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MULTIPLE MECHANISMS ENABLE INVASIVE SPECIES TO SUPPRESS NATIVE SPECIES[1]

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- *Premise of the study*: Invasive plants represent a significant threat to ecosystem biodiversity. To decrease the impacts of invasive species, a major scientific undertaking of the last few decades has been aimed at understanding the mechanisms that drive invasive plant success. Most studies and theories have focused on a single mechanism for predicting the success of invasive plants and therefore cannot provide insight as to the relative importance of multiple interactions in predicting invasive species' success.
- *Methods*: We examine four mechanisms that potentially contribute to the success of invasive velvetgrass *Holcus lanatus*: direct competition, indirect competition mediated by mammalian herbivores, interference competition via allelopathy, and indirect competition mediated by changes in the soil community. Using a combination of field and greenhouse approaches, we focus on the effects of *H. lanatus* on a common species in California coastal prairies, *Erigeron glaucus*, where the invasion is most intense.
- *Key results*: We found that *H. lanatus* had the strongest effects on *E. glaucus* via direct competition, but it also influenced the soil community in ways that feed back to negatively influence *E. glaucus* and other native species after *H. lanatus* removal.
- *Conclusions*: This approach provided evidence for multiple mechanisms contributing to negative effects of invasive species, and it identified when particular strategies were most likely to be important. These mechanisms can be applied to eradication of *H. lanatus* and conservation of California coastal prairie systems, and they illustrate the utility of an integrated set of experiments for determining the potential mechanisms of invasive species' success.

Key words: California coastal prairie; competition; *Holcus lanatus*; interference competition; invasive species; mammalian herbivory; plant-soil feedbacks..

Understanding the mechanisms that drive invasive plant success has become a major scientific undertaking in the last few decades (Catford et al., 2009). The majority of studies focus on a limited array of mechanisms that include release from native enemies, disturbance, or allelopathy but rarely, if ever, look at their interactions. However, the success of most introduced species and subsequent loss of native species may result from a combination of factors (Weiher, 2007). Understanding these mechanisms will both further our understanding of plant competition and community ecology as well as aid in the control of introduced species and promote the conservation and restoration of native communities.

A great deal of recent research has demonstrated that invasive species can strongly affect soil communities, and these changes can influence coexistence and success of native neighbors. Introduced species have been shown to negatively affect arbuscular mycorrhizal (AM) fungal density, which can generate negative feedback for neighboring native species (reviewed in van der Putten et al., 2007; Pringle et al., 2009). In addition, changes in plant community composition and litter layers associated with invasive plant species are known to change decomposer communities (reviewed in Ehrenfeld, 2003; van der Putten et al., 2007), which in turn can alter soil nutrient cycles (reviewed in Ehrenfeld, 2003). Changes in soil community composition associated with invasive species have been shown to negatively influence the performance of neighboring native species (Gillespie and Allen, 2006; Batten et al., 2008).

Soil feedback effects take place in a broader context that includes other factors likely to influence effects of invasive species on native species, such as disturbance (Richardson et al., 1994; Colautti et al., 2006; Kulmatiski, 2006), nutrient availability (reviewed in Davis et al., 2000), and grazing (reviewed in DiTomaso, 2000). In grassland systems, mammalian herbivory often strongly affects plant communities by removing palatable phenotypes or species (reviewed in Townsend et al., 2003). For example, voles have been shown to prefer grasses and forbs lacking endophyte infection, and endophyte-infected grasses dominate in the presence of herbivores (Clay, 1988; Fortier et al., 2000; Clay and Schardl, 2002). In a California coastal prairie system, reintroduced native elk have been shown to prefer and thereby reduce populations of the invasive velvetgrass *Holcus lanatus* L. (Poaceae) (Johnson and Cushman, 2007). As a result, in grassland and rangeland systems, mammalian herbivores can have strong indirect and direct impacts on abundance of invasive species and native species and can alter the structure of plant communities.

Velvetgrass (*H. lanatus*) has been identified as a serious threat to the California coastal prairie, and it serves as an excellent study system for exploring various mechanisms of plant competition. The California Invasive Plant Council lists *H. lanatus* as a moderate threat with severe local impacts in areas such as coastal prairies (http://www.cal-ipc.org/ip/inventory/weedlist.php?#key).

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For several decades, this Eurasian perennial grass has been forming dense monocultures in these coastal prairies and locally eliminating almost all other plant species (Kotanen, 2004). Seed production by *H. lanatus* in California coastal prairies has been reported to be greater than four times that of the native range (as discussed in Thomsen et al., 2006b), and abiotic factors such as increased spring moisture (Thomsen et al., 2006b) or elevation (Thomsen and D'Antonio, 2007) can enhance *H. lanatus* seedling establishment and competitive success. In addition, introduced perennial grasses (including *H. lanatus*) have been increasing in abundance in California coastal prairies over the last decade, and they outcompete both annual and perennial native plant species (Corbin and D'Antonio, 2010). The increasing proportion of California coastal prairie covered in monocultures of introduced *H. lanatus* highlights our need to understand the variety of mechanisms via which *H. lanatus* eliminates native species from these habitats, and it provides an ideal system for studying the role of plant competition in structuring plant communities. In addition, our study will aid in the conservation of numerous areas of California coastal prairie currently threatened by *H. lanatus* invasion.

The Bodega Marine Reserve (BMR), a University of California reserve located in Bodega Bay, California, USA, where this research was conducted, is an example of a site currently threatened by heavy *H. lanatus* invasion, and we therefore used it to study the effects of *H. lanatus* on native species at BMR. The terrestrial portion of the reserve is dominated by three habitats: salt marsh, sand dune communities, and California coastal prairie. *Holcus lanatus* has been officially recorded on the reserve since 1973, when it had a relatively limited distribution (Barbour et al., 1973). Today, more than 30% of the coastal prairie is covered with dense stands of *H. lanatus*, a product of rapid expansion in the last 10 yr (personal communication, J. Sones, BMR land manager).

Erigeron glaucus Ker Gawl. (Asteraceae) is an abundant native perennial at BMR found in habitats ranging from rocky seaside bluffs to richer areas of the coastal prairie. Clones of *E. glaucus* can live for decades (R. Karban, S. Y. Strauss, unpublished data) but are vulnerable to *H. lanatus* invasion in less rocky habitats. For example, 40 naturally occurring clones were observed by S. Y. Strauss for more than a decade. Before the invasion of *H. lanatus* into these areas, only one clone had died (from valley pocket gopher herbivory). Since 2003, more than 20 *E. glaucus* clones have been overgrown by *H. lanatus*, a process that results in eventual death of *E. glaucus* clones. As a result, *E. glaucus* is a model native species to use in examining the competitive effects of *H. lanatus* on neighboring native species.

The *H. lanatus* invasion at BMR also occurs within the context of extensive mammalian herbivory and gopher disturbance. The reserve contains a wide range of small mammalian herbivores, including the very abundant valley pocket gopher (*Thomomys bottae*) and three common, large mammalian herbivores: black-tailed jackrabbit (*Lepus californicus*), brush rabbit (*Sylvilagus bachmani*), and black-tailed deer (*Odocoileus hemionus columbianus*) (Bodega Marine Reserve Species Inventory:Mammals; http://www-bml.ucdavis.edu/bmr/Mammal_List.PDF). Exclosure studies have demonstrated that these mammalian herbivores can have large impacts on vegetation and community composition at BMR (Maron and Simms, 1997, 2001; Warner and Cushman, 2002).

Previous research and observations at BMR and other California coastal prairies have identified some potential mechanisms via which *H. lanatus* could hinder its native neighbors:

1. Direct competition. The dense growth (Thomsen et al., 2006a; Corbin and D'Antonio, 2010), great propagule pressure (Thomsen et al., 2006b), and thick litter layer (Bastow et al., 2008) associated with *H. lanatus* stands could inhibit germination and establishment of native species.

2. Changes in soil community abundance and diversity. Previous research conducted at BMR comparing soil beneath the remaining native flora and soil beneath *H. lanatus* has revealed an increase in the bacteria to fungi ratio, available nitrogen (Muir, 2009), and litter layer (Bastow et al., 2008) and a decrease in the number of macroinvertebrate detritivores (Bastow et al., 2008) and number and diversity of arbuscular mycorrhizal fungi (A.E.B., unpublished data). These changes in the soil community could alter plant–soil feedbacks and the success of native flora.

3. Indirect competition via herbivore effects. If the current herbivore assemblage prefers native plants, an increase in herbivore pressure on native plants could occur in areas where *H. lanatus* is abundant. In addition, native plant species occupy a decreasing area at BMR, which could increase herbivore pressure within the remaining native habitat. Deer are more frequently observed grazing within uninvaded than invaded areas, though deer are not observed avoiding *H. lanatus* stands (A.E.B., S.Y.S., and J. Sones, personal observations). *Holcus lanatus* has been shown to be more abundant in sites without cattle grazing (Hayes and Holl, 2003), and reintroduced elk also have previously been shown to limit *H. lanatus* populations (Johnson and Cushman, 2007). However, no cattle grazing occurs at BMR, and elk have not yet been reintroduced there.

4. Interference competition via allelopathy. Mild allelopathic effects of *H. lanatus* on seed germination and plant growth and establishment have been reported in *H. lanatus* populations in New Zealand (Wardle et al., 1992) and the United Kingdom (Newman and Rovira, 1975; Gilliland and Hayes, 1982). If allelopathic effects are active in the *H. lanatus* population at BMR, they could be suppressing germination and establishment of native plants.

In this paper we explore how these multiple factors (direct competition, mammalian herbivory, and soil-mediated effects and feedbacks) influence the impact of *H. lanatus* on a common, native perennial seaside daisy (*Erigeron glaucus*) using greenhouse and field experiments. We tested whether soils from beneath *H. lanatus*, native plant communities, and sites restored with the native grass *Calamagrostis nutkaensis* influenced *E. glaucus* germination, establishment, and growth in the greenhouse and the field. Using experimental exclosures, we also tested for effects of mammalian herbivory, direct competition from *H. lanatus*, and their interaction on *E. glaucus* germination and establishment in the field.

MATERIALS AND METHODS

Both the seed (from *H. lanatus* and *E. glaucus*) and soil for the greenhouse experiments described later were collected from the populations at BMR, and all field experiments were conducted in the invaded and uninvaded portions of the coastal prairie located at the reserve.

Greenhouse Experiment 1: growth response to different soil communities—To test whether *E. glaucus* responds differently to the various soil communities at BMR and whether this difference is related to AM fungi or other soil microbes, we conducted a greenhouse experiment in which *E. glaucus* was grown with a pure AM fungal culture or with soil collected from within the *H. lanatus* invasion or the uninvaded coastal prairie.

Seeds of *E. glaucus* and *H. lanatus* were allowed to germinate on a mist bench in sterile potting soil. Soil was collected from across the invaded and uninvaded coastal prairie, homogenized within each site, and transported from BMR to the greenhouses at University of California, Davis. To control for differences in nutrients between the two soil types, a background soil was created from both invaded and uninvaded sites by combining both soils, mixing it 1 : 1 with sand, and steam sterilizing it.

We filled 600-mL Deepots (Stuewe & Sons, Tangent, Oregon, USA) with a combination of the steam-sterilized soil mixture and inocula created from either the invaded or uninvaded site. Six inocula treatments were used: (1) an unmodified live invaded soil, (2) an unmodified live uninvaded soil, (3) a commercial AM fungal inocula containing *Glomus intraradices*, *G. etunicatum*, and *G. mosseae* (Mycorrhizal Applications, Grants Pass, Oregon, USA), (4) a microbial wash extracted from invaded soil, (5) a microbial wash extracted from uninvaded soil, and (6) a sterilized mixture of both soil types. Microbial washes were extracted from 100 mL of live soil from each site by passing the soil through a 25-mm sieve and a 40-μm sieve to remove AM fungal spores, followed by vacuum filtration through Whatman filter paper (Whatman, Maidstone, UK) to remove AM fungal hyphae (Ames et al., 1987). With the exception of the microbial wash, 100 mL of inoculum was added to each pot, and all inocula were mixed with the sterile background soil in the center of the pot to ensure maximum contact with plant roots. After the creation of the microbial wash, the filtrate containing the microbial community (the microbial wash) was added to pots containing a mixture of sterilized soil types by pouring 50 mL of the filtrate into each pot within the microbial wash treatment.

Ten 2-week-old seedlings of both *H. lanatus* and *E. glaucus* then were transplanted singly into pots within each soil treatment, producing a total of 120 pots. Plants were randomly assigned into four blocks and allowed to grow for 5 mo (the average length of the growing season) before aboveground and belowground biomass were harvested. The vast majority of plants in the experiment were not root bound. Roots were dried, weighed, rehydrated, and stained with trypan blue and mycorrhizal infection was assessed with the grid-intersect method (McGonigle et al., 1990).

Results were analyzed with the Proc GLM procedure of SAS 9.1 (SAS Institute, Cary, North Carolina, USA) in which log-transformed biomass and arcsin square-root transformed percent root colonization (to meet the normality assumptions of the analysis) were tested across the independent variables of block and soil treatment. Root biomass was used as a covariate in the analysis of percent root colonization to control for differences in plant size between treatments and plant hosts. A priori contrasts were conducted to compare between soils containing and lacking AM fungi and between soils from different sites.

Greenhouse experiment 2:* E. glaucus *germination in different soil communities—Several soil factors could limit *E. glaucus* germination, including *H. lanatus*–induced abiotic changes, changes in soil microbial communities (biota), or possibly allelopathic compounds in seeds or soil. To distinguish between abiotic and biotic factors influencing germination (such as, but not limited to, differences in nitrogen availability [Muir, 2009]), we grew *E. glaucus* seeds in both sterile and live soil. To test whether *H. lanatus* seeds are the source of allelopathic compounds that could hinder germination (as in Wardle et al., 1992), we added and removed *H. lanatus* seed. To determine whether biota or allelopathic effects are influenced by the soil in which they are located, we conducted all these comparisons in a background soil from either the invaded or uninvaded environment.

Thus we allowed *E. glaucus* seeds to germinate in 16 different soil environments. These soil environments consisted of two sterilized background soils (sterile *H. lanatus* invaded soil or sterile uninvaded soil) inoculated with one of two inocula (*H. lanatus* invaded soil or uninvaded soil) that have been manipulated in one of four ways (unmodified, sterilized, *H. lanatus* seed added, or all seeds removed by hand). Thus we germinated *E. glaucus* in two sterile soils inoculated with two soils that were manipulated in one of four ways, producing a total of 16 treatments ($2 \times 2 \times 4 = 16$).

Soil inocula modifications were created in the following ways: (1) Unmodified treatments contained live field soil. (2) Sterilized treatments (and background soils) were sterilized via two rounds of steam sterilization. (3) *Holcus lanatus* seed was added to both live invaded and uninvaded soil. Invaded soil naturally contained 4.5 mL of *H. lanatus* seed per liter of soil, and so 9 mL of *H. lanatus* seed per liter was added to the seed addition treatments to ensure effects of seed addition would be detected. (4) To determine whether germinating *H. lanatus* seeds negatively influenced *E. glaucus* germination, we removed *H. lanatus* (and all other) seeds from soil by hand. We mixed 100 mL of a given soil inoculum with 500 mL of sterile background soil and added it to 600-mL trays.

Eleven *E. glaucus* seeds were placed in trays filled with each soil environment, and each soil environment was replicated at the tray level 10 times, for a total of 200 trays and 2200 *E. glaucus* seeds. *Erigeron glaucus* seeds were glued (using Elmer's glue [Elmer's Products, Inc., Columbus, Ohio, USA], which dissolves in water) 1 cm from the tip of a toothpick, and 11 toothpicks were placed in each tray. Toothpicks have previously been used in a field study to quickly identify seedlings germinated from experimentally introduced seeds (Baack et al., 2006; Leger et al., 2009; Strauss et al., 2009), and they were used here to enable us to quickly distinguish between germinated seeds introduced experimentally and seedlings germinated from the seed bank. Toothpicks were placed so that seeds were barely below the soil surface. Treatments were divided evenly between five blocks, and trays were randomly assigned within each block. Trays were weeded regularly to eliminate nonexperimental germinants (recently germinated seeds) from the seed bank within all the live inocula treatments, and the number of germinants per tray was recorded after 1 mo.

Results were analyzed with the Proc GLM procedure of SAS (SAS Institute, 2002), in which the arcsin square-root transformed percent of germination (per tray) was tested across the full model, including the independent variables of block, background soil (invaded or uninvaded), soil inocula (invaded or uninvaded), soil inocula treatment (unmodified, sterilized, seed added, and seed removed), and the two- and three-way interactions among all terms except block.

Field experiment 1:* E. glaucus *germination in different environments—Although greenhouse experiment 2 enabled us to evaluate the effect of several soil communities on the germination of *E. glaucus* in a controlled setting, we also wanted to determine the factors that influence germination and establishment under field conditions. A previous study demonstrated that cryptic early herbivory can obscure germination success by removing germinants within days of germination, thus preventing detection of germination if field plots are not sampled at least every 2 d (Strauss et al., 2009). Dense vegetation or litter (like that produced by *H. lanatus*) from competitors may signal seeds to remain dormant or may increase attack by pathogens or herbivores, factors that also may reduce germination. To determine whether soil factors, competition, or larger mammalian herbivory influenced the germination of seeds in the field in invaded and uninvaded sites, *E. glaucus* seeds glued to skewer sticks were planted in 15 × 13 cm plots of 20 seeds across three areas: invaded, uninvaded, and a "restoration area." in which *H. lanatus* had been removed and *Calamagrostis nutkaensis* had been planted as an experimental restoration strategy (Thomsen and D'Antonio, 2007). To alter competition, plots were either weeded to remove all but the focal plants throughout the experiment or were undisturbed, and to vary grazer impacts, plots were either caged with chicken wire or left open. Each of these four treatments (two herbivory treatments × two competition treatments) was replicated 5 times within each soil area (invaded, uninvaded, or restored). The seeds were planted in December, when germination is occurring in the coastal prairie system, and elevation of all plots was recorded.

Chicken-wire cages 18 × 15 cm and 15 cm tall were placed over caged treatments. Cages were designed to protect plants from large mammalian herbivory, but very small mammals and insects could still access the plots.

Seeds were planted by gluing seeds 3 cm below the tip of a wooden skewer stick (like those used to create kabobs) and inserting skewers in a hexagonal array within a 15 × 13 cm planting grid that ensured each seed was equidistant (5 cm) from every other seed. Skewers were placed so that seeds were barely below the soil surface. Skewers were used as "large toothpicks" to aid in the identification of germinated seeds in plots that often contained tall, dense vegetation. The grid included additional skewers with seeds inserted following the same pattern surrounding the monitored seeds to control for edge effects.

Plots were monitored daily for 1 mo (December) during peak germination and monthly for an additional 8 mo (January through August) to record late germinants.

Results were analyzed with the Proc glm procedure of SAS (SAS Institute, 2002), in which the arcsin square-root transformed percent of germination (per plot) was tested across the independent variables of site (invaded, uninvaded, and restored), competition (weeded or unweeded), and herbivory (caged or uncaged) and the interactions among these variables. An effect size analysis was conducted to determine which factors influenced germination most strongly.

Field experiment 2:* E. glaucus *seedling establishment in different environments—To determine whether soil factors, competition, or larger mammalian herbivory influenced the establishment of seedlings in invaded, uninvaded, and restored sites, 5-wk-old *E. glaucus* seedlings in sterile potting media were planted in 30 × 25 cm plots of 20 seedlings across the invaded, uninvaded, and

TABLE 1. ANOVA analyses of *Erigeron glaucus* and *Holcus lanatus* biomass (log transformed) and proportion of root length colonized by arbuscular mycorrhizal (AM) fungi (arcsin square-root transformed).

	E. glaucus biomass			*E. glaucus* AMF			*H. lanatus* biomass			*H. lanatus* AMF		
	df	F	*P*	df	F	*P*	df	F	*P*	df	F	*P*
Block	1	6.87	**0.0124**	1	1.79	0.1892	1	0.98	0.3270	1	0.32	0.5728
Inoculum treatment	5	20.45	**<0.0001**	5	136.80	**<0.0001**	5	1.79	0.1363	5	92.43	**<0.0001**
Non-AMF vs. AMF	1	97.28	**<0.0001**	1	660.84	**<0.0001**	1	0.15	0.7007	1	330.58	**<0.0001**
Invaded + AMF vs. noninvaded	1	5.43	**0.0250**	1	0.14	0.7073	1	0.03	0.8666	1	0.94	0.3384
Noninvaded + AMF vs. invaded	1	1.78	0.1901	1	5.38	**0.0257**	1	5.49	**0.0242**	1	87.35	**<0.0001**
Error	39			39			40			40		

Note: Contrasts are indented and italicized below the variable in which they are contained. AMF = commercial three-species AM fungal mixture.

restored areas. To alter competition, plots were either weeded to remove all but the focal plants throughout the experiment or were unweeded, and to vary herbivory, plots were either caged with chicken wire or left open. Each of these four treatments (plots) was replicated 3 times within each soil site (invaded, uninvaded, or restored). The seedlings were planted in December, when germination is occurring in the coastal prairie system, and elevation of all plots was recorded.

Seedlings were planted in a manner similar to that used for the seeds in field experiment 1. Seedlings were planted within a 30 × 25 cm planting grid equidistant (10 cm) from every other seedling. Additional seedlings were planted surrounding monitored seeds to control for edge effects. Chicken-wire cages that were larger but otherwise identical to those in field experiment 1 (33 × 28 cm and 15 cm tall) were placed over caged treatments. Plots were monitored daily for 1 mo (December) and monthly for an additional 8 mo (January through August).

Erigeron glaucus is perennial and does not flower during the first year. Thus we used the number of leaves and the total leaf area as a metric of performance. This protocol is supported by greenhouse experiment 1, in which the log of *E. glaucus* leaf number and the log of total *E. glaucus* biomass at the end of the experiment were highly correlated ($r = 0.85$, df = 44, $P < 0.0001$). At the end of 8 mo, the number of leaves and the length and width of leaves were measured

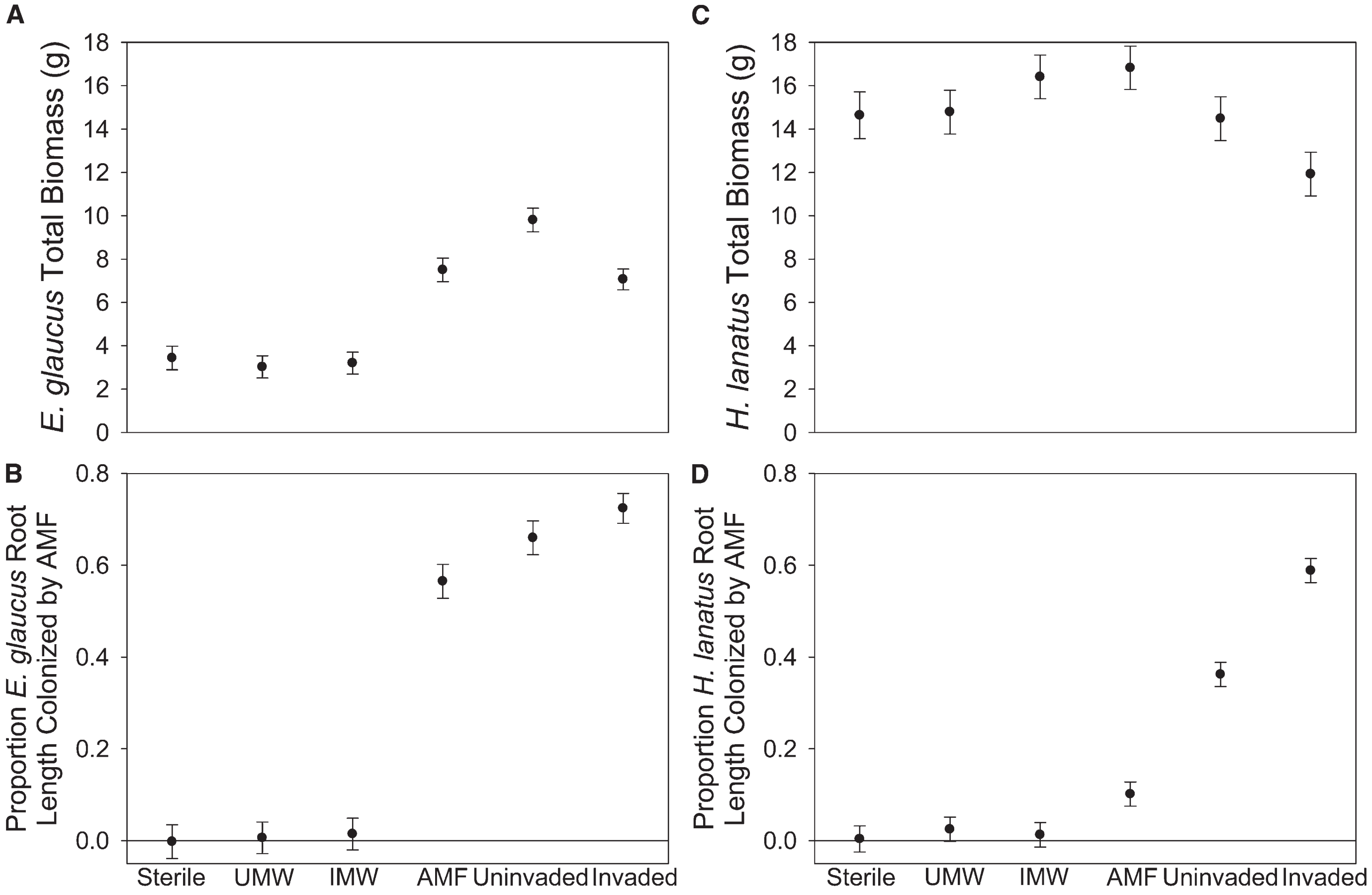

Fig. 1. Biomass of (A) *Erigeron glaucus* and (C) *Holcus lanatus* plants and proportion of (B) *E. glaucus* and (D) *H. lanatus* root length colonized by arbuscular mycorrhizal (AM) fungi of plants grown with six different soil communities. "Sterile" soil is the sterilized mixture of invaded and uninvaded soils, "Invaded Microbe Wash" (IMW) refers to the microbial wash created from invaded soil, "Uninvaded Microbe Wash" (UMW) refers to the microbial wash created from uninvaded soil, "AMF" refers to the commercial three-species AM fungal mixture, "Invaded" refers to whole soil collected from the invaded site, and "Uninvaded" refers to whole soil collected from the uninvaded site. All pots contained a background soil consisting of sterilized mixture of Invaded and Uninvaded soil to which the above six inocula were added. Error bars represent one standard error.

on all surviving seedlings. Total leaf area for each of the plants was calculated from these measurements by using the assumption that *E. glaucus* leaves are relatively oval in shape and applying the formula for oval area: L × W × 0.8.

Plot 10 within the invaded site was eliminated from the analysis because although this plot did not look different from other plots at the time of set-up, differences between this site and the other sites became apparent later in the season. Primarily, the behavior of both *H. lanatus* and the *E. glaucus* seedlings in the plot was dissimilar to other plots. Extremely vigorous growth of *E. glaucus* occurred at this site, which was confirmed by the Grubbs' test for outliers (Sokal and Rohlf, 1995) on the total leaf area of plants in each plot ($\text{Value}_{\text{Plot 10}} = 5.80$ greater than $\alpha_{0.005} = 3.33$, n = 36). Plot 10 also appeared to be an unsuitable habitat for *H. lanatus* persistence. The plot was located at the top of a ridge near the edge of the *H. lanatus* invasion, had virtually no litter layer, and after the initial weeding, *H. lanatus* never re-established in the plot, and no maintenance weeding was needed. This difference may have been due to drier soils within the plot, which have previously been shown to limit *H. lanatus* (Thomsen and D'Antonio, 2007). This area may represent a "refuge" habitat in which *H. lanatus* does poorly and could be further studied in the future to identify the characteristics that suppress *H. lanatus* persistence.

Seedling survival for the remaining plots was analyzed with a repeated-measures analysis within the Proc glm procedure of SAS (SAS Institute, 2002), in which the arcsin square-root transformed proportion survived (per plot) was tested across the independent variables of site (invaded, uninvaded, and restored), competition (weeded or unweeded), and herbivory (caged or uncaged) and the interactions among these variables and elevation for every month for which a census was conducted. Although previous studies have demonstrated that elevation plays a role in *H. lanatus* competitive effects (Thomsen and D'Antonio, 2007), elevation did not explain any variation in the model and was removed from further analyses. Results of Mauchly's sphericity test ($\chi^2 = 209.496$, $P < 0.0001$, df = 35) were significant, suggesting that the sphericity assumption for a repeated-measures analysis was not met, and *P* values would need to be adjusted. There was also a high Huynh-Feldt epsilon value (0.6401), so *P* values were adjusted by using the Huynh-Feldt method. Total number of leaves of plants surviving to the end of the experiment was also analyzed using Proc glm within SAS on the log-transformed number of leaves by using the above model. Because of the limited number of surviving plants, the three-way interaction and the insignificant interaction between site and competition were not included in the model.

Total leaf area collected from the majority of plants surviving to the end of the experiment also was analyzed with Proc glm within SAS (SAS Institute, 2002) on the log-transformed calculation of total leaf area. Independent variables included site, competition, herbivory, and the interactions among these variables, and effect sizes were calculated for each term. Because of the limited number of surviving plants (particularly in unweeded plots), the three-way interaction and the insignificant interaction between site and competition were not included in the model.

RESULTS

Greenhouse experiment 1: growth response to different soil communities—*Erigeron glaucus* growth strongly depended on inoculum treatment and particularly on the presence of AM fungi (Table 1; Fig. 1A). No significant difference existed between soils lacking AM fungi (sterile, invaded microbial wash, or uninvaded microbial wash). However, the biomass of *E. glaucus* plants doubled in the presence of AM fungi, and biomass of *E. glaucus* with uninvaded inocula was significantly greater than the biomass of *E. glaucus* associated with the commercial AM fungal inocula and invaded inocula (Table 1; Fig. 1). We found no AM fungal colonization in the sterile, invaded microbial wash, and uninvaded microbial wash treatments, and colonization by AM fungi from the whole invaded soil was greater than colonization by the commercial AM fungal inocula and the whole uninvaded soil inoculum (Table 1; Fig. 1B).

In contrast to *E. glaucus*, *H. lanatus* biomass did not differ between the various soil treatments (Table 1; Fig. 1C). Again, no AM fungal colonization occurred in sterile, invaded microbial wash, and uninvaded microbial wash; however, significantly greater AM fungal root colonization occurred in *H. lanatus* roots from invaded soil compared with uninvaded and commercial AM fungal inocula results (Table 1; Fig. 1D). Unlike *E.*

TABLE 2. ANOVA analyses of the arcsin square-root transformed values of the proportion of *Erigeron glaucus* germination in invaded and uninvaded soils.

	df	F	*P*
Block	4	2.55	**0.0416**
Inocula	1	15.12	**0.0002**
Treatment	3	9.41	**<0.0001**
Background	1	5.41	**0.0215**
Inocula × background	1	1.26	0.2629
Inocula × treatment	3	3.41	0.8651
Background × treatment	3	2.02	0.1140
Inocula × background × treatment	3	0.49	0.6915
Error	140		

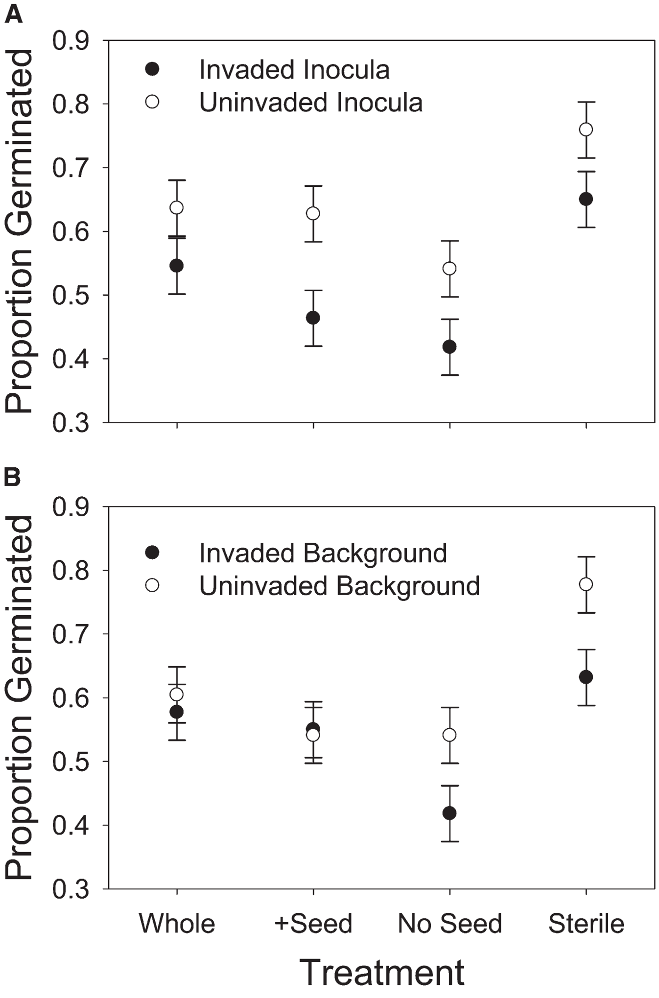

Fig. 2. The proportion of *Erigeron glaucus* seeds that germinated among the different soil treatments in (A) invaded or uninvaded inocula and (B) invaded or uninvaded background soil. Filled circles represent invaded (A) inocula or (B) background soil, whereas open circles represent uninvaded (A) inocula or (B) background soil. Background soils consist of (1) sterile *Holcus lanatus* invaded soil or (2) sterile uninvaded soil. Background soils were inoculated with one of two inocula: (1) live *H. lanatus* invaded soil or (2) live uninvaded soil. Error bars represent one standard error.

glaucus, *H. lanatus* root colonization patterns did not correspond to plant growth.

Greenhouse experiment 2:* E. glaucus *germination in different soil communities*—Erigeron glaucus* seed germination was always greater in uninvaded soil (Table 2, Fig. 2A). This was true regardless of whether live inocula (Fig. 2a) or sterile background soil (Fig. 2B) was examined. Germination was greater in sterilized soil than unmodified (live) soil treatments (Table 2; Fig. 2). There were no significant interactions, and neither seed removal nor seed addition had an impact on germination.

Field experiment 1:* E. glaucus *germination in different environments—Whereas the greenhouse trial suggested that *E. glaucus* preferred to germinate in uninvaded soil, in the field, *E. glaucus* seed germination was greatest in the weeded (no competition) plots but did not differ between sites (Table 3, Fig. 3). Competition explained 42% of the variation in germination, whereas the interaction between competition and site explained 11% and site alone explained 6% of the variation in germination. A trend was seen in the site by competition interaction, suggesting that weeding may provide less benefit in uninvaded areas than in invaded and restored areas (Table 3; Fig. 3).

Field experiment 2:* E. glaucus *seedling establishment in different environments—Over the course of the 8-mo observational period, effects varied by month, the interaction between month and site, and the interaction between month and weeding (competition) (Table 3, Fig. 4). Weeding (competition) was a significant predictor of survival beginning in February (Table 3, Fig. 4B) and explained 22–41% of the variation in establishment. During April through June, survival tended to be higher in restored sites (Fig. 4A), which often had bare patches (and thus less competition). Between April and June, site explained 26–44% of the variation in establishment (Fig. 4A). At the end of the experiment, reduced competition (weeding) and protection from mammalian herbivory (cage) resulted in bigger plants (as determined by leaf number), whereas total leaf area of seedlings was increased in sites with increased competition (plants in invaded and uninvaded sites were larger than plants in the restored site) (Table 3; Fig. 4C). Competition had the greatest influence on plant size (leaf number), explaining 24% of the variation, whereas mammalian herbivory explained 9% and site explained 2% of the remaining variation. Thus, all three factors (competition, mammalian herbivory, and site) influenced *E. glaucus* establishment and growth in the field to varying degrees.

DISCUSSION

These results clearly demonstrate that *H. lanatus* is hindering *E. glaucus* germination, growth, and establishment, resulting in alterations of plant community structure. In the field, the direct competitive effects of *H. lanatus* severely limited *E. glaucus* germination and establishment as evidenced by the increased number of germinants 815% in weeded relative to unweeded plots in the invaded area vs. the other sites. Direct competition was clearly a major factor in both field experiments. The competitive ability of *H. lanatus* is likely derived from its rapid, dense growth aboveground and belowground (Thomsen et al., 2006a) as well as the dense litter layer (Bastow et al., 2008) and high propagule pressure (Thomsen et al., 2006b) associated with *H. lanatus* stands.

Direct competitive effects are likely the strongest factor eliminating *E. glaucus* in the field; however, overall herbivore pressure and indirect effects such as soil-mediated effects, also played a role in seed germination and establishment. Plant size was reduced 71% by the presence of mammalian herbivory, and total leaf area depended on site or soil source. Plants in the invaded and uninvaded sites were 84% and 74% larger, respectively, than plants in the restored site, though no significant difference existed between plants in the invaded and uninvaded sites. As a result, survival was primarily a function of direct competition, whereas growth rate was more dependent on mammalian herbivores and soil source.

Mammalian herbivory decreased plant size across all sites, but no interaction was found between mammalian herbivory and site in either the germination or establishment experiments. The lack of an interaction between herbivory and site suggests that mammalian herbivore pressure was equal across sites regardless of invasion status. We had hypothesized that there might be an indirect effect of invasion through mammalian herbivory due to increased herbivore pressure in the shrinking uninvaded habitat (as *H. lanatus* is likely unpalatable to local herbivores). This hypothesis was not supported by our results. These results suggest that the mammalian herbivory at BML

Table 3. ANOVA analyses of *Erigeron glaucus* germination (arcsin square-root transformed) from field experiment 1 and repeated-measures ANOVA analyses of survival and ANOVA analyses of leaf number and total leaf area from field experiment 2.

	Germination			Survival			Leaf number			Leaf area		
	df	F	*P*	df	F	*P*	df	F	*P*	df	F	*P*
Time				8	128.23	**<0.0001**						
Site	2	1.68	0.1971	16	5.13	**<0.0001**	2	0.48	0.6206	2	5.43	**0.0092**
Weed	1	36.12	**<0.0001**	8	2.38	**0.0417**	1	15.52	**0.0004**	1	64.40	**<0.0001**
Cage	1	0.05	0.8184	8	0.63	0.6806	1	5.55	**0.0246**	1	0.15	0.7028
Site × weed	2	3.08	**0.0550**	16	0.50	0.8937						
Site × cage	2	0.85	0.4330	16	1.60	0.1141	1	0.00	0.9908	1	0.13	0.7195
Weed × cage	1	0.02	0.8858	8	0.83	0.5326	1	1.17	0.2881	1	0.39	0.5367
Site × weed × cage	2	0.15	0.8615	16	1.23	0.2637						
Error	49			184			33			33		

Note: Time = month since planting; site = plots located within the invaded, uninvaded, or restored areas; weed = plots in which competition was reduced by weeding; cage = plots that were caged to reduce mammalian herbivory. *P* values for the repeated-measures analysis of survival were adjusted using the Huynh-Feldt method.

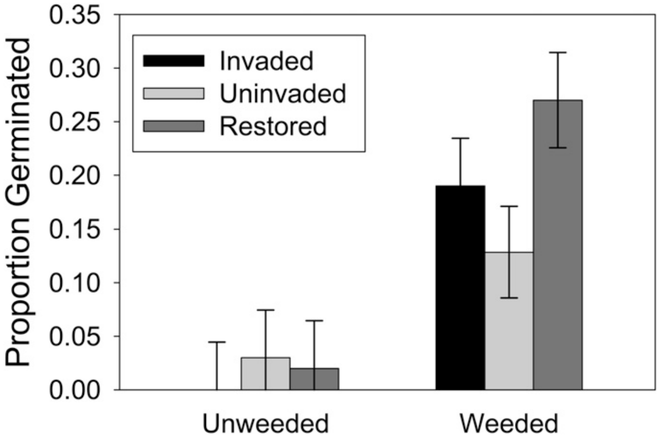

Fig. 3. The proportion of *Erigeron glaucus* seeds that germinated in the field within the different sites and competition treatments. Solid black bars represent seeds that germinated in the invaded area, light-gray bars represent seeds that germinated in the uninvaded area, and medium-gray bars represent seeds that germinated in the restored area.

(deer and rabbit herbivory) differs from mammalian herbivory of *H. lanatus* at previously studied sites (cattle and elk herbivory), which appears to limit *H. lanatus* (Hayes and Holl, 2003; Johnson and Cushman, 2007). This may be due to the stronger trampling effect of larger mammalian herbivores such as cattle and elk (Thompson and Turkington, 1988). As a result, although mammalian herbivory has been shown to favor native species (Hayes and Holl, 2003; Johnson and Cushman, 2007) such as *E. glaucus* at other sites, mammalian herbivory does not appear to favor native species at BMR.

While soil community effects do not seem to play a large role in the outcome of current *H. lanatus* and *E. glaucus* interactions in the field, soil effects are likely to play a large role after the removal of *H. lanatus*. Several studies have addressed the negative effects of the influence of introduced species on soil communities and the strong negative feedback on neighboring native species in those communities (e.g., Gillespie and Allen, 2006; Vogelsang and Bever, 2009). However, most of these studies have been conducted in greenhouse settings, and very few have tested the relative strength of competition and soil-mediated effects in the field.

Our field tests identified the presence of negative soil feedback on native plants by *H. lanatus* but also demonstrated that these negative soil effects are likely to be swamped out by *H. lanatus* direct competition, since weeding had the greatest impact on *E. glaucus* performance. Instead, the strong negative soil-mediated effects are likely to be most important after *H. lanatus* removal, when direct competitive effects are eliminated. *Holcus lanatus* alters the AM fungal community in a way that reduces the benefit of association with AM fungi for *E. glaucus* but not for *H. lanatus* itself. This change in AM fungal community is likely to persist after *H. lanatus* removal and to hinder establishment of *E. glaucus*. Invasive species–induced changes in soils are often long lived and have been shown to leave a legacy for future plants growing in that habitat (reviewed in Corbin and D'Antonio, 2004; Vogelsang and Bever, 2009).

Both germination and growth of *E. glaucus* were inhibited in soil that previously hosted *H. lanatus*. As a result, *H. lanatus* is dealing *E. glaucus* and the native species in the California coastal prairie a double blow: reduced establishment now through direct competitive effects and reduced future establishment mediated by abiotic and biotic changes in the soil caused by *H. lanatus*.

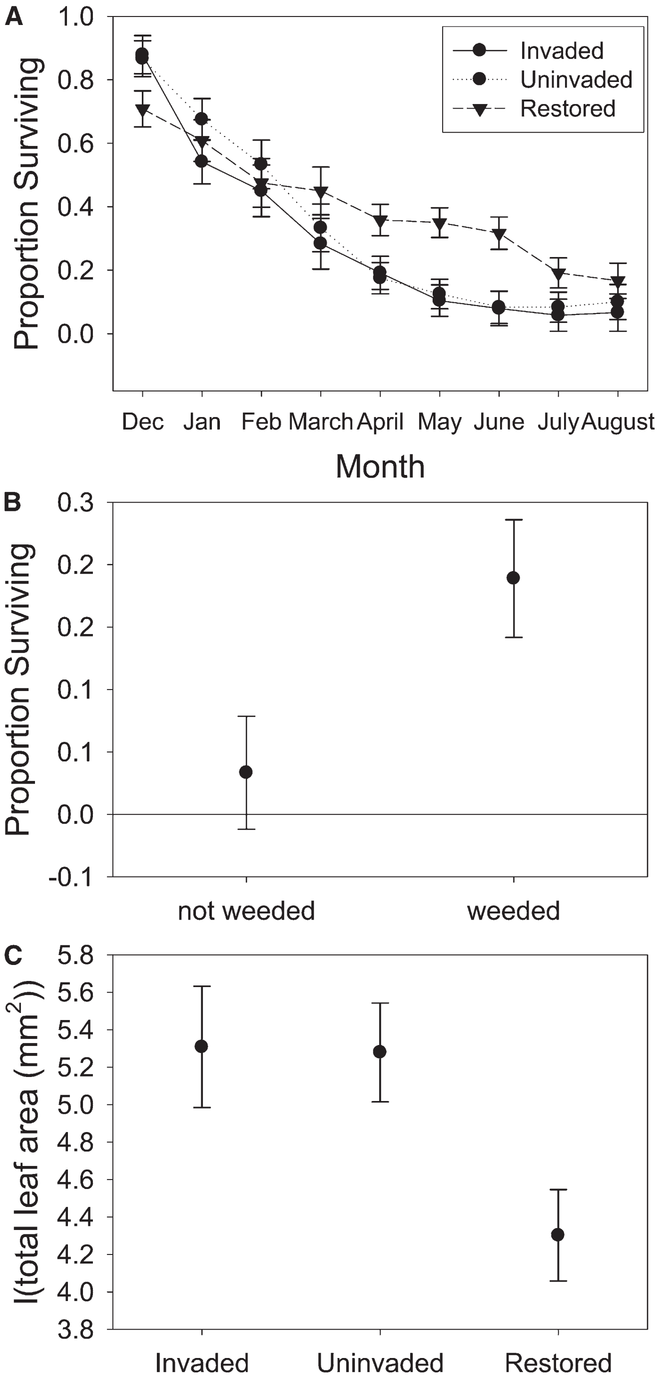

Fig. 4. The proportion of *Erigeron glaucus* seedlings that survived in the field (A) within the different sites by month and (B) within competition treatments at the end of the experiment (August) and (C) the log of total leaf area of surviving seedlings at the end of the experiment. In graph (A), invaded plots are represented by a solid line and circles, uninvaded plots are represented by a dotted line and circles, and restored plots are represented by triangles and dashed lines. In all plots, error bars represent one standard error.

The restoration plots containing *C. nutkaensis* had mixed effects on the success of *E. glaucus*. Survival of *E. glaucus* plants in the restoration plots planted with *C. nutkaensis* was greater, but plants in these plots were significantly smaller than plants in either the invaded or uninvaded sites. Thus, *C. nutkaensis* may allow the restoration process to get started by effectively outcompeting *H. lanatus* in locations where it is established; however, it may slow down the restoration process by hindering growth of native species replacing *H. lanatus*. These effects may be soil community driven as well.

Here we tested multiple potential factors (direct competition, indirect competition via mammalian herbivory, and indirect competition via changes in soil) that could influence *H. lanatus* dominance and competitive elimination of *E. glaucus* from the California coastal prairie. Examination of all these factors allowed us to identify three factors (direct competitive effects, mammalian herbivory, and changes in soil abiotic and biotic conditions) that contribute to the establishment and growth of *E. glaucus* and to measure their relative importance. This approach also allowed us to identify when these factors are likely to be most important. Here we show that the direct competitive effects of *H. lanatus* are most important during the invasion process, and they have the greatest effect on plant community structure. Reduction of the direct competitive effects of *H. lanatus* should aid in native plant community conservation. In contrast, plant community structure after the removal of *H. lanatus* is still likely to be altered by the legacy effects of *H. lanatus* on the soil community, and these legacy effects are likely to hinder restoration.

We see great opportunities for using a multifactor approach in future studies of competitive interactions and community ecology of plants. Our study reinforces other studies that have shown that dual processes are critical to plant community assembly—direct competition influences establishment (reviewed in Sommer and Worm, 2002), while soil communities cultivated by competitive dominants further alter establishment and growth of other species (Ehrenfeld, 2003; Gillespie and Allen, 2006; van der Putten et al., 2007; Batten et al., 2008; Pringle et al., 2009). We contribute to this previous work by demonstrating that, at least in this system, whereas direct competition may be the most important factor, the removal of competitors without attention to soil legacies may result in failure of native species to re-establish. Model systems involving introduced and native species, like *H. lanatus* and *E. glaucus*, can be used to tease apart the various different components of competitive exclusion. In addition, these studies can aid plant conservation, better identify the factors that contribute to invasive plant success and native species loss (Weiher, 2007), and identify factors that land managers can manipulate to reduce introduced species and conserve or restore native communities.

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