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Seed and establishment limitation contribute to long-term native forb declines in California grasslands

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Abstract. The effects of exotic species invasions on biodiversity vary with spatial scale, and documentation of local-scale changes in biodiversity following invasion is generally lacking. Coupling long-term observations of local community dynamics with experiments to determine the role played by exotic species in recruitment limitation of native species would inform both our understanding of exotic impacts on natives at local scales and regional-scale management efforts to promote native persistence. We used field experimentation to quantify propagule and establishment limitation in a suite of native annual forbs in a California reserve, and compared these findings to species abundance trends within the same sites over the past 48 years. Observations at 11 paired sites (inside and outside the reserve) indicated that exotic annual plants have continued to increase in abundance over the past 48 years. This trend suggests the system has not reached equilibrium >250 years after exotic species began to spread, and 70 years after livestock grazing ceased within the reserve. Long-term monitoring observations also indicated that six native annual forb species went extinct from more local populations than were colonized. To determine the potential role of exotic species in these native plant declines, we added seed of these species into plots adjacent to monitoring sites where plant litter and live grass competition were removed. Experimental results suggest both propagule and establishment limitation have contributed to local declines observed for these native forbs. Recruitment was highest at sites that had current or historical occurrences of the seeded species, and in plots where litter was removed. Grazing history (i.e., location within or outside the reserve) interacted with exotic competition removal, such that removal of live grass competition increased recruitment in more recently grazed sites. Abundance of forbs was positively related to recruitment, while abundance of exotic forbs was negatively related. Thus, exotic competition is likely only one factor contributing to local declines of native species in invaded ecosystems, with a combination of propagule limitation, site quality, and land use history also playing important and interactive roles in native plant recruitment.

Key words: California, USA grasslands; community assembly; competition; exotic species; invasion; livestock grazing; propagule limitation; recruitment limitation.

INTRODUCTION

Although biological invasions pose one of the greatest threats to global biodiversity, regional diversity may increase following exotic species invasions, due to fewer species extinctions than naturalizations (Sax et al. 2002, Davis 2003). Native and exotic diversity can be positively or negatively correlated at local scales (Sax 2002, Fridley et al. 2007), and long-term data are necessary to document local extinctions; thus extinction vs. naturalization rates at fine scales remain uncertain. Documenting native abundance over time in invaded systems is also important in the face of "extinction debt" and "invasion debt," where exotic species may not fully affect natives until generations after the initial invasion due to the timescale of processes leading to native extinction and the speed of invaders' spread to their potential distribution, respectively (Davis 2003, Seabloom et al. 2006, Kuussaari et al. 2009). Coupling long-term observations of local community dynamics with experiments to determine the role of exotic species in recruitment limitation of natives would thus inform both our understanding of local-scale exotic impacts on natives and regional-scale efforts to conserve native species.

Community assembly theory predicts that a combination of dispersal, abiotic conditions, and biotic interactions lead to membership in or exclusion from a community (Keddy 1992). Plant or animal recruitment, and thus population size and persistence, may be limited by a combination of processes where insufficient propagules are produced and dispersed (propagule limitation) and environmental conditions at a site are unsuitable for recruitment (establishment limitation)

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(Clark et al. 2007). Exotic invasion may increase propagule- and/or establishment limitation of native species by reducing native fecundity (Borer et al. 2007), introducing novel biotic interactions (Mitchell et al. 2006), or altering ecosystem processes (D'Antonio and Vitousek 1992). However, negative correlations often observed between native and exotic diversity (Fridley et al. 2007) may not be caused by exotic species, especially given a lack of evidence for extinctions caused solely by competition (Davis 2003, Seabloom et al. 2003*b*, Sax and Gaines 2008). We have a unique opportunity to evaluate the roles of community assembly processes in recruitment limitation by conducting a seed addition and competition removal experiment within long-term monitoring sites where species' historical occurrences are known.

Eurasian annual grasses and forbs began invading the California grasslands in the mid-1700s, and have dominated the system since at least the mid-1800s (Heady 1977, Hamilton 1997, Mensing and Byrne 1998). Though few native plant extinctions have been documented relative to the high floral diversity in California (Stein et al. 2000, California Native Plant Society 2011), natives remain limited in regional abundance and are often locally restricted to habitat refugia or sites with certain land use history (Stromberg and Griffin 1996, Seabloom et al. 2003*a*). A variety of recruitment limitation mechanisms may be responsible for these patterns, including seed limitation (Seabloom et al. 2003*a*, *b*, Seabloom 2011, but see Moore 2009), high spatial heterogeneity in the abiotic environment that limits the suitable sites within species' geographic ranges (Sax and Brown 2000, Moore 2009), disturbance and the consumer community (Stromberg and Griffin 1996, Hayes and Holl 2003*a*, *b*, Orrock et al. 2008, 2009, Seabloom et al. 2009), and competition from exotic species (D'Antonio and Vitousek 1992, Dyer and Rice 1999, Coleman and Levine 2007). Because California has few native annual grasses, the exotic community may have altered the competitive environment for native seedlings in two primary ways. First, early-fall germination and rapid shoot growth of annual grasses may reduce light and moisture resources for native seedlings (Dyer and Rice 1999, Coleman and Levine 2007). Second, plant litter build-up in annual grass-dominated communities may inhibit native seed germination and establishment (D'Antonio and Vitousek 1992, Foster and Gross 1998, Coleman and Levine 2007), as well as ultimately affecting rare native forb persistence (Levine and Rees 2004). Land use history, such as livestock grazing, may interact with exotic competition to affect native seedling establishment. Grazing may affect recruitment either directly (e.g., via seed limitation caused by herbivory) or indirectly (e.g., via altering plant community composition and biomass, reducing plant litter) (Hayes and Holl 2003*a*, *b*). Distinguishing the roles of recruitment limitation processes, including different mechanisms of exotic annual grass competition, would thus inform existing theory about biotic interactions within the system and effective management strategies to promote native recruitment.

We quantified changes in grassland plant abundances over 48 years in monitoring sites at the Hastings Natural History Reservation (HNHR) in central California. Within HNHR, total richness of native and exotic plants in local communities was relatively stable from 1963 to 2010; however exotics were becoming increasingly dominant in abundance (Brandt and Seabloom 2011). Furthermore, long-term local population trends appear idiosyncratic, regardless of provenance (native or exotic), life history, or functional group (grass or forb) (A. J. Brandt, *unpublished data*). Recruitment dynamics may thus be continuing to change in these local grassland communities even a century after exotic dominance, and decades after HNHR was established and livestock grazing ceased. Experimental investigation of the roles played by exotic species competition and land use change in native recruitment would elucidate their effects on observed local community dynamics relative to confounding changes in environmental conditions across the system, such as long-term climate change and fire suppression.

We conducted a seed addition experiment within long-term monitoring sites inside and outside the boundary of HNHR to determine the roles of seed limitation, habitat suitability, biotic interactions, and land use history in limiting recruitment of native species declining within those sites. We added seed of six native annual forb species that had declined in multiple monitoring sites to experimental plots in which exotic annual grass competition via the growth of grass seedlings and build-up of plant litter was removed in a factorial design. We made the following predictions about recruitment limitation of these native forbs:

Prediction 1.—If seed limitation drives local abundance, then seeded species will recruit across sites and experimental treatments, regardless of observed abundance trends within a site, exotic species competition, or land use history.

Prediction 2.—If habitat suitability drives local abundance, then seeded-species recruitment will be greater in sites occupied by those species during monitoring. Seeded-species recruitment will be greatest in monitoring sites with stable occupation or newly colonized sites, and will increase with observed abundance of the species during monitoring.

Prediction 3.—If exotic annual grass competition drives local abundance, then seeded-species recruitment will be greater in competition removal plots. Recruitment is expected to be greatest in plots where both live grass and plant litter are removed.

Prediction 4.—If land use history drives local abundance, then seeded-species recruitment and the effect of exotic competition will differ inside vs. outside the reserve. Because livestock grazing was the primary land

TABLE 1. Long-term occupancy trends for six native annual forb species in 11 paired monitoring transects located inside and outside of the reserve boundary in the Hastings Natural History Reservation in central California, USA.

	Number of transects inside reserve boundary				Number of transects outside reserve boundary			
Species	Stable	Extinct	Colonized	Absent	Stable	Extinct	Colonized	Absent
Castilleja exserta	3	4	0	4	2	5	0	4
Clarkia purpurea	8	2	1	0	10	1	0	0
Collinsia heterophylla	5	0	0	6	5	0	0	6
Nemophila menziesii	2	2	0	7	2	1	2	6
Plantago erecta	1	3	0	7	1	5	0	5
Trifolium microcephalum	10	0	0	1	10	0	0	1

Notes: "Stable" occupancy was documented presence during both the early monitoring period (1963–1978) and the late monitoring period (2005–2010). Species documented only during the early monitoring period were considered to be "extinct" in the transect, and species documented only during the late monitoring period were considered to have "colonized" the transect. Species never documented within a transect were considered "absent."

use and most monitoring sites are now ungrazed, recruitment differences inside vs. outside the reserve boundary will represent effects of the relative time lag since grazing ceased. Recent release of annual grasses from grazing pressure is expected to lead to reduced seeded-species recruitment on control sites outside the reserve, but competition removal treatments may be most effective at increasing recruitment in those sites.

METHODS

Work was conducted at the Hastings Natural History Reservation (HNHR), Monterey County, California, USA (36°22′ N, 121°32′ W), a 1000-ha reserve established in 1937 as part of the University of California's Natural Reserve System. Historical accounts describe grazing by a variety of livestock occurring on HNHR land until 1937, as well as on the adjacent land since it was first settled by European-Americans in the mid-1800s (J. M. Linsdale, *unpublished data*). A grazing-effects study was established in 1963 and sampled from 1963 to 1966, 1978, and 2005–2010. The sampling design consisted of 11 pairs of transects along the reserve boundary, one inside and one outside of the reserve, in a range of open-canopy to oak woodland habitats (visually estimated canopy cover ranging from 0 to 99%, with a mean of 46%), to monitor understory plant community composition. In 1963, cattle grazing occurred at all sites outside the reserve boundary. Since 1963, the reserve acquired lands containing two sites, and cattle grazing ceased on others; thus only three sites were still grazed in 2005–2010 (M. R. Stromberg and W. D. Koenig, *personal communication*). Presence of each species was recorded in 20 to 120 quadrats, each 20 × 50 cm, regularly spaced throughout each site during peak biomass (April–June), and percentage cover of each species was visually estimated from 2005 to 2010 (Brandt and Seabloom 2011). Permanent 40-m transects were marked at each site in 2006. Abundance of all annual plant species within transects was estimated for provenance (native and exotic) and functional groups (grass and forb) as a total frequency, where the number of quadrats in which a species was observed was divided by the total number of quadrats sampled per transect to obtain frequency, and frequencies of species of like provenance and functional group were summed. Abundances of six native annual forbs, *Castilleja exserta* (Scrophulariaceae), *Clarkia purpurea* (Onagraceae), *Collinsia heterophylla* (Scrophulariaceae), *Nemophila menziesii* (Hydrophyllaceae), *Plantago erecta* (Plantaginaceae), and *Trifolium microcephalum* (Fabaceae), were estimated as frequency per transect. These species were chosen because they were present in many monitoring sites, had declined in abundance at multiple sites, represented a variety of families, and had commercially available seed. A factor variable was created for each transect to describe the long-term occupancy trend for each species. "Stable occupancy" was defined as documented presence of a species during both the early (1963–1978) and late (2005–2010) monitoring periods. "Extinction" was defined as documentation of a species' presence only during the early monitoring period, "colonization" as documentation of presence only during the late monitoring period, and "absence" as no documentation of presence during monitoring (Table 1).

A seed addition experiment was nested within the long-term monitoring design to determine the relative role of several factors in limiting species recruitment. The same six native annual forbs were seeded into competition removal plots located adjacent to monitoring transects in a two-way factorial design in September 2009 (see Appendix A for a picture of the design). A block of four 1 × 1.5 m treatment plots was placed at the end and middle of each transect on each side of the reserve boundary ($n = 264$ plots). Each species was seeded into one of six, regularly spaced, randomly assigned locations within a 0.5 × 1 m cover quadrat and marked with a plastic cocktail sword, such that each species was sown a minimum distance of 30 cm from other seeded species. Species with laboratory trial germination rates >25% (*Clarkia* and *Collinsia*) were seeded at a rate of 30 seeds per plot, while the other four species were seeded at a rate of 40 seeds per plot. Seed was purchased from native seed suppliers in California (Larner Seeds, Bolinas; Pacific Coast Seed, Livermore; and Rana Creek Nursery, Carmel Valley); only *Castilleja* seed had been wild-collected in the local area of HNHR. Competition removal treatments were randomly assigned to plots within blocks, applied in a factorial design, and consisted of removing plant litter via hand clipping or removing live grass via herbicide. Litter was removed when plots were established, dried to constant mass, and weighed. Grass-specific herbicide (sethoxydim, with N-90 nonionic surfactant) at a concentration of 8 mL/L of water was applied at a rate of 100 mL per plot following initial germination of annual grasses in October–November 2009 and again in late December 2009. During herbicide application, seed-sowing locations within plots were covered with plastic containers and then grass underneath these containers was brushed with herbicide. Numbers of individuals of each seeded species were counted and the maximum height of each focal species at the seed-sowing locations was measured in March and May 2010. Additionally, seeded species were clipped at the soil surface at each seed-sowing location in May 2010, the number of reproductive individuals counted, and the plants dried to constant mass and weighed. The percentage cover of all grasses and forbs within each cover quadrat was visually estimated in October–November 2009, December 2009, and March 2010, and percentage cover of individual species was estimated in May 2010. All statistical analyses were conducted in R 2.11.1 (R Development Core Team 2010), and mixed-effects models used the nlme R library (Pinheiro et al. 2010) or the lme4 R library for models with Poisson errors (Bates and Maechler 2010).

Analyses of long-term monitoring trends

The effects of time as a continuous variable, land use history (i.e., location inside or outside the reserve), native status (i.e., native or exotic), and functional group (i.e., grass or forb) on total frequency of species within each provenance-functional group of annual plants in long-term monitoring transects were determined with mixed-effects models. The sampling design was incorporated into each model as nested random effects, with year nested within the two transects (inside or outside reserve) and transect nested within site. The full model included a four-way interaction between all predictors. Models were estimated using maximum likelihood, and model simplification was performed using ANOVA to find the model with the lowest AIC.

The effects of time, land use history, community composition, and canopy cover on frequency of the six native annual forb species of interest in long-term monitoring transects were determined with mixed-effects models. The sampling design was incorporated into each model as nested random effects, with year nested within the two transects (inside or outside the reserve) and transect nested within site. The full model included an interaction between time and land use history and was estimated using restricted maximum likelihood. Community composition predictors consisted of the first two axes of a Principal Components Analysis (PCA) using mean percentage cover from 2005 to 2010 of each species observed in each monitoring transect, and performed using the labdsv library in R (Roberts 2010). Percentage canopy cover was visually estimated at the ends and middle of each transect in 2008 and averaged.

Analyses of focal species recruitment

The effects of competition removal treatments, land use history, long-term occupancy trends at a transect, the mean mass of litter removed from a block, plant community composition, and canopy cover over a block on both total recruitment of seeded forb species and recruitment of individual species were determined with mixed-effects models with Poisson errors because responses were counts. The experimental design was incorporated into each model as nested random effects, with sampling date nested within block, block nested within the two transects (inside or outside reserve), and transect nested within site. Full models included a three-way interaction between land use history and competition removal treatments. Orthogonal contrasts of long-term occupancy trends for models of individual species responses were used to compare (1) sites with at least a single documentation of presence vs. sites where a species was never observed, (2) sites where a species had gone extinct vs. sites with documented presence during the late monitoring period, and (3) sites with stable occupancy vs. newly colonized sites. Results were robust to including only stands with more than one year of observed presence, and with more than one year of observed presence in both early and late periods, in experimental recruitment analyses. Community composition predictors included percentage cover of all forbs, exotic forbs, and all grasses within a plot, and the first two axes from the PCA described above. Models were estimated using maximum likelihood, and model simplification was performed based on AIC. Mixed-effects models were also used to describe effects of these predictors on the number of reproductive individuals (using Poisson errors), maximum height at seeding locations, and seeded-species biomass, both for summed responses within a plot and for each seeded species.

The effects of competition removal treatments, land use history (i.e., location inside or outside the reserve), functional group (i.e., grass or forb), and sampling date (i.e., fall, winter, early spring, or late spring) on percentage cover of vegetation were determined with mixed-effects models. The experimental design was incorporated into each model as nested random effects, with sampling date nested within plot, plot nested within block, block nested within the two transects (inside or outside reserve), and transect nested within site. The full model included three-way interactions between competition removal treatments and each of the other predictors, and all two-way interactions between land use history, functional group, and sampling date. Orthogonal contrasts for sampling date were used to compare responses (1) before and after the initial herbicide application (i.e., fall vs. other sampling dates),

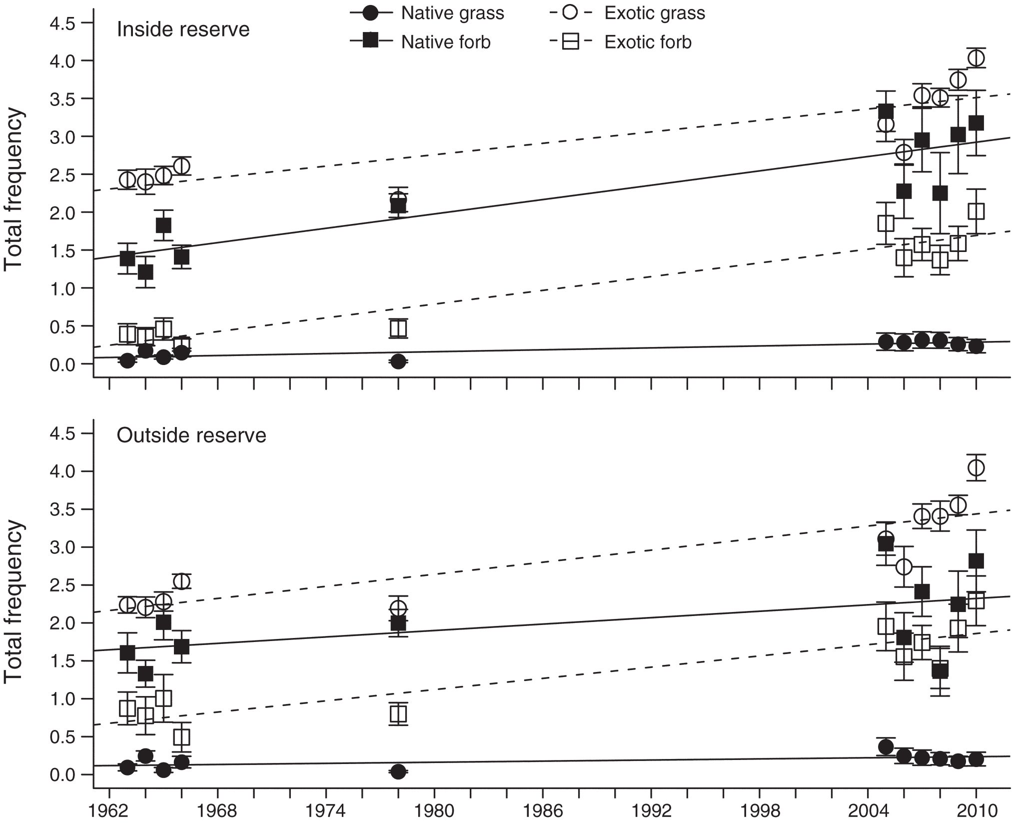

FIG. 1. Abundance (mean ± SE) of annual plants along 11 paired monitoring transects located inside and outside the reserve boundary in the Hastings Natural History Reservation in central California, USA. Abundance within each provenance functional group (e.g. native grasses) was estimated as the total frequency along each transect, or the number of quadrats in which a species within that group occurred divided by the total number of quadrats sampled, summed over all species within that group.

(2) before and after the second herbicide application (i.e., winter vs. both spring sampling dates), and (3) early vs. late spring. Model simplification was based on AIC.

RESULTS

Long-term monitoring trends

Total frequency of exotic annual plants increased in monitoring transects over 48 years (coefficient = 0.02, $P < 0.0001$), and exotic forb increases were greater inside the reserve boundary compared to outside (coefficient = 0.008, $P = 0.05$ for the interaction between year and land use history, and coefficient = −0.01, $P = 0.01$ for the interaction between year, land use history, and functional group; Fig. 1). Native annual forbs increased in total frequency to a lesser extent, particularly outside the reserve boundary (coefficient = −0.008, $P = 0.03$ for the interaction between year and native status, and coefficient = 0.008, $P = 0.09$ for the interaction between year, land use history, and native status). Frequency of the single native annual grass species, *Vulpia microstachys*, remained relatively stable (coefficient = −0.02, $P = 0.0001$ for the interaction between year, native status, and functional group).

Six native annual forb species differed in their site occupancy (Table 1) and frequency within occupied sites over time (Fig. 2; see Appendix B). The six focal species were observed in many long-term monitoring transects at some point during both the early and late monitoring periods (Table 1). However, observed extinctions from transects were more common than colonizations of new transects. Overall declines in frequency over time were observed for *Castilleja* (coefficient = −0.001, $P = 0.0001$) and *Plantago* (coefficient = −0.001, $P = 0.0008$), while general increases were observed for *Clarkia* (coefficient = 0.002, $P = 0.03$), *Collinsia* (coefficient = 0.001, $P = 0.07$), and *Trifolium* (coefficient = 0.002, $P = 0.004$).

Focal species abundances and temporal trends differed by land use history for two species. Mean *Castilleja* abundance was greater outside the reserve boundary (coefficient = 2.80, $P = 0.007$), but also declined over time outside the reserve boundary (coefficient = −0.001, $P = 0.0002$ for the interaction between year and land use history). Mean *Collinsia* abundance was greater inside the reserve boundary (coefficient = 3.95, $P = 0.007$) and increased more over time than outside the reserve boundary (coefficient = 0.002, $P = 0.0001$ for the interaction between year and land use history). Land use history did not affect mean

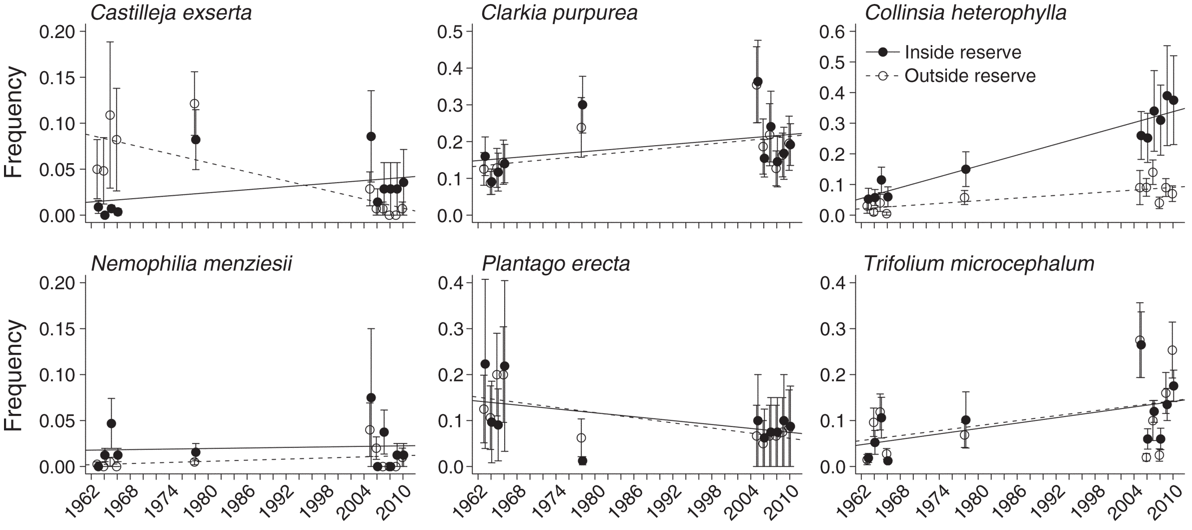

FIG. 2. Abundance (mean ± SE) of six native annual forbs along 11 paired monitoring transects located inside and outside the reserve boundary. Abundance was estimated as frequency of observations, or the number of quadrats in which a species was observed divided by the total number of quadrats sampled. Mean yearly abundances were calculated using only transects in which the species was observed in at least one year over the course of monitoring (see Table 1 for sample sizes).

frequencies or temporal trends in frequency of the other species ($P > 0.2$).

Abundance of four focal species also varied with community composition and canopy cover. In general, 11 exotic annual grasses and forbs, one native annual forb, one native perennial forb, the native annual grass, and one native perennial grass contributed to variation in community composition among monitoring transects (see Appendix C for species correlations with the first two axes from a PCA). The first PCA axis accounted for 25.2% of the variation in species composition; the second axis accounted for 23.4%. *Clarkia* was positively associated with *Aira caryophyllea*, *Bromus arenarius*, *B. hordeaceus*, *Erodium* spp., *Hypochaeris glabra*, *Lotus scoparius*, and *Nassella pulchra*; negatively associated with *B. diandrus*, *Lolium temulentum*, *Madia elegans*, *Torilis nodosa*, and *Vulpia microstachys*; and had a complex relationship with *Avena fatua* and *Lolium multiflorum* (coefficient = 0.004, $P = 0.03$ for positive relationship with PCA axis 1 and coefficient = −0.01, $P = 0.002$ for negative relationship with PCA axis 2). *Collinsia* and *Nemophila* were positively associated with *B. diandrus*, *Lolium* spp., *M. elegans*, *T. nodosa*, and *V. microstachys*; and negatively associated with *Aira caryophyllea*, *Avena fatua*, *B. arenarius*, *B. hordeaceus*, *E. cicutarium*, *H. glabra*, *Lotus scoparius*, and *N. pulchra* (coefficient = 0.001, $P = 0.07$ and coefficient = 0.001, $P = 0.09$ for positive relationship with PCA axis 2, respectively). *Trifolium* abundance increased with increasing canopy cover (coefficient = 0.002, $P = 0.06$).

Focal species recruitment

Multiple predictors affected recruitment of the seeded plants, and species-specific recruitment responses were largely idiosyncratic (see Appendices D, F, G, and H for predictor coefficients and significance in final mixed-effects models for number of individuals, number of reproductive individuals, maximum height, and mass, respectively). Overall trends in different recruitment metrics were similar; thus only results for the number of individuals are presented here.

Though overall recruitment was low, five of the six seeded species recruited at all sites, and long-term occupancy trends were good predictors of recruitment for four of the six species. Overall recruitment was 6.2% of total seeds sown, with 1.2% recruitment of *Castilleja*, 6.7% of *Clarkia*, 7.4% of *Collinsia*, 5.1% of *Nemophila*, 11.4% of *Plantago*, and 6.2% of *Trifolium*. *Castilleja* did not recruit in 4 of the 22 transects, which were located at three sites, both inside and outside the reserve boundary, where populations were either never documented or went extinct, according to monitoring data. Long-term occupancy trends along a transect were good predictors of species recruitment for *Castilleja* (coefficient = 0.58, $P = 0.04$ for increased recruitment in transects it had occupied compared to transects in which it was never documented), *Collinsia* (coefficient = 0.72, $P = 0.02$ for increased recruitment in transects it had occupied compared to transects in which it was never documented), *Nemophila* (coefficient = 1.74, $P < 0.0001$ for increased recruitment in transects with stable occupancy compared to colonized transects), and *Plantago* (coefficient = 0.32, $P = 0.009$ for increased recruitment in transects it had occupied compared to transects in which it was never documented) (Fig. 3; Appendix D). However, mean observed frequency of a species in a transect over the course of monitoring was rarely a good predictor of recruitment (see Appendices).

Competition removal increased total recruitment of seeded species in some cases, though removal of litter

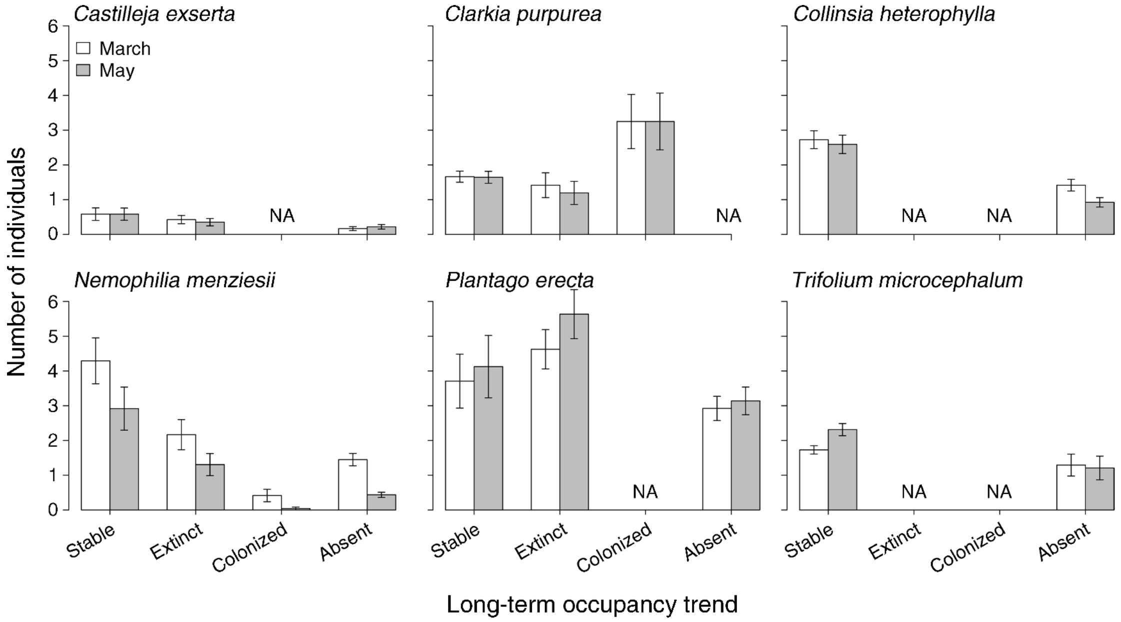

FIG. 3. Number of recruits (mean ± SE) recorded in March and May in seed addition plots located along long-term monitoring transects with various long-term occupancy trends for each of the six native annual forb species. Sample size for each trend category and definitions of these categories are provided in Table 1. NA indicates that no transects displayed that occupancy trend for that species.

and application of herbicide interacted with each other and with land use history to affect recruitment. (See Fig. 4 for total recruitment response and Appendices D–H for species-specific responses.) Total recruitment was generally greater when litter was removed (coefficient = 0.16, $P = 0.002$), and this positive effect was stronger for plots inside the reserve boundary (coefficient = 0.23, $P = 0.002$ for the interaction between litter treatment and land use history; Appendix D). Recruitment was lowest at sites with the greatest litter mass at the beginning of the experiment (coefficient = –0.003, $P = 0.007$; Appendix D), and annual grass cover at a site in the spring correlated positively with mean mass of litter removed from that site in the fall ($r = 0.65$, $P = 0.001$). Litter mass does not fully explain the interaction between litter removal and land use history, however, because the mass of litter removed did not differ across the reserve boundary (coefficient = 9.20, $P = 0.7$ from mixed-effects model including nested random effects of block within land use history within site).

Removal of live grass competition via herbicide application had a complex effect on seeded-species

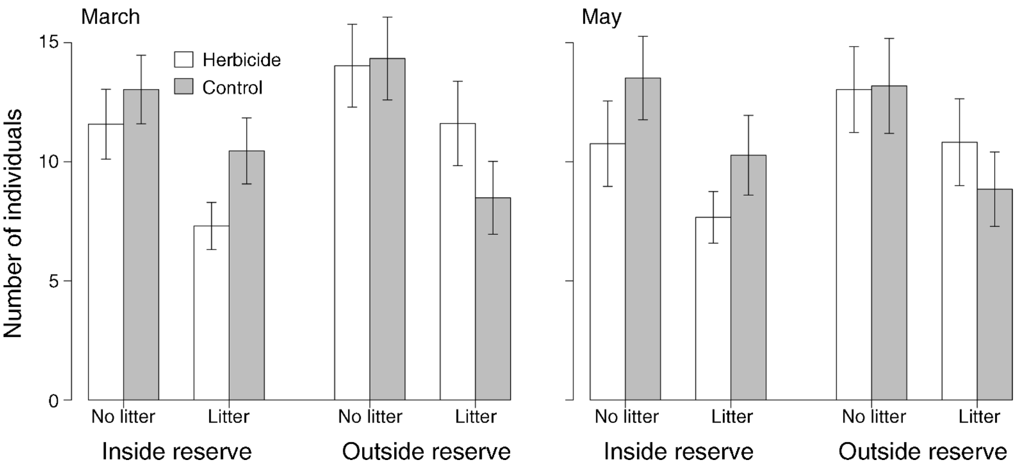

FIG. 4. Total number of recruits per plot (mean ± SE) of six native annual forb species recorded in March and May. Seeds for each species were sown into competition removal plots located along long-term monitoring transects inside and outside the reserve boundary. Litter removal and grass-specific herbicide treatments were applied in a factorial design.

recruitment, potentially due to compositional differences across the reserve boundary and the effects of herbicide on plant community composition. Herbicide application increased recruitment in plots outside the reserve where litter was not removed, and decreased recruitment in plots inside the reserve, particularly where litter was not removed (coefficient = –0.29, $P = 0.0001$ for the interaction between litter and herbicide treatments; coefficient = 0.16, $P = 0.02$ for the interaction between herbicide treatment and land use history; and coefficient = 0.41, $P = 0.0001$ for the three-way interaction between herbicide treatment, litter treatment, and land use history; Appendix D). Competition removal treatments, especially herbicide application, significantly reduced grass cover and increased forb cover (coefficient = 4.66, $P = 0.003$ for the interaction between litter removal and functional group, and coefficient = 30.19, $P < 0.0001$ for the interaction between herbicide application and functional group; see Appendix I). Grass cover was generally greater in plots located inside the reserve boundary and forb cover was greater outside the reserve (coefficient = 5.01, $P = 0.001$ for the interaction between functional group and land use history). In particular, exotic forb cover in the spring was greater outside the reserve (coefficient = 9.56, $P = 0.02$) and herbicide increased exotic forb cover (coefficient = 11.05, $P < 0.0001$). Thus the highest cover of exotic forbs was observed in plots outside the reserve boundary where herbicide was applied (coefficient = 6.08, $P = 0.003$ for the interaction between herbicide application and land use history). The herbicide application may have affected seeded-species recruitment, in part via its effects on the plant community, because recruitment increased with total forb cover (coefficient = 0.007, $P < 0.0001$), but decreased with increasing cover of exotic forbs (coefficient = –0.005, $P = 0.01$; Appendix D).

The complex and apparently contradictory relationships between seeded-species recruitment, herbicide application, and plant community composition may be due to other site characteristics and the abundance of certain species in the community. For example, total seeded-species recruitment decreased as canopy cover increased (coefficient = –0.005, $P = 0.03$; Appendix D). *Nemophila* recruitment decreased along PCA axis 1 (coefficient = –0.03, $P = 0.02$), which correlates with its observed positive relationship with *Bromus diandrus*, *Madia elegans*, *Torilis nodosa*, and *Vulpia microstachys*, and negative relationship with *B. hordeaceus* during long-term monitoring (see PCA axis correlations in *Analyses of long-term monitoring trends*, and Appendix B). *Plantago* recruitment increased along PCA axis 2 (coefficient = 0.04, $P = 0.03$), though long-term observations were not related to community composition.

DISCUSSION

California grassland communities appear to be exhibiting long-term transience, centuries after exotic plant invasion and decades after livestock grazing ceased. Increasing abundances of certain annual plant groups and declines in certain native annual forbs demonstrate that the system is continuing to change. Interannual variability in community composition has been observed here and in other California grassland communities, likely as a response to the system's highly variable precipitation patterns (Pitt and Heady 1978, Michaelsen et al. 1987, Elmendorf and Harrison 2009). Growing season precipitation at HNHR ranged from 304 to 861 mm (mean of 538 mm) during monitoring years (1963–1966, 1978, 2005–2010), with mean precipitation in the late monitoring period almost 50 mm lower than in the early period. Long-term community trends may thus be due in part to general drying, and expanding the temporal scale of observation may alter or reverse observed trends (Hobbs et al. 2007). However, long-term trajectories of increasing exotic abundances within transects seen here suggest that an "invasion debt" remains to be paid, even within sites where certain exotic species have been present for a century (Seabloom et al. 2006). Several confounding factors have been changing on a similar timescale with invasion of the system, including altered fire regimes, agricultural practices, nitrogen deposition, and climate change, and many of these changes promote invaders (D'Antonio et al. 2007). Thus, though we cannot definitively isolate the roles of abiotic changes, anthropogenic effects, and invasion on native species declines, it seems likely the effects of exotic invaders on the system, including their role in limiting native species recruitment, will continue to increase.

Population extinctions were more common than colonizations for certain native forbs over the past 48 years at HNHR, suggesting that they may eventually be extirpated from the reserve without management intervention. Native diversity is highly spatially variable across this reserve after accounting for temporal variability (Brandt and Seabloom 2011). Further demographic work with more extensive surveys of these species' populations would determine if declines might be balanced by immigration from neighboring populations that are increasing, thus promoting regional persistence (Hanski et al. 1996). Determining how common local declines are among native species, and how land use history or increasing exotic species abundance are associated with those declines, would be an important step to understanding the generality of results obtained for these focal species.

Five of six native forbs recruited across all sites, suggesting that populations are seed limited (Clark et al. 2007). Using commercial seed may have contributed to low recruitment if these species are locally adapted; however seed from the lowest recruiter, *Castilleja*, was collected near HNHR and low recruitment is typical of seed limitation (Clark et al. 2007). Seed limitation is common for plants, especially annuals (Turnbull et al. 2000, Clark et al. 2007), has been documented for several California grassland species, including *Plantago erecta* (Seabloom et al. 2003*a*, *b*, Seabloom 2011), and is likely exacerbated by other factors causing population declines, such as anthropogenic disturbance and biological invasions. Although we did not explicitly investigate potential mechanisms leading to seed limitation for these species, small-mammal consumers and seed bank dynamics are important factors to consider in future work (Orrock et al. 2008, 2009, Moore 2009). By examining annual plants, we were able to document recruitment to adulthood, but determining whether seed addition leads to self-sustaining populations is an important next step (Turnbull et al. 2000).

Identifying and measuring all important habitat attributes for a species is rarely feasible, and suitable habitat is likely patchy given the varying scales of spatial heterogeneity in abiotic and biotic environmental gradients (Sax and Brown 2000, Moore 2009). Here, higher recruitment at historically or currently occupied sites suggests that focusing restoration on sites known to be suitable (i.e., population augmentations) may be more effective than attempting restoration on other apparently similar sites (i.e., population introductions). Overall, population abundance was a poor predictor of recruitment, perhaps because it is the combined result of all processes acting on recruitment. Other community characteristics, such as composition and canopy cover, were poor predictors of site suitability in general, because they did not relate to both observed population trends and recruitment following seed addition. Long-term monitoring can thus help identify suitable habitat that is currently unoccupied, but determining which mechanisms limit establishment of declining species may still be necessary to promote population persistence. Further investigation of abiotic factors that increase recruitment may improve predictions of and elucidate mechanisms driving site suitability.

Competition removal generally increased native recruitment, though removal of litter and live grass had interactive effects, unlike the results that others have observed (Coleman and Levine 2007). Exotic competition that affects key demographic stages, such as recruitment of species with an annual life history, likely has the greatest impact on native populations. Furthermore, novel forms of competition may increase exotic effects on natives due to a lack of coevolutionary history between species (Mitchell et al. 2006). Here, competitive effects of exotic annual grasses were largely mediated by plant litter (but see Coleman and Levine 2007), which was correlated with exotic annual grass abundance. Native California forb diversity and abundance tend to be negatively associated with litter (Hayes and Holl 2003*a*, Coleman and Levine 2007), and litter is a key component influencing germination and recruitment of native forbs in models of the system (Levine and Rees 2004). Thus, in systems where exotic invaders are biased toward a specific novel plant group, such as annual grasses, novel competitive mechanisms may drive recruitment limitation, and mitigating such effects may most effectively restore native species.

Alternatively, exotic species that are ecologically similar to natives may more strongly limit recruitment (Fargione et al. 2003, Moore 2009). Live grass removal increased native recruitment only in certain conditions, which may be due in part to increases in exotic forb cover following herbicide application. Early-season forbs may enhance competition for certain soil resources relative to grasses or late-season forbs (Hooper and Vitousek 1997), and exotic forb competition reduces native forb diversity and seedling survival in other mediterranean systems (Castro and Badano 2010). Thus, exotic forbs that share traits and resource acquisition strategies may be stronger competitors with native forbs than exotic annual grass seedlings. Effects of the plant community on native recruitment were more complex, however, because recruitment increased with total forb abundance. Determining the relative and directional impact of native species' interactions with different members of the exotic and native community and their similarity in environmental preferences may elucidate these apparently contradictory results.

Increasing local abundance of some species during monitoring suggested that exotic competition did not limit their recruitment (*Collinsia* and *Trifolium*) and our experimental results affirmed this. However, recruitment of *Clarkia* increased with competition removal, though its populations were generally increasing. Thus, similar long-term abundance patterns may not be generated by the same recruitment limitation mechanisms. Litter accumulation in a variety of herbaceous plant systems often decreases recruitment, but can differentially affect species within the community and along gradients of productivity (Facelli and Pickett 1991, Foster and Gross 1998, Suding and Goldberg 1999). Our focus on mechanisms driving species declines biases our conclusions on the overall effect of exotic species on native recruitment. Litter can reduce moisture stress for emerging seedlings, which might lead to facilitation by exotic grasses and explain increasing native abundance at certain sites and increased native recruitment in some cases in the presence of grass or litter (Suding and Goldberg 1999, Fridley et al. 2007).

The role of exotic competition on native species recruitment from year to year may also change with temporal environmental heterogeneity (Moore 2009). This experiment was conducted in the wettest year of the late monitoring period, and conducting seed addition studies over multiple years with varying precipitation would test the robustness of our results. California annual plant richness increases overall in cool, wet years (Elmendorf and Harrison 2009), and exotic species abundance appears especially sensitive to temporal environmental variability (Brandt and Seabloom 2011). Thus, it is difficult to predict how the competitive environment into which seeded species recruit, and thus the relative roles of recruitment limitation processes, might change under different climatic conditions.

Our results suggest that grazing can produce a legacy effect, such that differences in community composition and recruitment limitation processes may persist on a site after grazing has ceased. Grazing history at our sites often reversed the effect of live grass competition removal on native recruitment. Although litter abundance was not lower outside the reserve, as is expected on currently grazed sites (Hayes and Holl 2003*a*), grass and forb abundances differed across the reserve boundary, and were related to focal species recruitment as described earlier. Certain types of anthropogenic land use can persistently affect plant community dynamics (Stromberg and Griffin 1996), and disturbance effects are often context dependent (Hayes and Holl 2003*b*). Our findings thus highlight the need to include land use history and additional abiotic variables in future work examining time lags in the effects of invasions.

Species-specific effects of site occupancy, competition, and grazing history on recruitment deserve further study because native annual forbs are often considered ecologically similar a priori. Different abiotic and biotic constraints on recruitment, and different population responses to grazing history and temporal environmental variation, suggest these species are "niche-limited" (Moore 2009). Evolutionary history may better encompass relevant species differences than provenance or functional group, and improve predictions, particularly if recruitment responses are more similar within than among clades (Cavender-Bares et al. 2009). Certain species traits, such as hemiparasitism, may provide unique insight into clade-specific responses. A lack of adequate hosts may have contributed to *Castilleja exserta*'s low recruitment, though it can benefit from a variety of host species as well as survive and reproduce autotrophically (Atsatt and Strong 1970). Growth benefits provided by hosts (i.e., facilitation) may explain *C. exserta*'s higher recruitment in control plots and plots with higher forb cover.

Exploring the potential time lags to extinction and shifts in native–exotic abundance following invasion are two important areas of study that can be addressed by coupling long-term monitoring with experiments (Sax and Gaines 2008). Although we focused on a single site over one year, exploring mechanisms governing local-scale recruitment improves our understanding of invasion impacts and informs management (Sax et al. 2002, Sax and Gaines 2008). We observed extinction of local native plant populations to be more common than establishment of new populations, and recruitment to be higher in sites with a history of species occupancy, suggesting that maintenance of documented populations is critical to long-term species persistence (Hanski et al. 1996). Both seed and establishment limitation processes affected native plant recruitment, with certain mechanisms of exotic plant competition providing a general recruitment barrier. Competition from exotic species likely plays a complex role in recruitment limitation of native forbs and may continue to increase in impact, as exotic invaders are predicted to continue expanding in range and abundance (Seabloom et al. 2006). Thus, multifaceted restoration approaches may be most effective for native plant conservation, including mitigation of novel exotic interaction mechanisms. Finally, our work suggests that community dynamics in predominantly annual systems may remain transient centuries after exotic invasion and decades after anthropogenic disturbance. Continued monitoring and biodiversity management is thus critical as systems may still be facing an "invasion debt," and certain native species an "extinction debt" (Seabloom et al. 2006, Kuussaari et al. 2009).

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Supplemental Material

Appendix A

Diagram of nested experimental design for seed addition plots (*Ecological Archives* E093-127-A1).

Appendix B

Statistical results for observed temporal trends in native forb abundance (*Ecological Archives* E093-127-A2).

Appendix C

Species correlations with principal components analysis axes constructed from monitoring transect plant cover data (*Ecological Archives* E093-127-A3).

Appendix D

Statistical results for native forb recruitment into competition removal plots (*Ecological Archives* E093-127-A4).

Appendix E

Recruitment of each native forb species by competition removal treatment and land use history (*Ecological Archives* E093-127-A5).

Appendix F

Statistical results for number of reproductive individuals of native forbs seeded into competition removal plots (*Ecological Archives* E093-127-A6).

Appendix G

Statistical results for maximum height of native forbs seeded into competition removal plots (*Ecological Archives* E093-127-A7).

Appendix H

Statistical results for biomass of native forbs seeded into competition removal plots (*Ecological Archives* E093-127-A8).

Appendix I

Grass and forb cover by competition-removal treatment and land use history (*Ecological Archives* E093-127-A9).