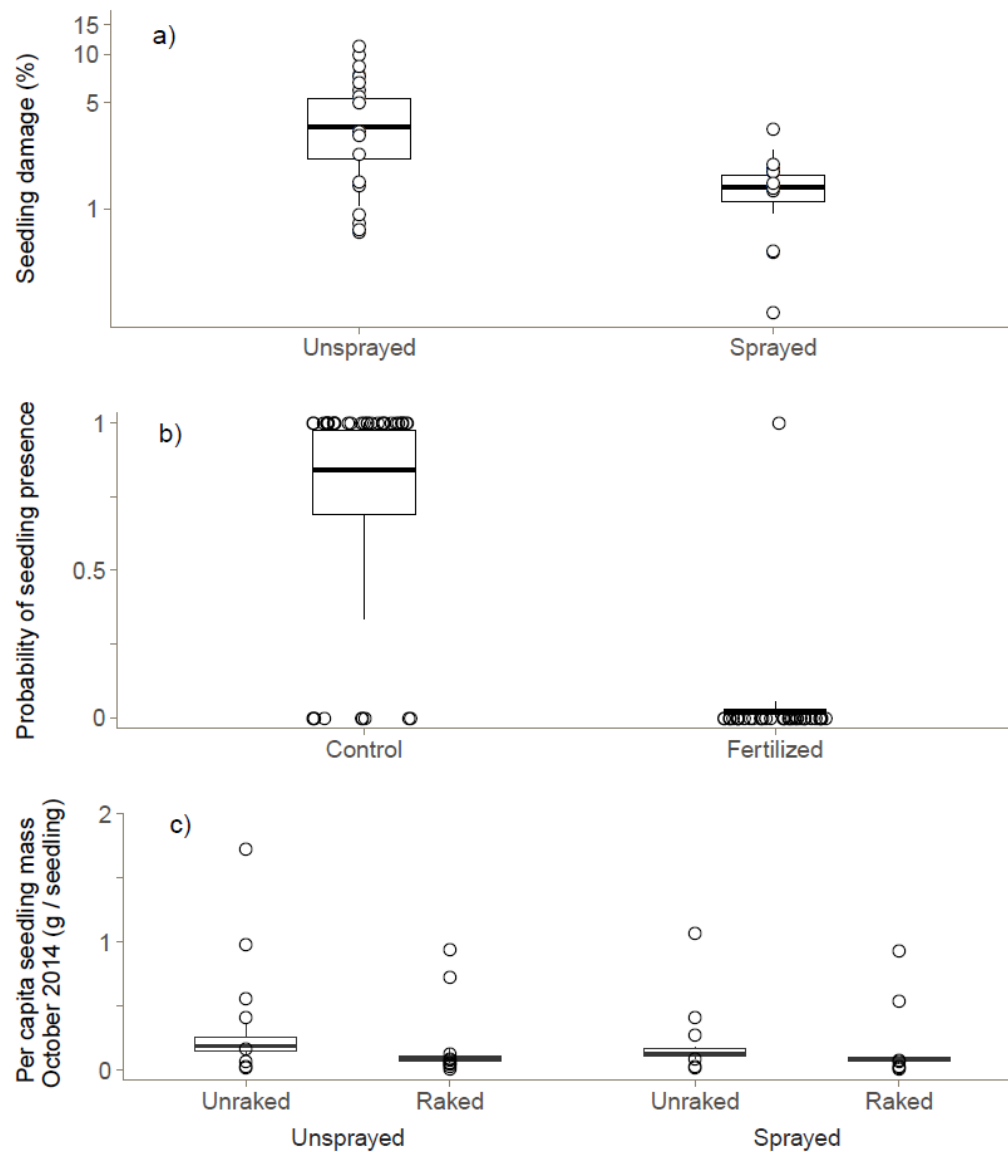


Fig. 4.6 Effects of A) nutrient addition, colonizer foliar N content, fungal disease and insect herbivory to the resident community (community damage), soil water availability, and light availability, and B) nutrient addition, enemy exclusion, litter removal, and colonizer foliar N content, on the number of seedlings surviving until September, 2014. Black points show means and grey lines show 95% credibility intervals of parameter estimates. This analysis included eight species for which we collected foliar N content in the greenhouse (*Andropogon virginicus*, *Apocynum cannabinum*, *Paspalum floridanum*, *Solidago pinetorum*, *Sorghastrum nutans*, *Steinchisma hians*, *Tridens flavus*, and *Verbesina alterniflora*); it did not include two species that had relatively high germination in the field (*Asclepias syriaca* and *Symphyotricum pilosum*), but that did not grow well in the greenhouse.

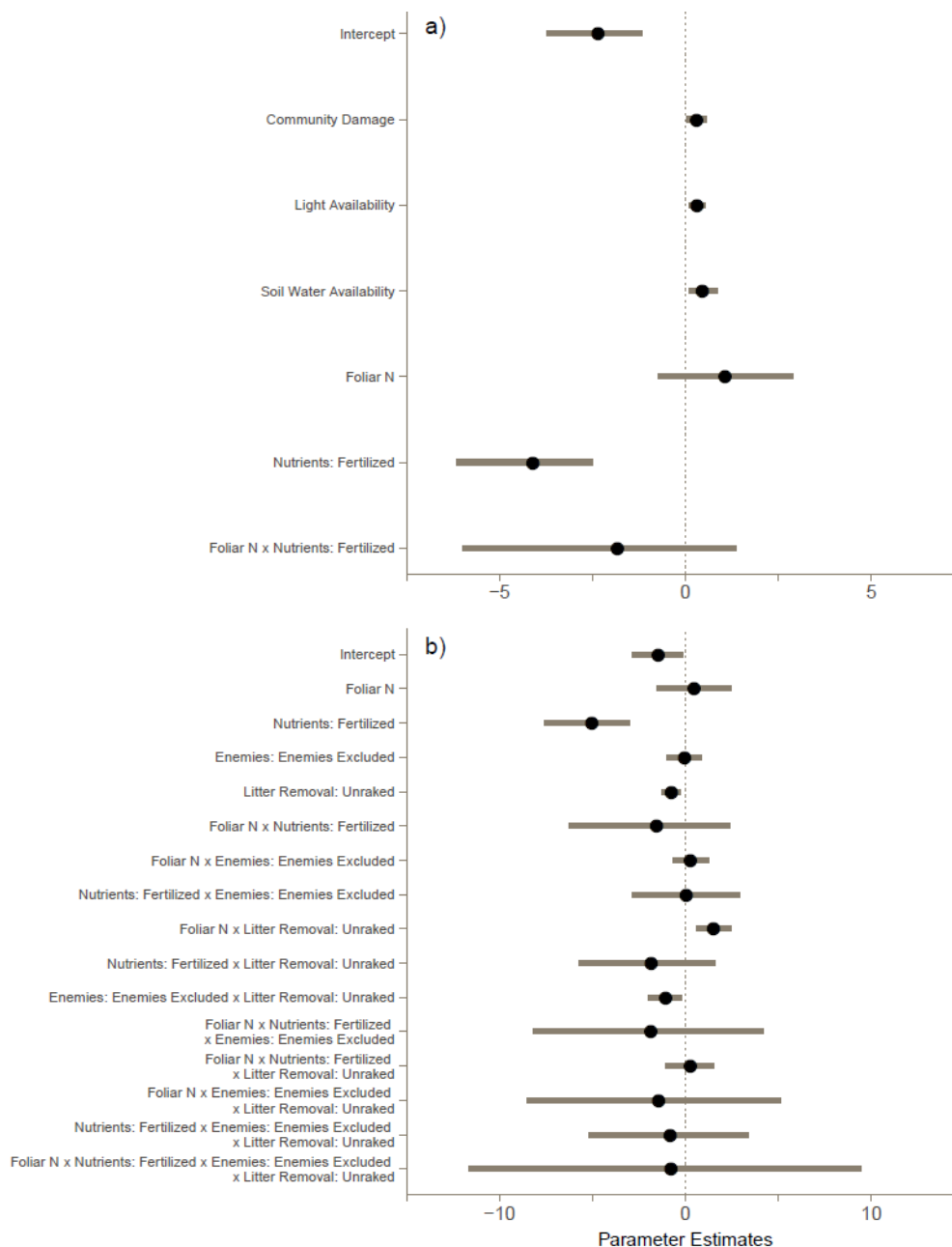
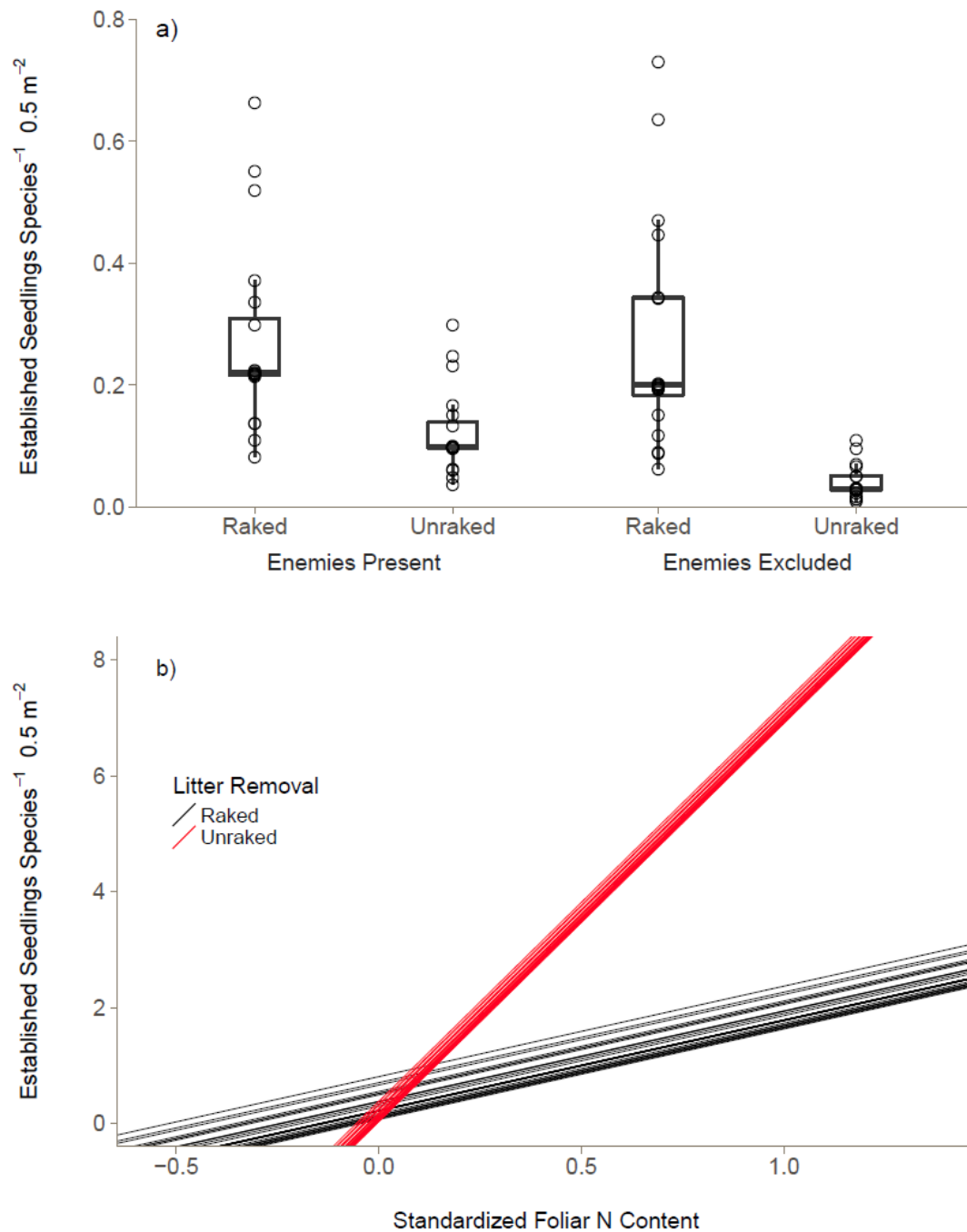


Fig. 4.7 Effects of A) enemy exclusion and litter removal, and B) standardized foliar N content and litter removal, on seedling establishment by species (seedlings species⁻¹ subplot¹). In panel A), individual points show fitted values, and box plots show the distribution of fitted values; the middle line represents the median, the edges of the box represent the first and third quartiles, and whiskers extend up to 1.5× the interquartile range. In panel B), each line represents an individual whole plot.



CHAPTER V. EFFECTS OF NATIVE DIVERSITY, SOIL NUTRIENTS, AND NATURAL ENEMIES ON EXOTIC INVASION IN EXPERIMENTAL PLANT COMMUNITIES

Introduction

Exotic plant invasions have many possible drivers, commonly including release from natural enemies, increased resource supply, and low richness of the resident community (Levine and D'Antonio 1999, Davis et al. 2000, Keane and Crawley 2002, Catford et al. 2009). These factors are often studied alone (e.g., Huenneke et al. 1990, Naeem et al. 2000, Davis and Pelsor 2001, Kennedy et al. 2002, Mitchell and Power 2003, DeWalt et al. 2004), but may act in concert to influence exotic success (Tilman 2004, Blumenthal 2006, Maron and Marler 2008, Turnbull et al. 2010, Mattingly and Reynolds 2014). Furthermore, single-factor explanations for exotic success often implicitly require other co-occurring drivers. In particular, species-rich communities may draw down resource availability, potentially reducing exotic success in these communities following resource addition (Levine and D'Antonio 1999, Tilman 2004). Alternatively, species-rich communities may reduce exotic success because these communities are less regulated by pathogens and herbivores (Turnbull et al. 2010). Meanwhile, high resource environments may allow exotic species to benefit more from enemy release (Blumenthal 2006, Blumenthal et al. 2009). Thus, considering multiple factors in combination may be necessary to explain exotic success. In this study, we experimentally tested the individual and interactive contributions of three potential drivers of exotic success: natural enemies, species richness, and resource supply.

Empirical and theoretical studies suggest that increasing species richness can reduce exotic success by two separate pathways (Levine and D'Antonio 1999). One pathway states that species-poor communities, where species typically occur at high density, may be more strongly regulated by herbivores and pathogens than species-rich communities, where most species occur at lower density, thereby reducing the potential of each species to support large assemblages of natural enemies (Maron et al. 2011, Schnitzer et al. 2011). Moreover, exotic species often lose their important herbivores and pathogens upon introduction to a new range (Mitchell and Power 2003, DeWalt et al. 2004, Torchin and Mitchell 2004, but see Parker and Hay 2005, Halbritter et al. 2012). Consequently, exotic species may realize an even larger advantage over their heavily-regulated native competitors in species-poor communities (Turnbull et al. 2010). Enemy exclusion experiments (e.g., fencing, biocide spraying) can be used to examine the influence of natural enemies on plant performance and test whether exotic species benefit from enemy release (Keane and Crawley 2002, Blumenthal 2006, Mitchell et al. 2006). If exotic species benefit from enemy release relative to co-occurring natives, excluding enemies could mitigate this effect and indirectly benefit native species. Therefore, excluding enemies from species-poor communities—where the difference between native and exotic species in regulation by enemies might be largest—should reduce exotic success more than excluding enemies from species-rich communities. We know of only one study to test this mechanism: in a greenhouse experiment, the effect of richness on exotic success depended on soil microbes (Liao et al. 2015).

Increasing species richness may also reduce exotic success by increasing niche overlap among residents, potentially allowing species-rich communities to reduce resource availability more than species-poor communities (Levine and D'Antonio 1999, Tilman 2004). When resource supply is high, such as following resource addition, species-rich communities may draw down

resource availability more rapidly and thoroughly than species-poor communities, which are unable to use as much of the resource supply (Mattingly and Reynolds 2014). With fewer resources available for exotics to use, exotic success should be lower in species-rich communities (Levine and D'Antonio 1999, Fargione et al. 2003, Tilman 2004). Thus, if resource availability drives the negative relationship between species richness and exotic success, adding resources should increase exotic success more in species-poor communities than in species-rich communities. Empirical support for this prediction has been inconsistent; in some studies, the effects of resource addition overwhelm the effects of increasing species richness (Renne et al. 2006, Mattingly and Reynolds 2014); in others, the effects of increasing species richness are independent of changes in resource supply (Maron and Marler 2007, 2008).

In addition to interacting with species richness, resources and enemies may also jointly influence exotic success (Blumenthal 2005, Blumenthal 2006). Successful invaders often grow in high-resource environments (van Kleunen et al. 2010, Leishman et al. 2014), and these invaders often experience more disease and herbivory in their native ranges (Coley et al. 1985, Fine et al. 2004, Lind et al. 2013). Upon introduction to a new range lacking their important natural enemies, exotic species in high resource environments may gain the benefits of high resource supply without being subject to increasing regulation by pathogens and herbivores (Blumenthal et al. 2009). As a result, these high-resource species may realize greater benefits from enemy release than exotic species in low-resource environments (Blumenthal 2005, Blumenthal 2006). Thus, if resource supply and enemy release interact to promote exotic success, excluding enemies should reduce exotic success most in fertilized communities (Blumenthal 2006). To our knowledge, this prediction has been tested only twice at the community level, each time with partial support. In one study, excluding vertebrate herbivores reduced exotic success, regardless

of resource supply to intact communities (Seabloom et al. 2015). In another study, adding resources to intact communities increased exotic success only when vertebrate herbivores had access to communities; however, adding resources increased exotic success regardless of fungal pathogen and insect herbivore access to communities (Heckman et al. 2016).

In addition to pairwise interactions between resources, enemies, and diversity, all three of these drivers might interact (Shea and Chesson 2002, Liao et al. 2015). Based on the theoretical and empirical evidence presented above—including both pathways hypothesized to explain why exotic success declines with increasing species richness—we predict that spraying will strongly reduce the benefit of fertilization to exotic species in species-poor communities; this effect will be weaker in species-rich communities. Furthermore, manipulating these three drivers of invasion factorially within a single study may reveal previously unknown aspects of their interactive and independent effects.

Some mechanisms proposed to explain exotic success should also promote the success of native species when colonizing a community, while other mechanisms should not. For example, mechanisms driven by resource supply should apply equally to exotic and native colonizers (Davis et al. 2000, Davis and Pelsor 2001, Fargione et al. 2003, Tilman 2004, van Kleunen et al. 2010, Leishman et al. 2014), whereas mechanisms driven by potential differences in enemy regulation (i.e., enemy release) should apply solely to exotic species (Keane and Crawley 2002, Shea and Chesson 2002). Meanwhile, either mechanism, resources or enemies, may drive the effects of species richness on exotic success (Levine and D'Antonio 1999, Tilman 2004, Turnbull et al. 2010). Consequently, we expect resource-based mechanisms to affect exotic and native colonizers similarly, but expect enemy-based mechanisms to affect only exotic colonizers. Thus, examining both native and exotic colonizers can clarify the mechanisms driving exotic success.

Despite the growing evidence that interactions between resource supply, species richness, and natural enemies can influence exotic success, the relative importance of these interactions, and of the three factors independently, remains untested. To test the individual and interactive contributions of these three factors, we here report results of the first experiment to manipulate resource supply, species richness, and natural enemies simultaneously. To better distinguish their effects, we examined not only exotic success, but also the success of native colonizers.

Methods

We performed this study at Widener Farm, an old field in Duke Forest (Orange County, NC, USA) that produced row crops until 1996. Since 1996, the site has been mowed to produce hay. It is dominated by perennial species, including many native species common in North Carolina Piedmont old fields (Oosting 1942), and several exotic species, including *Lespedeza cuneata*, *Lonicera japonica*, and *Schedonorus arundinaceus*.

The study employed a randomized complete block design with three factorial treatments: we manipulated native plant richness with multiple native community compositions at each level of richness; access by foliar fungal pathogens and insect herbivores; and soil nutrient supply. This yielded a study that comprised 240 plots (5 replicate blocks \times 2 nutrient supply levels \times 2 enemy access levels \times 2 richness levels \times 6 native community compositions).

We assigned each study plot to one of two levels of species richness: monoculture or 5-species polyculture. From a pool of six species, we assembled twelve planted communities: six monocultures and six 5-species polycultures where one species was excluded from each polyculture community. We omitted one species from each of the six polyculture communities to isolate the effects of planted species identity. All six species were represented equally at each

diversity level to better account for potential selection effects that can occur in planted biodiversity experiments (Huston 1997, Loreau and Hector 2001, Schmid et al. 2002).

In May 2011, we established five spatial blocks, each 15×15 m (225 m^2). Within each block, we established 64 plots, each 1×1 m with 1 m aisles between plots. In each block, 16 plots were not planted and are not included in this study. We removed all vegetation from plots by applying glyphosate herbicide (Riverdale® Razor® Pro, Nufarm Americas Inc, Burr Ridge, IL) to each plot in May 2011. We did not apply herbicide to aisles between plots in order to promote colonization. Two weeks after herbicide application, we removed dead vegetation and covered all plots with landscape fabric.

Species were selected from a pool of six native herbaceous perennials already present at Widener Farm. We chose only perennial species because perennials constituted > 95% of intact vegetative cover at Widener Farm. Furthermore, we selected species that were present locally to ensure site suitability and to increase the likelihood that pathogens and herbivores capable of exploiting them were present locally. Our species pool included three grasses—*Andropogon virginicus*, *Setaria parviflora*, *Tridens flavus*, and three forbs—*Packera anonyma*, *Scutellaria integrifolia*, *Solidago pinetorum* (see Appendix D2.1 for seed sources and species descriptions).

We grew all six species in the greenhouse at the University of North Carolina at Chapel Hill for 8-12 weeks before planting them in the field between June and September 2011. Because species germinated and grew at different rates, we transplanted each species into the field when the majority of individuals reached a size adequate to survive transplant stress; each species was transplanted in 1-2 days. In order to minimize recruitment from the seedbank and facilitate establishment of our planted species, we transplanted each species as follows. We cut a small hole in the landscape fabric covering the plot, dug a small hole, and planted the individual plant.

Each plot contained 41 individual plants, spaced approximately 10 cm from its nearest neighbors in a checkerboard pattern (Fig. 5.1). Polycultures contained 9 individuals of one randomly chosen species and 8 individuals of the other 4 species. In early summer 2012, we replaced all individual plants that had not survived the winter. *Setaria parviflora* was planted in 2012, but not in 2011 because it replaced a species (*Asclepias syriaca*) that we had originally planted in 2011 which failed to establish in any plots. In July 2012, we removed landscape fabric from all plots, removed non-planted individuals by hand, and let natural colonization proceed for the duration of the study. Because the goal of this study was to examine how exotic abundance changes over time, we did not weed plots to maintain richness (Fargione and Tilman 2005). Thus, the species richness treatments represent initial conditions and not necessarily the richness of a plot after July 2012.

We began enemy access and nutrient supply treatments in July 2012, soon after we completed planting. To manipulate access by foliar fungal pathogens and insect herbivores, each plot was assigned to one of two enemy access treatments (sprayed with fungicide and insecticide vs. not sprayed), hereafter referred to as the spraying treatment. We performed the spraying treatment from July 2012 through September 2015 by spraying non-systemic broad-spectrum biocides on the aboveground portion of all plants every two to three weeks throughout the growing season, from April to October. Neither the fungicide (mancozeb, Dithane[®] DF, Dow AgroSciences, Indianapolis, IN) nor the insecticide (es-fenvalerate, Asana[®] XL, Dupont, Wilmington, DE) had any non-target effects on plant growth under greenhouse conditions, and together they reduced foliar damage (insect herbivory + fungal disease) to intact communities by > 80% (see Heckman et al. 2016 for details). In this study, spraying reduced foliar damage to communities by > 55% ($P < 0.001$, Appendix D3.1, Appendix D1.1). Furthermore, after using

the ‘mvt’ method in the lsmeans package (Lenth 2013) to adjust for multiple comparisons among the six planted species, spraying reduced foliar damage marginally to one species (*S. pinetorum*, $P = 0.065$) and significantly to four of the six species (Appendix D3.2, Appendix D1.2).

To manipulate soil nutrient supply, each plot was assigned to one of two nutrient supply treatments (fertilized with $10 \text{ g N m}^{-2} \text{ yr}^{-1}$ as slow-release urea, $10 \text{ g P m}^{-2} \text{ yr}^{-1}$ as triple super phosphate, and $10 \text{ g K m}^{-2} \text{ yr}^{-1}$ as potassium sulphate vs. not fertilized), hereafter referred to as the fertilization treatment. This level of fertilization has been used in other field studies (e.g., Borer et al. 2014a). In 2012, we fertilized plots in July, and in subsequent years, we fertilized plots in early May. We applied slow-release forms of each nutrient in order to alleviate nutrient limitation within experimental communities through the growing season.

We visually quantified the percent cover of each plant species in each plot in September from 2012 through 2015 using a modified Daubenmire method (Daubenmire 1959, Borer et al. 2014a). To account for plot-level edge effects, we quantified the absolute cover of each species in a marked $0.75 \times 0.75 \text{ m}$ subplot in the center of each plot. Because we measured absolute cover of each species, the sum of covers for a plot could exceed 100%. To maximize accuracy and consistency of measurement, the same three researchers jointly assessed cover in each plot.

After identifying each species, we used the USDA Plants Database (and other sources, as needed) to classify species as exotic or native to eastern North America. A small number of species, amounting to less than 1% of total cover in any plot, were unidentifiable and were excluded from analyses. We then assessed the dominance of exotic species in response to our treatments (hereafter, exotic dominance), the ratio of the absolute exotic cover to the total cover of all plants within a plot. Exotic dominance is a measure of exotic success that is especially useful for comparing treatments that differ in productivity or species richness (i.e., where total

cover can vary) (Catford et al. 2012, Seabloom et al. 2013, Colautti et al. 2014). To determine whether changes in exotic dominance are due to changes in the absolute abundance of exotic species or of native species, we separated the cover of each community into three classes: native residents, native colonizers, and exotic colonizers. Native residents were species that we had initially planted in a plot in 2011 – 2012. Native colonizers were native species that we had not planted in a plot. Thus, species within our planted species pool were included as native colonizers only in plots in which they were not planted. We distinguished between these groups because resident individuals were not subject to the same natural dispersal or establishment limitations as colonizing individuals. Furthermore, resident individuals could benefit from priority effects that could cause their responses to the experimental treatments to differ from the responses of colonizing individuals. We then examined the absolute abundance of each group.

We analyzed all data in R version 3.2.2 (R Foundation for Statistical Computing, Vienna 2015). To model the effects of community richness, fertilization, enemy access, and the interactions between these factors on exotic dominance and absolute abundance by cover groups (native residents, native colonizers, exotic colonizers), we used the nlme package for linear mixed effects models (Pinheiro et al. 2016). In order to meet assumptions of homoscedasticity and normality of residuals, we logit-transformed exotic dominance and square-root transformed absolute abundance. Each model included block, fertilization, spraying, species richness, year of observation, and interactions between these factors as categorical fixed effects. In the model of absolute abundance, we also included the effect of cover group nested within plots to account for non-independence between groups. We modelled year categorically rather than continuously to avoid assuming that exotic dominance and absolute abundance would change linearly over time. To limit the number of comparisons of absolute abundance by cover groups, we focused on

pairwise comparisons of ecological relevance. Specifically, we used the `pairs` function in the `lsmeans` package (Lenth 2013) to test for differences between treatments within each combination of cover group and year (e.g., the effect of fertilization on native residents in 2013).

To account for temporal autocorrelation, we included an ARMA 1, 1 autocorrelation structure in each model (Zuur et al. 2009). This structure combined an autoregressive (AR) structure and a moving average (MA) structure, both of order 1. Following Schmid et al. (2002) and others (e.g., Hector et al. 2011), we included planted community composition as a random effect in each model. This is a more conservative test because it allows us to ascribe differences to richness *per se* only when differences in a response *within* a diversity level (i.e., polycultures or monocultures) are smaller than differences *between* diversity levels (Schmid et al. 2002). Except for the main effect of species richness, parameter estimates from models without the random effect of planted community composition did not differ qualitatively from models including this random effect.

Results

Spraying reduced exotic dominance overall, (Spraying: $P < 0.001$, Appendix D3.3, Fig. 5.2a), and particularly from the second year of the study onward (Spraying \times Year: $P < 0.001$). Exotic dominance was lower in sprayed plots primarily because spraying slowed the decline in abundance of native residents beginning in the second year of the study (Tukey HSD: 2013, $P = 0.01$; 2014, $P < 0.001$; 2015, $P < 0.001$; Appendix D3.4, Fig. 5.3a). In contrast, spraying only reduced exotic abundance in 2014 (Tukey HSD: $P = 0.013$, Fig. 5.3a). Furthermore, spraying did not affect the abundance of native colonizers in any year. Overall, these results suggest that enemies had little influence on the success of colonizers, whether exotic or native, but enemies suppressed native residents, and thereby increased exotic dominance.

Fertilization strongly increased exotic dominance throughout the experiment (Fertilization: $P < 0.001$, Appendix D3.3, Fig. 5.2c), and the magnitude of this effect increased over time (Fertilization \times Year: $P < 0.001$). Fertilization also increased exotic abundance (Tukey HSD: $P < 0.001$) particularly from the second year of the study onward, with the largest effect occurring in 2014 (Tukey HSD: $P < 0.001$). Interestingly, fertilization increased the abundance of native colonizers in 2012 (Tukey HSD: $P < 0.001$), then had no significant effect in 2013 (Tukey HSD: $P = 0.99$), and marginally reduced it in 2014 and 2015 (Tukey HSD: 2014, $P = 0.052$; 2015, $P = 0.067$). Furthermore, fertilization decreased the abundance of native residents beginning in the second year of the study (Tukey HSD: 2013, $P < 0.001$; 2014, $P < 0.001$; 2015, $P < 0.001$, Fig. 5.3c). Overall, these results suggest that fertilization increased the performance of exotic colonizers, allowing them to more rapidly outcompete both native colonizers and native residents, increasing exotic dominance.

Increased diversity did not significantly influence exotic dominance (Diversity: $P = 0.35$, Appendix D3.3, Fig. 5.2b). Similarly, diversity did not influence exotic abundance (Tukey HSD: $P = 0.93$). In contrast, diversity decreased the abundance of native colonizers each year (Tukey HSD: $P < 0.001$, Appendix D3.4, Fig. 5.3b). This may have occurred because diversity increased the abundance of native residents (Tukey HSD: $P < 0.001$, Appendix D3.4, Fig. 5.3b). Overall, these results suggest that increased diversity allowed native residents to resist colonization by other native species, but not by exotics, leading to no net effect on exotic dominance.

Even though fertilization and spraying each independently influenced exotic dominance, we found no evidence that any of the three potential drivers interacted to influence exotic dominance. Spraying and diversity did not interact to affect exotic dominance (Spraying \times Diversity: $P = 0.83$, Appendix D1.3), nor did fertilization and diversity (Fertilization \times Diversity:

$P = 0.20$, Appendix D1.4), nor fertilization and spraying (Fertilization \times Spraying: $P = 0.46$, Appendix D1.5). Furthermore, fertilization, spraying, and diversity did not interact either (Fertilization \times Spraying \times Diversity: $P = 0.10$). In whole, this indicates that nutrients and enemies acted additively and were the strongest drivers of exotic dominance.

Discussion

Overall, we found no evidence that native richness, resource supply, and enemy access to communities interacted to influence exotic success. Rather, resource addition and enemy exclusion each independently influenced exotic success, while native species richness did not strongly influence exotic success. Together, these results suggest that the effects of soil resources and enemies are additive and do not interact in our study system.

Several studies have demonstrated that community regulation by natural enemies, particularly soil pathogens, increases substantially with decreasing species richness (Maron et al. 2011, Schnitzer et al. 2011, Kulmatiski et al. 2012); this effect is attributed to species in species-rich communities being at lower densities, leading to weaker regulation by density-dependent herbivores and pathogens. Yet we know of only one study to extend this to plant invasions (Liao et al. 2015). Here we found no evidence for this negative relationship. There are several potential reasons for this. First, increasing species richness could increase regulation by pathogens and herbivores if one community member is a competent host that increases disease or herbivory within the entire community (Power and Mitchell 2004, Barbosa et al. 2009). Second, increasing regulation by pathogens in species-poor communities has primarily been observed with soil pathogens (e.g., Maron et al. 2011, Schnitzer et al. 2011, Kulmatiski et al. 2012), whereas our study manipulated aboveground enemies. Third, increasing species richness might have been unable to reduce regulation by enemies among native species enough to overcome the lack of

regulation by enemies among exotic species, altering the outcome of competition between native and exotic species too little to change community composition.

Theory also predicts that species-rich communities will reduce resource availability more than species-poor communities (Tilman 2004) and prior experimental evidence supports this (e.g., Kennedy et al. 2002, Roscher et al. 2009). Based on this empirical and theoretical evidence, species-rich communities should better resist exotic invasion following resource addition than species-poor communities. But here, we found no evidence for this relationship. One possible explanation for the lack of relationship would be if the rate of nutrient supply was greater than the rate at which even the species-rich communities were able to draw down nutrient availability, thus providing additional nutrients for exotics regardless of native richness (Renne et al. 2006, Mattingly and Reynolds 2014).

We found no evidence that enemy exclusion only reduces exotic success in fertilized communities. This is consistent with recent results from two studies that examined the effects of different enemy guilds on exotic success at different levels of ecological organization (Dawson et al. 2014, Seabloom et al. 2015). Neither Dawson et al. (2014), who manipulated access by insect herbivores to individual plants, nor Seabloom et al. (2015), who manipulated access by vertebrate herbivores to intact plant communities, found that exotic species in high resource environments benefitted more from enemy release relative to native competitors. A third study, performed at the same site as our study, largely agreed with Seabloom et al. (2015) and Dawson et al. (2014) when access by foliar fungal pathogens and insect herbivores to intact communities was manipulated. But when access by vertebrate herbivores to communities was manipulated, fertilization increased exotic success only when vertebrate herbivores were present (Heckman et al. 2016). Together, these studies suggest that across a range of natural enemy guilds,

fertilization does not often interact with enemies to influence the success of individual exotic plants, or exotic populations in intact or assembled communities. Instead, these effects of resource supply and enemy exclusion appear to be more often additive and independent.

In this study, enemy exclusion affected each measure of exotic success—exotic dominance and absolute exotic abundance—differently. This may have occurred because exotic dominance uses more information than absolute exotic abundance to explain the influence of enemy exclusion on the outcome of competition between natives and exotics (Keane and Crawley 2002, Mitchell et al. 2006). Although the enemy release hypothesis was originally intended to describe how biogeographic differences in regulation by enemies could influence exotic success (i.e., differences in regulation between the native and introduced range), more recently the hypothesis has been extended to explain exotic success within local communities (Keane and Crawley 2002, Heger and Jeschke 2014). Thus, if exotics are less heavily regulated by enemies than natives, enemy exclusion should indirectly influence exotics via competition from native species (Keane and Crawley 2002). This hypothesized interaction chain includes both direct and indirect effects of enemies (Mitchell et al. 2006). The hypothesized direct effects of enemy exclusion on natives, which should be detected quickly, only influence changes in exotic dominance (via its denominator, which includes absolute native abundance). In contrast, the hypothesized indirect effects of enemy exclusion on exotics, which should be detected more slowly, influence both exotic dominance and absolute exotic abundance. Thus in 2013, even though spraying directly benefitted native residents, reducing exotic dominance, this effect may not have been strong enough to detectably alter absolute exotic abundance. By 2014, the cumulative benefits of enemy exclusion to native residents that had built up over nearly three growing seasons may have been large enough to allow natives to slow the increase in absolute

exotic abundance. However, this did not continue in 2015, perhaps because although the increase in exotic abundance was slowed in unsprayed plots, it had still reached its maximum by 2015.

Unlike many other experimental studies (Knops et al. 1999, Naeem et al. 2000, Kennedy et al. 2002, Fridley et al. 2007), in this study increasing species richness had only a small effect on exotic dominance. This may have occurred because the planted native species and exotic colonizers exhibited large niche differences. Specifically, the most abundant exotic species at our site—a C3 grass (*Schedonorus arundinaceus*), a legume (*Lespedeza cuneata*), and a woody vine (*Lonicera japonica*)—might occupy different niches from our planted native species, C4 grasses and non-leguminous forbs. Our site, like many old field communities in the region (Oosting 1942), had few native species of C3 grasses, legumes, or woody vines, and those that were present occurred at low abundance. Because of this, our native planted species pool comprised species from the more common C4 grass and non-leguminous forb functional groups. While there was little effect of species richness on exotic dominance, species-rich communities strongly suppressed native colonizers, many of which were also C4 grasses and non-leguminous forbs, including *Dichanthelium* spp., *Apocynum cannabinum*, and *Solanum carolinense*. Together, these results are consistent with the idea that residents are better able to reduce colonization by species occupying more similar niches (Shea and Chesson 2002, Fargione et al. 2003, Tilman 2004), and that increasing species richness reduces exotic success by increasing niche overlap between native residents and exotics (Levine and D'Antonio 1999, Tilman 2004, MacDougall et al. 2009). Conversely, our results emphasize that increasing native richness may not reduce exotic success in systems where natives and exotics occupy different niches. If large niche differences between exotics and native residents are common to many plant communities, then species richness may be a poor predictor of exotic success and predictions for exotic success that

are based on richness may need to be reconsidered (e.g., Levine and D'Antonio 1999, Fridley et al. 2007).

Contrary to predictions of some invasion hypotheses, multiple interacting drivers were not necessary to explain exotic success in our system. Nor could exotic success be explained by any single driver. Instead, exotic success was driven by both nutrient addition and exclusion of enemies, which most strongly influenced native resident species, with each driver acting independently. Nutrient addition predominantly benefitted exotic species, allowing them to outcompete natives. On the other hand, excluding enemies primarily benefitted resident native species directly, while having subtler indirect negative effects on exotics. Thus, excluding enemies slowed the replacement of native resident species by exotics. Together, our results demonstrate that single-driver hypotheses may be insufficient and multiple interacting drivers may be unnecessary to explain invasions; within a single system, exotic success may be determined by multiple drivers acting independently.

Fig. 5.1 Planting arrangement of polycultures. This community has 9 *Andropogon virginicus* (AV) individuals and 8 individuals of *Tridens flavus* (TF), *Scutellaria integrifolia* (SI), *Packera anonyma* (PA), and *Solidago pinetorum* (SP). *Setaria parviflora* was excluded.

SP		TF		AV		SP		AV
	SI		TF		PA		PA	
SP		AV		SI		PA		AV
	PA		SI		AV		SP	
TF		TF		SP		AV		PA
	AV		SP		SI		TF	
SP		SI		AV		PA		TF
	PA		SP		TF		AV	
TF		PA		SI		SI		SI

Fig. 5.2 Effects of a) fungal and insect enemy exclusion (spraying), b) initial community richness, and c) fertilizer application, on exotic dominance over four years (2012 – 2015) calculated using restricted maximum likelihood estimation and back-transformed from a logit transformation. Error bars represent 95% confidence intervals. Within each year, asterisks denote significant treatment effects.

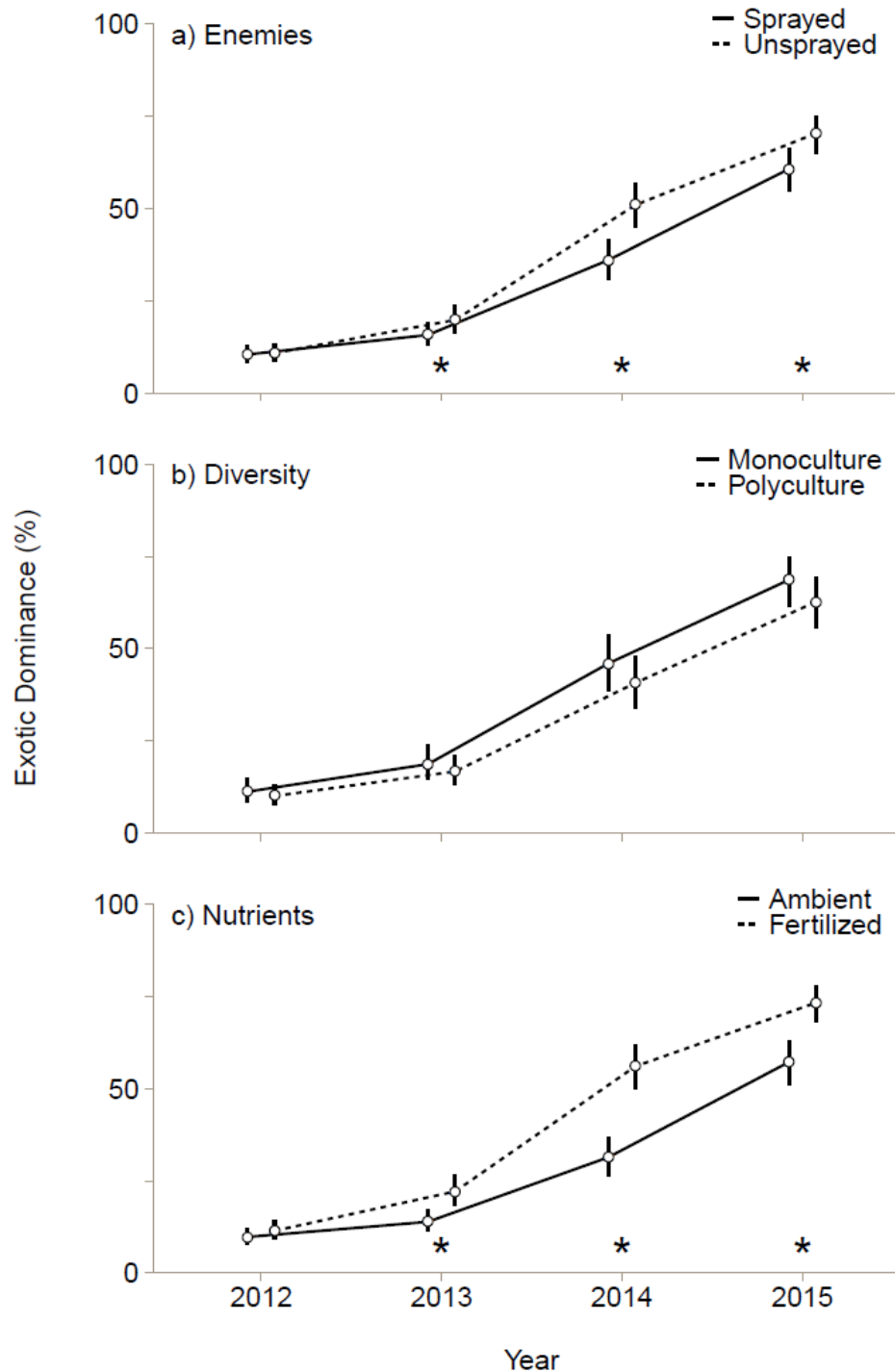
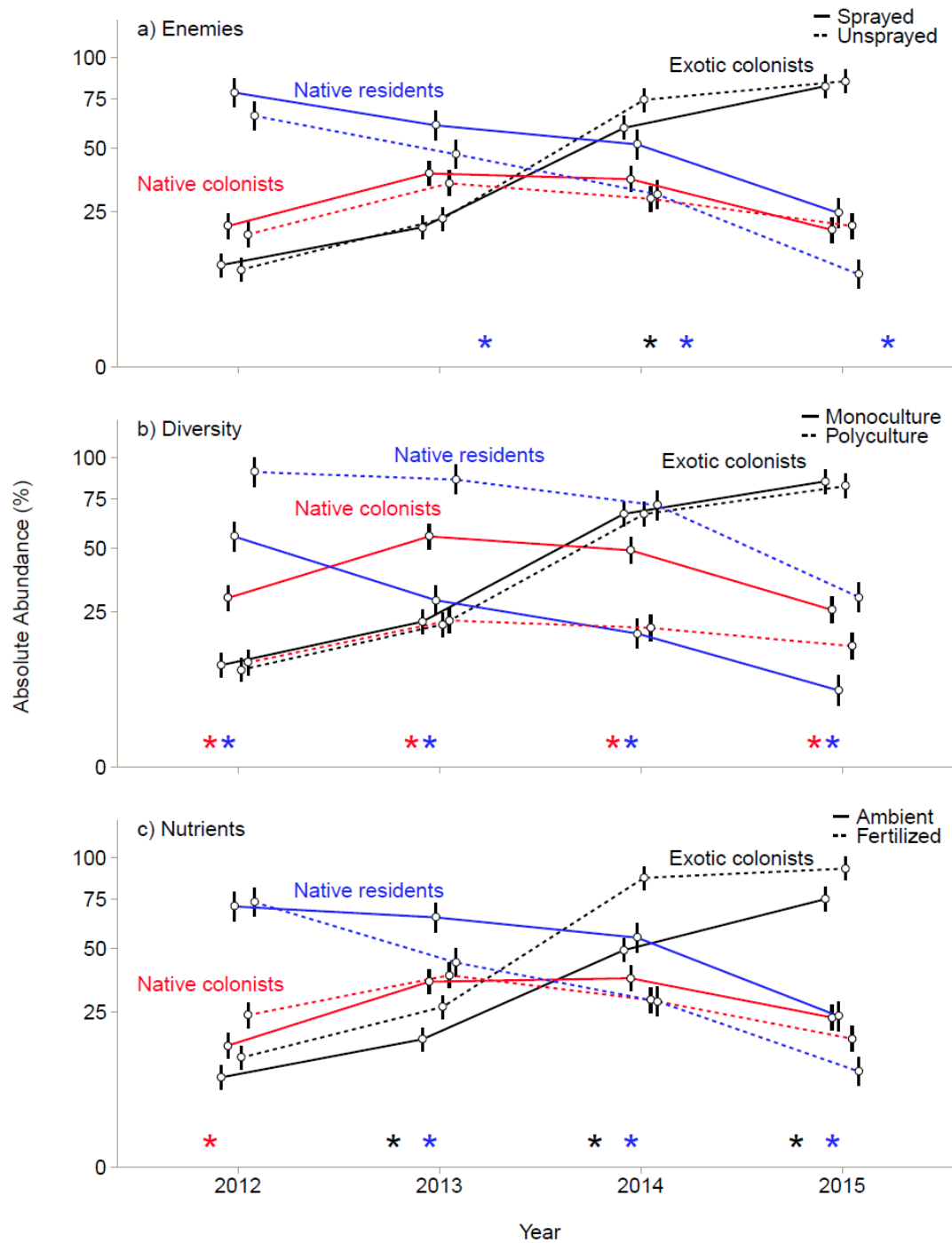


Fig. 5.3 Effects of a) fungal and insect enemy exclusion (spraying), b) initial community richness, and c) fertilizer application, on absolute abundance (%) of exotic colonizers (black line), native colonizers (red line), and native residents (blue line) over four years (2012 – 2015) calculated using restricted maximum likelihood estimation and back-transformed from a square-root transformation. Error bars represent 95% confidence intervals. Asterisks are colored by cover group; within each year, they denote significant treatment effects on that cover group.



CHAPTER VI. CONCLUSIONS

Exotic plant invasions continue to increase worldwide, as do the factors hypothesized to explain invasions (Shea and Chesson 2002, Mitchell et al. 2006, Catford et al. 2012). The proliferation of single-factor invasion hypotheses, many of which explain invasions over a narrow range of conditions, highlights the importance of integrating these hypotheses to explain invasions more generally. Here I tested the conditions and prediction for one of the most integrative hypotheses of plant invasions, the Resource-Enemy Release Hypothesis (R-ERH). The R-ERH predicts that natural enemies will promote dominance of the plant community by exotic species, particularly when resource availability is high (Blumenthal 2005, Blumenthal 2006). In this dissertation, I tested the underlying conditions and predictions of R-ERH with intact communities, assembled communities, and individual plants. Overall, I have found limited support for R-ERH. Instead, the major drivers of invasion tended to operate independently.

One of these major invasion drivers is increased resource supply (Huenneke et al. 1990, Davis et al. 2000, Shea and Chesson 2002, Tognetti and Chaneton 2015, Heckman and Carr 2016). Fertilization strongly and consistently increased exotic success relative to co-occurring natives. This increase in exotic success occurred in intact communities (Chapter 2) and in assembled native communities (Chapter 5). By reducing light and water availability, fertilization of exotic-dominated communities also reduced native seedling establishment (Chapter 4). Finally, exotic grasses often had stronger biomass responses to fertilization than native species (Chapter 3), probably because successful exotic species often possess traits associated with rapid growth in resource-rich environments (Baker 1986, Dawson et al. 2012, Leishman et al. 2014).

Differences in pressure from natural enemies on native and exotic species also often facilitates invasion (Keane and Crawley 2002, Mitchell and Power 2003, Liu and Stiling 2006, Heger and Jeschke 2014). Although enemies in this study did not always affect exotic and native species as R-ERH predicted, some effects of natural enemies on communities were consistent. Specifically, enemies had stronger effects on resident species than colonizing species. Excluding enemies reduced native establishment (Chapter 4). This occurred even though exotics were the dominant resident species, and enemy exclusion reduced disease and herbivory on colonizing species. Thus, enemy exclusion benefitted the resident community more than colonizing seedlings, reducing native establishment. As predicted, in Chapter 5 enemy exclusion reduced the abundance of exotic colonizers. However, this did not primarily occur by the predicted mechanism. Instead, enemy exclusion limited declines in the abundance of native residents, while having only minor effects on exotic colonists. In Chapter 2, where exotics and natives began at similar abundances, excluding fungal pathogens and insect herbivores reduced exotic success primarily in unfertilized plots. These results suggest that natural enemies acted in a density-dependent manner. Natural enemies impacted colonizers much less than resident species, regardless of provenance.

Because of the apparent density-dependence of plant-enemy interactions, enemies may help explain exotic establishment. Results from Chapter 4 and Chapter 5 suggest that establishment in intact perennial-dominated communities (where residents are large, well-established individuals, and bare ground is minimal) is difficult. But enemies may less effectively explain exotic success in communities they dominate. Once exotics dominate communities, their superiority in nutrient-rich environments may limit native re-establishment, even if enemies negatively impact exotics at that stage. Restoration of native-dominated habitats

may prove difficult, given the ease with which exotics colonized assembled communities (Chapter 5) and the difficulty with which natives colonized exotic-dominated intact communities (Chapter 4).

Results from Chapter 3 highlight the difficulty in using individual-level studies to explain community-level phenomena, like invasions. While native grasses did not systematically differ from exotic grasses in response to fertilization and enemy exclusion, communities are often dominated by a few species. Thus, within a community, the response of a few dominant species may drive the group-level responses of exotics and natives, whereas each species in an individual-level study contributes equally to the group-level response (Catford et al. 2012, Seabloom et al. 2013). In fact, the species with the strongest response to fertilization was *Schedonorus arundinaceus*, one of the dominant species of this community. To the contrary, several of the more common native grasses had more muted responses to these treatments (e.g., *Andropogon virginicus*, *Tridens flavus*). Moreover, processes at the individual level may not scale up to the population level. For instance, across an entire population, multiple individuals can compensate for reductions in individual performance, particularly across the full life-history of the plant, resulting in smaller population-level responses to perturbation than would be expected based on individual-level responses. (Alexander and Mihail 2000).

These results may have also occurred because grasses typically possess fewer qualitative defenses than other taxonomic groups. Because qualitative defenses are important deterrents of non-specialist enemies (Joshi and Vrieling 2005), taxonomic groups with fewer qualitative defenses may be more susceptible to evolutionarily-naïve pathogens and herbivores. Consequently, release from enemies may be weaker in grasses than in some other groups.

In conclusion, these results demonstrate that both nutrient supply and enemy pressure are important drivers of invasion. But contrary to prediction, these drivers rarely interact. Instead, they primarily act independently.

APPENDICES

Appendix A Supplementary Material for Chapter 2.

Appendix A1. Description of greenhouse biocide experiment

Biocide selection

We chose a fungicide (mancozeb, Dithane[®] DF, Dow AgroSciences, Indianapolis, IN) and an insecticide (es-fenvalerate, Asana[®] XL, Dupont, Wilmington, DE) that have been commonly used in ecological studies (e.g., Mitchell 2003, Rogers and Siemann 2004, Parker and Gilbert 2007, Cronin et al. 2010a). Mancozeb is a broad-spectrum non-systemic fungicide that has no known direct effects on photosynthesis, leaf longevity, shoot growth or root growth (Lorenz and Cothren 1989, Kope and Trotter 1998, Parker and Gilbert 2007), nor does it affect mycorrhizal fungi when applied at recommended rates (Trappe et al. 1984, Parker and Gilbert 2007). Es-fenvalerate is a broad-spectrum non-systemic insecticide which has had no direct effects on shoot or root biomass on terrestrial plants (Siemann et al. 2004, Agrawal et al. 2012).

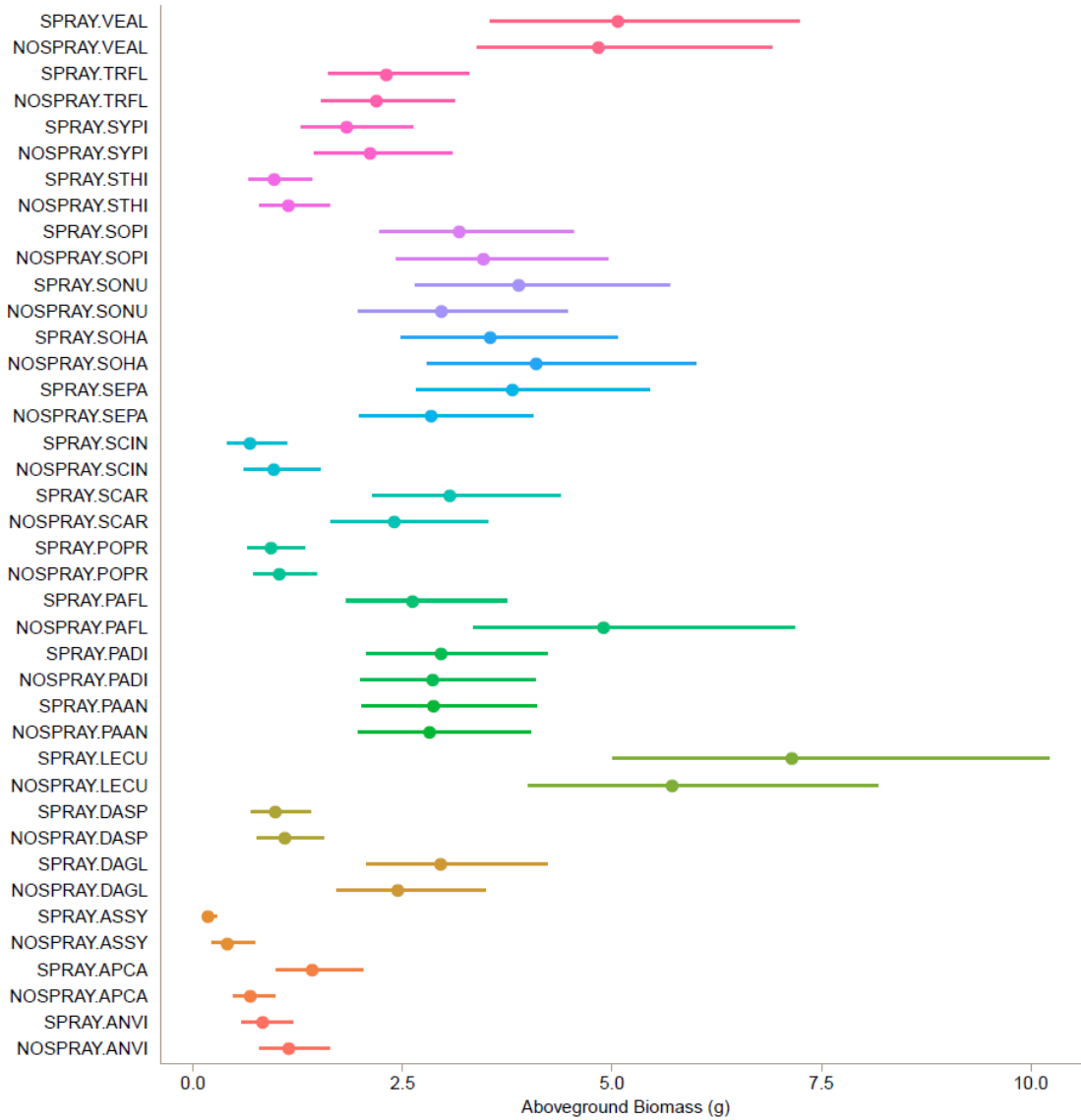
Greenhouse biocide experiment

We also tested for phytotoxic and any other direct effects of these biocides on 20 herbaceous species commonly found at Widener Farm. We sowed seeds of each of these 20 species into pots (938 mL D60 Deepots, Stuewe and Sons Inc, Tangent, OR) containing a 4:3:3 mixture of homogenized field soil, Fafard[®] 3B and Metro-Mix 360 potting media (both from Sun Gro Horticulture, Agawam, MA) on February 13, 2013. On May 20, 2013, we randomly assigned 8 pots of each species to a biweekly spraying treatment with both mancozeb and es-fenvalerate; the other 8 pots per species were controls. We harvested all aboveground biomass from each pot on July 15, 2013, then dried and weighed each sample.

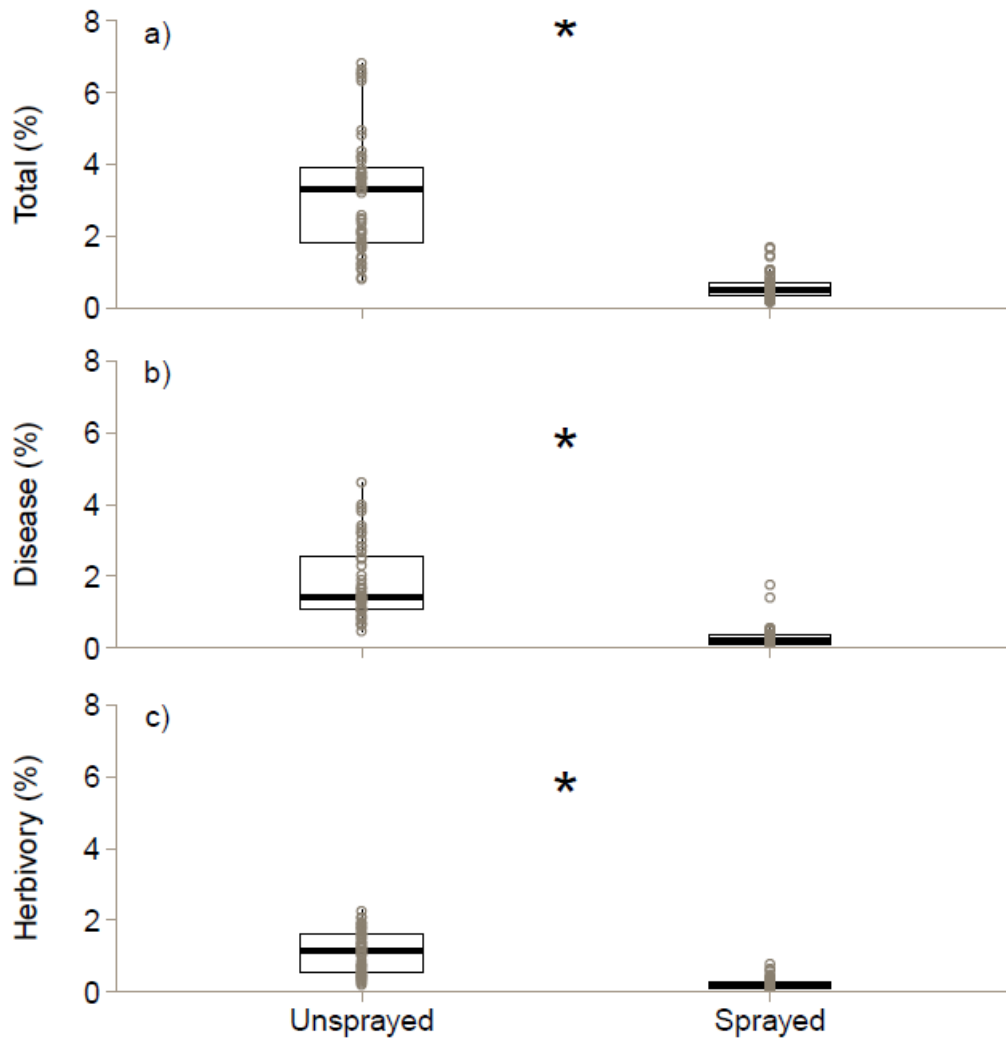
We analyzed these data with species and spraying treatments as fixed effects using the glm function with gamma errors and a log link (Zuur et al. 2013) in R version 3.2.2 (R Core Team 2016). We found no main effect of spraying on above-ground biomass ($P = 0.85$). To determine the effects of spraying on each species, we compared above-ground biomass of sprayed and unsprayed plants within a species using the lsmeans package (Lenth 2016) and controlled for multiple comparisons with the Tukey adjustment. After adjustment, only *Apocynum cannabinum*, which did not occur in this field experiment, had a marginally significant positive response to spraying ($P = 0.085$).

A2. Supplementary Figures

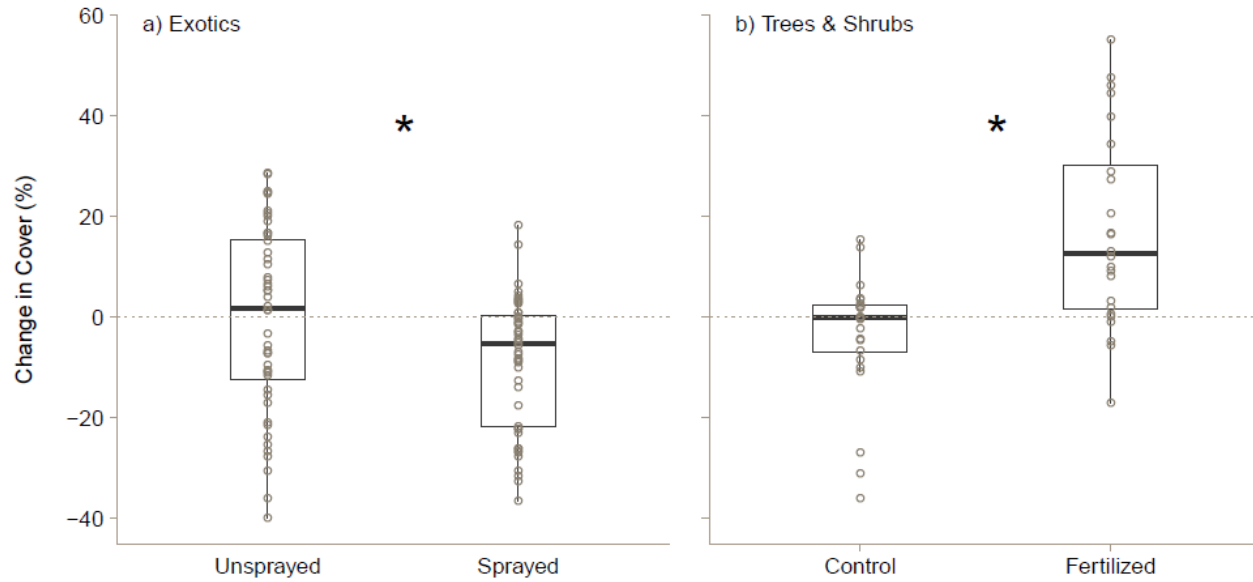
A2.1 Aboveground mean biomass (g) of 20 herbaceous species growing in greenhouse conditions. Plants were either sprayed biweekly with fungicide and insecticide (SPRAY) or left unsprayed (NOSPRAY). Species abbreviations are included in Table S2. Error bars represent 95% confidence intervals. Species did not differ in their response to spraying (Spraying \times Species: $P = 0.11$), nor did spraying have an effect on biomass (Spraying: $P = 0.85$) based on likelihood ratio tests.



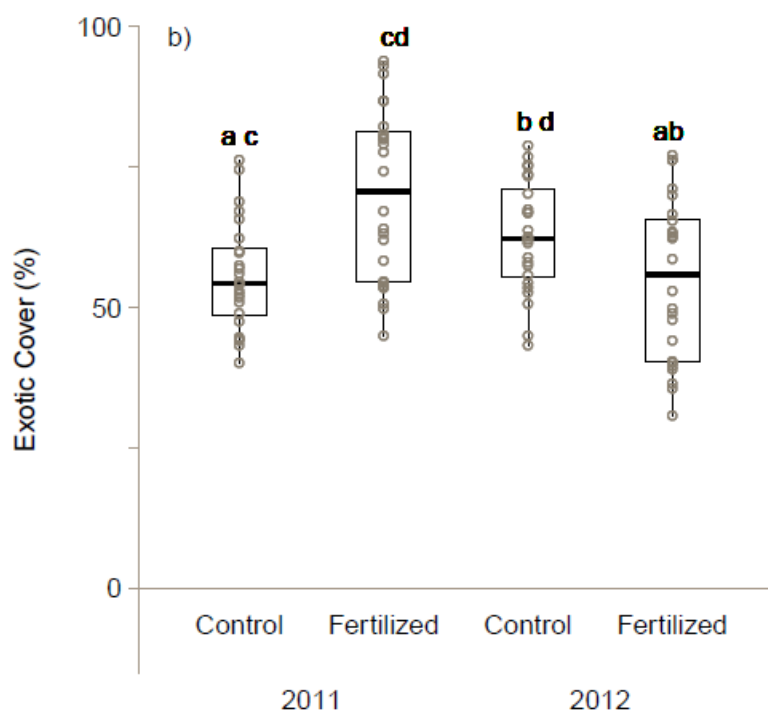
A2.2 Effects of fungal and insect enemy exclusion (spraying) on mean A) community total damage, B) community disease, C) community herbivory, calculated using restricted maximum likelihood estimation and back-transformed from a cube-root transformation. Points represent fitted values from the model. In each panel, * denotes a significant difference between treatments.



A2.3 A) Effects of fungal and insect enemy exclusion (spraying) on mean change in exotic cover from 2011 to 2012, B) effects of fertilization on mean change in tree and shrub cover from 2011 to 2012, calculated using restricted maximum likelihood estimation. Points represent fitted values from the model; * denotes a significant difference between treatments.



A2.4 Effects of fertilizer application on mean exotic cover in 2011 and 2012, calculated using restricted maximum likelihood estimation. Points represent fitted values from the model, shared letters denote no significant differences between treatments based on Tukey HSD.



A3. Full Statistical Models

A3.1 Comparisons of above-ground biomass for sprayed and unsprayed individuals within each species used in the greenhouse pesticide experiment. P-values were adjusted for multiple comparisons (20). We collected seeds locally in Duke Forest (Orange Co., NC) and purchased seeds from Ernst Conservation Seeds (Meadville, PA) or Everwilde Farms, Inc. (Sand Creek, WI).

Species	Abbreviation	Family	Seed Source	Adjusted P
<i>Andropogon virginicus</i>	ANVI	Poaceae	Ernst	0.99
<i>Apocynum cannabinum</i>	APCA	Apocynaceae	Ernst	0.085
<i>Asclepias syriaca</i>	ASSY	Asclepiadaceae	Ernst	0.44
<i>Dactylis glomerata</i>	DAGL	Poaceae	Field collected	1
<i>Danthonia spicata</i>	DASP	Poaceae	Everwilde	1
<i>Lespedeza cuneata</i>	LECU	Fabaceae	Ernst	1
<i>Packera anonyma</i>	PAAN	Asteraceae	Field collected	1
<i>Paspalum dilatatum</i>	PADI	Poaceae	Field collected	1
<i>Paspalum floridanum</i>	PAFL	Poaceae	Ernst	0.32
<i>Poa pratensis</i>	POPR	Poaceae	Ernst	1
<i>Schedonorus arundinaceus</i>	SCAR	Poaceae	Field collected	1
<i>Scutellaria integrifolia</i>	SCIN	Lamiaceae	Field collected	1
<i>Setaria parviflora</i>	SEPA	Poaceae	Field collected	1
<i>Sorghum halepense</i>	SOHA	Poaceae	Field collected	1
<i>Sorghastrum nutans</i>	SONU	Poaceae	Field collected	1
<i>Solidago pinetorum</i>	SOPI	Asteraceae	Field collected	1
<i>Steinchisma hians</i>	STHI	Poaceae	Field collected	1
<i>Symphyotrichum pilosum</i>	SYPI	Asteraceae	Ernst	1
<i>Tridens flavus</i>	TRFL	Poaceae	Ernst	1
<i>Verbesina alternifolia</i>	VEAL	Asteraceae	Field collected	1

A3.2 Model of A) community total damage, B) community disease, C) community herbivory with Spraying, field experiment.

		A) Community total damage		B) Community disease		C) Community herbivory	
	DF	F-value	P-value	F-value	P-value	F-value	P-value
Intercept	1, 47	623.88	<.001	275.14	<.001	487.99	<.001
Fertilization	1, 9	8.06	0.020	0.88	0.373	16.11	0.003
Spraying	1, 34	67.71	<.001	75.26	<.001	29.14	<.001
Month	1, 47	0.12	0.729	5.33	0.026	1.54	0.221
Fencing	1, 9	1.99	0.192	1.33	0.279	1.16	0.309
Fertilization ×Spraying	1, 34	1.88	0.179	3.09	0.088	1.11	0.300

A3.3 Model of A) community total damage, B) community disease, C) community herbivory.

		A) Community total damage		B) Community disease		C) Community herbivory	
	DF	F-value	P-value	F-value	P-value	F-value	P-value
Intercept	1, 46	517.93	<.001	241.65	<.001	257.16	<.001
Fertilization	1, 9	6.64	0.030	1.00	0.344	8.20	0.019
Provenance	1, 22	39.64	<.001	3.07	0.094	17.51	<.001
Month	1, 46	1.88	0.178	0.12	0.734	3.64	0.063
Fencing	1, 9	0.10	0.765	0.93	0.361	0.02	0.890
Fertilization ×Provenance	1, 22	7.28	0.013	0.70	0.413	10.45	0.004

A3.4 Model of exotic cover

	DF	F-value	P-value
Intercept	1, 136	371.79	<.001
Fertilization	1, 8	4.45	0.068
Fencing	1, 8	4.93	0.057
Spraying	1, 32	0.70	0.411
Year	1, 136	4.46	0.036
Fertilization × Fencing	1, 8	13.26	0.007
Fertilization × Spraying	1, 32	0.01	0.923
Fencing × Spraying	1, 32	0.37	0.545
Fertilization × Year	1, 136	35.45	<.001
Fencing × Year	1, 136	0.23	0.635
Spraying × Year	1, 136	3.61	0.059
Fertilization × Fencing × Spraying	1, 32	0.34	0.566
Fertilization × Fencing × Year	1, 136	0.92	0.340
Fertilization × Spraying × Year	1, 136	3.01	0.085
Fencing × Spraying × Year	1, 136	0.19	0.668
Fertilization × Fencing × Spraying × Year	1, 136	0.07	0.797

A3.5 Model of change in exotic cover

	DF	F-value	P-value
Intercept	1, 47	2.31	0.135
Fertilization	1, 8	11.15	0.010
Fencing	1, 8	0.02	0.904
Spraying	1, 32	5.07	0.031
Month	1, 47	2.55	0.117
Fertilization × Fencing	1, 8	0.19	0.672
Fertilization × Spraying	1, 32	3.91	0.057
Fencing × Spraying	1, 32	0.13	0.723
Fertilization × Fencing × Spraying	1, 32	0.09	0.770

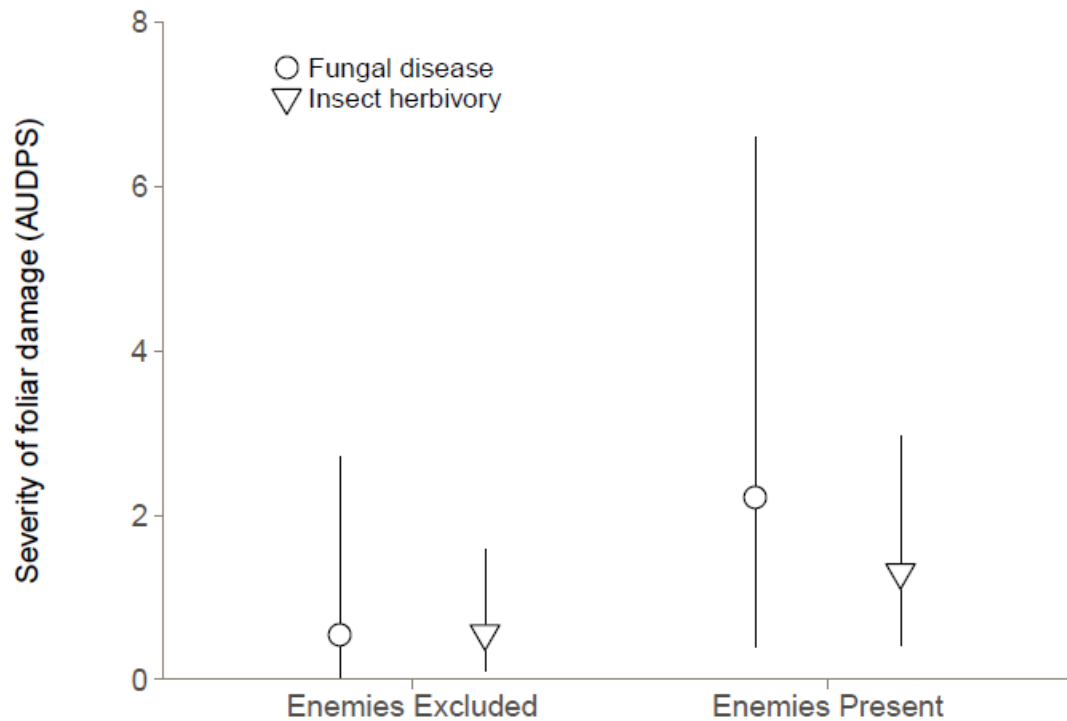
A3.6 Model of change in tree and shrub cover

	DF	F-value	P-value
Intercept	1, 32	7.90	0.008
Fertilization	1, 8	18.94	0.002
Fencing	1, 8	0.96	0.357
Spraying	1, 32	0.84	0.366
Fertilization × Fencing	1, 8	0.01	0.929
Fertilization × Spraying	1, 32	1.78	0.192
Fencing × Spraying	1, 32	1.95	0.172
Fertilization × Fencing × Spraying	1, 32	0.01	0.933

Appendix B Supplementary Materials for Chapter 3

B1. Supplementary Figures

B1.1 Effects of enemy exclusion on foliar fungal disease (open circles) and insect herbivory (open triangles), calculated using restricted maximum likelihood estimation and back-transformed from a cubed-root transformation. Points represent fitted values from the model, error bars represent 95% confidence intervals.



B2. Full Statistical Models

B2.1 Linear mixed models examining effects of nutrient supply, enemy exclusion, and provenance on severity of foliar damage (area under disease progress stairs) of A) fungal disease, B) insect herbivory, C) total damage.

A) Fungal disease	SumSq	MeanSq	NumDF	DenDF	F	P
Nutrients	0.707	0.7070	1	15.994	1.78	0.201
Enemies	11.171	11.1712	1	15.971	28.17	<0.001
Provenance	0.202	0.2019	1	9.004	0.51	0.494
Nutrients × Enemies	0.007	0.0074	1	15.938	0.02	0.893
Nutrients × Provenance	0.002	0.0019	1	177.933	0.01	0.945
Enemies × Provenance	0.366	0.3658	1	177.889	0.92	0.338
Nutrients × Enemies × Provenance	0.030	0.0302	1	177.809	0.08	0.783
B) Insect herbivory						
Nutrients	4.736	4.7362	1	16.360	4.30	0.054
Enemies	2.023	2.0225	1	16.352	1.84	0.194
Provenance	1.370	1.3702	1	9.166	1.25	0.293
Nutrients × Enemies	0.295	0.2953	1	16.340	0.27	0.612
Nutrients × Provenance	0.004	0.0035	1	179.092	0.01	0.955
Enemies × Provenance	1.315	1.3152	1	178.933	1.20	0.276
Nutrients × Enemies × Provenance	0.022	0.0220	1	178.684	0.02	0.888
C) Total damage						
Nutrients	6.422	6.4219	1	16.454	8.21	0.011
Enemies	13.928	13.9275	1	16.437	17.81	0.001
Provenance	0.115	0.1149	1	9.038	0.15	0.710
Nutrients × Enemies	0.009	0.0092	1	16.412	0.01	0.915
Nutrients × Provenance	0.229	0.2285	1	178.369	0.29	0.590
Enemies × Provenance	0.054	0.0542	1	178.303	0.07	0.793
Nutrients × Enemies × Provenance	0.004	0.0044	1	178.192	0.01	0.940

B2.2 Linear mixed models examining effects of nutrient supply, provenance, and foliar N content on severity of total foliar damage (area under disease progress stairs) across all plants (damaged and undamaged).

A) Total Damage	Sum Sq	Mean Sq	NumDF	DenDF	F	P
Nutrients	3.6841	3.6841	1	6.748	4.29	0.079
Provenance	0.2192	0.2192	1	9.205	0.26	0.626
Foliar N	0.5250	0.5250	1	57.029	0.61	0.438
Nutrients × Provenance	0.0001	0.0001	1	82.569	0.01	0.992
Nutrients × Foliar N	5.9467	5.9467	1	59.587	6.92	0.011
Provenance × Foliar N	1.9867	1.9867	1	84.356	2.31	0.132
Nutrients × Provenance × Foliar N	0.0168	0.0168	1	87.312	0.02	0.889

Appendix C Supplementary Materials for Chapter 4

C1. Full Statistical Models

C1.1 Parameter estimates from final confirmatory factor analysis.

Response	Predictor	Estimate	Std Error	P Value
Community Damage	Enemy Exclusion: Excluded	-0.8791	0.2276	<0.001
Community Damage	Litter Removal: Raked	0.4259	0.1623	0.012
Soil Moisture	Nutrients: Fertilized	-1.1278	0.2477	<0.001
Light	Litter Removal: Raked	1.7475	0.1310	<0.001
Light	Nutrients: Fertilized × Litter Removal: Raked	-0.4941	0.1853	0.011
Light	Nutrients: Fertilized	-0.3301	0.1853	0.083
Seedling Establishment	Nutrients: Fertilized	-2.5816	0.4053	<0.001
Seedling Establishment	Light	0.4211	0.0737	<0.001
Seedling Establishment	Community Damage	0.2878	0.0815	<0.001
Seedling Establishment	Soil Moisture	0.3676	0.1373	0.007

C1.2 Model of seedling damage using a linear mixed model.

	DF	F Value	P Value
Intercept	1, 16	395.13	<0.001
Enemy Exclusion	1, 16	5.89	0.027
Litter Removal	1, 11	0.06	0.811
Enemy Exclusion × Litter Removal	1, 11	1.35	0.270

C1.3 Model of seedling presence using a generalized linear mixed model with binomial errors.

	DF	X ²	P Value
Nutrients	1	11.70	<0.001
Enemy Exclusion	1	2.66	0.103
Litter Removal	1	2.21	0.137

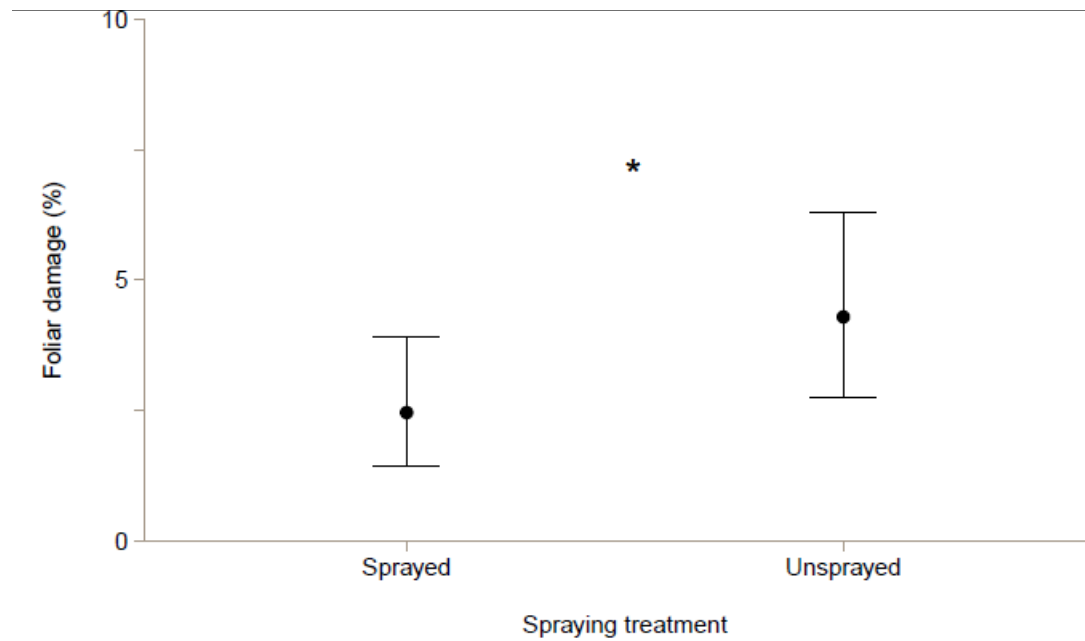
C1.4 Model of seedling per capita biomass using a linear mixed model.

	DF	F Value	P Value
Intercept	1, 16	54.24	<0.001
Enemy Exclusion	1, 16	0.07	0.788
Litter Removal	1, 11	1.59	0.234
Enemy Exclusion × Litter Removal	1, 11	0.08	0.778

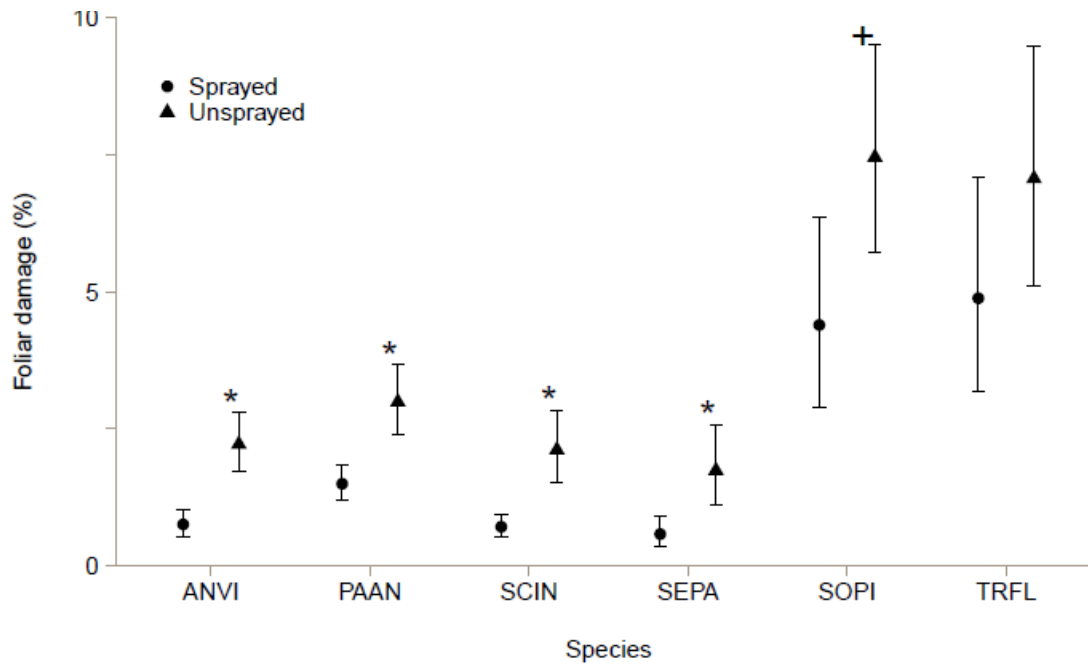
Appendix D Supplementary Materials for Chapter 5

D1. Supplementary Figures

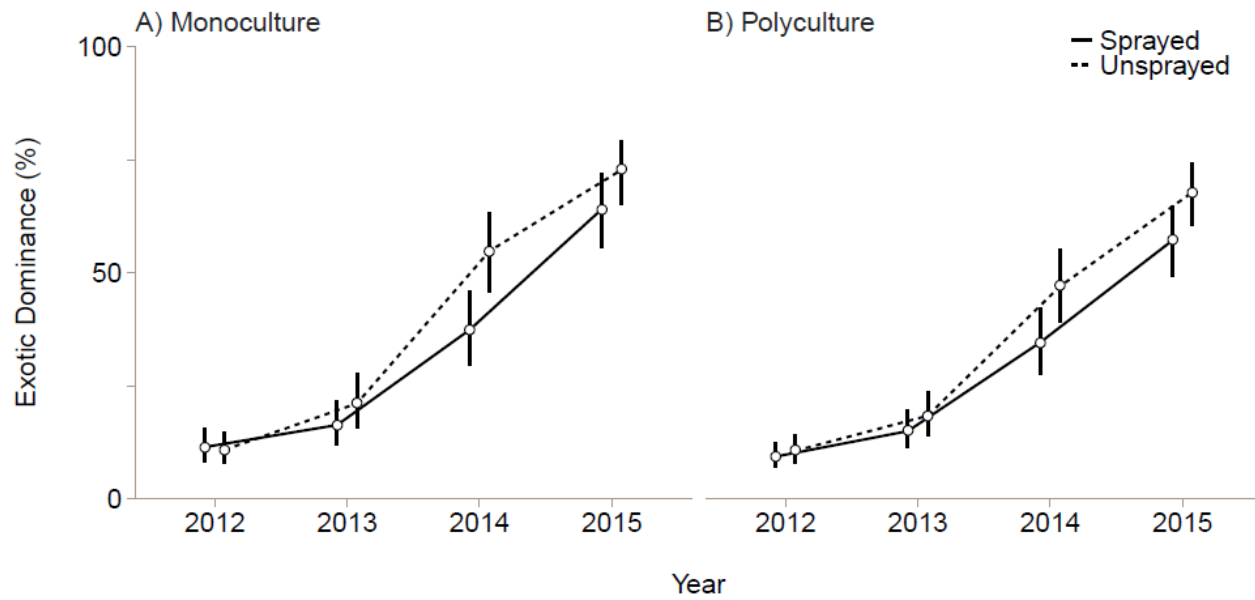
D1.1 Effects of fungal and insect enemy exclusion (spraying) on foliar damage (insect herbivory + fungal disease) in 2012 calculated using restricted maximum likelihood estimation and back-transformed from a cubed-root transformation. Error bars represent 95% confidence intervals. Significant differences ($P < 0.05$) between treatments are denoted by *.



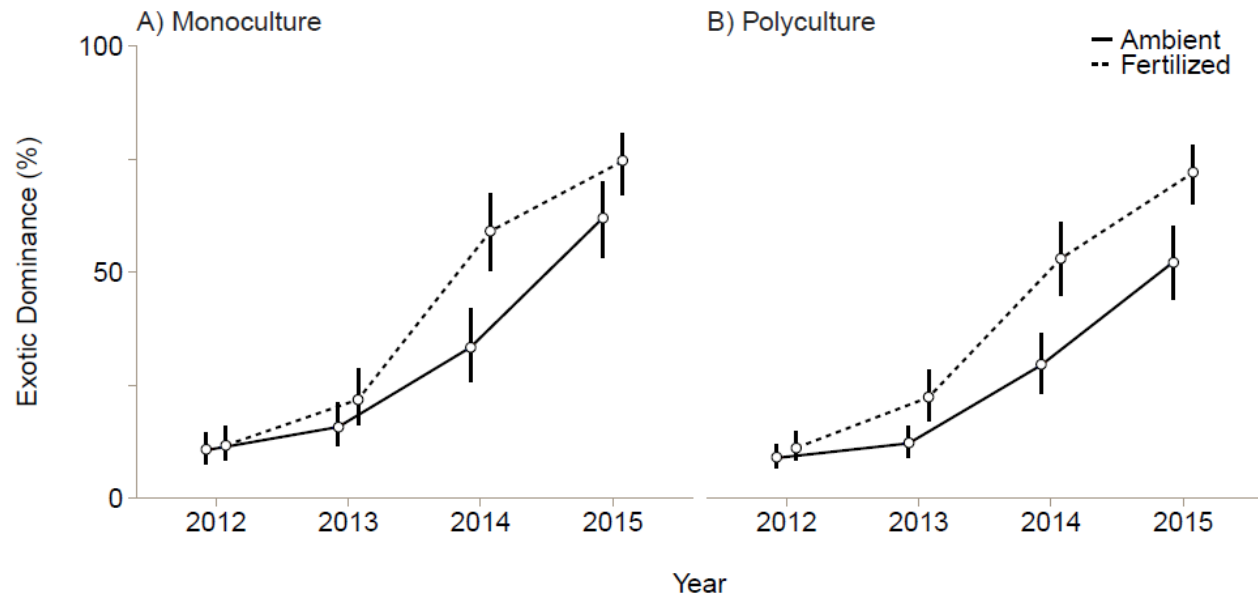
D1.2 Effects of fungal and insect enemy exclusion (spraying) on foliar damage (insect herbivory + fungal disease) by planted species (ANVI = *Andropogon virginicus*; PAAN = *Packera anonyma*; SCIN = *Scutellaria integrifolia*; SEPA = *Setaria parviflora*; SOPI = *Solidago pinetorum*; TRFL = *Tridens flavus*) calculated in 2012 using restricted maximum likelihood estimation and back-transformed from a cubed-root transformation. Error bars represent 95% confidence intervals. Within each species, * denotes significant differences ($P < 0.05$) and + denotes marginally significant differences ($0.1 > P > 0.05$) between treatments after adjusting for multiple comparisons with the ‘mvt’ method in the lsmeans package in R.



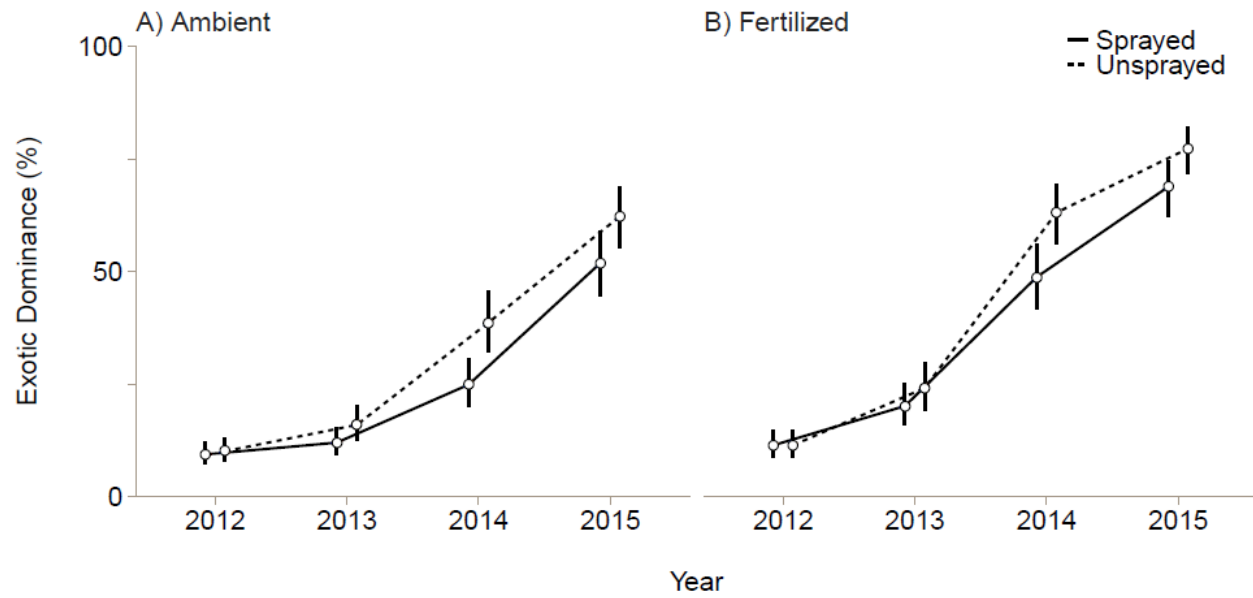
D1.3 Effects of fungal and insect enemy exclusion (spraying) in a) plots planted as monocultures and b) plots planted as five-species polycultures, on exotic dominance over four years (2012 – 2015) calculated using restricted maximum likelihood estimation and back-transformed from a logit transformation. Error bars represent 95% confidence intervals.



D1.4 Effects of fertilizer application in a) plots planted as monocultures and b) plots planted as five-species polycultures, on exotic dominance over four years (2012 – 2015) calculated using restricted maximum likelihood estimation and back-transformed from a logit transformation. Error bars represent 95% confidence intervals.



D1.5 Effects of fungal and insect enemy exclusion (spraying) in a) unfertilized (ambient) and b) fertilized communities, on exotic dominance over four years (2012 – 2015) calculated using restricted maximum likelihood estimation and back-transformed from a logit transformation. Error bars represent 95% confidence intervals.



D2. Supplementary Tables

D2.1 Species, family, and seed source of the six perennial herbaceous species used in this study. Seeds from two species were purchased from Ernst Conservation Seed (Meadville, PA), seeds from three species were collected from natural populations growing locally in Orange County, NC, and seeds of one species were collected from natural populations in Avery County, NC.

Species	Family	Seed Source
<i>Andropogon virginicus</i> L.	Poaceae	Ernst Conservation Seed
<i>Packera anonyma</i> (Alph. Wood) W.A. Weber & Á. Löve	Asteraceae	Field collected, Avery, NC
<i>Scutellaria integrifolia</i> L.	Lamiaceae	Field collected, Orange, NC
<i>Setaria parviflora</i> (Poir.) Kerguélen	Poaceae	Field collected, Orange, NC
<i>Solidago pinetorum</i> Small	Asteraceae	Field collected, Orange, NC
<i>Tridens flavus</i> (L.) Hitchc.	Poaceae	Ernst Conservation Seed

D3. Full Statistical Models

D3.1 Model of community-level foliar damage.

	Num DF	Den DF	F-value	P-value
Intercept	1	224	232.05	<0.001
Block	1	224	0.31	0.58
Spraying	1	224	28.93	<0.001

D3.2 Model of foliar damage by species.

	Num DF	Den DF	F-value	P-value
Intercept	1	454	4900.42	< 0.001
Block	1	224	0.27	0.60
Species	5	454	53.28	< 0.001
Spraying	1	224	95.17	< 0.001
Species \times Spraying	5	454	0.52	0.76

D3.3 Model of exotic dominance

	Num DF	Den DF	F-value	P-value
Intercept	1	690	78.93	< 0.001
Block	4	217	15.84	< 0.001
Fertilization	1	217	78.08	< 0.001
Spraying	1	217	22.42	< 0.001
Diversity	1	10	0.97	0.35
Year	3	690	700.34	< 0.001
Fertilization \times Spraying	1	217	0.54	0.46
Fertilization \times Diversity	1	217	1.65	0.20
Spraying \times Diversity	1	217	0.05	0.83
Fertilization \times Year	3	690	15.85	< 0.001
Spraying \times Year	3	690	6.42	< 0.001
Diversity \times Year	3	690	0.44	0.72
Fertilization \times Spraying \times Diversity	1	217	2.71	0.10
Fertilization \times Spraying \times Year	3	690	0.07	0.98
Fertilization \times Diversity \times Year	3	690	1.91	0.13
Spraying \times Diversity \times Year	3	690	0.07	0.36
Fertilization \times Spraying \times Diversity \times Year	3	690	0.21	0.89

D3.4 Model of absolute abundance by cover group

	Num DF	Den DF	F-value	P-value
Intercept	1	2070	6759.91	< 0.001
Block	4	217	1.45	0.22
Cover Type	2	462	86.28	< 0.001
Fertilization	1	217	2.49	0.12
Spraying	1	217	10.10	0.002
Diversity	1	10	0.15	0.71
Year	3	2070	166.15	< 0.001
Cover Type \times Fertilization	2	462	35.54	< 0.001
Cover Type \times Spraying	2	462	17.37	< 0.001
Fertilization \times Spraying	1	217	0.30	0.59
Cover Type \times Diversity	2	462	159.99	< 0.001
Fertilization \times Diversity	1	217	0.08	0.78
Spraying \times Diversity	1	217	0.22	0.64
Cover Type \times Year	6	2070	371.10	< 0.001
Fertilization \times Year	3	2070	10.90	< 0.001
Spraying \times Year	3	2070	0.47	0.70
Diversity \times Year	3	2070	2.90	0.03
Cover Type \times Fertilization \times Spraying	2	462	0.70	0.50
Cover Type \times Fertilization \times Diversity	2	462	1.09	0.34
Cover Type \times Spraying \times Diversity	2	462	0.82	0.44
Fertilization \times Spraying \times Diversity	1	217	0.01	0.94
Cover Type \times Fertilization \times Year	6	2070	19.38	< 0.001
Cover Type \times Spraying \times Year	6	2070	7.21	< 0.001
Fertilization \times Spraying \times Year	3	2070	0.22	0.95
Cover Type \times Diversity \times Year	6	2070	14.49	< 0.001
Fertilization \times Diversity \times Year	3	2070	0.78	0.51
Spraying \times Diversity \times Year	3	2070	0.38	0.77
Cover Type \times Fertilization \times Spraying \times Diversity	2	462	0.88	0.42
Cover type \times Fertilization \times Spraying \times Year	6	2070	0.51	0.80
Cover Type \times Fertilization \times Diversity \times Year	6	2070	2.83	0.01
Cover type \times Spraying \times Diversity \times Year	6	2070	1.00	0.43
Fertilization \times Spraying \times Diversity \times Year	3	2070	0.23	0.95
Cover type \times Fertilization \times Spraying \times Diversity \times Year	6	2070	0.89	0.50

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