

Realistic variation in species composition affects grassland production, resource use and invasion resistance

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Abstract We investigated the effects of realistic variation in plant species and functional group composition, with species occurring at realistic abundances, on ecosystem processes in exotic-dominated California grassland communities. Progressive species removals from microcosm communities, designed to mimic nested variation in diversity observed in the field, reduced grassland production, resistance to intentional invasions, and resistance to natural colonization by new species. Three lines of evidence point to the particular importance of intensified competition within a single functional group—late-active forbs—in explaining the observed effects of realistic species loss order on community resistance. First, reduced success of naturally colonizing species in more diverse assemblages was dominated by declining colonization by late-active forbs. Second, increasing late-active forb biomass appeared to reduce the biomass of intentionally introduced yellow starthistle (*Centaurea solstitialis*, a late-season forb) both within and across diversity levels. Finally, starthistle addition reduced biomass of resident late-season forbs but not of any other functional group. Increasing diversity increased light levels and soil moisture availability

in spring and summer, providing a proximate mechanism linking our realistic species loss order to decreased community resistance. Starthistle addition reduced light and soil moisture availability but not N across richness levels, mirroring the apparent effects of the additional late-active forb species present in higher diversity treatments. Species losses that entail the early loss of whole or key functional groups could, through mechanisms like those we explore, have greater ecosystem consequences than those suggested by randomized-loss experiments.

Keywords California grassland · *Centaurea solstitialis* · Diversity · Species composition · Invasion · Nestedness

Introduction

In the last decade, a great many studies have examined the effects of biodiversity on ecosystem processes by directly manipulating the former in model systems such as grasslands (Loreau et al. 2002). Few such studies, however, have attempted to manipulate species diversity in ways that mirror actual changes or variation in diversity occurring as a result of human activity (Lyons and Schwartz 2001; Smith and Knapp 2003). Exploring actual or realistic patterns of variation and change in species richness is critical to under-

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standing their consequences because species with particular traits appear to be especially vulnerable to decline (Fox 1987; McDonald and Brown 1992; Lawler et al. 2001; Henle et al. 2004) or prone to expansion (Mack 1996; Rejmanek and Reichard 2001). Traits associated with vulnerability or invasiveness could also be, or correlate with, traits with particular effects on ecosystem functioning. It follows that in communities affected by human activity, functional consequences of actual species loss or gain may differ systematically from the consequences of the randomized or hypothetically structured biodiversity treatments typical of experimental studies in this area.

A number of experiments have found that functional diversity (or the particular functional characteristics of species), rather than species richness *per se*, predicts the magnitude and variation of particular ecosystem processes (Hooper and Vitousek 1997; Wardle et al. 1997; Symstad 2000; Dukes 2001; Von Holle and Simberloff 2004). Observation of actual patterns of richness changes through time or space could suggest how functional groups are lost as species richness declines. At the neighborhood scale, competitive interactions might dictate that functionally different species can most easily coexist with one another and that species are lost in order of greatest similarity to persisting neighbors (Fox 1987; Walker et al. 1999). In this case, functional groups might continue to be represented longer in declining communities than expected based on randomized species removals. Alternatively, it is possible that species with particular response and/or effect traits (Hooper et al. 2005) will share degrees of vulnerability. If this is the case, whole functional groups might disappear earlier than expected from an assumption of randomized species losses, leading to more rapid changes in ecosystem functioning.

To examine the effects of realistic changes in species richness on ecosystem functioning, we designed an experiment that based the composition of each of several species richness levels on 4 years of observed spatial variation in grassland species richness at the neighborhood scale (Zavaleta and Hulvey 2004). Patterns of functional group diversity (defined *sensu* Hooper and

Vitousek 1997) therefore emerged directly as a result of this observed order of species gains and losses. Because relative abundances of species in real communities generally vary by orders of magnitude and species that occur only in the most diverse assemblages are often rare (Magurran 1988), we based species' relative abundances in our richness treatments on field observations rather than using the more common design of equal species abundances (Loreau et al. 2002). This allowed us to examine whether additional species can contribute significantly to ecosystem processes at the abundances they typically occur at rather than at artificially high abundances.

Our design differs from previous experiments on the effects of species and functional group diversity through the incorporation of both field-based abundances and a quantitative rationale for including particular species at each diversity level. A tradeoff between randomized experimental designs and our approach is that while the former do not distinguish the effects of random from realistic diversity declines, the latter confounds species richness and composition effects through focus on a particular order of species removals. The goals of each approach are different. The randomized diversity approach can tackle the effects of species richness *per se*, an important theoretical question; our approach can pinpoint the effects of likely or ongoing species losses by focusing on particular sequences of diversity change, an important conservation question. In the context of our study, change and variation in diversity refer not to diversity *per se* but to the particular, observation-based order of diversity change that we focused on.

This paper build on previous reported results about invasion resistance from this experiment (Zavaleta and Hulvey 2004) by examining in detail the mechanistic links underlying the responses of invasion and two additional sets of ecosystem functions, aboveground production and resource availability, to the realistic order of species loss in our grassland system that we simulated. We hypothesized that patterns of functional group loss correlated with our diversity treatments (Table 1) would explain the effects of this realistic species loss order on ecosystem processes. We specifically explore the role of a key functional

Table 1 Species compositions at each experimental species richness level

Species	Richness level						Functional group
	3	6	9	12	15	21	
Number of species	3	6	9	12	15	21	
Number of functional groups	2	3	3	4	4	5	
<i>Avena barbata</i>	206	149	110	98	74	24	Early grass
<i>Bromus hordeaceus</i>	41	41	41	41	41	41	Early grass
<i>Geranium dissectum</i>	21	20	21	20	21	21	Early forb
<i>Bromus diandrus</i>		13	13	12	12	12	Early grass
<i>Lolium multiflorum</i>		35	28	24	18	7	Early grass
<i>Vicia sativa</i>		10	10	10	10	10	Early forb
<i>Avena fatua</i>			34	33	34	33	Early grass
<i>Erodium botrys</i>			9	9	9	9	Early forb
<i>Vulpia microstachys</i>			2	2	2	2	Early grass
<i>Anagallis arvensis</i>				10	27	63	Late forb
<i>Briza minor</i>				7	10	15	Early grass
<i>Epilobium brachycarpum</i>				2	3	5	Late forb
<i>Crepis vesicaria</i>					2	2	Perennial (forb)
<i>Torilis arvensis</i>					3	3	Late forb
<i>Trifolium hirtum</i>					2	2	N-fixer
<i>Danthonia californica</i>						5	Perennial (grass)
<i>Hemizonia congesta</i>						2	Late forb
<i>Hordeum murinum</i>						2	Early grass
<i>Medicago polymorpha</i>						2	N-fixer
<i>Phalaris aquatica</i>						2	Perennial (grass)
<i>Rumex acetosella</i>						6	Perennial (forb)

Values are target numbers of stems for each species in a given treatment. Functional types were defined as early-active grasses, early-active forbs, nitrogen-fixers, late-active forbs, and perennials. Exotic species are in italics.

group in our California grassland study system, late-active annual forbs (see also Dukes, 2001), in explaining responses to our field-based species loss order of production, resource use, and community resistance to invasion and colonization. We chose to explore the role of late-active forbs for two reasons. First, many California grasslands are now strongly dominated by early-active annual exotic species that senesce in the spring. In this context, late-active species, which remain active throughout spring and early summer and senesce in mid-late summer, are uncommon and likely to exert a distinct influence on ecosystem processes. Second, yellow starthistle (*Centaurea solstitialis*), a novel exotic species of high management concern in California grasslands, is a late-active annual forb species. Previous work suggests that such an invader could be resisted more effectively by communities that already contain one or more functionally similar species (Fargione et al. 2003).

We distinguish (1) natural colonization of our assemblages by volunteers dispersed from a

nearby grassland and (2) invasion by yellow starthistle intentionally introduced to our assemblages. California grassland management involves both promoting native populations and preventing new exotic invasions within the context of dominance by long-naturalized, exotic Mediterranean grasses intractable to eradication at large scales (e.g. Hatch et al. 1999). Against this shifted baseline (Pauly 1995) of exotic annuals, the relative newcomer yellow starthistle (YST) causes considerable economic and ecological harm and is considered undesirable by a range of stakeholders (Bossard et al. 2000; Jetter et al. 2000). We investigated the effects of ordered richness declines in this system on YST invasion due to the real-world value placed on enhancing resistance to its spread. We also investigated richness effects on natural colonizations into our assemblages from the regional species pool as a general measure of community resistance to change and a means of exploring relationships between functional traits of resident and colonizing species.

Methods

Site

All work took place outdoors at the Jasper Ridge Biological Preserve near Palo Alto, California, USA (37°24' N, 122°14' W, 120 m elevation). The study site has a Mediterranean-type climate with summer droughts and winter rains and is dominated by exotic annual herbs. Mean annual rainfall for 1975–2004 was 652 mm at the study site. The starthistle experimental array was supported in a wood and wire frame on a flat cement pad approximately 50 m from the Jasper Ridge Global Change Experiment (JRGCE) site (Zavaleta et al. 2003).

Starthistle invasion experiment

We replicated planted communities in 30.5-cm diameter \times 1 m deep plastic PVC containers filled, tamped, and topped off with homogenized sandstone soil collected ~200 m away at depths of 0.1–1 m. We replicated treatments containing 3, 6, 9, 12, 15, and 21 species ten times each (Table 1) and invaded half the replicates with 25 seeds of yellow starthistle (*Centaurea solstitialis* L., Asteraceae) to produce a total of twelve treatments replicated five times each in a randomized block design.

Composition of each species richness level

We used 4 years of species presence/absence and relative abundance data from 136 0.78 m² grassland plots at the JRGCE site (Zavaleta et al. 2003). Plots were grouped in adjacent sets of four, with the sets evenly distributed across the site several meters from each other. A species list was assembled yearly (1998–2001) for each plot from the combined results of (i) a biomass harvest, (ii) a 50-stem pin tally, and (iii) exhaustive searches. Relative species abundances were compiled from the results of (ii) and (iii) when possible and from (i) for species appearing only in the biomass harvest.

We used the Nestedness Temperature Calculator (NTC) (Atmar and Patterson 1995) to (i) analyze plot compositions for nested structure

and (ii) identify the best-fit order of species losses from plots of progressively lower diversity at our study area. The NTC provides a measure T of the degree to which a set of species assemblages varying in richness are nested, with a perfectly nested series consisting of assemblages that all contain only proper subsets of the species found in more species-rich assemblages. A highly nested distribution pattern implies a predictable order of species loss as one moves from more to less diverse assemblages (Atmar and Patterson 1993), whose differing compositions are assumed to reflect outcomes of local colonization and extirpation events shaped by underlying differences in site conditions (Wright and Reeves 1992). The NTC also provides an ordination of the species that maximizes the degree of nestedness among the assemblages. Species are ranked from those that occur (or are expected to occur) in all assemblages, including the least speciose ones, to species that occur only in the most species-rich assemblages. NTC software and examples of its output are freely available (see (Atmar and Patterson 1995).

We generated T values and species rankings for each year of data (1998–2001). T values $< 25^\circ$ for all years indicated significantly nested species occurrence patterns among plots (NTC $p < 0.0001$) (Jonsson 2001; Fischer and Lindemayer 2002). We then compared nested orders and associated uncertainty values across years to check the stability of nested orders and restricted selection of experimental species to those with stable positions. From this subset, we chose experimental species based on place in the nested order (Table 1). We skipped over four species (*Sisyrinchium bellum*, *Convolvulus arvensis*, *Euphorbia peplus*, and *Nassella pulchra*) low in the nested order because their seeds were not available. Our experimental species loss order is therefore based on spatial patterns of species loss and gain that we treat as proxies for temporal patterns of loss and gain at a similar spatial scale.

The three-species treatments contained the first three species in the nested order; the six-species treatment contained the first six species in the nested order; and so on (see Zavaleta and Hulvey 2004 for additional details). We sowed all microcosms with locally collected seed on Nov 6,

2002 using available data or results of germination trials to guide seed numbers. All communities had target density of 4,125 stems/m² based on 1998 field means and the observation that stem density did not vary with plot species richness. We used our 1998 field data to set target abundances for each species, with a minimum of two stems per species per treatment. For species with positive ($n = 4/21$) or negative ($n = 2/21$) trend ($p < 0.20$, OLS regression) between abundance and patch richness, we varied stem number across species richness treatments based on the best-fit regression equation for that species (stem density or % stems = $a + b(\text{richness})$). Each 0.073 m² microcosm (except 0-species treatments) contained a target of 268 stems. Species abundances in our constructed communities followed a log-series-like distribution like that typically observed in a variety of communities including California grasslands near our study site.

After planting, we pulled cylindrical aluminum mesh screens 15 cm. above the rim of each microcosm to simulate shading by standing litter and provide protection against seed loss. We covered the entire array of microcosms with plastic bird netting for two weeks after planting to minimize seed predation. We watered all microcosms with 0.3 l tap water on Nov. 19 during extended hot, dry weather to prevent seedling mortality. Otherwise, communities received only ambient rainfall.

We measured soil moisture approximately every 7 days during peak drydown period, from early April through early August. Our soil moisture measurements integrated from surface to 15, 30, and 45 cm using permanently installed probes and time-domain reflectometry (Topp et al. 1980). Soil drydown occurred faster from our microcosms than from adjacent field plots, but the observed timing of microcosm drydown was within the normal range for the site (Zavaleta et al. 2003). We measured plant-available ammonium and nitrate at 13 cm depth using ion exchange resin bags exchanged bimonthly, extracted in 2 M KCl, and analyzed on an autoanalyzer (Lachat FIA 8000). Bags made of nylon stocking materials and closed with a cable tie each contained 2.5 g oven-dried resin, comprising anion (AG-1 X8, Bio-Rad Laboratories, Hercules,

California, USA) and cation (HCR-W2 H + form, 16–40 mesh, J.T. Baker, Phillipsburg, NJ, USA) resin beads mixed to achieve a 1:1 ratio of exchange capacities. We soaked bags in 5 M NaCl for several hours, rinsed them in DI water, and placed them at the bottom of capped PVC sections 1.27 cm in diameter installed vertically in the soil surface of each microcosm so that bags uniformly contact 1.27 cm² of soil inside the microcosms.

We measured leaching losses of water using plastic collection bottles installed beneath each microcosm and sampled on 2/28, 4/8 and 6/9. Leachate N concentrations were not measured because many microcosms never produced any leachate. We measured % photosynthetically active radiation (PAR) with a Sunfleck ceptometer (SF-80, Delta-T Devices, Ltd.) at the soil surface monthly from April to August, averaging six subcanopy measurements in each microcosm. We took a seventh PAR measurement immediately above the canopy to allow data to be expressed as %PAR reaching the soil surface. Computed values of %PAR over 200% were discarded because they suggested a change in light conditions during measurements. Values between 100% and 200% were recoded as 100%. Discarding these values did not noticeably affect results.

Responses of species composition and yellow starthistle

We weeded down invaded pots to five and four starthistle individuals in January and May 2003, respectively, to maintain equal numbers of invading stems across pots and isolate treatment effects on per-plant invader responses. We estimated aboveground net primary production as end-season aboveground biomass harvested Aug. 14, 2003, sorted to species, oven-dried, and weighed. We measured abundances for all species and per-individual flower production for starthistle from these samples. We allowed natural colonizations into our communities to occur during the course of the experiment. Colonizers increased realized species richness above target levels in all but the zero- and 21-species treatments but contributed little biomass. Realized

richness levels were strongly related to target levels ($R^2_{\text{adj}} = 0.77$, $F = 193$, $p < 0.0001$).

Because we timed our aboveground harvest to capture peak YST biomass, much of the non-YST biomass (including most early-active annuals) had already shattered and senesced. *Avena barbata* and *A. fatua* therefore could not be reliably distinguished and were categorized as “*Avena* spp.,” most individuals of other species were identifiable. We oven-dried all samples at 60°C for at least 72 h and weighed them to the nearest 0.001 g. We counted YST flowers and categorized them as pre-flowering (buds closed), flowering (flower attached, partially or completely open) or post-flowering (flower no longer attached). We term all planted species except starthistle “resident”, and refer to naturally colonizing (non-YST) species as “colonizers”.

Analysis

All statistical analyses were performed using Systat v10.2 (Systat Software Inc, 2002). Response variables were log transformed as needed to satisfy distributional assumptions of the tests used. To test diversity and YST effects on total and forb colonizer biomass, resident biomass, and naturally colonizing (non-YST colonizer) biomass by functional group (grasses, early-active forbs, late-active forbs, N-fixers), we used ANOVA with target species richness and YST presence as categorical variables and an interaction term ($N = 60$). We omitted blocks from all analyses after verifying that no significant block effects occurred. We used Bonferroni corrections to account for multiple tests on non-YST biomass by functional group ($n = 4$ tests; we did not break down other components of biomass by functional group for similar repeated testing). To test the effects of species richness on YST biomass, we used ANOVA with target species richness as the categorical variable ($N = 30$). To test the effect of species richness on the proportion of total biomass made up of forbs in the non-YST communities, we used ANOVA with forb biomass/total biomass as the response variable ($N = 30$). To test the relationship between resident + naturally invading late-active forb biomass and YST biomass, we used simple Pearson correlation ($N = 30$). To test the rela-

tionship between resident late-active forb biomass and YST biomass in 21-species communities, we used simple Pearson correlation ($N = 5$).

To analyze responses of soil moisture to YST and diversity, we used a repeated-measures ANOVA for the 14 measurement dates after the last rain event of the season (April 28) ($N = 60$). We analyzed nitrate and ammonium data using repeated-measures ANOVA on the four measurement dates (January, March, May and August 2003) with species richness and YST as independent variables ($N = 60$). We used repeated measures ANOVA to test richness effects on PAR across the three measurement dates (May, June and July 2003) ($N = 30$). We tested YST effects on PAR with a single t -test of July PAR, omitting the earlier dates when YST plants remained small in stature ($N = 30$).

Results

Community responses

Resident plant production, measured as peak aboveground biomass, increased with diversity across communities with and without YST ($F_{\text{rich}} = 4.842$, $p = 0.001$; $F_{\text{rich} \times \text{YST}} = 1.30$, $p > 0.15$; Fig. 1a). In contrast, biomass of intentionally introduced YST declined with increasing diversity ($F_{\text{rich}} = 19.0$, $p < 0.001$; Fig. 1b). Nearly all locally occurring annual grass species were included as residents in our treatments with diversity > 6 species, so biomass of naturally colonizing grasses necessarily declined from 3- and 6- species treatments to treatments > 6 species. We therefore focus on the biomass response of naturally colonizing forbs rather than all naturally colonizing species (forbs + grasses) to richness. Naturally colonizing forb biomass declined with increasing richness ($F_{\text{rich}} = 2.379$, $p = 0.05$; $F_{\text{rich} \times \text{YST}} = 1.67$, $p > 0.15$; Fig. 1a), but did so less strongly in treatments > 12 species. This overall decline was driven by decreases in the biomass of naturally colonizing late-active forbs; perennial and early-active forbs made up small proportions of overall colonizing forb biomass and responded idiosyncratically to richness treatments (Fig. 2).

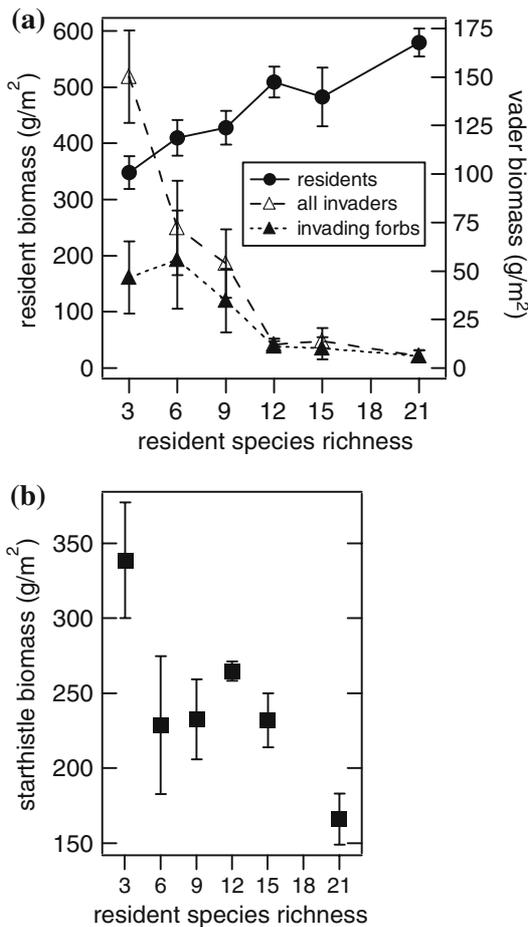


Fig. 1 Effects of ordered species richness changes on (a) aboveground biomass of resident vegetation (closed circles), naturally colonizing forbs (closed triangles), and all naturally colonizing vegetation (open triangles) in communities without introduced yellow starthistle ($N = 30$), and (b) yellow starthistle biomass in intentionally invaded communities ($N = 30$)

As richness increased in communities without yellow starthistle, the proportion of community biomass made up of forbs increased and the proportion made up of grasses declined ($F_{rich} = 3.69, p = 0.013$; Fig. 3). These differences in functional composition appeared to affect intentionally introduced YST biomass. Across richness treatments, biomass of late-active forb species (resident + naturally colonizing) was negatively correlated with intentionally introduced YST biomass (Pearson correlation coefficient = $-0.612, \chi^2 = 12.9, p < 0.001$; Fig. 4a). Late-active forbs were resident only in 12- to 21-species treatments. Within the 21-species

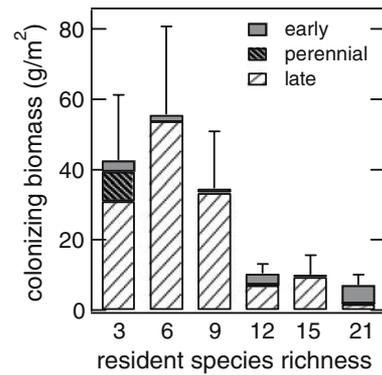


Fig. 2 Aboveground biomass of naturally colonizing forbs in communities without yellow starthistle. Forbs are divided into early-active, late-active and perennial functional groups

replicates, so that the effect of richness treatment is removed, resident late-active forb biomass and YST biomass were also negatively correlated (Pearson correlation coefficient = $-0.932, \chi^2 = 5.08, p = 0.02$; Fig. 4b).

Intentionally introduced YST reduced total biomass of both the resident community ($F_{YST} = 18.2, p < 0.001$) across richness levels and natural colonizers mainly at lower richness levels ($F_{YST} = 7.99, p_{adj} = 0.014; F_{rich \times YST} = 2.85, p = 0.025$). These effects were driven by YST suppression of other late-active forb species ($F_{YST} = 42.9, p_{adj} < 0.001$). YST suppression of resident and naturally colonizing late-

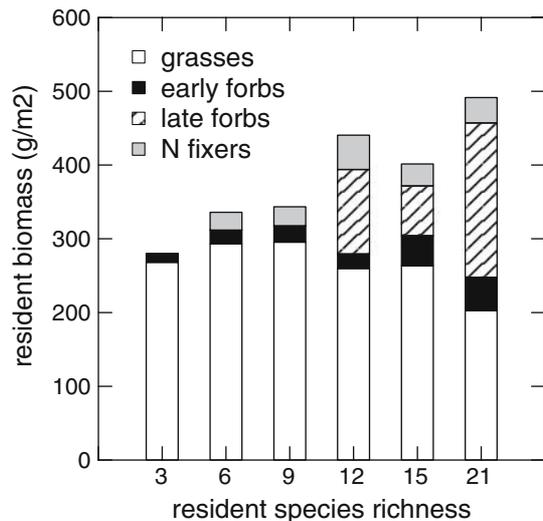


Fig. 3 Functional composition of resident biomass in communities without yellow starthistle (YST) ($n = 30$)

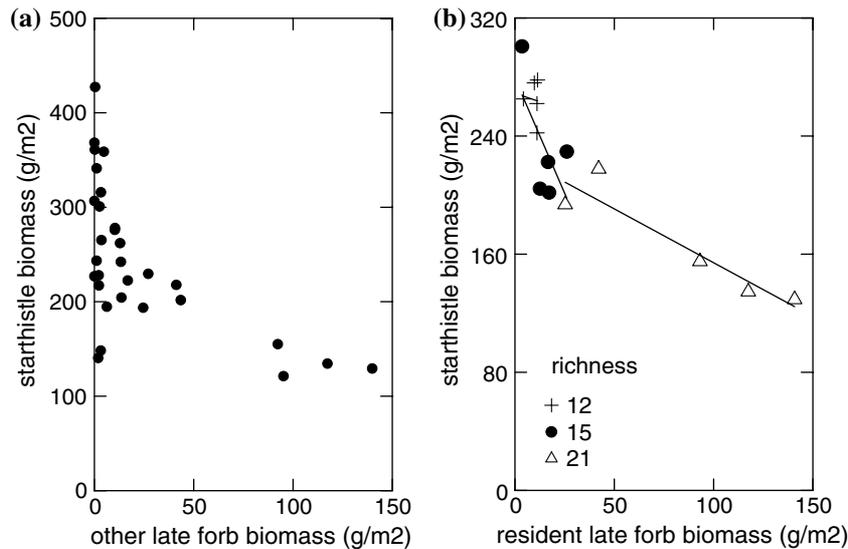


Fig. 4 Effects on intentionally introduced yellow starthistle biomass of (a) resident + naturally colonizing late-active forb biomass in communities planted with three to 21 species, and (b) resident late-active forb biomass in 12- to 21-species communities. Only 12- to 21-species

communities include planted late-active forbs; late forbs in 3- to 9-species communities are all natural colonizers. In (a), Pearson correlation coefficient = -0.612 , $\chi^2=12.9$, $p < 0.001$; for 21-species communities in (b), Pearson correlation coefficient = -0.932 , $\chi^2=5.08$, $p = 0.02$

active forbs increased with increasing richness ($F_{\text{rich}} \times \text{YST} = 25$, $p_{\text{adj}} = 0.05$). YST, a late-active forb itself, had no significant effect on biomass of resident + naturally colonizing grasses, early-active forbs, or N-fixers (all $p_{\text{adj}} > 0.05$).

Resource responses

At the soil surface, %PAR tended to decline with realized species richness across dates and invasion treatments (repeated measures $F_{\text{rich}} = 3.73$, $p = 0.06$). This trend was driven by a richness effect that was significant on June 2 but not on April 24 or July 2 (6/2: $F_{\text{rich}} = 7.36$, $p = 0.01$, $R^2_{\text{adj}} = 0.18$, uninvaded pots only). There was no relationship between light in any month and late forb biomass, across or within YST treatments.

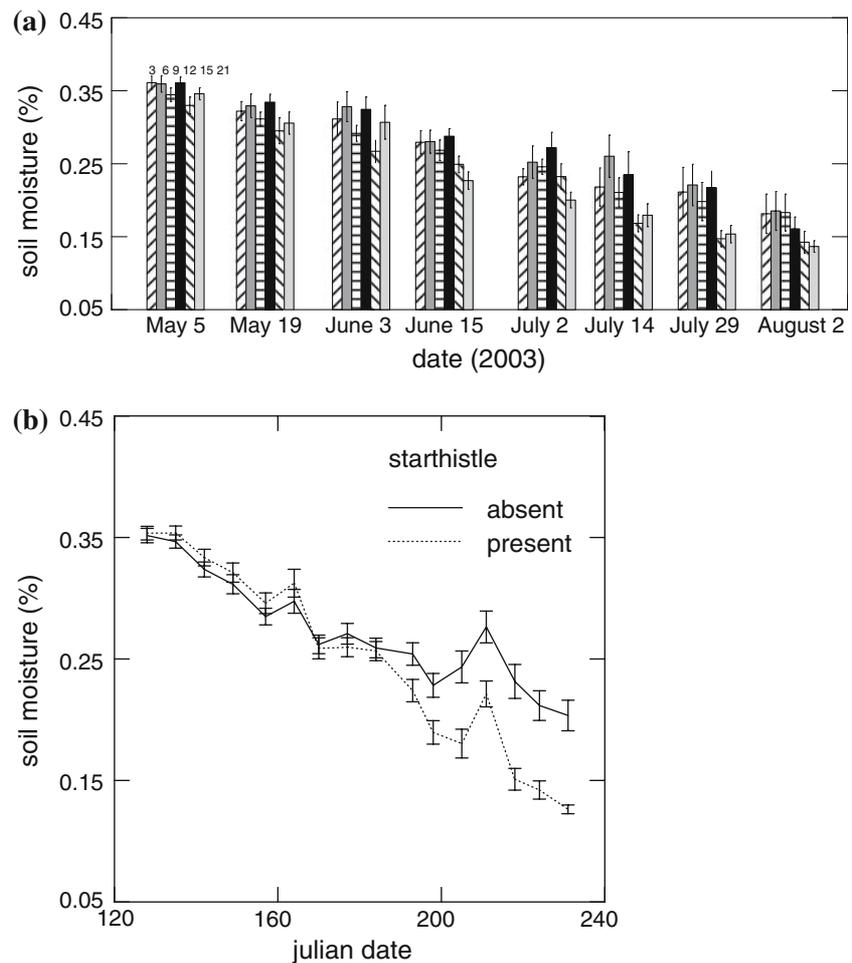
Across richness treatments > 0 species, YST did not affect soil surface %PAR until July, when YST-invaded pots received less PAR ($9.8 \pm 5.2\%$) than non-YST pots ($14.3 \pm 9.1\%$) (t -test, $n = 30$, $t = 2.36$, $p = 0.02$). The degree of YST effect did not depend on species richness in any month. YST effects on soil surface %PAR were much more marked in bare pots in all three months (repeated measures within-subjects

$F = 13.3$, $p < 0.005$), indicating either that YST individuals were markedly less able to intercept PAR in vegetated pots or that YST displaced native canopy biomass rather than added to it. This is consistent with the overall negative effects that YST had on resident biomass (see above).

Soil moisture (0–45 cm) was lower in higher richness treatments after April 28, the date of the last rain event in spring 2003 ($n = 14$ dates, repeated measures $F = 2.79_{\text{rich}}$, $p = 0.03$; Fig. 5a). The richness effect appeared earlier in shallow soil (0–15 cm) then extended to deeper soil (30–45 cm) in mid-July, when only late-active forbs remained green in the communities. A significant interaction term between richness and date ($F = 1.37_{\text{rich}} \times \text{date}$, $p = 0.04$) indicated that the richness effect on soil moisture depletion increased as the dry season progressed. Many pots never yielded any leached water below the soil profile, with large variation in volume among those that did release leachate. We detected no effects of richness on leachate volume.

Soil moisture levels before the last rain of the season on April 28, 2003 were not affected by YST. After April 28, soil moisture declined faster in YST-invaded pots than uninvaded pots ($n = 14$

Fig. 5 Effects on soil moisture of (a) species richness and (b) yellow starthistle invasion after the last rain event of the season on April 28, 2003. Soil moisture is volumetric % integrated from 0–45 cm. depth. In (a), bars within each date from left to right indicate 3-, 6-, 9-, 12-, 15-, and 21-species treatments, respectively. For readability, data are shown for every other measurement date in (a)



dates, $F_{YST} = 5.26$, $p = 0.03$; Fig. 5b). A significant interaction term between date and YST invasion ($F_{YST \times \text{date}} = 14.215$, $p < 0.001$) indicated that the difference between YST and non-YST treatments grew as the dry season progressed. Intermediate (15–30 cm) and deep (30–45 cm) soil moisture responded later than shallow (0–15 cm) soil moisture to YST invasion. The magnitude of YST effects on soil moisture did not vary with species richness.

Plant-available soil nitrate (NO_3) (uninvaded pots only) declined from January to August across richness levels ($F = 287$, $p < 0.001$), while soil ammonium (NH_4) varied among dates but without consistent pattern ($F = 15.2$, $p < 0.001$). There were no richness effects on plant-available nitrate or ammonium across dates ($p > 0.15$). Significant date-by-richness interactions for both nitrate ($F = 2.18$, $p = 0.01$) and

ammonium ($F = 1.80$, $p = 0.04$) reflected greater variation in plant-available N, but without consistent pattern, among richness levels on some dates. We observed no relationship between late forb biomass and soil N in any month, across or within YST treatments. There were no effects of YST on leachate volumes, soil nitrate or ammonium availability ($p > 0.25$).

Discussion

Grassland production, water and light use levels, and resistance to invasion all decreased with non-random, nested declines in species and correlated functional group richness in our study system. These responses occurred despite the low relative abundances of species absent from higher diversity levels, illustrating that uncommon species can

nevertheless exert substantial effects on ecosystem functioning if they possess unique functional characteristics. Previous experimental studies have identified positive diversity effects on production (Hector et al. 1999; Reich et al. 2001; Tilman et al. 2001; Smith and Knapp 2003; van Ruijven and Berendse 2003; Spehn et al. 2005), resource use (Tilman et al. 1996; Reich et al. 2001; Zak et al. 2003), and invasion resistance (Knops et al. 1999; Levine 2000; Kennedy et al. 2002; Fargione et al. 2003; van Ruijven and Berendse 2003) in a variety of ecosystem types. Most, however, manipulated all species at equal abundances, and none varied species diversity based on observations of natural diversity gradients or changes. The results we report indicate that non-random diversity declines can strongly affect ecosystem processes even when the dominant species remain unchanged across diversity levels and the species added and removed are rare.

The few, existing studies that explore effects of rare species removals on grassland processes treat local rarity as a direct surrogate for extinction risk (Smith and Knapp 2003; Lyons and Schwartz 2001). The field observations on which our experimental design was based, however, indicate that local rarity does not necessarily correspond to the order in which species disappeared from grassland neighborhoods. For example, the most abundant species in our 21-species mixtures rarely occurred in the field anywhere other than the most speciose patches (Table 1). Although our treatments were based on observations of variation and change in diversity at small spatial scales and over only a few years, our design provides insight into how actual patterns of variation and change in both species richness and species abundances can affect ecosystem processes. Additional diversity-function experiments that base species removals on observed patterns of variation and change in species richness, composition and abundance could further our understanding of the effects of ongoing biodiversity change in natural ecosystems.

Other studies have pointed to the strong effects of particular functional groups on production and invasion resistance (Tilman et al. 2001; Fargione et al. 2003; Lambers et al. 2004). Our observed responses could also be the result of changes in

functional diversity that accompanied richness declines, with one functional group—late-active forbs—playing a particular role in affecting the invasion response. Because our experimental design was based on observed variation in richness in the field, species and functional diversity covaried, generating a sampling effect (Wardle 2001) in which only high-diversity treatments contained late-active forb species. This complicates the task of separating the effects of species and functional group diversity (Reich et al. 2004). Still, four of our findings support the interpretation that late-active forbs played an important role: (1) the increase in biomass of naturally invading forbs as species diversity declined was driven by increases in the biomass of invading late-active forbs, without consistent responses by species in other functional groups. (2) Invasion by yellow starthistle (YST, a late-active forb) suppressed the biomass of resident and naturally invading late-active forbs, but not of other functional groups. (3) Within and across diversity levels, YST biomass was negatively correlated with the biomass of late-active forbs. (4) Light and soil moisture responses to diversity and to YST addition were most apparent in June and later, after other dominant functional groups had entirely (early grasses, early forbs) or largely (N-fixers) senesced and therefore were no longer contributing to resource use levels.

Zavaleta and Hulvey (2004) found that YST increasingly replaced biomass in communities of higher richness rather than adding to it. We show that this occurred specifically through replacement of resident and naturally colonizing late-active forbs, which were increasingly prevalent at higher diversity levels and subject to being displaced by a functionally similar invader, YST. The suppressive effects of species richness on natural colonizations were also restricted to species functionally similar to those added at higher richness levels—late-active forbs. In other words, the richness effect appears mediated not by a general increase in resource use or resident production but by a particular effect of the species present at higher richness levels on functionally similar colonizers. This mechanism is further supported by a lack of relationship between resident and colonizing biomass within richness

levels (even though resident biomass varies considerably within richness levels); such a relationship exists only across richness levels, with the addition of new species—not just new biomass. An important caveat to this apparent pattern is the possibility that biomass of colonizing stems was underestimated because we could not distinguish between resident and colonizing stems of a single species. The effects of species richness on resource use levels, particularly in summer, point to a possible proximate mechanism for the observed richness effects on production and invasion resistance. The persistence of light and moisture responses into summer months when mainly late-active species remained alive indicates a key role of late-active forbs in producing more complete resource consumption through space and time.

Our findings emphasize the importance of species functional traits and identity in addition to species richness *per se* (Hooper et al. 2005), and we caution against the interpretation that “more”, regardless of provenance, is “better”. Both exotic and native species appeared to contribute to community resistance in our study. This illustrates that communities with established exotics as well as native species can be managed towards the goal of enhancing resistance to further invasions. Co-occurring exotics and natives could have contrasting effects, though, on a range of other desirable ecosystem processes not addressed by our study, such as the maintenance of native soil biota, herbivores, and pollinator communities. We suggest that mixed systems like our California grassland site can provide important insights into aspects of species interactions, community dynamics, and biotic controls over ecosystem processes. However, their condition provides a shifted baseline that from a management perspective is likely neither the most nor the least desirable state (Pauly 1995).

Determining what factors underlie the spatial patterns of species loss that we observed in our grassland study area is beyond the scope of this study. Previous work on determinants of species vulnerability indicates both taxonomic and trait correlates of endangerment and rarity in animals at scales from local to global (Foufopoulos and Ives 1999; del Monte-Luna and Lluch-Belda 2003; Lips et al. 2003; Davies et al. 2004; Sekercioglu

et al. 2004), though plants are poorly studied by comparison. If traits associated with vulnerability are also associated with particular functional contributions to ecosystem processes, it is likely that progressive species losses often entail the early loss of particular functional types and their ecological roles. If species loss patterns and biotic controls over ecosystem processes at larger spatial and longer time scales resemble what we observed in this plot-scale experiment, ongoing species losses and declines could have greater ecosystem consequences than those suggested by randomized-loss experiments. Further research linking species losses, changes in evenness and functional composition, and ecosystem processes would shed light on this critical area.

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