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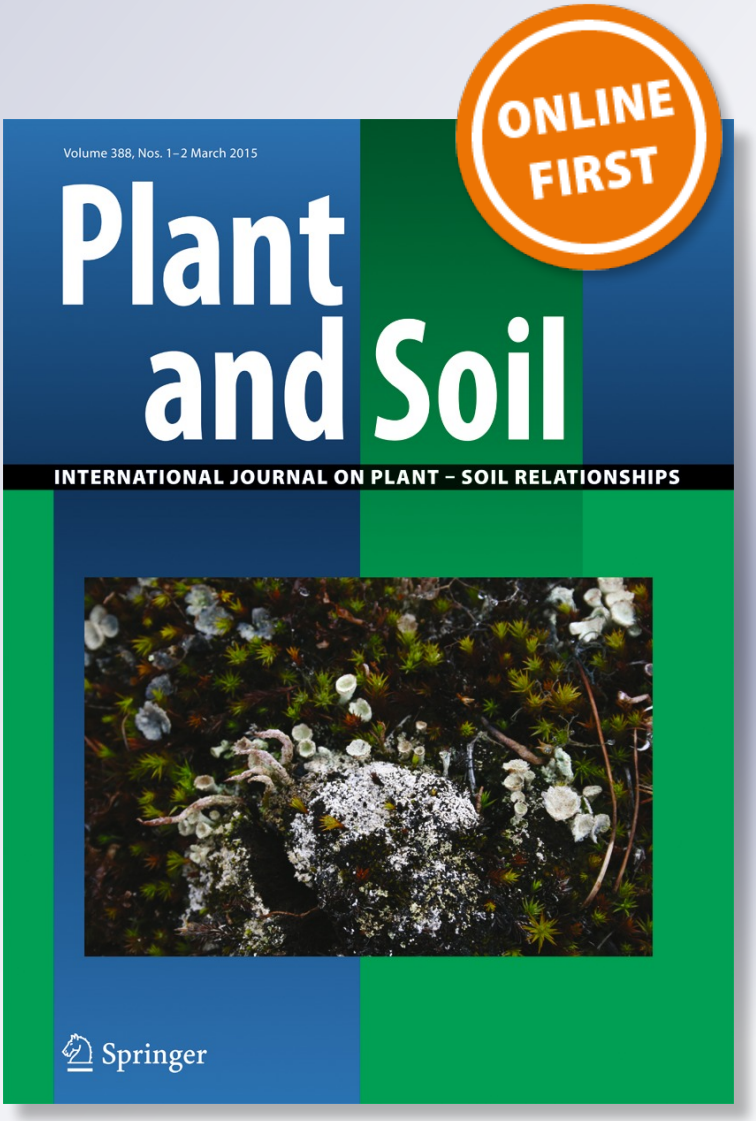

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REGULAR ARTICLE

Plant-soil feedbacks and competitive interactions between invasive *Bromus diandrus* and native forb species

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Abstract
Background and aims Feedback between plant and soil microbial communities plays a key role in plant invasions. We examined feedback in native and invasive plants growing in monoculture and mixture, to determine soil microorganisms' role in *Bromus diandrus* invasion.
Methods Four native forb species were grown in monoculture and in competition with *Bromus* and with different microbial inocula. Inoculum consisted of 20 g of soil collected from the rhizosphere of native or invasive plants used to create treatments of (1) whole soil, (2) filtrate containing non-mycorrhizal microbes, and (3) arbuscular mycorrhizal fungi (AMF) spores.
Results Native species in monoculture experienced neutral to positive feedback with whole soil and filtrate inoculum. Feedback in *Bromus* grown in monoculture varied in direction and magnitude with different soil microbial fractions. Fine AMF (*Glomus tenue*) in filtrate inoculum appeared to cause observed positive feedback effect in native and invasive species, even with pathogenic fungi in roots. Feedback in mixture was more positive than in monoculture for some species.
Conclusions Our study highlights the difficulty of extending feedback results in monoculture to the community level, and the importance of fine AMF, which has received little attention, interacting with pathogens in plant invasion.

Keywords Abandoned agriculture · Coarse arbuscular mycorrhizal fungi · Fine arbuscular mycorrhizal fungi · *Glomus tenue* · Oomycetes · Plant invasion

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Introduction

While many mechanisms have been proposed to explain the success of invasive species, plant-soil feedback has been widely proposed and tested over the past two decades (Klironomos 2002; Callaway et al. 2004b; van Grunsven et al. 2007; Batten 2008; van der Putten et al. 2013). Plant-soil feedback is defined as plant-influenced changes to the soil microbial community that then positively or negatively affects subsequent plant growth (Bever 1994; Bever et al. 1997). Much of the plant-soil feedback research has approached soils as a black box, and explanations of invasiveness assume the role of either soil-borne pathogens or mutualists though they are seldom observed (reviewed in van der Putten et al. 2013, but see Klironomos 2002 and Callaway et al. 2011).

Biogeographical comparisons of plant species often detect more negative effects of soil biota from plants' native vs. non-native ranges (Callaway et al. 2004b, Callaway et al. 2011). Invasive species may establish in a novel environment due to a release from soil-borne

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pathogens (Keane and Crawley 2002; Bezemer and Van der Putten 2007; Kardol et al. 2007; Reinhart et al. 2010). Alternatively, invasive species can alter the soil biota in invaded ranges creating positive feedback effects that promote invasion (Richardson et al. 2000, Vogelsang and Bever 2009). Associated native species form either positive or negative feedback (Klironomos 2002), and the direction of the feedback may affect interspecific competition and plant community composition.

Both feedback effects and the potential role of competitive interactions are significant in plant invasion but seldom studied together (Hodge and Fitter 2013). Soil mutualists (Callaway et al. 2004b) and pathogens (van der Putten et al. 1993, van der Putten and Peters 1997) affect competitive interactions, and in the context of competition feedback effects may change in direction and magnitude (Shannon et al. 2012). Stabilizing mechanisms of species coexistence would suggest plant species in intraspecific competition experience a greater negative growth response than in interspecific competition (Chesson 2000; Casper and Castelli 2007). Therefore, invasive species may experience more negative feedback effects over time as they continue to dominate a plant community. However, Casper and Castelli (2007) found no evidence that intraspecific competition results in greater negative growth response, and the combined effects of competition and the strength of the growth response was different among species. This suggests that plant responses to soil biota when grown in intraspecific competition cannot adequately predict plant responses to soil biota when grown in interspecific competition (Allen and Allen 1984). Studies examining soil biota in invasions need to examine growth responses of the invasive species both in intraspecific competition and in competition with the native species it displaces.

Bromus diandrus is a Mediterranean annual grass invading much of the remaining coastal sage scrub and native forbland communities in southern California (Barbour et al. 2007; Minnich 2008). Invasive grasses have been shown to alter soil dynamics that contributes to their overall success in coastal sage scrub (Dickens et al. 2013), and host a different assemblage of arbuscular mycorrhizal fungi (AMF) from native plants (Hawkes et al. 2006; Siguenza et al. 2006; Busby et al. 2013). Exotic grasses in coastal sage scrub are predominately infected with fine AMF, often identified as *Glomus tenue*, whereas native shrubs they displace are infected mainly with coarse AMF and infection by fine AMF is infrequent (Siguenza et al. 2006). *Glomus tenue*, the fine AMF, has been reported to infect numerous grass species (Molina et al. 1978; Powell 1979; Rabatin et al. 1993) and be more frequent in pioneer plants (Blaschke 1991). It is commonly found in a wide range of soils including agricultural and forest soils, at a wide range of altitudes, often recorded in lowlands and high mountain soils (Abbott and Robson 1977; Molina et al. 1978; Blaszkowski 1994), and is especially common in degraded soils (Gucwa-Przepióra et al. 2013). While some studies suggest the fine endophyte is the main root colonizer in the absence of other AMF, the ecology of the fine AMF is poorly understood and its role in invasion is virtually unstudied (but see Siguenza et al. 2006).

The influence of *Bromus diandrus* on the soil community and subsequent impacts on native forb growth and interspecific competition is unknown. We examined the role of soil microbial feedbacks in the competitive dominance of the invasive grass *Bromus diandrus*. More specifically, we (1) examined plant-soil feedback effects from native and invasive plants on conspecific and interspecific growth, (2) tested different microbial fractions to evaluate which groups of fungi influence plant-soil dynamics and, (3) determined whether native or invasive inoculum affect growth and competition between *Bromus diandrus* and native forbs.

Material and methods

Study site

Soils for this study were collected at Riverside County Habitat Conservation Agency lands near Lake Mathews, in Riverside, CA (33°36′29.80 N, 117°02′00.81 W) in September 2012. The site is abandoned citrus agriculture that was formerly coastal sage scrub (CSS) and annual forbland (Minnich 2008), and is currently dominated by the exotic annual grass *Bromus diandrus*. Citrus trees were removed some 5 years prior to our study when the land was acquired as a conservation reserve. Bulk soil to be used as a greenhouse growth medium was collected in an adjacent 2 ha native CSS community. Soils from both the citrus agricultural site and the adjacent CSS site are in the Porterville cobbly clay series (Nelson et al. 1919). The soil was cut 50 % with silica sand to improve drainage (a common practice for inoculum studies in fine-textured soil, e.g., Johnson et al. 2008), steam-sterilized for 24 h, held at room temperature for 24 h, and sterilized for another 24 h. The resulting soil

contained total KCl-extractable N (NO_3^--N plus NH_4^+-N) of 17.0 μg/g soil, and 18.1 μg/g bicarbonate-extractable P. This soil mix was placed into 800 ml Conetainer® pots, and seed mixes and soil inocula with or without biota as described below were added to pots.

Soils and inoculum material

Inoculum soil for the greenhouse experiment was collected directly from the field to assure that field-cultured microbial species were present. Native CSS inoculum was taken in the 2 ha remnant stand from underneath 15 *Artemisia californica* shrubs, whose understory consists of a mixture of native annual forb species including all of the native annuals in this study, to a depth of high fine root activity (10 cm) and mixed. Therefore, our native inoculum contains the soil microorganisms from a natural CSS community where shrub and forb species co-occur in a matrix, and changes to the soil from that matrix may have consequences for the growth or fitness of the species within the matrix. Invasive inoculum was collected underneath 15 *Bromus diandrus* plants from the abandoned citrus orchard. By collecting inoculum directly from the field we assured that organisms that represented the legacy of abandoned citrus agriculture, including oomycetes and *Fusarium* spp., and native CSS were included in the inoculum (Allen et al. 1993).

Soil feedback in native versus invasive plants was determined using additions of soil inoculum with or without soil biota from different microbial fractions. Seven soil microbial fractions were created from 20 g of inoculum soil for each replicate pot: 1) sterile soil, 2) native whole soil, 3) invasive whole soil, 4) native filtrate, 5) invasive filtrate, 6) native AMF spores, and 7) invasive AMF spores. Twenty grams of soil per pot were passed through a 2 mm sieve for whole soil inoculum, or a 20 μm sieve to create a filtrate that excludes AMF spores >20 μm and includes potential pathogens (Klironomos 2002). AMF spores were collected using the sucrose extraction method (Allen et al. 1979), and were surface sterilized with 5.25 % sodium hypochlorite. An average number of 435 AMF spores occurred in 20 g of inoculum from native CSS species, whereas 239 spores were found in 20 g of *Bromus* inoculum. Pots each received 20 g of steam-sterilized inoculum from the other source and sterile soil received 20 g of steam-sterilized inoculum soil from each of the two inoculum sources (40 g total) to balance nutrients in soil from native and former agricultural land.

Greenhouse experimental design

In a controlled greenhouse environment, four native forbs and the exotic annual grass *Bromus diandrus* were grown from seed in monocultures in the seven soil treatments described above for 6 weeks (n=10). Additionally, native forbs were grown in competition with *Bromus* in native and invasive whole soil inoculum, and sterile soil (n=10). Based on vegetation surveys completed in 2010 at Lake Mathews (Allen unpublished), we selected two common forbs (*Amsinckia menziesii* and *Layia platyglossa*) and two uncommon forbs (*Plantago erecta* and *Lasthenia californica*). Seeds of the native forb species were from regional collections from S&S Seed Co. (Carpinteria, California), and seeds of *Bromus diandrus* were collected at Lake Mathews in September 2011. Seeds of all five species were planted and thinned to two individuals of the same species for monocultures, and one native forb individual with one *Bromus* individual for mixtures. The resulting 470 pots were arranged in a complete randomized design to control for potential temperature gradients in the greenhouse.

Microbial assessment for feedback

After 6 weeks, plants were harvested for aboveground biomass and root biomass. Biomass was determined after drying at 60 ° C for 48 h. Plant-soil feedback was calculated in whole soil inoculum, filtrate, and AMF spore treatments using the following equation: soil feedback = [aboveground biomass of plant grown in inoculum fraction − aboveground biomass of plant grown in sterile soil]. Dried root biomass was rehydrated and mycorrhizal/non-mycorrhizal fungi colonization was assessed (prior observations showed that drying did not change percent colonization of mycorrhizal or pathogenic fungi). To assess fungal colonization, roots were washed from soil, cleared overnight in 2.5 % KOH, acidified in 1 % HCl, and stained in 0.05 % trypan blue (Kormanik and McGraw 1982, Koske and Gemma 1989). Percent colonization was estimated using a modified magnified intersection method (McGonigle et al. 1990). Roots were mounted in PVLG on microscope slides and 80 intercepts per replicate were observed at 400× magnification. Root fragments were examined for coarse AMF hyphae, fine endophytic AMF hyphae, pathogenic/saprophytic hyphae, oomycete hyphae, vesicles, and arbuscules. Coarse AMF hyphae are aseptate, 2–10 μm in diameter, and characterized by defining

features such as dichotomous branching at a 60° angle and knobby hyphal walls that stain dark (Rillig et al. 1999; Siguenza et al. 2006). Fine endophyte AMF have thinner hyphae, <2 μm in diameter, and lightly stained walls in these roots (Siguenza et al. 2006). Hyphae of the Ascomycota are characterized as having septa at regular intervals and sometimes staining blue while other times non-staining. Fungi of the Ascomycota range from purely saprophytic to obligate pathogens, and include important plant pathogens such as *Fusarium* sp. (Webster and Weber 2007). Previous culturing from this field site identified two *Fusarium* species *Fusarium equiseti* and *Fusarium pseduograminerarum* (Hilbig, unpublished). Both species are known pathogens. Dikaryotic hyphae of the Basidiomycota are characterized as having distinct clamp connections, or lateral bulges in the hyphae, at regular intervals (Webster and Weber 2007). Oomycetes are morphologically identified by coenocytic hyphae with walls that lack chitin and therefore fail to stain with trypan blue. Additionally, oomycetes are determined morphologically by distinct lemon-shaped sporangia, 10–20 μm in width (Webster and Weber 2007).

Statistical analysis

Biomass data were analyzed using separate one-way ANOVA for each species, with soil treatment as a fixed factor. Soil treatments were compared using least significant difference ($L.S.D._{0.05}$). All data were checked for homogeneity of variances using Levene's tests, and for normality using the Shapiro-Wilk test. For all species, total biomass data was ln transformed to meet the assumptions of normality for ANOVA. Percent root colonization data failed to meet the normality assumption even after a log transformation, and were analyzed using Kruskal-Wallis rank sum test for each species with soil treatment as a fixed factor. All statistical analyses were performed using R version 3.0.2 (R Development Core Team 2013).

Feedback was modeled in a Bayesian framework to incorporate different variances among species-soil treatment combinations. Biomass within each species-inoculum treatment was modeled using a normal distribution and its own variance. Feedbacks were calculated for each species within the model as $\text{Aboveground Biomass}_{\text{microbial fraction}} - \text{Aboveground Biomass}_{\text{sterile}}$. *P* values were calculated as the probability that the posterior probability distributions of these feedbacks overlapped zero, with significant values≤0.05 (corresponding to 95 % credible intervals that did not overlap zero). All mean and variance parameters were given non-informative priors, models were run for 20,000 iterations, and convergence was assessed by visual inspection of three independent chains after a brief burn-in period. Models were fit using OpenBUGS version 3.2.2 rev 1063 called from R using the R2OpenBUGS package (R Developing Core Team, Sturtz et al. 2005). Feedback was calculated using aboveground biomass due to the difficulty of separating root biomass by species when plants were grown in mixture. In monoculture, where root biomass was measured, we compared feedback calculated with total biomass to feedback calculated with aboveground biomass. Feedback did not change significantly in direction in any case, and in both filtrate and whole soil treatments significant feedback was observed in the same species regardless of the biomass data used. In AMF inocula treatments 4 of the 10 species treatment combinations shifted from trending to significant or vice versa. We therefore used aboveground biomass so that feedback could be compared between monoculture and mixture.

Statistical comparisons of feedback in monoculture and mixture for each species-inoculum treatment were done by modeling Difference = $(\text{Aboveground Biomass}_{\text{microbial fraction}} - \text{Aboveground Biomass}_{\text{sterile}})$ in monoculture - $(\text{Aboveground Biomass}_{\text{microbial fraction}} - \text{Aboveground Biomass}_{\text{sterile}})$ in competition. *P* values were calculated as the probability that the posterior probability distributions of feedback differences overlapped zero, with significant values≤0.05 (corresponding to 95 % credible intervals that did not overlap zero). All mean and variance parameters were given non-informative priors, models were run for 20,000 iterations, and convergence was assessed by visual inspection of three independent chains after a brief burn-in period. Models were fit using OpenBUGS version 3.2.2 rev 1063 called from R using the R2OpenBUGS package (R Developing Core Team, Sturtz et al. 2005).

Results

Monocultures

Aboveground biomass of *Amsinckia* and *Plantago* did not differ significantly by soil treatment when grown in monoculture (Fig. 1a and d). *Lasthenia* grown in soil with native AMF spores and invasive whole soil inocula

had greater aboveground biomass than *Lasthenia* grown with sterile soil, native whole soil or invasive AMF spores inocula (F=5.231, P<0.0001; Fig. 1b). Similarly, *Layia* grown in soil with native whole soil inoculum and native AMF spores had greater aboveground biomass than *Layia* grown in sterile soil and filtrate from native inoculum (F=4.509, P<0.001; Fig. 1c). Aboveground biomass of *Bromus* was smaller when plants were grown with native filtrate inocula than all other soil treatments except invasive AMF spores inocula (F= 5.877, P<<0.0001; Fig. 2a).

Competition with *Bromus diandrus*

Across all native species, plant biomass was smallest in sterile soils when grown in competition with *Bromus* (Fig. 1e–h). *Amsinckia, Layia* and *Plantago* grown in competition with *Bromus* had increased aboveground biomass with whole soil inoculum from both inoculum sources compared to sterile treatments (Fig. 1e, g and h). *Lasthenia* had greater aboveground biomass in invasive than native whole soil inoculum (Fig. 1f; F=8.78, P= 0.0014). *Bromus* aboveground biomass was significantly greater in whole soil inocula than sterile soil when grown with all native forb species, except *Lasthenia* (Fig. 2b–e).

Soil feedback

Calculated feedback for each species is graphically represented with absolute values (Figs. 3 and 4) and biomass was not standardized for comparisons across species. Feedback in all four native species grown in monoculture experienced neutral to positive feedback (Fig. 3). Significant positive feedback was observed in both *Lasthenia* and *Layia* when grown with native AMF spores (P=0.007, P=<0.0001), invasive filtrate (P= 0.005, P=0.017), and native whole soil (P=0.047, P<0.0001). *Lasthenia* also had a positive feedback when grown with native filtrate (P=0.012) and invasive whole soil inoculum (P=0.010). *Bromus* had a positive feedback when grown with native AMF spores (P= 0.003) and invasive filtrate (P=0.042), and negative feedback when grown with native filtrate (P=0.006). *Amsinckia* and *Plantago* had no significant feedback across all soil treatments at α=0.05.

In competition with *Bromus*, *Layia* and *Plantago* had significant positive feedback when grown with whole soil inoculum from both inoculum sources (native whole soil: P<<0.001, P<<0.001; invasive whole soil: P=0.009, P<<0.001 respectively). Calculated feedback with invasive whole soil inoculum was stronger when plants were grown in mixture than in monoculture for both *Layia* and *Plantago* (P=0.048, P=0.023 respectively; Fig. 4a). *Amsinckia* had a positive feedback in invasive whole soil only (P=0.002; Fig. 4a). *Bromus* grown with *Amsinckia* and *Plantago* had positive feedback with whole soil inoculum from both sources (Fig. 4b). These feedbacks were significantly stronger than the positive feedback observed in *Bromus* grown in monoculture under the same soil conditions (native whole soil: P = 0.013 (with *Amsinckia*), P<<0.001 (with *Plantago*); invasive whole soil: P = 0.015 (with *Amsinckia*), P = 0.013 (with *Plantago*))

Percent root colonization

Both coarse and fine AMF hyphae were found colonizing the roots of all five species, although native forb species were colonized more by fine AMF hyphae when grown with invasive inoculum and more heavily colonized by coarse AMF in native inoculum (Table 1, Fig. 5). For example, in *Amsinckia* grown with native whole soil inoculum 72 % of the total mycorrhizal colonization was by coarse AMF compared to 75 % of the total mycorrhizal colonization by fine AMF colonization when grown with invasive whole soil inoculum. Similarly, the majority of root colonization (65 %) of *Lasthenia* grown with native whole soil inoculum was by coarse AMF, whereas in the invasive whole soil inoculum 70 % of the total mycorrhizal colonization came from fine AMF colonization. *Bromus* had the lowest total AMF root infection on average across all five species, and was predominately infected by fine AMF in both native and invasive inoculum (Fig. 5). It had significantly greater colonization of fine AMF when grown with invasive whole soil inoculum and invasive AMF spore inoculum compared to all other soil treatments (H=16.3713, df=6, P=0.0119; Table 1). *Layia* had the highest total percent AMF root colonization across all five species, with up to 53 % of roots infected when grown with invasive whole soil (Table 1). Similarly, high percent AMF infection was found in *Layia* grown with invasive AMF spores, invasive filtrate, and native AMF spores (about 30 % each treatment). Individuals of *Plantago* had high percent root colonization of AMF when grown with AMF spores from both

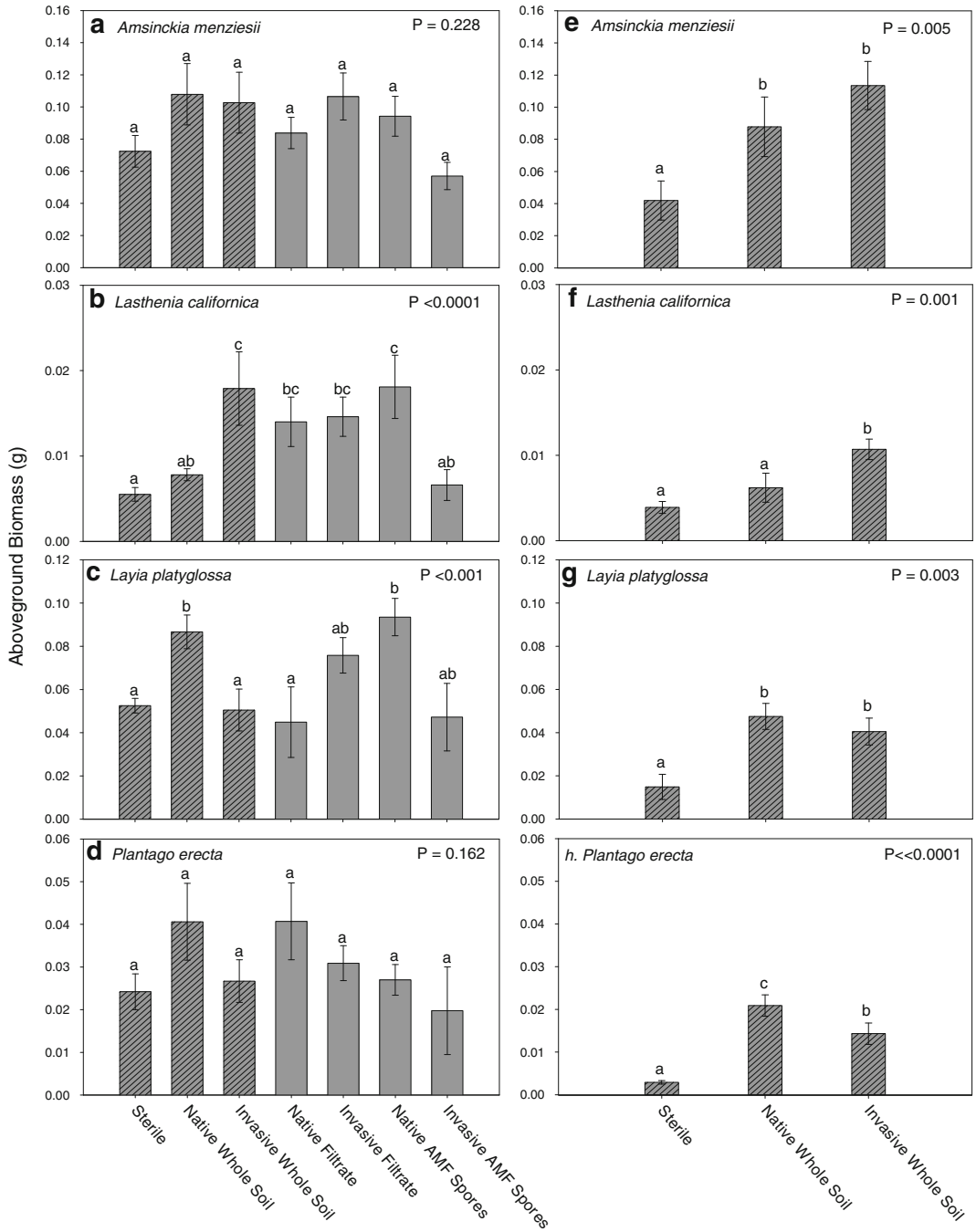

Fig. 1 Aboveground biomass of native forbs in monoculture (**a**–**d**) and mixture with *Bromus* (**e**–**h**) grown under different soil inoculum conditions. *Patterned bars* represent soil treatments that occur in both monoculture and mixture. Significance was determined at $\alpha=0.05$

inocula sources and whole soil inoculum from both sources (Table 1). Filtrate treatments from both inocula contained the fine AMF (spores <20 μm), and *Layia* grown with invasive filtrate had up to 30 % of roots colonized by fine AMF. Virtually all of the colonization was by AM hyphae, with no more than 2 % vesicles and no arbuscules in any treatment.

In every observation, hyphae that morphologically appeared to be ascomycetes were the dominant form of non-mycorrhizal hyphae. Overall, the greatest

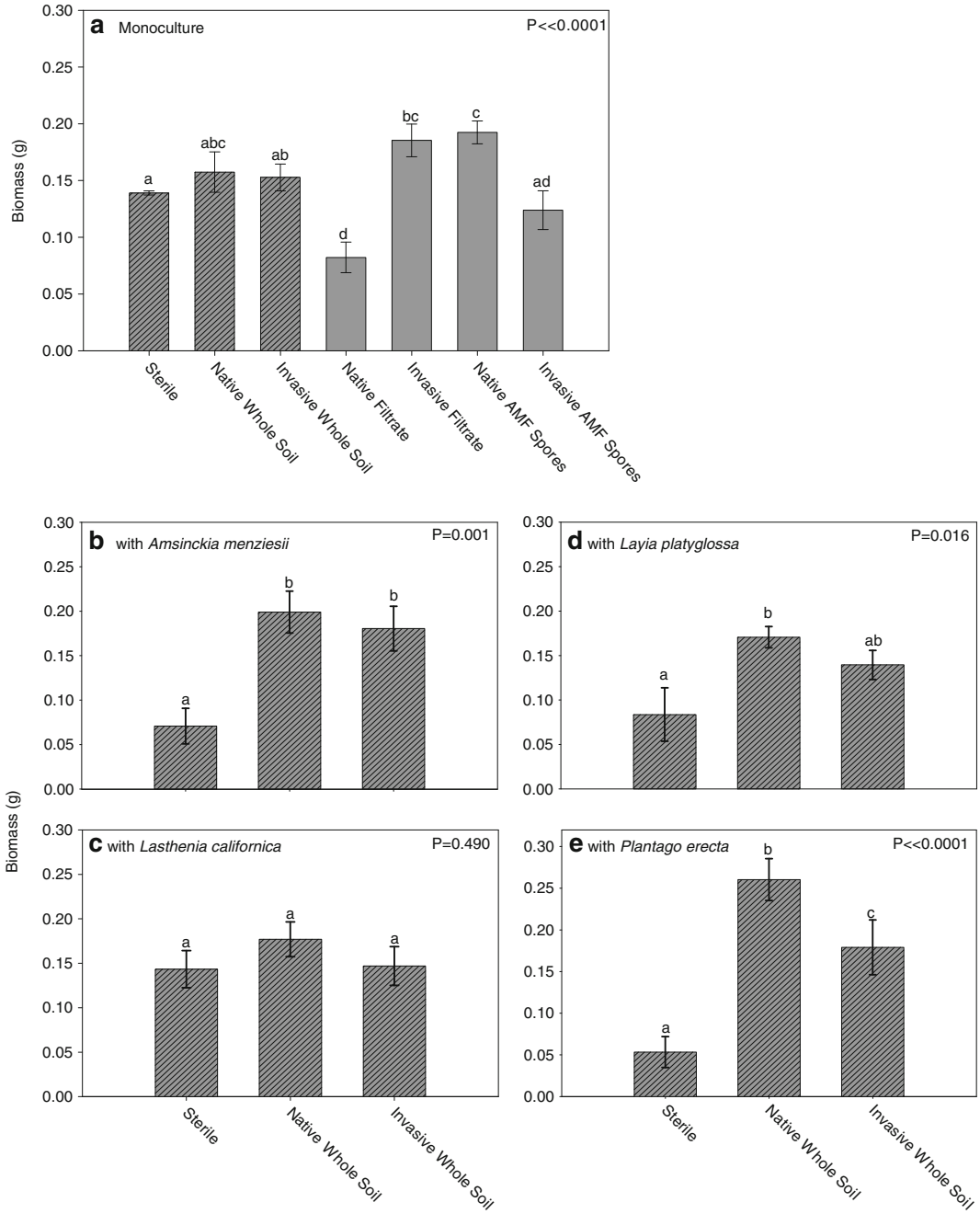

Fig. 2 Aboveground biomass for *Bromus* grown in monoculture (**a**) and in mixture with four native forb species (**b**–**e**) in different soil microbial fractions analyzed using separate one-way ANOVA. *Patterned bars* are those soil treatments that occur in both monoculture and mixture. Significance was determined at $\alpha=0.05$

colonization by non-mycorrhizal fungi occurred in species grown in invasive whole soil inoculum. *Lasthenia* had the highest percent non-mycorrhizal fungi colonization (30.1 %) when grown in invasive whole soil inoculum. Root colonization of *Amsinckia* by non-mycorrhizal fungi was significantly higher in invasive whole soil inoculum than all other soil treatments ($H=23.88$, df=6, $P<0.001$; Table 1). High percent root colonization by non-mycorrhizal fungi was found in individuals of *Plantago* grown with invasive whole soil,

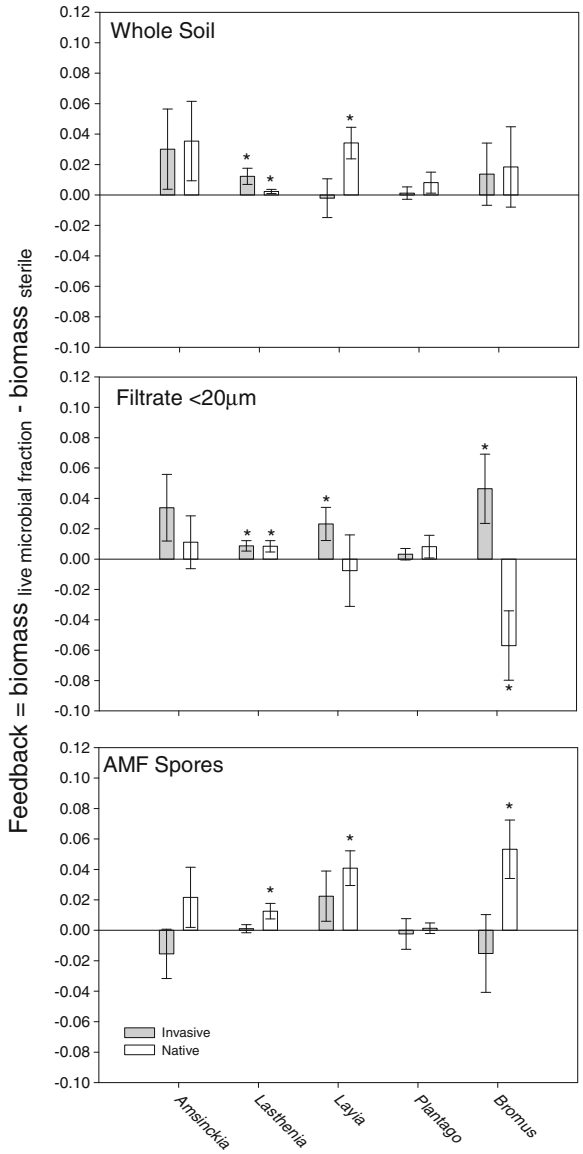

Fig. 3 Feedback calculated for all five species grown in monoculture by [aboveground biomass of plant grown in inoculum fraction—aboveground biomass of plant grown in sterile soil]. The *open bars* are feedback calculated from microbial fractions collected from the rhizosphere of *Artemisia californica*. The *gray bars* are feedback calculated from microbial fractions collected from the rhizosphere of *Bromus diandrus*. *Asterisks* represent significant feedback at α=0.05

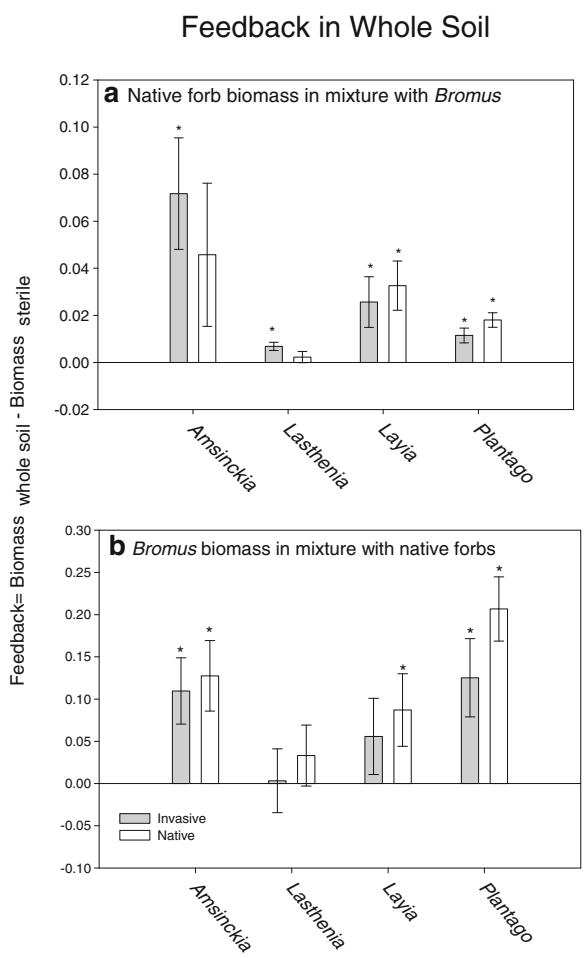

Fig. 4 Feedback calculated for four native forb species grown in mixture with *Bromus* (**a**) as [aboveground biomass of in whole soil—aboveground biomass in sterile soil]. *Bromus diandrus* feedback in mixture with native forbs (**b**) calculated by [aboveground biomass of *Bromus* in whole soil—aboveground biomass of *Bromus* in sterile soil]. The *open bars* are feedback calculated from microbial fractions collected from the rhizosphere of *Artemisia californica*. The *gray bars* are feedback calculated from microbial fractions collected from the rhizosphere of *Bromus diandrus*. *Asterisks* represent significant feedback at α=0.05

native whole soil, and invasive filtrate. Similarly, a high percentage of non-mycorrhizal fungi were found colonizing the roots of *Bromus* in whole soil inoculum from both inocula sources. A low percentage of oomycete hypha was found in the roots of the four forb species, but not *Bromus*, in the invasive AMF spore inoculum and invasive whole soil inoculum. *Layia* had the greatest infection of oomycetous hyphae among the forbs (Table 1). There was some contamination in sterile treatments, but oomycete hyphae were never found colonizing the roots of plants grown with native inoculum fractions.

Discussion

Co-existence theory predicts that co-occurring species experience negative feedback that prevents species dominance and contributes to ecosystem stability (Chesson 2000; Bever et al. 2012; Reinhart 2012).

Table 1 Percent Root colonization in all five species and seven soil treatments for plants grown in monoculture at week 6

Treatment	Species	Fine AMF		Coarse AMF		Non-mycorrhizal Fungi		Oomycetes	
		Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.
Sterile	*Amsinckia menziesii*	5.0	4.1	0.0	0.0	1.5	1.2	0.0	0.0
	Lasthenia californica	0.3	0.3	0.0	0.0	1.5	1.5	0.0	0.0
	Layia platyglossa	8.3	3.0	0.0	0.0	2.3	0.6	7.4	3.2
	Plantago erecta	3.0	1.6	0.0	0.0	0.8	0.5	0.3	0.3
	Bromus diandrus	1.8	0.8	2.0	0.8	1.8	0.5	0.0	0.0
Native Whole Soil	*Amsinckia menziesii*	4.9	1.8	**12.8**	**3.5**	**13.9**	**1.5**	0.0	0.0
	Lasthenia californica	**7.0**	**1.8**	**13.1**	**2.3**	**22.7**	**4.0**	0.0	0.0
	Layia platyglossa	1.5	0.8	**13.8**	**0.7**	9.7	1.5	0.0	0.0
	Plantago erecta	4.7	0.7	**14.7**	**2.8**	**19.1**	**2.2**	0.0	0.0
	Bromus diandrus	5.9	1.7	4.3	1.9	**15.6**	**4.2**	0.0	0.0
Invasive Whole Soil	*Amsinckia menziesii*	9.2	1.1	2.9	1.3	**21.6**	**4.3**	0.0	0.0
	Lasthenia californica	**10.0**	**2.0**	**4.4**	**1.9**	**30.1**	**5.9**	0.0	0.0
	Layia platyglossa	**33.8**	**7.7**	**19.5**	**10.9**	12.0	3.5	**0.3**	**0.3**
	Plantago erecta	6.4	0.6	**4.5**	**0.6**	**26.2**	**4.1**	0.0	0.0
	Bromus diandrus	**9.5**	**1.9**	1.1	0.5	**15.5**	**6.3**	0.0	0.0
Native Filtrate	*Amsinckia menziesii*	1.1	0.8	0.0	0.0	7.5	2.0	0.0	0.0
	Lasthenia californica	3.5	2.3	0.3	0.3	5.9	1.0	0.0	0.0
	Layia platyglossa	4.4	1.7	0.0	0.0	9.6	1.9	0.0	0.0
	Plantago erecta	3.4	2.6	0.3	0.3	**8.6**	**1.6**	0.0	0.0
	Bromus diandrus	5.4	2.2	0.3	0.3	10.4	2.0	0.0	0.0
Invasive Filtrate	*Amsinckia menziesii*	4.1	0.5	1.3	0.8	**13.1**	**2.9**	0.0	0.0
	Lasthenia californica	3.0	0.6	0.0	0.0	**18.1**	**1.9**	0.0	0.0
	Layia platyglossa	**29.6**	**4.6**	0.0	0.0	16.2	7.8	0.0	0.0
	Plantago erecta	3.9	1.9	0.5	0.3	**13.6**	**2.9**	0.0	0.0
	Bromus diandrus	6.8	1.1	0.0	0.0	4.7	1.4	0.0	0.0
Native AMF Spores	*Amsinckia menziesii*	2.8	1.5	**9.9**	**2.2**	2.4	0.8	0.0	0.0
	Lasthenia californica	4.7	0.8	4.1	1.4	3.8	2.9	0.0	0.0
	Layia platyglossa	22.1	7.5	8.1	2.5	9.1	3.7	0.0	0.0
	Plantago erecta	**21.4**	**4.8**	**7.3**	**2.0**	4.2	0.7	0.0	0.0
	Bromus diandrus	4.0	0.5	2.7	0.9	1.8	1.0	0.0	0.0
Invasive AMF Spores	*Amsinckia menziesii*	4.8	1.0	3.6	1.0	5.3	1.3	**0.5**	**0.3**
	Lasthenia californica	**12.3**	**4.1**	**9.8**	**2.7**	4.8	1.1	**3.8**	**1.5**
	Layia platyglossa	**28.4**	**4.4**	2.1	0.8	14.9	4.5	3.9	3.6
	Plantago erecta	**9.8**	**2.7**	1.8	0.8	3.3	2.0	0.0	0.0
	Bromus diandrus	**9.6**	**2.9**	0.5	0.3	0.3	0.3	0.0	0.0

Bold numbers denote values significantly different than the sterile treatment for each species and each microbial fraction at $P<0.05$

Invasive species often benefit from positive feedback (Richardson et al. 2000) while native species experience negative feedback contributing to an invasive species overall dominance. However, in our study all four native species experienced neutral to positive feedback. The unexpected positive feedback of native species may be explained by their annual life history. Mixed populations of native annual forbs in the understory of coastal sage scrub change in abundance annually with fluctuating rainfall characteristic of semi-arid Mediterranean

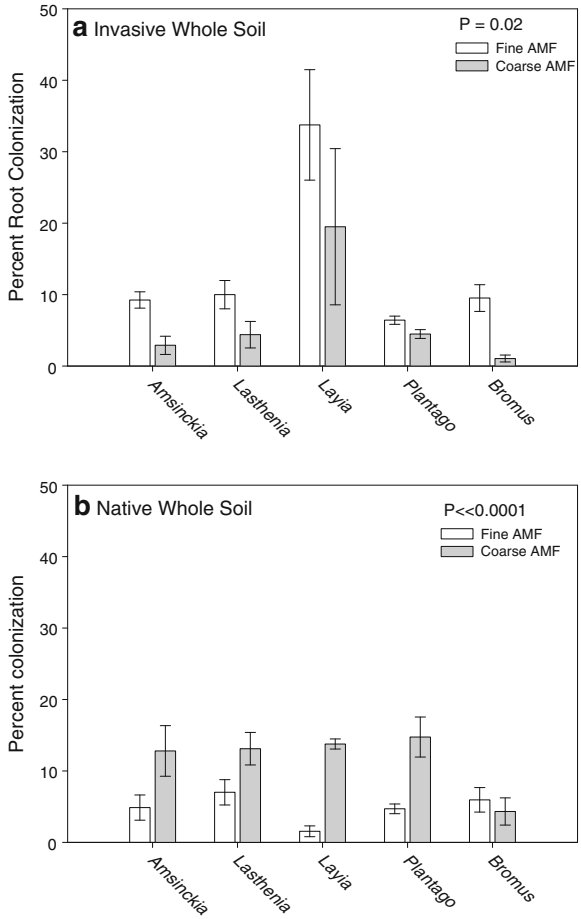

Fig. 5 Percent colonization of coarse and fine AMF in **a** invasive whole soil and **b** native whole soil for all five species. See Table 1 for percent colonization values for all species treatment combinations

climates (Heady 1958). Inoculum soil contained inputs from a mixture of native annual and shrub roots, including the annual species tested. The predominant negative feedback of native species described in other studies (Kulmatiski et al. 2008) may not occur in unstable populations of annuals. The observed positive feedback could contribute to the instability of this invaded annual system where the competitively superior invasive species experiences positive feedback leading to its dominance.

Fine AMF may also contribute to the unexpected positive feedback in native forbs and *Bromus*. The use of microscopy and morphological identification of fungal groups revealed that the soils of our *Bromus*-dominated, recently abandoned agricultural site have a high load of fine AMF, often identified as *Glomus tenue*. AMF are obligate mutualists (Smith and Read 2008). Therefore, the shift in AMF colonization in native forbs from coarse to fine AMF when grown with native and invasive inoculum respectively suggests that culturing of fine AMF by *Bromus* shifts the mycorrhizal community and de-stabilizes the system. Other studies have demonstrated significant shifts in AMF communities following invasion (Mummey and Rillig 2006), and these shifts may confer a competitive advantage to the invasive species.

Little is known about the taxonomy, physiology and ecology of the fine AMF, although a few studies examining plant responses to infection by fine AMF exist (Powell 1979; Rabatin et al. 1993; Siguenza et al. 2006; Zubek et al. 2009). Our results suggest that the fine AMF is important in the success of *Bromus* through neutralizing negative impacts of potential pathogens. This is demonstrated through positive feedback in *Bromus* when grown in soil inoculated with the invasive filtrate treatment described above and negative feedback when grown in soil inoculated with the native filtrate. Feedback is the net combination of mutualists and pathogens, and any potential negative impact of non-mycorrhizal fungi may be offset by positive responses to fine AMF. In native filtrate, higher percent root colonization by pathogens than by fine AMF resulted in a significant negative feedback. Whereas, when fine AMF infection was greater than pathogen infection in invasive filtrate, *Bromus* experienced positive feedback. Other studies have demonstrated positive plant growth responses in native (Powell 1979) and invasive (Siguenza et al. 2006) species to *Glomus tenue*. Positive feedback in *Bromus* grown with native AMF spores is due to the combination of coarse and fine AMF. While we predict *Bromus* would benefit from a positive feedback with invasive AMF spores due to infection by fine AMF, high within-treatment variation results in a non-significant neutral feedback that overlaps zero. Variation in growth within treatment cannot be explained by differences in fungal colonization, but may be related to factors not explicitly studied here such as seed size or germination timing. Further understanding of the effects of fine AMF on plant growth will require plants to be grown with single species of AMF.

At this point we do not know to what extent the fine AMF has been introduced with exotic grasses, or if the fine AMF is locally native and increasing in abundance because the most abundant plant species is culturing it. While a dominant native CSS shrub, *Artemisia californica*, had little fine AMF colonization in the field

or greenhouse, even when grown in mixtures with exotic grasses (Siguenza et al. 2006), in our study all four native forbs were colonized by fine AMF and experienced neutral to positive feedback. However, in competition with *Bromus*, native forb species, with the exception of *Amsinckia*, experienced reduced biomass relative to intraspecific competition regardless of the inoculum source. Whole soil inoculum resulted in greater forb biomass than sterile soil when in competition with *Bromus*, suggesting that though native forbs are poor competitors with *Bromus* AMF may partially alleviate the negative competitive effects of *Bromus*. The fact that *Amsinckia* does not have reduced biomass in mixture with *Bromus* suggests that it is a better competitor with the invasive grass than other natives, and in fact *Amsinckia* is more abundant than other native annuals at our site (unpublished observations) as well as at other invaded California annual grasslands (Pantone et al. 1995).

The high frequency of fine AMF in our soils collected from the rhizosphere of *Bromus* demonstrates that the traditional methods in plant-soil feedback studies to partition out non-mycorrhizal fungi in a microbial filtrate by using a 20 μm sieve (Klironomos 2002; Agrawal et al. 2005; Kardol et al. 2007; Callaway et al. 2011) may not always work as expected. Fine AMF spores have been observed to be as small as 10 μm in diameter (personal observation, Siguenza et al. 2006), and thus in our study the filtrate treatment allowed passage of both fine AMF and possible pathogens. Most studies report using 100× magnification to assess AMF (McGonigle and Fitter 1990), but because of the small diameter and poorly staining cell walls of fine AMF hyphae in our roots, they must be observed at 400X. It is possible that fine AMF is more prevalent than published literature would suggest and its ecological importance in plant community composition warrants further investigation.

Our study focused on soil fungi in *Bromus* invasion, although other microbes might affect plant-soil feedback including oomycetes, microfauna, and bacteria. Perhaps the most unexpected finding of this study was the presence of oomycetes in the invasive AMF spore inoculum. The field site is a former citrus orchard, and citrus is known for high incidence of root diseases (Kosola et al. 1995). We are not aware of reports of a high incidence of oomycetes in roots of native plants. In fact, they are thought to be highly host-specific, and not expected to infect the roots of native plants. The occurrence of oomycete hyphae in the invasive AMF spore treatment for some species may explain the neutral feedback, as the negative growth responses of known oomycete pathogens are balanced by the positive responses to AMF. Nematodes are often the most abundant microfauna, and can be readily observed on root surfaces or in sucrose spore extracts (Persmark et al. 1992). We did not observe nematodes in our sucrose spore extracts or microscope slides, therefore they are likely not abundant in these soils. Plant growth-promoting bacteria could result in positive growth responses in plants (Çakmakçi et al. 2006), but bacteria species would be similar among all fractions except sterilized soils and could not adequately explain different growth response in plants to different soil fractions. Thus our results are best explained by the balance of AMF and potential pathogens.

Lastly, we observed plant-soil feedback from fungi in interspecific competition and intraspecific competition. Feedback changed in magnitude in the context of competition, and in some species the feedback in mixture was more positive than the feedback in monoculture. Competition for mutualists in intraspecific competition may be stronger than in interspecific competition due in part to niche differentiation of AMF symbiosis. *Bromus* was predominately infected with fine AMF whereas native forbs were infected with both fine and coarse AMF. Thus individuals in intraspecific competition may experience greater competition for symbionts than individuals grown in interspecific competition, which may lead to a more positive feedback in interspecific competition. Others have suggested that in interspecific competition plants may benefit from common mycelium networks (Callaway et al. 2004a), but the mechanism behind shifts in the magnitude of feedback with competition is still poorly understood. Our study further demonstrates the difficulty of extrapolating the effects of feedback from monocultures to competition, and extending plant-soil feedback studies to the community assembly framework. A better mechanistic understanding of microbe-root interactions in monoculture and mixture will be needed to differentiate the effects of competition and feedback in plant-plant interactions.

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References

Abbott LK, Robson AD (1977) The distribution and abundance of vesicular arbuscular endophytes in some Western Australian soils. Aust J Bot 25:515–522

Agrawal AA, Kotanen PM, Mitchell CE, Power AG, Godsoe W, Klironomos J (2005) Enemy release? An experiment with congeneric plant pairs and diverse above- and belowground enemies. Ecology 86:2979–2989

Allen EB, Allen MF (1984) Competition between plants of different successional stages: mycorrhizae as regulators. Can J Bot Rev Can Bot 62:2625–2629

Allen MF, Moore TS, Christensen M, Stanton N (1979) Growth of vesicular-arbuscular mycorrhizal and non-mycorrhizal *Bouteloua gracilis* in a defined medium. Mycologia 71:666–669

Allen EB, Cannon JP, Allen MF (1993) Controls for rhizosphere microorganisms to study effects of VA mycorrhizae on *Artemisia tridentata*. Mycorrhiza 2:147–152

Barbour MG, Keeler-Wolf T, Schoenherr AA (2007) Terrestrial vegetation of California. University of California Press, Los Angeles

Batten KM (2008) Soil microbial community associated with an invasive grass differentially impacts native plant performance. Microb Ecol 55:220–228

Bever JD (1994) Feedback between plants and their soil communities in an old field community. Ecology 75:1965–1977

Bever JD, Westover KM, Antonovics J (1997) Incorporating the soil community into plant population dynamics: the utility of the feedback approach. J Ecol 85:561–573

Bever JD, Platt TG, Morton ER (2012) Microbial population and community dynamics on plant roots and their feedbacks on plant communities. Annu Rev Microbiol 66:265–283

Bezemer TM, van der Putten WH (2007) Ecology: diversity and stability in plant communities. Nature 446:E6–E7

Blaschke H (1991) Multiple mycorrhizal associations of individual calcicole host plants in the alpine grass-heath zone. Mycorrhiza 1:31–34

Blaszkowski J (1994) Arbuscular fungi and mycorrhizae (*Glomales*) of the Hel Peninsula, Poland. Mycorrhiza 5:71–88

Busby R, Stromberger M, Rodriguez G, Gebhart D, Paschke M (2013) Arbuscular mycorrhizal fungal community differs between a coexisting native shrub and introduced annual grass. Mycorrhiza 23:129–141

Çakmakçi R, Dönmez F, Aydın A, Şahin F (2006) Growth promotion of plants by plant growth-promoting rhizobacteria under greenhouse and two different field soil conditions. Soil Biol Biochem 38:1482–1487

Callaway RM, Thelen GC, Barth S, Ramsey PW, Gannon JE (2004a) Soil fungi alter interactions between the invader *Centaurea maculosa* and North American natives. Ecology 85:1062–1071

Callaway RM, Thelen GC, Rodriguez A, Holben WE (2004b) Soil biota and exotic plant invasion. Nature 427:731–733

Callaway RM, Bedmar EJ, Reinhart KO, Silvan CG, Klironomos J (2011) Effects of soil biota from different ranges on *Robinia* invasion: acquiring mutualists and escaping pathogens. Ecology 92:1027–1035

Casper BB, Castelli JP (2007) Evaluating plant-soil feedback together with competition in a serpentine grassland. Ecol Lett 10:394–400

Chesson P (2000) Mechanisms of maintenance of species diversity. Annu Rev Ecol Syst 31:343–366

Dickens SJM, Allen EB, Santiago LS, Crowley D (2013) Exotic annuals reduce soil heterogeneity in coastal sage scrub soil chemical and biological characteristics. Soil Biol Biochem 58:70–81

Gucwa-Przepióra E, Błaszkowski J, Kurtyka R, Małkowski L, Małkowski E (2013) Arbuscular mycorrhiza of *Deschampsia cespitosa* (Poaceae) at different soil depths in highly metal-contaminated site in southern Poland. Acta Soc Bot Pol 82(4): 251–258

Hawkes CV, Belnap J, D'Antonio C, Firestone MK (2006) Arbuscular mycorrhizal assemblages in native plant roots change in the presence of invasive exotic grasses. Plant Soil 281:369–380

Heady HF (1958) Vegetational changes in the California annual type. Ecology 39(3):402–416

Hodge A, Fitter AH (2013) Microbial mediation of plant competition and community structure. Funct Ecol 27:865–875

Johnson NC, Rowland DL, Corkidi L, Allen EB (2008) Characteristics of plant winners and losers during grassland eutrophication – importance of biomass allocation and mycorrhizal function. Ecology 89:2868–2878

Kardol P, Cornips NJ, van Kempen MML, Bakx-Schotman JMT, van der Putten WH (2007) Microbe-mediated plant-soil feedback causes historical contingency effects in plant community assembly. Ecol Monogr 77:147–162

Keane RM, Crawley MJ (2002) Exotic plant invasions and the enemy release hypothesis. Trends Ecol Evol 17:164–170

Klironomos JN (2002) Feedback with soil biota contributes to plant rarity and invasiveness in communities. Nature 417: 67–70

Kormanik PP, McGraw AC (1982). "Quantification of vesicular-arbuscular mycorrhizae in plant roots". In: Schenck NC (ed) Methods and principles of mycorrhizal research. St. Paul, Am Phytopathol Soc pp, 37–45

Koske RE, Gemma JN (1989) A modified procedure for staining roots to detect VA-Mycorrhizas. Mycol Res 92:486–505

Kosola KR, Eissenstat DM, Graham JH (1995) Root demography of mature citrus trees—the influence of *Phytophthora nicotianae*. Plant Soil 171:283–288

Kulmatiski A, Beard KH, Stevens JR, Cobbold SM (2008) Plant-soil feedbacks: a meta-analytical review. Ecol Lett 11:980–992

McGonigle TP, Fitter AH (1990) Ecological Specificity of Vesicular Arbuscular Mycorrhizal Associations. Mycol Res 94:120–122

McGonigle TP, Miller MH, Evans DG, Fairchild GL, Swan JA (1990) A new method which gives an objective-measure of colonization of roots by vesicular arbuscular mycorrhizal fungi. New Phytol 115:495–501

Minnich RA (2008) California's fading wildflowers: lost legacies and biological invasions. University of California Press, Berkeley

Molina RJ, Trappe JM, Strickler GS (1978) Mycorrhizal fungi associated with *Festuca* in the western United States and Canada. Can J Bot 56:1691–1695

Mummey DL, Rillig MC (2006) The invasive plant species *Centaurea maculosa* alters arbuscular mycorrhizal fungal communities in the field. Plant Soil 288:81–90

Nelson JW, Zinn CJ, Strahorn AT, Watson EB, Dunn JE (1919) Soil survey of the Los Angeles area, California. Advanced sheets-field operations of the bureau of soils, 1916. United States Department of Agriculture, Washington

Pantone DJ, Pavlik BM, Kelley RB (1995) The reproductive attributes of an endangered plant as compared to a weedy congener. Biol Conserv 71:305–311

Persmark L, Banck A, Andersson S, Jansson HB (1992) Evaluation of methods for extraction of nematodes and endoparasitic fungi from soil. Nematologica 38:520–530

Powell CLI (1979) Inoculation of white clover and ryegrass seed with mycorrhizal fungi. New Phytol 83:81–85

R Development Core Team (2013). R: a language and environment for statistical computing. R foundation for statistical computing, Vienna, Austria. http://www.R-project.org/

Rabatin SC, Stinner BR, Paoletti MG (1993) Vesicular-arbuscular mycorrhizal fungi, particularly *Glomus-tenue*, in Venezuelan bromeliad epiphytes. Mycorrhiza 4:17–20

Reinhart KO (2012) The organization of plant communities: negative plant-soil feedbacks and semiarid grasslands. Ecology 93:2377–2385

Reinhart KO, Tytgat T, Van der Putten WH, Clay K (2010) Virulence of soil-borne pathogens and invasion by *Prunus serotina*. New Phytol 186:484–495

Richardson DM, Allsopp N, D'Antonio CM, Milton SJ, Rejmanek M (2000) Plant invasions—the role of mutualisms. Biol Rev 75:65–93

Rillig MC, Field CB, Allen MF (1999) Fungal root colonization responses in natural grasslands after long-term exposure to elevated atmospheric CO2. Glob Chang Biol 5:577–585

Shannon S, Flory SL, Reynolds H (2012) Competitive context alters plant-soil feedback in an experimental woodland community. Oecologia 169:235–243

Siguenza C, Corkidi L, Allen EB (2006) Feedbacks of soil inoculum of mycorrhizal fungi altered by N deposition on the growth of a native shrub and an invasive annual grass. Plant Soil 286:153–165

Smith SE, Read D (2008) Mycorrhizal Symbiosis. Elsevier Ltd, London

Sturtz S, Ligges U, Gelman A (2005) R2WinBUGS: a package for running WinBUGS from R. J Stat Softw 12(3):1–16

van der Putten WH, Peters BAM (1997) How soil-borne pathogens may affect plant competition. Ecology 78:1785–1795

Van der Putten WH, Van Dijk C, Peters BAM (1993) Plant-specific soil-borne diseases contribute to succession in foredune vegetation. Nature 362:53–56

van der Putten WH, Bardgett RD, Bever JD, Bezemer TM, Casper BB, Fukami T, Kardol P, Klironomos JN, Kulmatiski A, Schweitzer JA, Suding KN, Van de Voorde TFJ, Wardle DA (2013) Plant-soil feedbacks: the past, the present and future challenges. J Ecol 101:265–276

van Grunsven RHA, van der Putten WH, Bezemer TM, Tamis WLM, Berendse F, Veenendaal EM (2007) Reduced plant-soil feedback of plant species expanding their range as compared to natives. J Ecol 95:1050–1057

Vogelsang KM, Bever JD (2009) Mycorrhizal densities decline in association with nonnative plants and contribute to plant invasion. Ecology 90:399–407

Webster J, Weber RWS (2007) Introduction to Fungi. Cambridge University Press, Cambridge, 841 p

Zubek S, Blaszkowski J, Delimat A, Turnau K (2009) Arbuscular mycorrhizal and dark septate endophyte colonization along altitudinal gradients in the tatra mountains. Arct Antarct Alp Res 41:272–279