

Effects of native diversity, soil nutrients, and natural enemies on exotic invasion in experimental plant communities

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Abstract. Many factors can promote exotic plant success. Three of these factors—greater pressure from natural enemies on natives, increased soil nutrient supply, and low native species richness—may interact during invasions. To test for independent and interactive effects of these drivers, we planted herbaceous perennial communities at two levels of native richness (monocultures and five-species polycultures). We then factorially manipulated soil nutrient supply and access to these communities by aboveground foliar enemies (fungal pathogens and insect herbivores), and allowed natural colonization to proceed for four years. We predicted that nutrient addition would increase exotic success, while enemy exclusion and increasing native richness would reduce exotic success. Additionally, we expected that enemy exclusion would reduce the benefits of nutrient addition to exotic species most in species-poor communities, and that this effect would be weaker in species-rich communities. In total, we found no evidence that nutrient supply, enemy access, and native richness interacted to influence exotic success. Furthermore, native richness had no effect on exotic success. Instead, nutrient addition increased, and enemy exclusion decreased, exotic success independently. As predicted, enemy exclusion reduced exotic success, primarily by slowing the decline in abundance of planted native species. Together, these results demonstrate that multiple drivers of exotic success can act independently within a single system.

Key words: *biological invasions; biotic resistance; bottom-up effects; diversity-invasibility; old fields; top-down effects.*

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 be less regulated by pathogens and herbivores, and thus, better resist exotic invasions (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, bioicide spraying) can be used to examine the influence of natural enemies on plant performance and test whether

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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 benefit native species indirectly, by reducing competitive asymmetry and directly, by reducing regulation of native populations. 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, 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, 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 in intact communities regardless of resource supply (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 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, North Carolina, 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).

Plant composition and species richness

We assigned each study plot to one of two levels of species richness: monoculture or five-species polyculture. From a pool of six species, we assembled 12 planted communities: six monocultures and six five-species polycultures, where one species was excluded from each polyculture community. We omitted one species from each of the six polycultures to isolate the effects of planted species identity. All six species were represented equally at each diversity level to better account for the influence of each species on community processes, which is often difficult to assess in planted biodiversity experiments (Huston 1997, Schmid et al. 2002).

In May 2011, we established five spatial blocks, each 15 \times 15 m (225 m^2). Within each block, we established 64 plots, each 1 \times 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, Burr Ridge, Illinois, USA) 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 S1: Table S1 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 d. 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. Polycultures contained nine individuals of one randomly chosen species and eight individuals of the other four 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 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.

Nutrient supply and enemy access treatments

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, Indiana, USA) nor the insecticide (es-fenvalerate, Asana XL; Dupont, Wilmington, Delaware, USA) 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 S1: Table S2a, Fig. S1). Furthermore, after using the mvt method in the lsmeans package (Lenth 2016) 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 S1: Table S2b, Fig. S2).

To manipulate soil nutrient supply, each plot was assigned to one of two nutrient supply treatments (fertilized with $10\text{ g N}\cdot\text{m}^{-2}\cdot\text{yr}^{-1}$ as slow-release urea, $10\text{ g P}\cdot\text{m}^{-2}\cdot\text{yr}^{-1}$ as triple super phosphate, and $10\text{ g K}\cdot\text{m}^{-2}\cdot\text{yr}^{-1}$ as potassium sulfate 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. 2014), and has been shown to alleviate limitation by N, P, and K across a range of grassland habitats (Fay et al. 2015). 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.

Colonization and quantification of species abundance

We visually quantified the percent cover of all plant species in each plot in September 2012–2015 using a modified Daubenmire method (Daubenmire 1959, Borer et al. 2014). To account for plot-level edge effects, we quantified the absolute cover of each species in a marked 0.75×0.75 m subplot in the center of each plot. Because we measured absolute cover, the sum of cover 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 <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.

Data analysis

We analyzed all data in R version 3.2.2 (R Development Core Group 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. Because we were unable to fully remove heteroscedasticity through transformation, we incorporated an identity variance structure into each model using the varIdent function in nlme, which allows residual variance to differ between treatment levels: by diversity for the exotic abundance analysis, and by cover group for the absolute abundance by cover group analysis (Zuur et al. 2009, Pinheiro et al. 2016). 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 2016) 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 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 (i.e., relative exotic abundance) by 20% overall, (spraying, $P < 0.001$), and particularly from the second year of the study onward, by 20%, 29%, and 13%, respectively (spraying \times year, $P < 0.001$; Appendix S1: Table S3; Fig. 1a). Exotic dominance was lower in sprayed plots primarily because spraying significantly slowed the decline in abundance of native residents beginning in the second year of the study (Tukey HSD, 2013, $P = 0.01$; 2014,

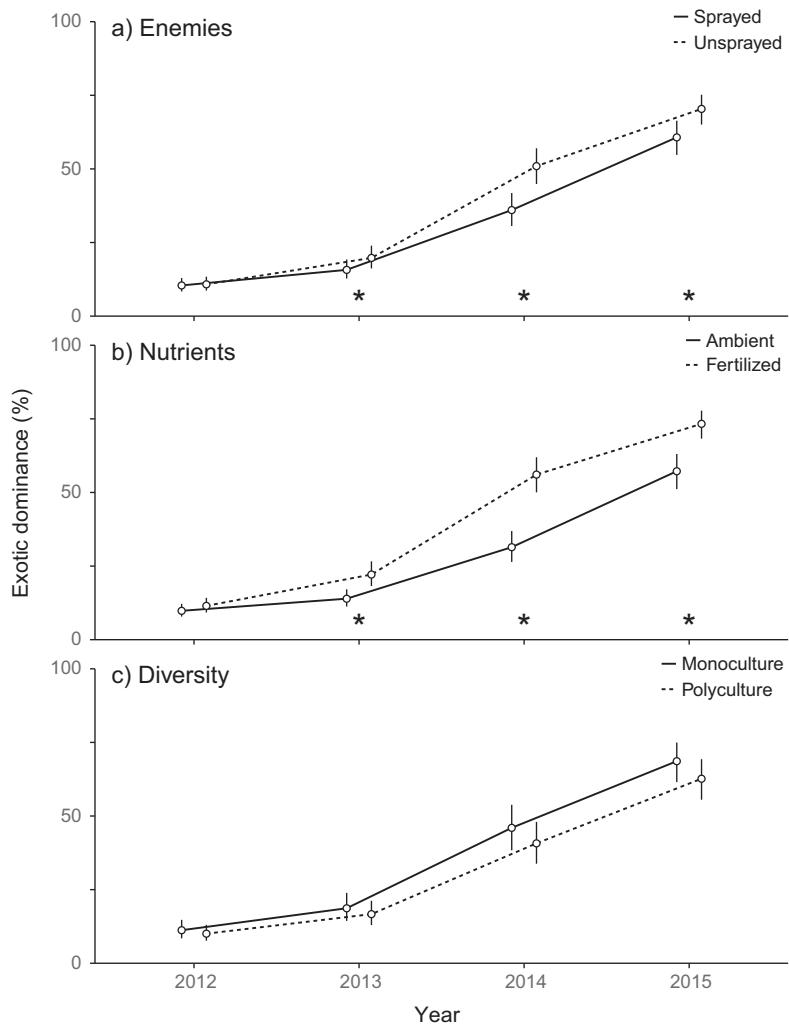


FIG. 1. Effects of (a) fungal and insect enemy exclusion (spraying), (b) fertilizer application, and (c) initial community richness on exotic dominance over four years (2012–2015) calculated using restricted maximum likelihood estimation and back-transformed from a logit transformation. Error bars represent 95% confidence intervals. Within each year, asterisks denote significant treatment effects ($P < 0.05$).

$P < 0.001$; 2015, $P < 0.001$; Appendix S1: Table S4; Fig. 2a). In 2013, 2014, and 2015, sprayed plots had 29%, 66%, and 176% higher abundance of native residents. In contrast, spraying only reduced exotic abundance significantly in 2014 (Tukey HSD, $P = 0.013$, Fig. 2a), reducing exotic abundance by 20%. 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 by >50% throughout the experiment (fertilization: $P < 0.001$), and the magnitude of this effect increased over time, 17%, 59%, 78%, and 28%, for 2012–2015, respectively (fertilization \times year, $P < 0.001$; Appendix S1: Table S3; Fig. 1b). 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, when fertilization increased exotic abundance by 77% (Tukey HSD, $P < 0.001$; Appendix S1: Table S4; Fig. 2b). Interestingly, fertilization increased the abundance of native colonizers in 2012 by 57% (Tukey HSD, $P < 0.001$), then had no significant effect in 2013 (Tukey HSD, $P = 0.99$), and reduced native colonizer abundance by 21% in 2014 and by 26% in 2015, but these effects were only marginally significant (Tukey HSD, 2014, $P = 0.052$; 2015, $P = 0.067$; Fig. 2b). Furthermore, fertilization decreased the abundance of native residents by at least 30% each year beginning in the second year of the study (Tukey HSD, 2013, $P < 0.001$; 2014, $P < 0.001$; 2015, $P < 0.001$; Fig. 2b). Overall, these results suggest that fertilization increased the performance of exotic colonizers, allowing them to more rapidly outcompete both

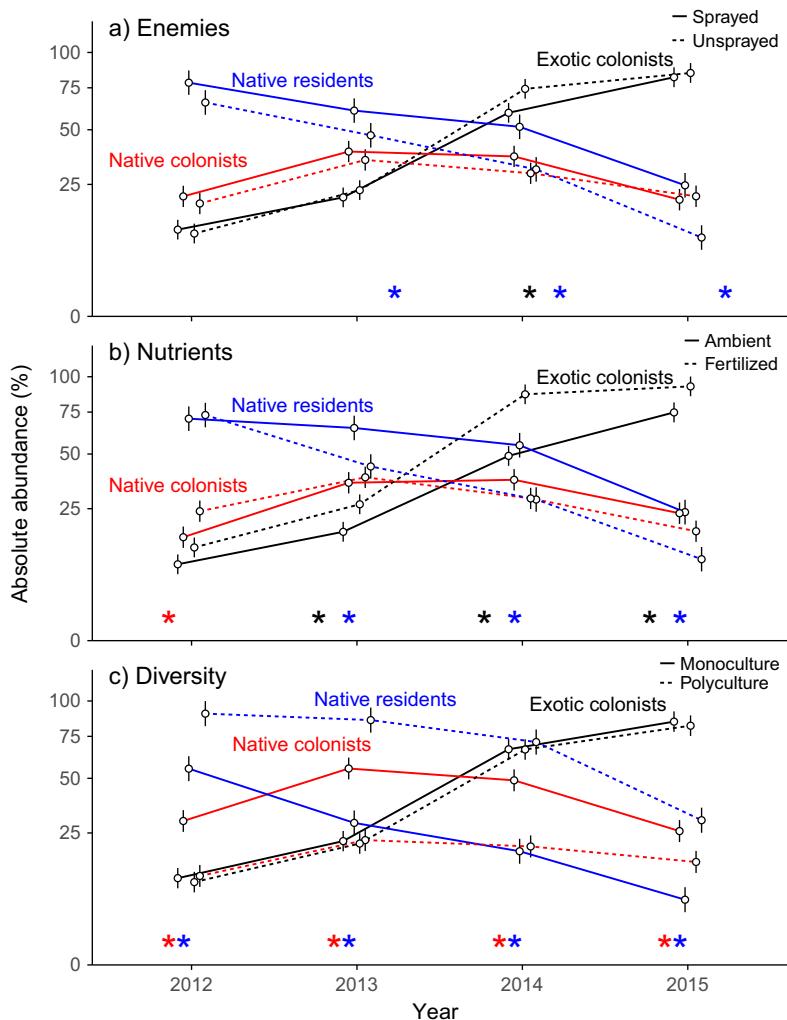


FIG. 2. Effects of (a) fungal and insect enemy exclusion (spraying), (b) fertilizer application, and (c) initial community richness on absolute abundance (%) of exotic colonizers (black line), native colonizers (red line), and native residents (blue line) over four years (2012–2015) calculated using 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 ($P < 0.05$).

native colonizers and native residents, increasing exotic dominance.

Increased diversity did not significantly influence exotic dominance (diversity, $P = 0.35$; Appendix S1: Table S3; Fig. 1c). Similarly, diversity did not influence exotic abundance (Tukey HSD, $P = 0.93$; Fig. 2c). In contrast, diversity decreased the abundance of native colonizers by at least 40% each year (Tukey HSD, $P < 0.001$; Appendix S1: Table S4; Fig. 2c). This may have occurred because diversity increased the abundance of native residents by at least 63% each year (Tukey HSD, $P < 0.001$; Appendix S1: Table S4; Fig. 2c). 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.

Although 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 S1: Table S3, Fig. S3), nor did fertilization and diversity (fertilization \times diversity, $P = 0.20$; Appendix S1: Table S3, Fig. S4), nor fertilization and spraying (fertilization \times spraying, $P = 0.46$; Appendix S1: Table S3, Fig. S5). Furthermore, fertilization, spraying, and diversity did not interact either (fertilization \times spraying \times diversity, $P = 0.10$; Appendix S1: Table S3). 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).

While fertilization and species richness did not interact, fertilization alone greatly increased exotic abundance. This effect was substantially larger than in a similar study that was replicated at 64 grassland sites worldwide (Seabloom et al. 2015). Several other studies that used higher fertilization levels than our study also found stronger responses from the exotic community

than we did (e.g., Renne et al. 2006, Tognetti and Chanton 2015, Heckman and Carr 2016). In our study, fertilization also increased the abundance of native colonizers in the first year, consistent with the idea that fertilization can increase colonization regardless of provenance (Davis et al. 2000). This trend did not continue in subsequent years, suggesting that native colonizer abundance stabilized at a lower level.

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 Dawson et al. (2014) and Seabloom et al. (2015) 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). 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, Prior et al. 2015). 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.

Several studies have shown that populations of exotic species benefit from excluding aboveground enemies (e.g., Schutzenhofer et al. 2009, Vasquez and Meyer 2011, Stricker and Stiling 2012). Importantly, a population-level increase in performance following enemy exclusion does not necessarily indicate that enemy release is not occurring. If exotics lose their specialist natural enemies and are still regulated to a lesser degree than natives, this could give exotics a competitive advantage. Thus, an important step forward in studying the role of enemies in plant invasions is determining how enemies influence competition between populations of native and exotic species within communities (e.g., Heard and Sax 2013, Kalisz et al. 2014, Stricker et al. 2016). The studies examining community-level enemy effects have had mixed results: excluding vertebrate herbivores for several years doubled native *Trillium* density and significantly reduced exotic *Alliaria petiolata* population growth (Kalisz et al. 2014); excluding foliar fungal pathogens increased the biomass of exotic *Microstegium viminum* by 39%, but had no effect on the biomass of the native community (Stricker et al. 2016); and excluding insect herbivores reduced the abundance of competitively superior exotic species (Heard and Sax 2013). Collectively, the results of these studies show that more work is needed to assess the community-level influences of enemies on plant invasions.

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. These planted species reflected the local species pool; our site, like many old field communities in the region (Oosting 1942), had few native C₃ grasses, legumes, or woody vines, and those that were present occurred at low abundance. This contrast between the functional traits of the exotic species and native species may stem from introduction bias,

in which exotic species that possess traits suited to a habitat are more likely to be introduced to that habitat, either accidentally or intentionally (Chrobock et al. 2011, Maurel et al. 2016). 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*. The effect of planted richness on native colonizers was of similar magnitude to other studies examining natural colonization of communities in which species richness was experimentally manipulated (Fargione and Tilman 2005, Roscher et al. 2009). This supports the ideas that residents are better able to reduce colonization by species occupying more similar niches (Shea and Chesson 2002, Fargione et al. 2003, Tilman 2004), like most of the potential native colonizers, 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). Thus, our results emphasize that increasing native richness may not reduce exotic success in systems where natives and exotics occupy distinct 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 benefited 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.

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LITERATURE CITED

- Barbosa, P., J. Hines, I. Kaplan, H. Martinson, A. Szczepaniec, and Z. Szendrei. 2009. Associational resistance and associational susceptibility: having right or wrong neighbors. *Annual Review of Ecology, Evolution, and Systematics* 40: 1–20.
- Blumenthal, D. 2005. Interrelated causes of plant invasion. *Science* 310:243–244.
- Blumenthal, D. M. 2006. Interactions between resource availability and enemy release in plant invasion. *Ecology Letters* 9:887–895.
- Blumenthal, D., C. E. Mitchell, P. Pysek, and V. Jarosik. 2009. Synergy between pathogen release and resource availability in plant invasion. *Proceedings of the National Academy of Sciences USA* 106:7899–7904.
- Borer, E. T., W. S. Harpole, P. B. Adler, E. M. Lind, J. L. Orrock, E. W. Seabloom, and M. D. Smith. 2014. Finding generality in ecology: a model for globally distributed experiments. *Methods in Ecology and Evolution* 5:65–73.
- Catford, J. A., R. Jansson, and C. Nilsson. 2009. Reducing redundancy in invasion ecology by integrating hypotheses into a single theoretical framework. *Diversity and Distributions* 15:22–40.
- Catford, J. A., P. A. Vesk, D. M. Richardson, and P. Pyšek. 2012. Quantifying levels of biological invasion: towards the objective classification of invaded and invasible ecosystems. *Global Change Biology* 18:44–62.
- Chrobock, T., A. Kempel, M. Fischer, and M. van Kleunen. 2011. Introduction bias: cultivated alien plant species germinate faster and more abundantly than native species in Switzerland. *Basic and Applied Ecology* 12:244–250.
- Colautti, R., J. Parker, M. Cadotte, P. Pyšek, C. Brown, D. Sax, and D. Richardson. 2014. Quantifying the invasiveness of species. *NeoBiota* 21:7–27. doi:10.3897/neobiota.21.5310.
- Coley, P. D., J. P. Bryant, and F. S. Chapin. 1985. Resource availability and plant antiherbivore defense. *Science* 230:895–899.
- Daubenmire, R. 1959. A canopy-coverage method of vegetational analysis. *Northwest Science* 33:43–64.
- Davis, M. A., J. P. Grime, and K. Thompson. 2000. Fluctuating resources in plant communities: a general theory of invasibility. *Journal of Ecology* 88:528–534.
- Davis, M. A., and M. Pelsor. 2001. Experimental support for a resource-based mechanistic model of invasibility. *Ecology Letters* 4:421–428.
- Dawson, W., A. Bottini, M. Fischer, M. van Kleunen, and E. Knop. 2014. Little evidence for release from herbivores as a driver of plant invasiveness from a multi-species herbivore-removal experiment. *Oikos* 123:1509–1518.
- DeWalt, S. J., J. S. Denslow, and K. Ickes. 2004. Natural-enemy release facilitates habitat expansion of the invasive tropical shrub *Clidemia hirta*. *Ecology* 85:471–483.
- Fargione, J., C. S. Brown, and D. Tilman. 2003. Community assembly and invasion: an experimental test of neutral versus niche processes. *Proceedings of the National Academy of Sciences USA* 100:8916–8920.
- Fargione, J. E., and D. Tilman. 2005. Diversity decreases invasion via both sampling and complementarity effects. *Ecology Letters* 8:604–611.
- Fay, P. A., et al. 2015. Grassland productivity limited by multiple nutrients. *Nature Plants* 1:15080.
- Fine, P. V. A., I. Mesones, and P. D. Coley. 2004. Herbivores promote habitat specialization by trees in Amazonian forests. *Science* 305:663–665.
- Fridley, J. D., J. J. Stachowicz, S. Naeem, D. F. Sax, E. W. Seabloom, M. D. Smith, T. J. Stohlgren, D. Tilman, and B. Von Holle. 2007. The invasion paradox: reconciling pattern and process in species invasions. *Ecology* 88:3–17.
- Halbritter, A. H., G. C. Carroll, S. Gusewell, and B. A. Roy. 2012. Testing assumptions of the enemy release hypothesis: generalist versus specialist enemies of the grass *Brachypodium sylvaticum*. *Mycologia* 104:34–44.
- Heard, M. J., and D. F. Sax. 2013. Coexistence between native and exotic species is facilitated by asymmetries in competitive ability and susceptibility to herbivores. *Ecology Letters* 16:206–213.
- Heckman, R. W., and D. E. Carr. 2016. Effects of soil nitrogen availability and native grass diversity on exotic forb dominance. *Oecologia* 182:803–813.
- Heckman, R. W., J. P. Wright, and C. E. Mitchell. 2016. Joint effects of nutrient addition and enemy exclusion on exotic plant success. *Ecology* 97:3337–3345.
- Hector, A., T. Bell, Y. Hautier, F. Isbell, M. Kéry, P. B. Reich, J. van Ruijven, and B. Schmid. 2011. BUGS in the analysis of biodiversity experiments: species richness and composition are of similar importance for grassland productivity. *PLoS ONE* 6:e17434.
- Heger, T., and J. M. Jeschke. 2014. The enemy release hypothesis as a hierarchy of hypotheses. *Oikos* 123:741–750.
- Huenneke, L. F., S. P. Hamburg, R. Koide, H. A. Mooney, and P. M. Vitousek. 1990. Effects of soil resources on plant invasion and community structure in California serpentine grassland. *Ecology* 71:478–491.
- Huston, M. A. 1997. Hidden treatments in ecological experiments: re-evaluating the ecosystem function of biodiversity. *Oecologia* 110:449–460.
- Kalisz, S., R. B. Spigler, and C. C. Horvitz. 2014. In a long-term experimental demography study, excluding ungulates reversed invader's explosive population growth rate and restored natives. *Proceedings of the National Academy of Sciences USA* 111:4501–4506.
- Keane, R. M., and M. J. Crawley. 2002. Exotic plant invasions and the enemy release hypothesis. *Trends in Ecology & Evolution* 17:164–170.
- Kennedy, T. A., S. Naeem, K. M. Howe, J. M. H. Knops, D. Tilman, and P. Reich. 2002. Biodiversity as a barrier to ecological invasion. *Nature* 417:636–638.
- Knops, J. M. H., et al. 1999. Effects of plant species richness on invasion dynamics, disease outbreaks, insect abundances and diversity. *Ecology Letters* 2:286–293.
- Kulmatiski, A., K. H. Beard, and J. Heavilin. 2012. Plant-soil feedbacks provide an additional explanation for diversity-productivity relationships. *Proceedings of the Royal Society B* 279:3020–3026.
- Leishman, M. R., J. Cooke, and D. M. Richardson. 2014. Evidence for shifts to faster growth strategies in the new ranges of invasive alien plants. *Journal of Ecology* 102: 1451–1461.
- Lenth, R. V. 2016. Least-Squares Means: The R Package *lsmeans*. *Journal of Statistical Software* 69(1):1–33. doi: 10.18637/jss.v069.i01.
- Levine, J. M., and C. M. D'Antonio. 1999. Elton revisited: a review of evidence linking diversity and invasibility. *Oikos* 87:15–26.

- Liao, H., W. Luo, S. Peng, and R. M. Callaway. 2015. Plant diversity, soil biota and resistance to exotic invasion. *Diversity and Distributions* 21:826–835.
- Lind, E. M., et al. 2013. Life-history constraints in grassland plant species: a growth-defence trade-off is the norm. *Ecology Letters* 16:513–521.
- MacDougall, A. S., B. Gilbert, and J. M. Levine. 2009. Plant invasions and the niche. *Journal of Ecology* 97:609–615.
- Maron, J., and M. Marler. 2007. Native plant diversity resists invasion at both low and high resource levels. *Ecology* 88:2651–2661.
- Maron, J. L., and M. Marler. 2008. Effects of native species diversity and resource additions on invader impact. *American Naturalist* 172:S18–S33.
- Maron, J. L., M. Marler, J. N. Klironomos, and C. C. Cleveland. 2011. Soil fungal pathogens and the relationship between plant diversity and productivity. *Ecology Letters* 14:36–41.
- Mattingly, W. B., and H. L. Reynolds. 2014. Soil fertility alters the nature of plant-resource interactions in invaded grassland communities. *Biological Invasions* 16:2465–2478.
- Maurel, N., J. Hanspach, I. Kühn, P. Pyšek, and M. van Kleunen. 2016. Introduction bias affects relationships between the characteristics of ornamental alien plants and their naturalization success. *Global Ecology and Biogeography* 25: 1500–1509.
- Mitchell, C. E., and A. G. Power. 2003. Release of invasive plants from fungal and viral pathogens. *Nature* 421:625–627.
- Mitchell, C. E., et al. 2006. Biotic interactions and plant invasions. *Ecology Letters* 9:726–740.
- Naeem, S., J. M. H. Knops, D. Tilman, K. M. Howe, T. Kennedy, and S. Gale. 2000. Plant diversity increases resistance to invasion in the absence of covarying extrinsic factors. *Oikos* 91:97–108.
- Oosting, H. J. 1942. An ecological analysis of the plant communities of Piedmont, North Carolina. *American Midland Naturalist* 28:1–126.
- Parker, J. D., and M. E. Hay. 2005. Biotic resistance to plant invasions? Native herbivores prefer non-native plants. *Ecology Letters* 8:959–967.
- Pinheiro, J., D. Bates, S. DebRoy, D. Sarkar, and R Core Team. 2016. nlme: linear and nonlinear mixed effects models. R package version 3.1-128. <http://CRAN.R-project.org/package=nlme>
- Power, A. G., and C. E. Mitchell. 2004. Pathogen spillover in disease epidemics. *American Naturalist* 164:S79–S89.
- Prior, K. M., T. H. Q. Powell, A. L. Joseph, and J. J. Hellmann. 2015. Insights from community ecology into the role of enemy release in causing invasion success: the importance of native enemy effects. *Biological Invasions* 17:1283–1297.
- R Development Core Group. 2015. R version 3.2.2. R Foundation for Statistical Computing, Vienna, Austria. www.r-project.org
- Renne, I. J., B. F. Tracy, and I. A. Colonna. 2006. Shifts in grassland invasibility: effects of soil resources, disturbance, composition, and invader size. *Ecology* 87:2264–2277.
- Roscher, C., H. Beßler, Y. Oelmann, C. Engels, W. Wilcke, and E.-D. Schulze. 2009. Resources, recruitment limitation and invader species identity determine pattern of spontaneous invasion in experimental grasslands. *Journal of Ecology* 97: 32–47.
- Schmid, B., A. Hector, M. Huston, P. Inchausti, I. Nijs, P. Leadley, and D. Tilman. 2002. The design and analysis of biodiversity experiments. Pages 61–75 in M. Loreau, S. Naeem, and P. Inchausti, editors. *Biodiversity and ecosystem functioning: synthesis and perspectives*. Oxford University Press, Oxford, UK.
- Schnitzer, S. A., et al. 2011. Soil microbes drive the classic plant diversity-productivity pattern. *Ecology* 92:296–303.
- Schutzenhofer, M. R., T. J. Valone, and T. M. Knight. 2009. Herbivory and population dynamics of invasive and native *Lespedeza*. *Oecologia* 161:57–66.
- Seabloom, E. W., et al. 2013. Predicting invasion in grassland ecosystems: Is exotic dominance the real embarrassment of richness? *Global Change Biology* 19:3677–3687.
- Seabloom, E. W., et al. 2015. Plant species' origin predicts dominance and response to nutrient enrichment and herbivores in global grasslands. *Nature Communications* 6:7710.
- Shea, K., and P. Chesson. 2002. Community ecology theory as a framework for biological invasions. *Trends in Ecology & Evolution* 17:170–176.
- Stricker, K. B., P. F. Harmon, E. M. Goss, K. Clay, and S. Luke Flory. 2016. Emergence and accumulation of novel pathogens suppress an invasive species. *Ecology Letters* 19:469–477.
- Stricker, K. B., and P. Stiling. 2012. Herbivory by an introduced Asian weevil negatively affects population growth of an invasive Brazilian shrub in Florida. *Ecology* 93:1902–1911.
- Tilman, D. 2004. Niche tradeoffs, neutrality, and community structure: a stochastic theory of resource competition, invasion, and community assembly. *Proceedings of the National Academy of Sciences USA* 101:10854–10861.
- Tognetti, P. M., and E. J. Chaneton. 2015. Community disassembly and invasion of remnant native grasslands under fluctuating resource supply. *Journal of Applied Ecology* 52:119–128.
- Torchin, M. E., and C. E. Mitchell. 2004. Parasites, pathogens, and invasions by plants and animals. *Frontiers in Ecology and the Environment* 2:183–190.
- Turnbull, L. A., J. M. Levine, A. J. F. Fergus, and J. S. Petermann. 2010. Species diversity reduces invasion success in pathogen-regulated communities. *Oikos* 119:1040–1046.
- van Kleunen, M., E. Weber, and M. Fischer. 2010. A meta-analysis of trait differences between invasive and non-invasive plant species. *Ecology Letters* 13:235–245.
- Vasquez, E. C., and G. A. Meyer. 2011. Relationships among leaf damage, natural enemy release, and abundance in exotic and native prairie plants. *Biological Invasions* 13:621–633.
- Zuur, A., E. N. Ieno, N. Walker, A. A. Saveliev, and G. M. Smith. 2009. *Mixed effects models and extensions in ecology with R*. Springer Science & Business Media, New York, New York, USA.

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