

Interplay of limiting factors explains context dependence in plant invasions

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August 2, 2022

Abstract

Context dependence impedes accurate prediction in ecology, including the extent and impact of invasion. Using a grassland experiment, we show that the interplay of limiting factors can explain why invasion trends vary with circumstance. By manipulating invader seed dose and physical disturbance and tracking cover of groups of fast and slow invaders in three communities over three years, we examined how seed-, resource- and growth rate-limitation affected the abundance and diversity impacts of invading plants. Evidence indicated that seeds primarily limited cover of resource-acquisitive fast invaders in early and mid successional communities, growth rate limited resource conservative slow invaders in early and mid succession, and resources limited invasion of both groups in late succession. Resident community diversity declined as invader cover increased, consistent with effects of resource limitation. A greater focus on limiting factors in invasion ecology will provide us with a structure for predicting invasion outcomes in different situations.

Introduction

Context dependence, where relationships differ in different situations, is widespread in ecology and invasion ecology (Catford *et al.* 2022; Pyšek *et al.* 2020). For example, invasion can have a positive, negative, neutral or unimodal relationship with disturbance (Moles *et al.* 2012). Such variation can make it difficult to generalise across studies, systems and species, can prompt questions about the validity of hypotheses, and can hamper invasive species management. Like other subfields of ecology (Rillig *et al.* 2019), most studies in invasion ecology consider only one or two factors (Jeschke *et al.* 2020; Moles *et al.* 2012; Zhang *et al.* 2022), and most invasion hypotheses only consider one or two processes (Latombe *et al.* 2021). However, multiple factors can limit species' growth, survival and reproduction in heterogenous environments (Farrior *et al.* 2013; Harpole *et al.* 2016; Harpole & Tilman 2007; Tilman 1982), and unless the primary limiting factor in a given situation is alleviated, a response to increases in other factors will be slight or not occur (Cade *et al.* 1999; Tilman 1990). For example, in a resource-limited system, increasing species' seed dose (or propagule pressure) will not result in increased invasion if resource availability, rather than seed availability, is the main factor constraining species' abundance (Fig. 1) (Kaiser *et al.* 1994). The concept of limiting factors (or environmental constraints (Tilman 1990) or Sprengel-Liebig's Law of the Minimum (van der Ploeg *et al.* 1999)) is central to thinking in community and restoration ecology (Farrior *et al.* 2013; Greet *et al.* 2022; Seabloom 2011; Tilman 1982) (but see Danger *et al.* 2008), as well as agronomy (van der Ploeg *et al.* 1999), but invasion ecology typically concentrates on drivers or correlates of invasion rather than on the factors that limit it (Catford *et al.* 2009; Taylor *et al.* 2016). In this paper, we argue that a greater focus on ecological mechanisms rather than ecological phenomena in invasion ecology, and a greater focus on limiting factors specifically, will help us better explain and anticipate context dependence and enable more accurate predictions of invasion and its impact on native diversity. Because species coexistence relies on species being

able to invade communities from which they are absent (Chesson 2000), increased mechanistic understanding of invasion is central to community ecology too.

Invasion by plant species requires seeds, resources, suitable abiotic conditions and biotic interactions, and sufficient time and space for populations to establish and grow; any of these factors could limit invasion when in short supply (Catford *et al.* 2009; Pearson *et al.* 2018). However, their relative scarcity – and thus importance – may vary from situation to situation depending on the species-site mixes in question. Variation in species’ life histories means that what may limit one species may not limit another (Catford *et al.* 2019; Tilman 1990). If a study considers and manipulates multiple factors in parallel, effectively alleviating multiple limiting factors, then the effect of these factors should become visible (Fig. 1). However, if invasion is co-limited by two essential factors but only one limiting factor is alleviated, then otherwise positive or negative relationships may appear neutral. Because of this, studies that investigate multiple factors and hypotheses in combination may be more likely to find evidence for underlying mechanisms than studies that manipulate one factor at a time. Considering factors that are likely to limit invasion in a given situation can help us to understand and predict context dependence. A species-site matrix provides a structure for considering the hierarchy and interplay of limiting factors and for making such predictions (Fig. 1).

Imagine we have two groups of species, “fast invaders” characterised by resource acquisitive traits, fast growth rates, high seed production and poor competitive ability, and “slow invaders” with conservative traits, slow growth rates, low seed production and high competitive ability (Craven *et al.* 2018; Reich 2014). These species might invade early, mid and late successional sites (Fig. 1a). Succession theory tells us that early succession should primarily be seed-limited and late succession resource-limited (Catford *et al.* 2012a; Huston & Smith 1987). However, the extent of limitation should differ between the two groups of species reflecting their life histories (and associated tradeoffs, Tilman 1990), and these differences may be most pronounced in mid-succession where seed- and resource-limitation are less extreme. We might thus expect that fast invaders will be resource-limited in mid succession, whereas slower-growing and less resource-demanding slow invaders will – in the initial years of invasion – primarily be limited by their intrinsic growth rates and seed availability. Once we have a species-site matrix portraying the hierarchy of limiting factors, we can then imagine how seed addition or disturbance might modulate these conditions, revealing underlying relationships (Fig. 1b). Where seed-limited, invader abundance should primarily be determined by seed dose, whereas species’ competitive ability for limiting resources and disturbance (or other actions that increase resource availability) should largely determine the extent of invasion when resources are limiting. Alleviating primary limiting factors can allow effects of other, sequentially limiting factors to be observed, thus revealing the interplay of limiting factors.

By considering factors that are likely to limit resident plant diversity and how invasion may affect those limiting factors, we can also use the concept of limiting factors to predict the conditions under which invasion may reduce resident diversity (cf community diversity made up of both residents and invaders). For example, in the very early stages of succession, resident diversity would likely be limited by resident seed availability and to a lesser extent by resident population growth rates. Resident diversity in mid succession will likely be limited by a combination of resources, seeds and growth rates, moving to resource-limitation in late succession (Fig. 1). In the first few years of invasion, especially when invasion is small-scale, invading plants are unlikely to alter the seed availability or population growth rates of resident plants, so invaders will primarily affect resident diversity through resource competition. By consuming resources, like space, soil nitrogen or water, plant invaders can increase the extent of resource limitation, and thus potentially reduce the occupancy and abundance of resident species. The diversity impacts of invasion will depend on context though, such that invasion may have little impact on resident species when invaders are poor competitors or when factors other than resources primarily constrain resident diversity (i.e. plant invaders will only reduce diversity if they effectively lower the capacity of Liebig’s barrel; Fig. 1a).

In this study, we use a seed addition experiment in Minnesotan grasslands to test whether the concept of limiting factors can help predict and explain the cover abundance of two groups of invading species and their impact on resident diversity in communities that vary in successional stage. Our three-year experiment

involves four treatments in a partially factorial design: i) successional stage of recipient community; ii) disturbance; iii) invader type; and iv) invader seed dose. Invader type and community successional stage provide a matrix of conditions where we expected the relative importance of seed-, resource- and growth rate-limitation on invader abundance and resident diversity to vary (Fig. 1). Invader seed dose, disturbance (which elevates resource availability) and invader type (fast vs slow invaders) enable us to effectively modulate invader seed-, resource- and invader growth rate-limitation respectively and to test our predictions. Variation in invader type and abundance in the invaded communities enabled us to test how invasion can reduce resident diversity via its effect on resource availability.

Through simultaneous invasion of up to 14 grassland species (10/14 native but all species formerly absent from the experimental sites), we also examine the importance of interactions among invaders, specifically whether resource acquisitive “fast” invaders constrain the abundance of more conservative and competitive “slow” invaders via niche pre-emption, or whether slow invaders constrain fast invaders via resource competition for soil N and soil water (Catford *et al.* 2012a). We use measures of resource availability (light at ground level, soil moisture, soil nitrate) and, where relevant, resident species richness to help understand the trends observed. We use “invasion” to describe the colonisation and establishment of previously absent (“invading”) plant species in this paper, using the term invasion in a broad mechanistic sense (Chesson 2000; Tilman 2004). The processes that we examine and discuss may thus relate to native species added for restoration, range-shifting and range-expanding species, as well as human-mediated alien species invasion and community ecology research (Seabloom *et al.* 2003). To avoid confusion, we use the term “factor” to refer to limiting factors only, and “treatment” to refer to the four factors manipulated in the experiment.

Methods

Study design

Our three-year grassland experiment involved four treatments in a partially factorial design: i) successional stage of recipient community (early, mid, late); ii) disturbance (disturbed, undisturbed); iii) invader type (fast invaders, slow invaders, both fast and slow invaders); and iv) seed dose of invader species (none, low, high). The invader type and seed dose treatments were not fully crossed for logistical reasons (Fig. S1). This resulted in a total of 42 treatment combinations (3 community levels x 2 disturbance levels x 7 invader levels), replicated four times, giving a total of 168 plots, which were surveyed over three growing seasons.

The experiment was carried out in three old field sites at Cedar Creek Ecosystem Science Reserve (45.4°N, 93.2°W), Minnesota in 2013-2015. Cedar Creek has nitrogen-limited sandy soils, annual precipitation of ~770 mm, mean summer temperatures of 27°C and winter lows of -14°C. In 2013, the late succession site was approximately 80 years old, the mid succession site was 23 years old, and the early succession site was 0 years old with no live standing vegetation when the treatments were imposed. Community composition was distinct among the three sites and soil fertility varied, reflecting the accumulation of organic carbon and total nitrogen through succession (Appendix S1).

In each site, 56 1 m x 1 m plots were permanently marked, separated by 1 m wide walkways (Fig. S2). Plots were randomly assigned disturbance (0, 1) and seed sowing treatments, which were enacted in May 2013 (disturbance first). Disturbance involved mowing vegetation to 10 cm followed by soil tilling, with the aim of killing approximately half of the standing vegetation. Litter and dead plants were left in the plots. Walkways were undisturbed.

We selected 23 grassland species for seed addition, all of which were native or naturalized at Cedar Creek but were absent or rare in the sites prior to seed addition. We separated the species into two groups (fast and slow invaders) based on time of colonisation during succession (Tilman 1988) and plant functional traits (Table S1, Figure S3). Within the groups, we aimed to balance functional type, origin and lifespan. We purchased seeds from local suppliers. We added 0.5 g and 4 g of viable seed per species for the low and high seed dose treatments respectively. Of 23 invader species sown, 15 were observed at least once (Table S1). *Strophostyles leiosperma* was only added to some of the intended plots, resulting in an imbalanced study design. Where present, it had mean cover of 0.3% (maximum 1%). It was hand-pulled to remove it from plots

when observed. Preliminary analysis showed that its inclusion or exclusion did not affect model outputs, so it was excluded from further analyses. This left 7 fast invaders and 7 slow invaders (Tables 1 & S1, Appendix S1).

Data collection

In early August each year, percent cover of all vascular plants, bare mineral soil, bryophytes and litter were estimated in the central 0.7 m x 0.7 m area of each plot by the same observer (JC). 106 plant taxa were recorded across all plots and years. We measured light availability and volumetric soil moisture content (surface and subsurface) four times throughout each growing season. We collected and mixed four soil cores (10 cm deep) from each plot in June and July each year, extracted the samples with 0.01 M KCl and measured extractable soil nitrate, the key limiting resource at Cedar Creek (Tilman 1990). Appendix S1 includes further details.

Statistical analysis

Step 1: Best performing models for invader cover and resident diversity

We used linear mixed-effects models to determine relationships between the four experimental treatments and invader cover, and between treatments, invasion and resident diversity. Our mixed-effect models were multilevel linear models with both ‘fixed’ and ‘random’ effects, implemented through R package ‘nlme’ (Pinheiro *et al.* 2022).

Response variables : We examined cover of the fast and slow invader groups separately and in combination (total cover). Group-level cover was not overly dominated by particular species (Fig. S4). Our invader cover analysis only included plots where invaders were sown and had colonised (Appendix S2). All invader covers were log-transformed.

We examined effects of invasion on resident effective species richness (following Tilman *et al.* 2001) (hereon resident diversity). Resident diversity was strongly correlated with resident richness ($r = 0.78$) but not with total resident cover ($r = 0.28$, Table S7). We focus on resident diversity and not resident cover as it corresponds more closely with our interests (Catford *et al.* 2012b). We define the resident community as all species that were not experimentally sown even if they colonised the plots during the experiment. Invaders rarely colonised plots where they were not sown (Table S2). The resident diversity model included all plots where invaders were sown, regardless of whether invaders were present. Resident diversity was square-root transformed.

Regression model structure : We considered invader cover as a function of disturbance, successional stage, seed dose, invader type and growth season. For resident diversity, we considered invader richness and total invader cover instead of invader seed dose. Plot was included as a random intercept and growth season as an interacting random slope to account for repeat measurements and variation in individual plots over time. From these fixed effects, we constructed two separate global models for each response variable. First, a base model where each treatment was considered as a non-interacting effect. Second, an interaction model where two-way interactions between the experimental treatments were considered (Appendix S2, Tables S3-S6 & S9-11).

Model selection : For each base and interaction model, we ranked the performance of the full model and all potential subsets using Bayesian Information Criterion (BIC). From this, we considered all models within 6 BIC points of the best performing model. For each best performing model (lowest BIC), we calculated marginal and conditional R^2 values, representing total variance explained by the entire model (fixed & random effects) and fixed effects only (Nakagawa & Schielzeth 2013). Based on BIC and marginal R^2 values, the interaction models performed better than base models, especially for invader cover (Tables S8-11). For each response variable, the competing models (<6 BIC points) were either subsets of the best model or had a better performing model nested within them. For simplicity and because the ecological interpretation of the competing models is effectively the same, we focus on the best interaction model for each response variable.

Step 2: Variance component analysis

Variance component analysis was used to compare the relative importance of each fixed effect included within the best performing models from Step 1. We built a Bayesian hierarchical multilevel linear model for each response variable considered in the mixed effects models, implemented through R package ‘R2Jags’ (Su & Yajima 2021), following Gelman & Hill (2007), Hector *et al.* (2011) and Catford *et al.* (2014). These models were produced by taking the structure of the best performing mixed-effects models and replicating this in JAGS (Just Another Gibbs Sampler) multilevel structure, with variable intercepts and slopes applied following Gelman & Hill (2007). Variance components were then calculated from these multilevel models and presented on a standard deviation scale to aid comparison between predictors (Gelman & Hill 2007; Hector *et al.* 2011).

Step 3: Resource availability and resident diversity across treatments

We calculated paired Pearson’s correlation coefficients between treatments (excluding invader type), resources (soil nitrate, soil moisture, light at ground level) and resident community (diversity, richness, total cover). We focus on conditions in 2013 as these would have affected invasion, whereas conditions in 2014 and 2015 would also reflect effects of invasion, complicating likely causal relationships.

All analysis was completed using R version 3.6.3 (R Core Team 2020) and JAGS version 4.3.0 (Plummer 2003). Appendix S2 includes additional details.

Results

Invader abundance

Results were largely consistent with predictions (Figs 1 & 5). Of the experimental treatments, fast invader cover was most strongly linked with successional stage (Fig. 2a), which itself was strongly linked with extractable soil nitrate (Fig. S6, Table S7), the dominant limiting resource in these grasslands (Tilman 1990). Limited soil N therefore likely explained the low invader cover in late successional communities (Fig. 2c), an effect that was partially alleviated when plots were disturbed (Fig. 2d), temporarily increasing soil N (Fig. S6). Early successional plots had consistently high levels of invasion through time despite marked declines in soil N (Fig. S6), suggesting that soil N did not limit fast invader cover in early succession, at least not in undisturbed plots. The non-conditional positive effect of seed dose demonstrated that seed dose was important across all conditions, including in early succession where fast invaders were otherwise unconstrained (Fig. 2a-c). Combined with lack of growth rate- or resource-limitation, this suggests that seeds were the key limiting factor in early succession for fast invaders, at least in undisturbed plots (Fig. 2e). Fast invader cover reached intermediate levels in mid succession, with trends consistent with seed- and some resource-limitation.

Slow invader cover was primarily limited by resources in late succession too, as demonstrated by effects of disturbance and trends across the three successional stages (Fig. 3c-d). However, unlike fast invaders, slow invader cover seemed to be limited by species’ growth rates in this short-term experiment, as the strong link between slow invader cover and time (Fig. 3a) and its marked temporal increase in early and mid successional plots demonstrated (Fig. 3c).

Most relationships involved interactions, consistent with effects of an interplay of limiting factors (Fig. 1b). Unless interactions were included, successional stage was not selected in the fast invader model despite it being the most influential treatment for fast invader cover (Tables S4 & S9, Fig. 2a). A similar situation occurred for slow invader cover where invader type and disturbance were absent from the best non-interaction model (Tables S5 & S10). The most marked interactions for both invader groups were between community successional stage and disturbance, and successional stage and time (Fig. 2a,c,d, Fig. 3a,c,d). These interactions reflected the varying importance of resource- and growth rate- limitation in different contexts (Figs 1 & 5). Invader type (both groups or target group only) was the only experimental treatment not included in the best model for fast invaders, which had a marginal R^2 of 0.44 and conditional R^2 of 0.80. All four experimental treatments plus time were included in the best model for slow invaders, which explained considerable

variance (marginal $R^2 = 0.83$; conditional $R^2 = 0.92$).

Impacts of invasion on resident diversity and other invaders

Resident diversity, as indicated by effective species richness, was negatively related to invader cover (Fig. 4b). The relationship did not depend on invader type, time, nor any other experimental treatment (Fig. 4, Tables S6 & S11). Resident diversity was highest in late succession and lowest in early succession, but these differences declined over time as richness decreased slightly in late succession and increased in mid and, to a lesser extent, early succession (Fig. 4c). Resident diversity was higher in disturbed than undisturbed plots (Fig. 4d). In general, resident diversity in plots with little or no invader cover was stable over time, whereas resident diversity in plots with high invader cover declined over time (Fig. S9). These trends are consistent with impacts of invasion on resident diversity rather than effects of resident diversity on invasion, though the relationship can go in both directions (Catford *et al.* 2020; MacDougall *et al.* 2014). Resident diversity was most strongly related to invader cover and community successional stage (Fig. 4a, marginal $R^2 = 0.50$, conditional $R^2 = 0.78$).

Evidence suggested that fast invaders limited cover of slow invaders (Fig. 3b) but co-occurring slow invaders did not affect fast invader cover (Fig. 2, Tables S9-10). Plots sown with only fast invaders had similar total invader cover to plots sown with both fast and slow invaders (Fig. S5d, Tables S3 & S8). Plots with only slow invaders had lower cover (Fig. S5d).

Resource availability

Based on measurements in 2013, community successional stage was positively correlated with resident diversity ($r = 0.679$) and negatively correlated with soil nitrate (-0.861 ; Table S7a). Effects of disturbance on soil N varied with successional stage (Table S7b-d, Fig. S6); disturbed plots had lower soil N than undisturbed plots in early succession ($r = -0.42$), but higher soil N in mid and late succession ($r = 0.64$ and 0.44 respectively). Light levels at ground level declined with successional stage and time, and were higher in disturbed than undisturbed plots ($r = 0.711$), especially in mid and late successional communities ($r = 0.90$ and $r = 0.95$ respectively; Table S7, Fig. S8). Soil moisture was highest in late successional communities and lowest in mid successional communities (Fig. S7). Disturbed plots had lower soil moisture than undisturbed plots, especially in mid successional communities ($r = -0.71$) where soil moisture was already very low (Fig. S7). Apart from soil moisture, these correlations provide evidence that competition generally increased and availability of key resources, namely soil nitrate, decreased with succession.

Discussion

Interplay of limiting factors explains context dependence in invader abundance

Using a grassland experiment, we show that the interplay of limiting factors can explain context dependence in plant invasions, including why relationships can oscillate between positive, negative and neutral depending on circumstances. Evidence suggested that seeds primarily limited fast invader cover in early and mid successional communities, resources limited invasion of both groups in late succession, and invader growth rate primarily limited slow invaders in early and mid succession (Fig. 5). The key limiting factor in each situation was only detectable, and a nuanced effect of each treatment only visible, when temporal trends and effects of the four treatments were collectively examined. For example, the strength of resource-limitation in late successional communities only became apparent through time, and the varied effects of disturbance illustrated changes in the relative importance of resource limitation with succession. By manipulating invader seed dose, imposing disturbance that altered resource availability and examining temporal trends of invaders with different growth rates, we were able to ascertain the hierarchy of limiting factors affecting cover abundance of two invader groups in three successional stages, and explain context dependence of the invasion relationships observed (Fig. 5).

Community successional stage and disturbance reveal importance of resource limitation on invader abundance
Consistent with resource competition and succession theory (Huston & Smith 1987; Tilman 2004), the less

diverse early successional communities experienced higher levels of invasion than mid or late successional communities, and their levels of invasion increased with time, especially for cover of slow invaders. Invasion trends in mid and late successional communities were more complicated, with their ranks changing depending on invader type and time. Collectively, these dynamics reflect invader life histories and the strength of competition in the recipient communities. Other studies have also found that invasion levels decline as the diversity of recipient communities increases (Beaury *et al.* 2020; Hector *et al.* 2001; Petruzzella *et al.* 2018) (although not universally, MacDougall *et al.* 2014) and as limiting resources become scarcer (Catford *et al.* 2020; Fargione & Tilman 2005; Seabloom 2011). Fast invaders were able to establish quickly, but their cover declined notably in late successional communities, presumably as resource limitation took effect (Figs S6 & S8). Slow invaders are more resource competitive and conservative and have slower rates of growth than fast invaders (Lauenroth & Adler 2008), attributes that enabled them to persist in late successional communities and gradually increase in cover in mid successional communities. The importance of resource limitation was further highlighted by interactive effects of disturbance and community successional stage on invader cover (Figs 2d & 3d).

Temporal trends indicated that disturbance facilitated invasion in late successional communities but inhibited invasion in early successional communities (Figs 2d & 3d). We expected that the facilitatory role of disturbance on invasion would strengthen with succession reflecting greater resource limitation in later stages of succession (Catford *et al.* 2012a). However, the negative disturbance-invasion relationship in early succession indicates that disturbance can hinder, as well as help, invasion depending on local conditions. Disturbance is usually hypothesised (and often found, Kempel *et al.* 2013; Seabloom 2011) to facilitate invasion because it decreases the biomass of competing established plants and because it increases resource availability (Catford *et al.* 2009; Davis *et al.* 2000), as was the case with soil N in mid and late succession. Resource competition tends to be weaker earlier in succession (Catford *et al.* 2012a; Clark *et al.* 2019; Lohbeck *et al.* 2014) and in less diverse communities (Catford *et al.* 2020; Fargione & Tilman 2005), which may restrict the positive effects of disturbance on invasion, enabling negative effects to be seen. By destroying standing biomass, disturbance can alter microclimates, potentially exposing seedlings to dry and hostile conditions and reducing the number of safe sites for recruitment (Wandrag *et al.* 2019). Indeed, disturbed plots had higher light and lower surface soil moisture than undisturbed plots. However, disturbance reduced soil N in our early successional communities in the peak growing season (June and July), which could plausibly be the explanation for the negative effect of disturbance on invader cover in early succession. We posit that disturbance had a largely neutral effect in mid successional communities because of its opposite effects on soil N and soil moisture (Figs S6 & S7, Table S7c). The benefit of increased N availability was annulled by reduced water availability (which was already very low in the mid successional plots), marking a shift from N- to water-limitation with disturbance (though water-limitation can itself cause N-limitation because of reduced access to nutrients in the soil, Bloom *et al.* 1985). Light is rarely limiting in unfertilised grasslands that occur on the sandy, low-nitrogen soils of Cedar Creek (Tilman 1990; Wilson & Tilman 1991), so any benefits of increased light availability with disturbance in this experiment were likely to be secondary to benefits of increased N availability.

Had we only considered main effects or only examined invasion in early successional communities, the effect of disturbance in our study would appear to contradict ecological theory when, in fact, our findings are consistent with hypothesised disturbance-invasion mechanisms. The succession-dependent effects of disturbance illustrate the importance of explicitly examining interaction effects (Catford *et al.* 2022) and considering how limiting factors will likely vary from situation to situation – in this case, when resource limitation, and thus a positive disturbance-invasion relationship, would be likely or unlikely.

Invaders impact community diversity and other invaders via resource competition

The observed decline in resident diversity with increasing invader cover is consistent with effects of resource competition and resource limitation. The invasion-diversity relationship did not vary with invader type, despite slow invaders being better able to persist in the more diverse late successional communities and their potential to reduce soil N and water to lower levels than fast invaders. Somewhat surprisingly, fast invaders appeared to limit cover of slow invaders when the groups were sown together. This trend likely reflects niche

pre-emption where the more abundant fast invaders colonised safe sites more rapidly than slow invaders (Catford *et al.* 2012a; Wandrager *et al.* 2019). With more time, and as their temporal trends suggest (Figs. 2c & 3c), slow invaders will likely replace or displace shorter-lived fast invaders (Lauenroth & Adler 2008) and have a greater impact on community diversity than fast invaders, provided that disturbance is rare and no more seeds are sown (Tilman 1990).

The negative relationship between invader cover and resident diversity was not conditional on invader type or local conditions and suggested that resource limitation was important under all conditions examined here. Our experimental design (including its temporal and spatial scale) meant that we were more likely to detect effects of resource limitation on resident diversity than effects of other factors that might be limiting. In other situations, invader impacts may be more variable, reflecting a greater diversity of factors that limit resident species and how invaders may affect those limiting factors (e.g. via enemies, mutualists, abiotic conditions) (Kempel *et al.* 2013; Levine *et al.* 2004; Petruzzella *et al.* 2020).

Conclusion

Most studies in invasion ecology aim to find the key drivers of invasion. Such a quest encourages us to focus on main effects and key correlates of invasion, but may poorly equip us to deal with interaction effects and associated context dependence. We contend that flipping our thinking and focusing on mechanisms and factors that *limit* invasion, rather than phenomena or factors that *drive* it, will encourage us to consider how the impacts of various factors vary with a particular context, giving us a stronger foundation for prediction and understanding. As demonstrated in this four-treatment experiment, different invading species experience different levels of seed-, resource- and growth rate-limitation and the hierarchy of limiting factors depends upon the community and sites that the species invade. We found that results largely matched our expectations (Fig. 5). Fast invaders were primarily seed- and resource-limited in early and late successional communities respectively, such that seed dose (propagule pressure) or disturbance would primarily determine their abundance in these two different contexts. Abundance of slow invaders in early and mid succession was limited by invader growth rate such that abundance shortly after introduction may belie long-term trends. As theory would predict, invasion in late succession was primarily resource-limited, such that disturbance that increased soil N strongly facilitated invasion in late succession. Invading plants reduced resident plant diversity via effects on resource limitation, a relationship that was not conditional. Much like in restoration and community ecology, a greater focus on limiting factors in invasion ecology will enable us to better understand why some treatments or hypotheses are important in some situations and not others, and will provide us with a structure for predicting invasion outcomes in different situations.

Acknowledgments

We thank Troy Mielke, Krysta Overlander, Jacob Miller, Mara Sagedahl, Peter Wragg, Adam Clark, Dan Bauhaudin, Susan Barrott, Kally Worm, Kyle Naish and many interns at Cedar Creek for help and support. This project has received funding from the European Research Council (ERC) under the European Union’s Horizon 2020 research and innovation programme (grant agreement No. [101002987]), the Australian Research Council (DE120102221) and the ARC Centre of Excellence for Environmental Decisions. Cedar Creek Ecosystem Science Reserve, the University of Minnesota and King’s College London provided further support.

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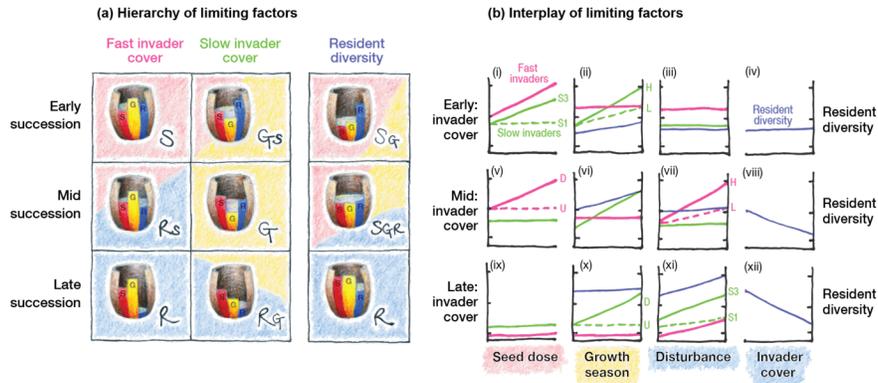


Figure 1: Limiting factors operate through a hierarchy to constrain the abundance and diversity of plants (a), and the interplay of limiting factors can result in relationships that are context dependent (b). *Left panel (a):* Effect of limiting factors illustrated using “Liebig’s barrel”, which shows the law of the minimum where an increase in plant abundance or diversity (represented as water in the barrel) will only occur when the primary factor limiting abundance or diversity is elevated (shortest stave of the barrel). The matrix shows hypothesised limiting factors (S: seeds, red; G: growth rate, yellow; R: resources, blue) after a single growing season for: fast and slow invader groups when added as seed to early, mid and late successional communities, and resident plant diversity in early, mid and late successional communities. R, S, and G indicate primary and important secondary/tertiary (subscript) limiting factors, e.g. slow invaders are hypothesised as primarily being growth rate-limited but also seed-limited in early succession (G_S). *Right panel (b):* Predicted responses of cover abundance of fast and slow invaders (left y-axis) to seed dose, disturbance and growth season, which reveals seed-, resource- and growth rate-limitation respectively, in early, mid and late successional communities (upper, middle and lower panels respectively). Also shown is resident community diversity (right y-axis) and its predicted response to disturbance, growth season and invader abundance, the latter of which reduces resource availability, increasing resource-limitation. Interactions are shown with solid and dashed lines: S1= growth season 1, S3 = growth season 3, U = undisturbed, D = disturbed, H = high seed dose, L = low seed dose. Seed dose is never 0 in these plots, so invasion is always possible. Interpretation: The extent of response to alleviation of a limiting factor depends on degree of limitation and on constraints from other limiting factors, e.g. if resources are the only limiting factor and resource-limitation is strong, disturbance should facilitate increased abundance, but if resources are not primarily limiting and primary limiting factor is unchanged, then increasing resource availability will have no effect, and if resources are not limiting at all, increasing resource availability would also have no effect). Limiting factors can vary from situation to situation and their relative strengths will determine the effect of a given experimental treatment, environmental gradient or perturbation. The higher the potential number of limiting factors (staves in a barrel), the higher the likelihood of observing context dependence. Example hypotheses: Because fast invaders are seed limited in early succession (S, left panel), their abundance increases with seed dose (i), but they show no response to growth season (ii) or disturbance (iii). Slow invaders are primarily growth rate-limited in early succession but also somewhat seed-limited (G_S , left panel), so they should increase with seed dose and growth season (i, ii), with the strength of the responses being conditional on alleviation of the other limiting factor (i.e. interaction effects).

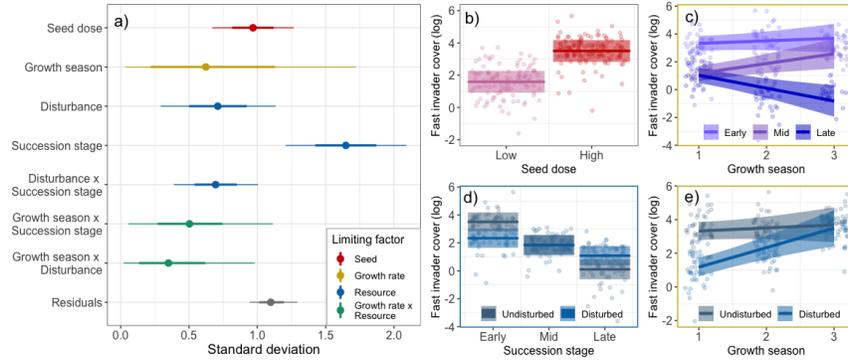


Figure 2: Variance components and fitted relationships between the cover of fast invaders and experimental treatments. *Left panel (a):* Variance components for the best performing model of fast invader cover (log-transformed). Point estimates are set on a standard deviation scale and represent the medians of the posterior distributions. Bars present 95% (wide) and 68% (narrow) intervals. Variance components were produced through Bayesian multilevel modelling. *Right panels (b-e):* Fitted relationships between fast invader cover (log-transformed) and b) seed dose of invaders; c) growth season (separated by successional stage); d) successional stage (separated by disturbance treatment) and e) growth season (separated by disturbance treatment). Envelopes represent 95% confidence intervals. Points represent partial residuals. Relationships are fitted from the best performing linear mixed-effects models.

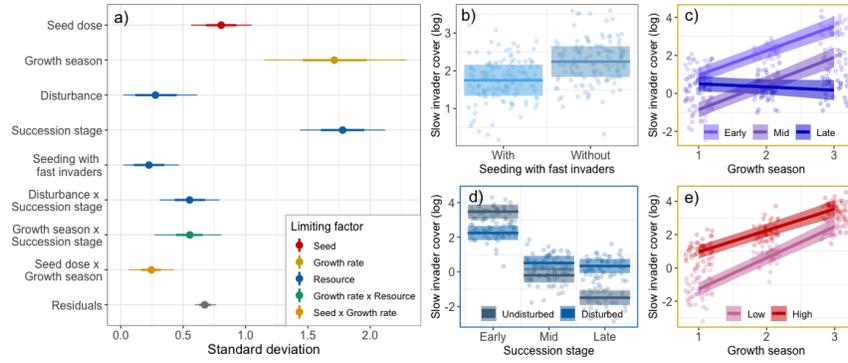


Figure 3: Variance components and fitted relationships between the cover of slow invaders and experimental treatments. *Left panel (a):* Variance components for the best-performing model of slow invader cover (log-transformed). Point estimates are set on a standard deviation scale and represent the medians of the posterior distributions. Bars present 95% (wide) and 68% (narrow) intervals. Variance components were produced through Bayesian multilevel modelling. *Right panels (b-e):* Fitted relationships between slow invader cover and b) seeding with fast invaders; c) growth season (separated by successional stage); d) successional stage (separated by disturbance treatment) and e) growth season (separated by seed dose) Envelopes represent 95% confidence intervals. Points represent partial residuals. Relationships are fitted from the best performing linear mixed-effects models.

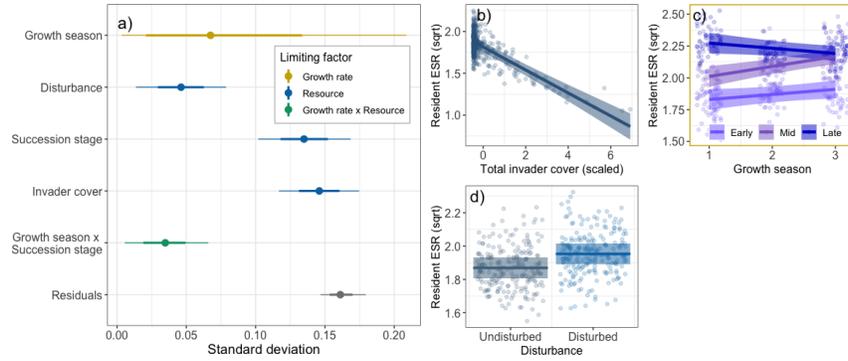


Figure 4: Variance components and fitted relationships between resident diversity, as indicated by resident effective species richness (ESR), and experimental treatments and consequent invader cover. *Left panel (a):* Variance components for the best performing model of resident ESR (sqrt-transformed). Point estimates are set on a standard deviation scale and represent the medians of the posterior distributions. Bars present 95% (wide) and 68% (narrow) credible intervals. Variance components were produced through Bayesian multilevel modelling. Point estimate scale varies compared to Fig 2a and 3a due to the square-root transformation of resident ESR. *Right panels (b-d):* Fitted relationships between resident diversity (square-root transformed) and b) total invader cover (scaled); c) growth season (separated by succession stage) and d) disturbance. Envelopes represent 95% confidence intervals. Points represent partial residuals. Relationships are fitted from the best performing linear mixed-effects models.

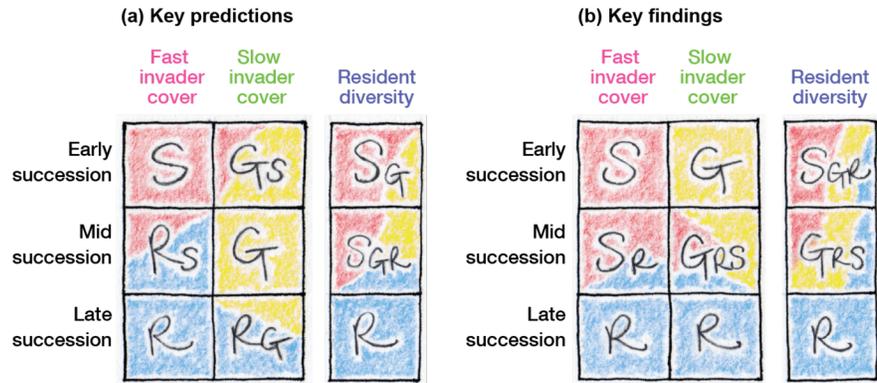


Figure 5: Schematic summary of a) key predictions and b) key findings regarding limiting factors (seeds, resources or growth rate) that constrain cover abundance of groups of fast and slow invaders and constrain diversity of resident species in early, mid and late successional communities. Large letters indicate factors that primarily limit plant cover or diversity; subscripts indicate secondary or tertiary limiting factors; e.g. S_R indicates that seeds are primary and resources secondary limiting factor. Key results are based on results shown in Figs 2-4.

Table 1: Characteristics and occupancy of 14 target invader species in our experiment. Occupancy was calculated as percentage of plots in which each species was recorded relative to the 96 plots where each species was sown. See Table S1 for mean and maximum abundances of the target invaders, and for details of other species that were sown but did not colonise the plots.

Species	Family	Functional group	Lifespan	Origin	Occupancy (%)
					2013
Fast invaders	Fast invaders	Fast invaders			
<i>Chamaecrista fasciculata</i>	Fabaceae	C3 legume	Annual	Native	57
<i>Lupinus perennis</i>	Fabaceae	C3 legume	Perennial	Native	69
<i>Medicago lupulina</i>	Fabaceae	C3 legume	Annual	Introduced	27
<i>Trifolium repens</i>	Fabaceae	C3 legume	Perennial	Introduced	66
<i>Rudbeckia hirta</i>	Asteraceae	C4 non-legume forb	Biennial	Native	85
<i>Lolium perenne L. ssp. multiflorum</i>	Poaceae	C3 grass	Annual	Introduced	32
<i>Phleum pratense</i>	Poaceae	C3 grass	Perennial	Introduced	0
Slow invaders	Slow invaders	Slow invaders	Slow invaders	Slow invaders	Slow invaders
<i>Amorpha canescens</i>	Fabaceae	C3 legume	Perennial	Native	41
<i>Astragalus canadensis</i>	Fabaceae	C3 legume	Perennial	Native	33
<i>Agastache foeniculum</i>	Lamiaceae	C3 non-legume forb	Perennial	Native	19
<i>Koeleria macrantha</i>	Poaceae	C3 grass	Perennial	Native	19
<i>Bouteloua gracilis</i>	Poaceae	C4 grass	Perennial	Native	92
<i>Muhlenbergia glomerata</i>	Poaceae	C4 grass	Perennial	Native	44
<i>Panicum virgatum</i>	Poaceae	C4 grass	Perennial	Native	1