Functional composition controls invasion success in a California serpentine grassland

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Summary

1. Recent debates about the role of biotic resistance in controlling invasion success have focused on effects of species richness. However, functional composition could be a stronger control: species already in the community with similar functional traits to those of the invaders should have the greatest competitive effect on invaders. Still, experiments assessing effects of functional similarity have found contradictory results.

2. We used experimental communities in a serpentine grassland in California, USA, to assess the extent to which functional composition and functional diversity influenced success of two different types of invading plants: early season annuals (E) and late-season annuals (L) that have been previously shown to differ in patterns of resource acquisition.

3. We seeded known quantities of seed of six different species (three in each functional group) into experimental plots containing established communities differing in functional composition and functional diversity. The experimental communities contained different combinations of E, L, perennial bunchgrass (P) and nitrogen fixer (N) functional groups, with functional diversity ranging from 0 to 4 groups. Each invading species was seeded into a separate quadrat within each plot to minimize competitive effects of invaders on each other. We measured both seedling and adult success of invaders for two full growing seasons to further understand mechanisms underlying biotic resistance.

4. More functionally diverse communities were less invaded overall, as measured by the average success of individual invaders. However, assessment of invaders by functional groups was more informative: Es in the extant community suppressed E invaders the most, and Ls in the extant community suppressed L invaders the most.

5. We observed a variety of interactions among extant functional groups in reducing invader success, including synergism, complementarity and ‘basement’ effects, where two or more groups negatively affected invaders, but combinations of groups were no more suppressive than single groups. The extant community influenced invaders more strongly through suppression of adult plant growth than through effects on seedling establishment.

6. Synthesis. Contrary to predictions from neutral theory, these results indicate that niche overlap was an important component of biotic resistance in these experimental plant communities and summed up to significant effects of species richness.

Key-words: biodiversity, community assembly, competition, functional diversity, invasive species, niche complementarity, phenology, plant functional groups

Introduction

The question of how resident communities influence the success of invading species has generated substantial controversy. Elton (1958) postulated that higher numbers of species would reduce success of invaders. The concept of biotic resistance suggests native species could repel invaders by either resource competition, natural enemies or the interaction between the two (Levine, Adler & Yelenik 2004; Blumenthal 2005). While small-scale studies have largely supported the pattern of greater resistance with more diversity, the functional traits of the species involved have had mixed success in explaining the underlying mechanisms (see Hector et al. 2001; Herben et al. 2004; Fridley et al. 2007 for reviews). In addition, some broad-scale studies question the effects of biotic resistance (Stohlgren,...
Barnett & Kartesz 2003; Stohlgren et al. 2006). Understanding mechanisms of biotic resistance will help better predict changes in community composition in response to global changes, and subsequent effects on ecosystem properties, which remains a knotty challenge in ecology (Suding et al. 2008). While natural enemies can also have strong effects on community composition (Schmitz 2006; van der Putten et al. 2009), here we focus on interactions among plants at small scales to better understand the potential for resource pre-emption to influence invader success and community assembly.

Research into the effects of biodiversity on ecosystem functioning suggests that diversity could influence the degree of competitive resistance to invasion in three ways. First, more diverse communities have a higher probability of including particularly competitive species (i.e. sampling or selection effects; Huston 1997; Loreau & Hector 2001; Wardle 2001). Second, consistent with theory on species coexistence (Chesson 2000; Shea & Chesson 2002; Tilman 2004), niche overlap among functionally similar species (limiting similarity) could cause natives to repel invaders with similar resource acquisition strategies more strongly than invaders with different resource strategies. Third, complementarity among multiple species with non-overlapping resource use strategies could lead to greater use of available resources than less functionally diverse communities (Theoharides & Dukes 2007). These mechanisms are not mutually exclusive. For example, Fargione, Brown & Tilman (2003) and Fargione & Tilman (2005) found evidence for multiple mechanisms in Minnesota grasslands, including competition, niche differences among invaders and resident species, and complementarity. Other theory suggests, however, that neutral processes, or even statistical determinism, could lead to the appearance of negative relationships between native and exotic species richness at small scales, even in the absence of functional mechanisms (Fridley, Brown & Bruno 2004; Herben et al. 2004). Differentiating among these hypotheses has important implications for understanding basic mechanisms of both community assembly and invader success.

However, even small-scale studies that have found evidence for biotic resistance differ in their support for potential mechanisms. Some studies have found effects of limiting functional similarity (Dukes 2001, 2002; Prieur-Richard et al. 2002a; Fargione, Brown & Tilman. 2003; Fargione & Tilman 2005, 2005), but others have not (Von Holle 2005; Emery 2007). van Ruijven, De Deyn & Berendse (2003) found strong effects of two species in the family Asteraceae on the success of two common invaders (non-exotic) also in this family. However, they hypothesized that the effects stemmed from indirect interactions via nematode grazers rather than direct competition for resources. Some studies have found that effects of dominant species outweigh other mechanisms in resisting invasion (Symstad 2000; Emery & Gross 2007; James et al. 2008). Yet others find that traits of particular species actually facilitate invaders, especially in stressful environments (Prieur-Richard et al. 2000; Bruno, Stachowicz & Bertness 2003; Fridley et al. 2007).

A number of experimental issues complicate interpretation of previous results. Few experiments have explicitly controlled for a variety of potentially confounding variables, such as order of propagule input, interactions among invaders, life-history stage of invaders, and consistency of responses among species of the same functional type. Many studies focus on only one or two species of invader, and so lack generality. On the other hand, those that have looked at more species often lack explicit control of propagule pressure because they assessed diversity, number of individuals, and/or biomass of species weeded from plots. Another issue with such designs is lack of control for invasion order, including potential effects of dominant invaders on other invading species. Because order of establishment can have strong effects on community composition (e.g. Lockwood et al. 1997; Samuels & Drake 1997), the effects on invader composition and diversity could result as much from initial invaders as from the original community. Yet another issue is that many studies assessed success of seedlings rather than adult plants, even though the mechanisms and niche characteristics influencing these different life-history stages could differ greatly (Grubb 1977; Prieur-Richard et al. 2000; Milbau et al. 2005; Maron & Marler 2007). Studies assessing a variety of mechanisms and also controlling for all these issues have been rare (but see Maron & Marler 2007).

Here, we report results of a small-scale experiment that explicitly tested for a variety of niche-based mechanisms of biotic resistance using a California serpentine grassland as a model system. We tested for the effects of plant functional diversity and composition under common environmental conditions, controlling for disturbance regime, seed input, soil resource availability, and interactions among invading species. We used plots with well-established experimental communities that differed in composition and diversity of four functional groups of native species: early season annuals, late-season annuals, perennial bunchgrasses and nitrogen fixers (Hooper 1998; Hooper & Dukes 2004). We used a total of six invader species: three species from each of two different functional groups (early season annuals and late-season annuals). This experimental design allowed us to test whether functional diversity reduced success of invaders through sampling effects, complementarity effects (Hooper & Dukes 2004) or a combination of the two (Fargione, Brown & Tilman 2003; Fargione & Tilman 2005). However, we also tested whether invading species had lower success in plots containing functionally similar species, independent of the diversity of the resident community. Finally, we tested whether effects of resident communities were consistent across all invaders within a functional group, and whether effects were strongest during establishment or adult phases of competition.

Materials and methods

Experimental plots

Site and initial experimental communities.

We directly tested the interactions between community diversity and invader traits by seeding species of two plant functional groups into experimental plots that differed in functional group composition and
richness. Experimental plots had been established in early 1992 on an area of serpentine soil in south San Jose, California, USA, near the Kirby Canyon Landfill, operated by Waste Management, Inc. (37°15' N, 121°45' W) (see Hooper 1998 for details). The climate at this site is Mediterranean-like, with cool wet winters and a summer dry season extending from approximately May to October.

In the initial communities, we used four different functional groups, each containing two or three species that naturally occurred in high abundance in the area. These were: (i) early season annuals (E): Lasthenia californica Lindley, Microseris douglasii (DC.) Schultz-Bip., ssp. douglasii and Plantago ereta E. Morris; (ii) late-season annuals (L): Helenium congetta ssp. hzuilafolia (DC.) Babc. & H.M. Hall and Lessingia micradenia E. Greene var. glabrata (Kecck) Ferris; (iii) perennial bunchgrasses (P): Nassella pulchra (Hitchece.) Barkworth and Elymus multisetus (J.G. Smith) Burtt Davy and (iv) nitrogen fixers (N): Lotus wrangelianus Fischer and C. Meyer and Astragalus gambeliiensis E. Sheldon. Nomenclature follows Hickman (1993).

Treatments were applied in a randomized complete block design, with 10 plant communities and six replicates, for a total of 60 plots. Plots were square, 1.5 m on a side, and separated by 0.5–1 m buffer strips. The treatments received either no plants (B; bare), a single functional group (E, L, P and N), two functional groups (EL, EP, LP), three functional groups (ELP) or all four functional groups (ELPN). This produced a full factorial combination of the E, L and P groups, plus two N treatments (alone and with all other groups). All species established successfully. We maintained species composition by weeding out unplanted species for 8 years until the start of the invasion experiment in the 1999–2000 growing season. However, we did not manipulate density or relative abundance of the planted species during that time, allowing natural rank abundance curves to develop for the species present. In 1998 and 1999, functional group diversity, measured as H', increased as intended with greater richness of planted functional groups. Biomass varied approximately threefold among treatments as a result of differences in functional group composition and richness (Hooper & Dukes 2004).

INVADING SPECIES

We used three species from each of two different functional groups as invaders (E and L; Table 1). We used species that were either natives from the surrounding serpentine grasslands or common exotics that had established local populations there, but which were neither included in the original experimental treatments nor previously found as weeds in the plots. Therefore, invasions were not limited by the invaders’ inability to survive the climate or peculiarities of serpentine soils (low nutrients, low Ca : Mg ratios, high Ni content; Kruckeberg 1984). For simplicity, these species are referred to by genus below. In December 1999, we seeded each invader into a designated 30 × 30 cm (0.09 m²) subplot within each experimental plot to prevent confounding interference from other seeded invaders. We added 100 seeds of each early season invader and 45 seeds of each late-season invader. For comparison, serpentine grasslands typically have about 4500 individuals m⁻² of early season annuals (405 individuals 0.09 m⁻²) and 500 individuals m⁻² of late-season annuals (45 individuals 0.09 m⁻²) (Gulmon et al. 1983). In December 2000, we re-seeded all invaders in each quadrat, supplementing any seed that had fallen naturally as a result of 1999–2000 establishment. Thus, 2000–2001 growing season invader success reflected equal outside propagule input plus differential success in each community.

<table>
<thead>
<tr>
<th>Functional group</th>
<th>Species name</th>
<th>Growth form</th>
<th>Native/exotic</th>
<th>Abbreviation</th>
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<tr>
<td>E</td>
<td>Avena barbata</td>
<td>Grass</td>
<td>Exotic</td>
<td>Av ba</td>
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<tr>
<td>E</td>
<td>Bromus madritensis L. ssp. rubens (L.) Husnot</td>
<td>Grass</td>
<td>Exotic</td>
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<tr>
<td>E</td>
<td>Lasthenia platyglossa (Fischer &amp; C. Meyer) A. Gray</td>
<td>Forb</td>
<td>Native</td>
<td>La pl</td>
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<tr>
<td>L</td>
<td>Centaurea solstitialis L.</td>
<td>Forb</td>
<td>Native</td>
<td>Ca mu</td>
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<tr>
<td>L</td>
<td>Epilobium brachycarpum C. Presl</td>
<td>Forb</td>
<td>Native</td>
<td>Ce so</td>
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**Original community composition**

To estimate cover of the original species in the absence of invaders, we sampled non-destructively by the point-quadrat method (Goodall 1952) in April (peak biomass of early season annuals) and August (peak biomass of late-season annuals). Maximum cover of each species across months was then used for one overall estimate of community composition. We used 40 regularly spaced points per plot. Points were located at least 10 cm from plot edges and outside of the invaded subplots. At each point, we recorded objects that were hit by the tip of a sharpened metal rod as it was lowered vertically toward the ground, identifying each species. Because the tip could encounter multiple individuals or parts of individuals as it passed through the grassland canopy, our estimates of cover can exceed 100%. They are roughly equivalent to leaf area index, but also record cover of stems and reproductive parts of the plants.

**Statistical analyses**

We tested for effects of functional diversity on total invader success (number of individuals × reproduction/individual) using linear and nonlinear regressions. For nonlinear regression, we used the hyperbolic model \( Y = \frac{\alpha}{b + X} \), where \( Y \) was total success of all invaders in a given year and \( X \) was the realized functional diversity for that year, expressed as \( e^{\beta X} \) (the number of equally abundant functional groups that would give that value of \( H^* \)). This nonlinear model is mechanistically compatible with a situation in which resource availability to an invader individual decreases proportionally to 1/(functional diversity) (Bolker 2008, pp. 90–92). We performed analyses both with and without bare plots included to see if bare plots alone drove any negative relationship with functional diversity. Where data did not fit the assumptions of least-squares regression (normal residuals, homoscedastic), we obtained bootstrapped estimators of regression parameters using 1000 randomizations in the Regression procedure of SYSTAT v. 12. Linear and nonlinear models were compared using AICc (Burnham & Anderson 2002). Only the best-fit models are shown.

To test for effects of the presence/absence of particular functional groups, we used the following analysis of variance (ANOVA) design in the General Linear Models routine of SYSTAT v. 12:

\[
Y = \text{Constant} + \text{BLOCK} + E + L + P + N + E \times L + E \times P + L \times P + E \times L \times P + E \times L \times P \times N + \text{error},
\]

where \( E, L, P \) and \( N \) were dummy variables coded as 0 or 1 for absence or presence, respectively, of the four planted functional groups. Because this is not a full factorial model, the \( E \times L \times P \times N \) term is a partial interaction that tests the hypothesis that adding nitrogen fixers to communities without them has a consistent effect (B−N = ELP−ELPN; Hooper & Vitousek 1998). Where the initial ANOVA indicated significant interactions, we used Scheffé corrections on post-hoc comparisons to determine significant differences among the agglomerated means (Neter, Wasserman & Kutner 1990). For example, with an \( E \times L \) interaction, we tested for differences among the following means: −E−L (average of B, N and P treatments), +E−L (average of E and EP treatments), −E+L (average of L and LP treatments) and +E+L (average of EL, ELP and ELPN treatments). Because different kinds of interactions signify meaningful differences in biological patterns, we categorized interactions into four main types (Fig. 1): (i) ‘basement’ or ‘ceiling’ effects; (ii) solo functional group effects; (iii) reduced main effects and (iv) synergistic effects. Basement or ceiling interactions (Fig. 1a) can occur if two functional groups do not differ in their effects on an invader and if those two groups together utilize no more resources than either group alone (i.e. lack of complementarity). Basement effects can also arise if presence of one particular functional group repels an invader more strongly than presence of any other groups and mixtures do no better at reducing invader success than the single best functional group. This could reflect sampling effects for the
Weiner diversity index calculated by functional group. eH plots (Y = 89.7–25.4 data in panel a, linear regression was also significant without bare point quadrat method) are for the original planted species only. For ods). Cover data (average number of canopy hits per grid point in the mean success of that species in the bare plots (see Materials and meth-

Effects of functional diversity of the original communities in 2001 on (a) aggregate invader success and (b) total cover minus invaders. Functional diversity is expressed as $eH^2$, where $H$ is the Shannon–Weiner diversity index calculated by functional group. $eH^2$ gives the number of equally abundant functional groups that would give the same value of $H$ as the original community. Aggregate invader success is the average, across all six species of invader, of each invader’s proportional success (reproduction or stem length) relative to the mean success of that species in the bare plots (see Materials and methods). Cover data (average number of canopy hits per grid point in the point quadrat method) are for the original planted species only. For data in panel a, linear regression was also significant without bare plots (Y = 89.7–25.4 X, $n = 51$, $R^2 = 0.25$, $P < 0.001$, $AIC_c = 511.5$), but nonlinear regression had lower $AIC_c$ (Y = 53.8/(-0.26 + X), $n = 51$, $R^2 = 0.29$, $AIC_c = 508.8$).

RESPONSES OF INDIVIDUAL INVADERS

We expected that all of the invaders within a functional group would respond similarly to any given resident community. Only $Layia$ did not follow the predicted functional group pattern; it responded more like the late-season annuals than the other early season annuals. Principal components

INVADER SUCCESS

Based on our previous work with these communities (e.g. Hoover & Vitousek 1998), we expected that if a resource-based explanation of invasibility was correct, the more diverse communities would have lower reproductive success of invaders.

In line with this expectation, we observed lower aggregate reproductive output in more functionally diverse plots, whether or not the analysis included bare plots (Figs 2 and S1). Functional diversity led to significantly greater plant cover in the original communities in both years as well. However, invaders differed in their responses to community composition. Early season annual invaders were most strongly suppressed by the presence of early season annuals in the resident community and were not influenced or were positively influenced by other functional groups (e.g. Ls in 2000, Ps in both years; Figs 3a and S2, Tables 2 and S1). In contrast, each of the functional groups individually reduced success of late-season annual invaders compared to bare plots, although resident Ls had the strongest effect among single functional group treatments (Figs 3b and S2; Tables 2 and S1). The one exception to this pattern occurred in 2001, when Ns did not affect L invaders. Success of L invaders was uniformly low in communities with two or more functional groups, whether or not resident Ls were present. E and P functional groups combined either additively (2000) or synergistically (in 2001) to reduce L invader success. However, functional group effects were never additive or synergistic beyond two groups (Figs 3b and S2; Tables 2 and S1).

EFFECTS OF DIVERSITY AND COMPOSITION ON INVADER SUCCESS

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analysis identified two distinct groups of invaders. The late-season annuals plus *Layia* loaded most strongly onto axis 1, whereas *Avena* and *Bromus* loaded most strongly onto axis 2 (Fig. 4a). *Centaurea* and *Calycadenia* loaded slightly negatively onto axis 2. Patterns were very similar in 2000 (Fig. S3) and for simple correlations among invaders (Table S2). Together, these two axes accounted for 70–80% of the total variance (51.4% for axis 1 and 21.9% for axis 2 in 2000; 52.6% for axis 1 and 27.3% for axis 2 in 2001) and were the only axes with eigenvalues greater than 1.

Community types differed in their susceptibility to invasion and, in contrast to predictions from neutral theory, in the invaders that were successful. Not surprisingly, communities with no or few functional groups and low cover (B, N, P) were highly invasible, particularly by the later-flowering species (*Ls + Layia*), as reflected by high axis 1 scores (Fig. 4b). L and LP treatments were most strongly invaded by the earlier-flowering *Avena* and *Bromus*, whereas E-only treatments were most strongly invaded by *Centaurea* and *Calycadenia* (strongly negative axis 2 scores). Mixtures that included Es generally had low abundances of all invaders, except in Block 4 and EP2 in 2001 (Fig. 4b).

**Table 2.** ANOVA results for normalized success of early season annual (E) and late-season annual (L) invaders (average success of all species in each group) in 2001. Treatment means shown in Fig. 3. Data for 2000 are shown in Fig. S2 and Table S1. Direction of effects: ↑, ↓ mean that addition of that functional group increased or decreased, respectively, relative success of that invader group; number of arrows reflects relative magnitude of main effects within that particular ANOVA only (visually estimated from figure). Interaction types (see Fig. 1 for illustration): E, L, P or N = effect of that functional group only when other group(s) were not present; B = ‘basement’ effect: negative effects of both groups, but not additive in mixture; S = synergism: effects of both groups in mixture stronger than expected based on effects in single group treatments; R = reduced main effect. 

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<th>All Ls</th>
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<td>E × L × P × N</td>
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†Reduced main effect: reduction of E invader success by Es in the original community (E, EP) was not as great with Ls also in the original community (EL, ELP, ELPN), but there was no positive effect of Ls alone (L, LP) on E invader success.

‡Baseline effect: E and L effects were not additive – adding Es (EL, ELP, ELPN) did not reduce invader success any more than Ls alone (L, LP).

§E, P effects were synergistic, i.e. greater reduction than expected by E or P effects alone.

*Basement effect: L, P effects were not additive – adding Ps (LP, EL, ELP, ELPN) did not reduce invader success any more than Ls alone (L, LP).
negative effects of Es alone. For seedling establishment of *Layia* and the L invaders, however, Ls had negative (*Layia* 2001, *Calycadenia* 2001 and *Centaurea* 2000, 2001) or no effects (*Layia* 2000, *Epilobium* 2000, 2001 and *Calycadenia* 2000). Typically, 49–64% of variance in seedling establishment for *Avena* and *Bromus* and 47–83% of variance for *Layia* and the L invaders were explained by functional group composition of the initial community. The exception was *Calycadenia* in 2000, for which none of the functional groups explained significant variance in seedling establishment (Tables 3 and S3). When effects were present, Ps had positive effects on *Avena* and *Bromus* and negative effects on *Layia* and L invaders. The latter often occurred in interaction with the presence of other functional groups, sometimes synergistically so with Es (e.g. *Epilobium* and *Centaurea* 2001).

Functional group composition of the initial community also had strong competitive effects on success of the invaders in the adult stage. For *Avena* and *Bromus*, only the presence of Es decreased invader success in both years, while Ps increased individual success but less consistently (Figs 6 and S5; Tables 4 and S4). In contrast, all functional groups, but particularly Es and Ls, decreased size or reproductive output for *Layia* and the L invaders. Typically Ls suppressed *Layia* and the L invaders most strongly, with a similar magnitude of effects in all L-containing mixtures as with Ls alone (significant ‘basement’ interactions). In several cases, however, Es and/or Ps had relatively limited effects alone, but had synergistic or additive effects in mixture with each other (e.g. *Epilobium* and *Calycadenia* 2000, *Epilobium* and *Centaurea* 2001). These interactions reduced success of *Layia* and the L invaders even if E main effects were not significant. Ns occasionally had negative effects on *Layia* and *Epilobium*, although these were not consistent across years.

Patterns for reproductive success per plant corresponded more closely with overall reproductive success than did patterns of seedling establishment in 2000 for *Bromus* and all the late-season annuals (Table 5). However, this association reversed in 2001, when seedling establishment correlated most closely with total success of the individual invaders. This reversal likely occurred because total seed input was consistent across all plots in 2000, but in 2001 it reflected both new seed input plus reproductive success in the previous year.

**Discussion**

**OVERVIEW**

We found strong biotic resistance to invasion among plant species in these experimental grassland communities. When the physical environment, propagule pressure and interactions among invaders were held constant, functional diversity in the extant community decreased overall invasion success. Our results are similar to those from other studies experimentally assessing the response of invasion to plant diversity and contrast with results of cross-community observational studies (see Fridley *et al.* 2007 for a review; Maron & Marler 2007). Differences in results between broad-scale surveys and plot-scale experiments likely arise because broad-scale surveys cut across many environmental gradients that can simultaneously affect diversity of both native and exotic species (e.g. environmental heterogeneity, soil fertility, disturbance history and propagule pressure) (Robinson & Dickerson 1984; D’Antonio & Mahall 1991; Burke & Grime 1996; Planty-Tabacchi *et al.* 1996; Levine 2000, 2001; Davies *et al.* 2005).

In our study, the net effect of reduced invasion at higher functional diversity resulted from multiple niche-based processes operating both solely and in various combinations, including complementarity among some functional groups in suppressing some invaders, strong effects of extant functional
groups on invaders with similar traits, effects of a dominant functional group (Es) on all invaders, and facilitation of some groups for some invaders. Results were consistent with a priori functional classification for all invader species except _Layia platyglossa_, which has the phenology of an early season annual but which responded to the extant communities like the late-season annual invaders. Because we assessed invader responses based on growth or reproductive output rather than species richness of invaders, we can rule out neutrality or statistical artefacts at small plot sizes (e.g. Fridley, Brown & Bruno 2004; Herben et al. 2004) as dominant mechanisms in this study. However, those mechanisms may influence observed native-exotic richness relationships in cross-community surveys. In the first year, patterns of overall invader success more closely matched those expected from competition of adult invaders for soil resources than those expected from suppression of seedlings, suggesting that functional classifications may vary depending on life-history


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**Table 3.** _ANOVA_ results for invader seedling establishment in 2001 (data shown in Fig. 5). Results for 2000 are in Fig. S4 and Table S3. Invader species are arranged by functional group and according to similarities in response to treatments as shown in the PCA (Fig. 4). Data analysed were maximum number of plants in any sampling period as an estimate of number of seeds getting established. All analyses were performed on natural log-transformed data to improve homoscedasticity. Symbols, interaction types and levels of statistical significance as in Table 2. Species abbreviations as in Table 1. Block effects are not shown.

<table>
<thead>
<tr>
<th>E invaders</th>
<th>PCA 2 invaders</th>
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†_Layia_ in 2001, basement effect on establishment for any combination of functional groups > 1.
‡E+P synergistic; additive effects of up to two functional groups, but not 3.
§N > B, but _ELPN_ < _ELP_.
* N < B, but _ELPN_ = _ELP._
stages. Questions remain as to how mechanisms of biotic resistance play out in natural systems across gradients of nutrient availability, disturbance, propagule pressure, environmental heterogeneity or other factors that can influence invader success across landscapes (Stohlgren et al. 1999; Fridley et al. 2007). Nevertheless, our results strongly support a role for biotic resistance, specifically competition, as a mechanism reducing invasion success.

MECHANISMS THROUGH WHICH DIVERSITY AND COMPOSITION AFFECT INVASIBILITY

We found evidence for simultaneous operation of most postulated niche-based mechanisms. Our analyses clearly indicate that invaders were most strongly suppressed by species in the same functional group. E invaders were only suppressed by presence of Es. While L invaders were suppressed by presence

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**Fig. 6.** Adult success of invaders in 2001 in response to functional group composition of the original community. Growth per plant is the maximum average reproductive success for individuals of each species at the time of peak biomass (April for early season annuals [upper panels], June–August for late-season annuals [lower panels]). Reproductive success is estimated differently for each species: seeds per plant for *Avena* and *Bromus*, flowering heads per plant for *Layia* and *Centaurea*, and total stem length for *Calycadenia* and *Epilobium*. Statistics shown in Table 4. Results for 2000 are shown in Fig. S5 and Table S4.

**Table 4.** ANOVA results for effects of functional group composition on adult success of invaders (growth and reproduction) in 2001 (data shown in Fig. 6). Data were total number of seeds (*Avena* and *Bromus*), number of flowering heads (*Layia* and *Centaurea*) or total stem length (*Calycadenia* and *Epilobium*) at peak biomass divided by the total number of individuals in that census period. Symbols as in Table 2, species abbreviations as in Table 1, grouping as in Table 3. All analyses on natural log-transformed data to improve homoscedasticity. Results for 2000 are shown in Fig. S5 and Table S4.

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†No interaction indicates that main effects are additive, i.e. complementary. ‡Additive effects for two-way functional group mixtures, but basement effect for functional groups > 2. §E × P synergism and L basement effect. *N < B, but ELP ≤ ELP. ††C, ‘ceiling effect’: positive effects of Ps and to some extent Ls, but the effects were not additive.
of all functional groups in the original community, this effect was much stronger for the presence of Ls than for the other groups (Table 1, Fig. 3). This pattern was fairly consistent when assessed for individual species as well. The early annual invaders *Avena* and *Bromus* consistently followed this pattern and were even facilitated to some extent by late-season annuals. *Layia* success was also strongly negatively affected by Es, but overall, its response resembled that of the late-season annuals more closely than expected. Ls, on the other hand, were quite consistent as a group. In addition, many of the interactions were ‘basement effects’, where presence of a single functional group reduced success as much as two functional groups together. This is consistent with results for the exotic *Centaurea solstitialis* studied in non-serpentine grassland microcosms, where competition for late-spring moisture appeared to be the dominant mechanism driving the response of *Centaurea* (Dukes 2001, 2002). We found this pattern consistently for two additional late-season annual species (both natives), suggesting that this pattern was not just a species-specific response of *Centaurea*.

Overall, our results support theory that suggests species are more likely to coexist with each other when their resource demands have minimal overlap, whether for exotics establishing in a native community or for community assembly more generally (Chesson 2000; Shea & Chesson 2002). Our results are also compatible with theory related to variation in resource availability promoting invasion (Davis, Grime & Thompson 2000), because the functional groups we used differ primarily in spatial and temporal patterns of resource acquisition.

Our results are consistent with some field studies, but not with others. Other grassland studies have also shown effects of functional similarity on reducing invader success (e.g. Piereur-Richard et al. 2002a; Fargione, Brown & Tilman 2003; Fargione & Tilman 2005; Maron & Marler 2008). Lack of consistency with other experiments could result for several reasons. First, some studies not finding consistent effects of ‘limiting similarity’ assessed success of invader seedlings (e.g. Emery 2007), which may or may not reflect the functional classification of the adults (see below). Second, when all invaders were seeded in together, interactions among invaders could also have reduced consistency with functional classification of the original community in that study. Third, potential for unmeasured axes of niche partitioning could lead to results inconsistent with original functional classifications (e.g. James et al. 2008). Finally, some communities or parts of communities (e.g. understory in forests; Von Holle 2005; Von Holle & Simberloff 2005) may be less structured by interspecific interactions than others, perhaps because of overriding effects of abiotic factors, such as disturbance (e.g. Symstad 2000). The first three issues speak to experimental protocols, whereas the last suggests that abiotic conditions in some communities may reduce the potential for effective biotic resistance. This possibility warrants further exploration.

We found evidence for complementary effects of extant functional groups, particularly in reducing success of the L invaders. The effects of Es and Ps in the original community were consistently additive or synergistic in reducing L invasion success overall (Tables 2 and S1), as well as for both *Epilobium* and *Centaurea* individually (Tables 3, 4, S3 and S4). We also saw occasional additive effects of E and L and of L and P in reducing adult success of *Epilobium* and/or *Calycadenia* (Table 4). Negative effects of Ns on L success were also additive with those from other functional groups in 2000. We did not, however, find evidence for complementarity for more than two groups at a time in reducing invader success. While we cannot rule out multitrrophic effects (e.g. van Ruijven, De Deyn & Berendse 2003; Schmitz 2006), these patterns are consistent with previous observations of complementary resource use and production in this system (Hooper 1998; Hooper & Vitousek 1998; Hooper & Dukes 2004), in which Es are strongly complementary with Ls and Ps. The greater plant cover observed at higher functional diversity also suggests greater total resource pre-emption as a primary mechanism reducing invader success. Other researchers have also found evidence for complementary effects that co-occur with effects of particular functional types (Fargione & Tilman 2005). These patterns support theory predicting that communities occupying a greater breadth of potential niche space lower the probability of establishment by new species (as reviewed in Chesson, Pacala & Neuhauser 2002; Levine, Kennedy & Naeem 2002).
Sampling effects can arise through the influence of a single group, or through the presence of multiple groups. In the first case, more groups in a community could result in a greater probability of including a particularly competitive species or functional group. Alternatively, if each group has the strongest effect on invaders like it, communities composed of more groups should reduce the success of more kinds of invaders. We argue that both of these could play a role in the reduction of invader success in more functionally diverse communities. Because both E and L invaders did worse in communities that included those same groups, the more diverse communities were able to resist invasion by more species – although this was partially offset in some cases by facilitation of some invaders by members of other functional groups (see below). In addition, early season annuals were particularly effective at reducing the success of both E and L invaders. Because more diverse plots had a higher probability of containing Es, invader success would be expected to decline (Huston 1997; Wagner, Wildl & Ewald 2000; Wardle 2001). Past research has shown that Es are particularly good competitors for nitrogen early in the growing season (Hooper & Vitousek 1998). Because of this, even L invaders in E-containing plots must initially withstand strong competition for nitrogen before they are able to take advantage of reduced competition later in the season when their roots are deeper and Es begin to senesce (Gulmon et al. 1983; Mooney et al. 1986). Once the dry season begins, however, Es die and competition among Ls for water becomes more important (Dukes 2001). Similar results were found in Great Plains grasslands, where total biomass of perennial bunchgrasses had a greater effect on reducing success of annual grass invaders than did overlap of nitrogen uptake patterns (James et al. 2008) (although that study did not investigate the potential for partitioning multiple resources). While some might insist that sampling effects are statistical artefacts of the experimental design, we argue that they may be an important component of diversity in natural systems where specific functional traits of all species are rarely known and where variation in invader abundance correlates with differences in native community composition (Hooper et al. 2005; Thomsen & D’Antonio 2007). Indeed, the traits that lead to strong competitive ability are likely to vary with environment (Chapin 1980; Grime 2001), such that fast growth and wide seed dispersal are effective strategies in resource-rich environments (Hamilton et al. 2005), but slow growth and resource-use efficiency are more effective in resource-poor environments (e.g. Funk & Vitousek 2007).

We found evidence for facilitation; both Ls and Ps facilitated success of some E invaders, but by different mechanisms. Perennial bunchgrasses increased success of E invaders (primarily Bromus and Avena), partially offsetting the negative effect of early season annuals in the extant community (Fig. 3, Tables 2-4, S3 and S4). Avena often grew in patches of decomposing P litter (even in the middle of live plants), indicating that Ps may have ameliorated low soil nutrient or water-holding conditions. Presence of Ls in the original community reduced negative effects of extant Es on success of E invaders in both years, particularly from enhanced establishment for Avena and Bromus (Tables 2, 3, S1 and S3). This could result from sheltering of seedlings from desiccation relative to the bare plots (e.g. Bruno, Stachowicz & Bertness 2003), or potentially from reduced herbivory (e.g. Prieur-Richard et al. 2000, 2002b). Similarly, some native species can facilitate invasive grasses in California coastal sage scrub (Thomsen & D’Antonio 2007). Finally, Ps sometimes facilitated invader establishment, but these effects were context specific: they depended on species of invader, stage of invasion (establishment versus growth), which other species were in the original community (ELPN interactions) and on year. Effects of Ps were also sometimes negative (e.g. on all Ls in 2000; Table S1). Where N effects on invader success were positive, competition for nitrogen between the invader and resident community may have been strongest. Others have noted the importance of facilitation in invasion success and community assembly in general (e.g. Bruno, Stachowicz & Bertness 2003; Fridley et al. 2007). In our experiment, facilitation occurred in the context of simultaneous competition, so although facilitation weakened biotic resistance, it did not overcome it in general.

We found little support for neutrality as a dominant driver of invasion patterns in this experiment (e.g. Hubbell 2001; Fridley, Brown & Bruno 2004; Herben et al. 2004). Neutral processes would predict no difference in responses of invader species to extant functional groups. Because all communities had several generations to saturate niche space under null sum gain assumptions of neutrality (Hubbell 2001; Holyoak & Loreau 2006), we would have expected that priority effects of the original species would predominate and invasion success would have been similar for all species in all communities. However, invader fitness in this experiment depended strongly on the functional traits of the invaders relative to the functional composition of the community, even at similar levels of functional diversity (Fig. 4). That is, even communities that were well established for over 8 years (eight generations for the annual-dominated plots) were readily invaded by species from functional groups not present. Our results clearly indicate that we cannot consider these species’ fitnesses to be equivalent and independent of the pre-existing community – a key assumption of neutrality (Hubbell 2001; Tilman 2004). That said, neutrality could still play a role in community assembly. For example, species within functional groups may more closely approximate the assumption of similar fitness, leading to stronger effects of history and dispersal constraints in determining species composition within groups (Tilman 2004; Gonzalez 2007).

**CONSISTENT RESPONSES OF SPECIES WITHIN FUNCTIONAL GROUPS?**

We saw strong, but not complete, consistency within our phenologically defined groups. Only Layia deviated markedly from initial expectations, responding more like the late-season invaders than the other early season invaders. A native forb, Layia platyglossa grows abundantly in intact grasslands surrounding the experimental plots. Layia is in the same tribe
(Tarweeds, or Madieae) within the Asteraceae as H. congesta ssp. laevisfolia, one of the L species in our resident communities. Layia is somewhat later phenologically than the other early season annuals (Mooney et al. 1986), and together the traits associated with these two differences may have made Layia more susceptible to suppression by extant late-season annuals in our plots. Alternatively, Layia and the other late-season annuals are all forbs, whereas Avena and Bromus are annual grasses. While traits associated with these morphotypes may explain the observed patterns, other studies have found little explanatory power in grass–forb functional types (Wright et al. 2006). We saw some spread in response among the L invaders, with Epilobium most similar to Layia in its responses and Calycadenia most different from the E invaders (Fig. 4). Other studies assessing the functional similarity hypothesis have reported mixed results. Emery (2007) saw substantial variation among species of invaders, with some acting consistently with respect to limiting similarity with dominant species and others not.

COMPETITION AT DIFFERENT LIFE STAGES:
ESTABLISHMENT VERSUS GROWTH OR REPRODUCTION

Unless seedling traits are explicitly addressed, we suggest that assessment of effects of functional similarity should be done on indices of adult fitness, rather than on seedling germination or diversity. Three of our analyses suggested that functional overlap at the adult stage corresponded most closely with our a priori expectations based on resource acquisition patterns. First, resident Ls had stronger effects on adult success per plant than seedling establishment of L invaders in both years (Tables 3, 4, S3 and S4). Second, the functional group having the greatest effect on seedling establishment of invaders was not necessarily the same as that having the greatest effect on adult success of invaders. Third, in 2000, total invader success correlated more strongly (higher r values) with adult success per plant than with seedling establishment for four of the six invader species (Bromus and all three L invaders) (Table 5). We suspect that the reason for the reversal of this pattern in 2001 was that the total number of seedlings represented both controlled seed input from that year plus seed input from successful adults in 2000. That is, 2001 seedling establishment was a combination of both variation in reproductive success in 2000 and variation in seedling mortality in 2001, whereas only seedling mortality contributed in 2000. Data from 2000 therefore represent a cleaner test of the establishment versus adult success hypothesis.

Our results indicate that studies using weeded seedlings as indices of invader success are likely to underestimate the influence of functional composition and resource pre-emption. Prieur-Richard et al. (2000) also found different effects of species richness, functional group richness and functional group composition on different life-history stages of two species of Conyza in Mediterranean oldfields. Similarly, in experimental communities of Montana grasslands, USA, species richness had no effect on seedling success but significantly reduced adult success of Centaurea maculosa (Maron & Marler 2007). It is not surprising then, that effects on first-year seedlings of perennial species may not show the same patterns as those expected for adults (Milbau et al. 2005; Emery 2007).

CONCLUSIONS – IMPLICATIONS FOR LARGER SCALES AND MANAGEMENT

A true test of the resource pre-emption hypothesis of species richness requires that seed inputs, levels of disturbance and overall resource availability are constant across levels of species richness (or arise because of differences in species richness and not vice versa). Under such controlled conditions, we saw a variety of effects of functional composition and diversity. Our study supports theory suggesting that the degree of niche overlap among species strongly influences invasion success and coexistence (Chesson 2000; Shea & Chesson 2002; Tilman 2004). The effects of functional composition were the strongest mechanism limiting invader success in this experiment. In addition, multiple functional groups exhibited complementarity (additive and synergistic effects), sampling effects (‘basement’ interactions) and also counteracting negative and positive effects on invader success. However, the net effect of all of these interactions was strongly reduced invader success in more functionally diverse communities, both for individual invaders and for all invaders considered together. These results correspond with several other studies assessing the effects of plant diversity on invader success in controlled, small-scale experimental studies (see reviews by Hector et al. 2001; Levine, Adler & Yelenik 2004; Maron & Marler 2007). The plethora of effects operating simultaneously on different species (and even the same species) may help explain why different studies find effects of different mechanisms (e.g. Fargione, Brown & Tilman 2003; Fargione & Tilman 2005; Emery & Gross 2007).

This study exclusively focused on small-scale processes, so patterns we observed do not necessarily predict patterns at larger scales (Stohlgren, Barnett & Kartesz 2003; Stohlgren et al. 2006). On the other hand, patterns at large scales (e.g. positive correlations between native and exotic richness) do not necessarily rule out potential mechanisms at small scales. It is entirely possible that competition with native species reduces success of exotics, but that disturbance (Davis, Grime & Thompson 2000; Hobbs, Yates & Mooney 2007), spatial heterogeneity in resources (Huenneke et al. 1990; Levine, Kennedy & Naeem 2002; Davies et al. 2005; Thomsen & D’Antonio 2007) or complex species interactions (e.g. between goatgrass, fungus and gophers; Eviner & Chapin 2003) come into play that allow ecosystems to be invaded despite their high richness (Fridley et al. 2007). However, an observational study investigating these patterns in the natural serpentine grasslands at Coyote Ridge near San Jose, CA, USA, suggests that exotic success is negatively correlated with native diversity, even after taking environmental variables into effect (D. Slakey & D. Hooper, unpub-
lished data). Whether this correlation reflects causality or covariance with yet other environmental variables remains to be determined. Similarly, high propagule pressure and competitive success of some new invaders can overwhelm native communities without appropriate management action (Maron & Marler 2008). Thus, while loss of local diversity could lead to greater invasion, the environmental changes leading to that local loss could have effects on invader success that are equal to or greater than those of local diversity itself. This study and others have clearly established the potential for biotic resistance to reduce the success of invaders via competition related to resource uptake. The task now is to identify how this process interacts with other mechanisms to determine patterns of invasion at larger scales, for particular problem species, and in potentially vulnerable ecosystems.

Acknowledgements

We thank Colleen Mohl for dedicated technical assistance, Julia Verville and Sarah Hobbie for fruitful discussions and help in the field, and Crystal Elliot, Dominic Chiarelli and Jesse Burns for lab and field assistance. This research was supported by the National Science Foundation (DEB99-74159). We thank Waste Management, Inc. for allowing us to conduct research at the Kirby Canyon Sanitary Landfill.

References

Functional composition and invasibility

Supporting Information

Additional Supporting Information may be found in the online version of this article:


Figure S1. Effects of functional diversity of the original communities in 2000 on (a) aggregate invader success and (b) total cover minus invaders.

Figure S2. Relative success of (a) early season annual and (b) late-season annual invaders in 2000 in response to plot functional group composition.

Figure S3. Loadings for PC axes 1 and 2 for all invader species in 2000.

Figure S4. Seedling establishment of invaders in 2000 in response to functional group composition of the original community.

Figure S5. Adult success of invaders in 2000 in response to functional group composition of the original community.

Table S1. ANOVA results for relative success of E and L invaders (average success of all species in each group) in 2000.

Table S2. Correlation coefficients for normalized reproductive success among individual invading species in 2000 and 2001.

Table S3. ANOVA results for invader seedling establishment in 2000 (data shown in Fig. S4).

Table S4. ANOVA results for effects of functional group composition on adult success of invaders (growth and reproduction) in 2000 (data shown in Fig. S5).

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