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ORIGINAL PAPER

Nitrogen enrichment contributes to positive responses to soil microbial communities in three invasive plant species

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Abstract Increased resource availability and feedbacks with soil biota have both been invoked as potential mechanisms of plant invasion. Nitrogen (N) deposition can enhance invasion in some ecosystems, and this could be the result of increased soil N availability as well as shifts in soil biota. In a two-phase, full-factorial greenhouse experiment, we tested effects of N availability and N-impacted soil communities on growth responses of three Mediterranean plant species invasive in California: *Bromus diandrus*, *Centaurea melitensis*, and *Hirschfeldia incana*. In the first phase, plants were grown individually in pots and inoculated with sterile soil, soil from control field plots or soil from high N addition plots, and with or without supplemental N. In the second phase, we grew the same species in soils conditioned in the first phase. We hypothesized growth responses would differ across species due to species-specific relationships with soil biota, but overall increased N availability and N-impacted soil communities would enhance plant growth. In the first phase, *Centaurea* had the greatest growth response when inoculated with N-impacted soil, while *Bromus* and *Hirschfeldia* performed best in low N soil communities. However, in phase two all species exhibited positive growth responses in N-impacted soil communities under high N availability. While species may differ in responses to soil biota and N, growth responses to soils conditioned by conspecifics appear to be most positive in all species under high N availability and/or in soil communities previously impacted by simulated N deposition. Our results suggest N deposition could facilitate invasion due to direct impacts of soil N enrichment on plant growth, as well as through feedbacks with the soil microbial community.

Keywords Nitrogen deposition · Plant–soil feedbacks · Arbuscular mycorrhizal fungi · Invasive species · Coastal sage scrub

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Introduction

Resource availability plays a key role in mediating nonnative plant invasion (Davis et al. 2000; Huenneke et al. 1990; Vitousek and Walker 1989), and elevated soil nitrogen (N) due to anthropogenic N deposition may increase community invasibility (Dukes and Mooney 1999; Fenn et al. 2003; Weiss 1999). Feedbacks between plants and soil biota can also influence invasion success (Callaway et al. 2004; Klironomos 2002; Pringle et al. 2009; Van der Putten et al. 2007), and N deposition might alter soil microbial communities in ways that

could further promote invasion (Egerton-Warburton and Allen 2000; Egerton-Warburton et al. 2001; Sigüenza et al. 2006a, b). However, the response of invasive plants to increased soil N and N-impacted soil communities is likely species-specific, and once established plants can exert strong effects on soil biota (Belnap et al. 2005; Hawkes et al. 2005; Kourtev et al. 2002) that might influence subsequent growth and performance of conspecifics. In this study, we asked how N deposition influences the response of three invasive plant species in southern California to native soil communities and how grow responses change once invasives have conditioned soils.

The invasion of ecosystems by nonnative species poses a serious ecological and economic threat worldwide (Pimentel et al. 2000; Vitousek et al. 1997b), and multiple hypotheses have been invoked in an attempt to identify generalizable mechanisms. The theory of fluctuating resource availability (Davis et al. 2000) posits that a community becomes more invasible as unused resource availability increases. Under this hypothesis, the release or enrichment of a resource is expected to enhance the performance of arriving invaders (Davis et al. 2000). Most terrestrial ecosystems are N limited, and multiple studies across biomes have found increased invasion with N addition (Brooks 2003; Burke and Grime 1996; Rao and Allen 2010; Wedin and Tilman 1996). As global rates of N deposition continue to rise (Galloway et al. 2004), this could increase the success of future invasions (Bradley et al. 2010; Dukes and Mooney 1999).

In addition to resource availability, invasion success can be strongly influenced by biotic factors, including soil fungi (Callaway et al. 2004; Klironomos 2002; Pringle et al. 2009; Reinhart and Callaway 2006). Species differ in their associations with soil biota, such as mutualists and pathogens, and therefore their effects on soil microbial communities, as well as their responses to these changes, resulting in plant–soil feedbacks (Bever et al. 1997). For example, a plant species may accumulate host-specific soil pathogens that inhibit the subsequent growth of conspecifics, resulting in a negative feedback (Mills and Bever 1998). Conversely, plants may promote the accumulation of beneficial soil mutualists, such as arbuscular mycorrhizal (AM) fungi, resulting in positive feedback (Zhang et al. 2010). Furthermore, the influence plants exert on soils will also impact the performance of other plant species in the community, and may be an important ecological driver of community-level processes, such as invasion (Bever et al. 1997; Klironomos 2002).

Feedbacks between plants and soil biota have been hypothesized to be an important driver of plant invasion, with many invasive species exhibiting positive responses to soil communities (Klironomos 2002; Levine et al. 2006; Reinhart and Callaway 2006). For example, de la Pena et al. (2010) found that despite initial biotic resistance of native soils to invasion by *Carpobrotus edulis* in Mediterranean sand dunes, once this species establishes, biotic resistance is diminished through the promotion of a soil community that enhances growth of this species, to the detriment of native plant species. Introduced plants may benefit from a lack of host-specific soil pathogens, promoting their invasiveness (Keane and Crawley 2002; Mitchell and Power 2003). However, while release from natural enemies belowground may contribute to positive feedback in invasive plants (Agrawal et al. 2005; Klironomos 2002; Kulmatiski et al. 2008), mutualists, such as AM fungi, can also be involved. These fungi form symbiotic relationships with plant roots, enhancing nutrient and water uptake in exchange for photosynthetic carbon (Smith and Read 2010). Arbuscular mycorrhizae play an important role in shaping plant community structure and diversity (van der Heijden et al. 1998), and may be critical determinants of invasion success (Callaway et al. 2004; Pringle et al. 2009).

Invasion can be influenced by AM fungi in a number of ways. Some nonnatives can reduce mycorrhizal density, and as invasive species are often less dependent on these associations, this results in increased performance relative to mycorrhizal natives (Callaway et al. 2008; Owen et al. 2013; Stinson et al. 2006; Vogelsang and Bever 2009). This interaction forms the basis of the "degraded mutualism" hypothesis (Vogelsang and Bever 2009). Alternatively, invading plant species may alter the mycorrhizal community by selecting for the most beneficial fungal species present, thereby reinforcing their competitive dominance, a phenomenon referred to as the "enhanced mutualism" hypothesis (Reinhart and Callaway 2006; Zhang et al. 2010). Finally, AM fungi can hinder invasion when a species promotes fungi that benefits heterospecifics more than conspecifics, contributing to a negative plant–soil feedback (Bever 2002). These responses will depend on a number of

factors, including the mycorrhizal status of the plant host, the identity of the host species and fungal symbiont(s) and the growth response of the plant (Pringle et al. 2009), as well as environmental conditions such as soil fertility (Hoeksema et al. 2010; Johnson et al. 1997).

Resource availability strongly influences interactions between plants and AM fungi (Hoeksema et al. 2010; Johnson et al. 1997), and this could impact the response of invading plants to native soil communities and the trajectory of plant invasions. Increased soil N due to anthropogenic N deposition may promote invasion via changes to the soil community. For example, high soil N may reduce root colonization and sporulation of some large-spored fungal species, thereby lowering mycorrhizal diversity and concomitantly increasing the proportion of small-spored fungal species, which has been shown to favor invasive annual grasses over native plant species (Egerton-Warburton and Allen 2000; Egerton-Warburton et al. 2001; Sigüenza et al. 2006b). Nutrient limitation may be a driver of local adaptation in mycorrhizae (Johnson et al. 2010), and increased soil N may also select for less beneficial fungi, resulting in a parasitic, rather than mutualistic, relationship (Johnson et al. 1997).

In southern California, anthropogenic N deposition has been implicated in the widespread invasion of coastal sage scrub (CSS) by annual grasses and forbs native to the Mediterranean Basin (Cox et al. 2014; Fenn et al. 2003; Goldstein and Suding 2014; Kimball et al. 2014; Talluto and Suding 2008). While elevated soil N appears to favor invasives in this system, there is also evidence that the soil microbial community plays an important role mediating these effects (Bozzolo and Lipson 2013; Egerton-Warburton and Allen 2000; Sigüenza et al. 2006a). Research in this ecosystem has demonstrated differential responses of both native and nonnative plant species to microbial inoculation and N addition (Padgett and Allen 1999; Yoshida and Allen 2001; Sigüenza et al. 2006a, b; Bozzolo and Lipson 2013), strongly suggesting a potential influence of N deposition on invasion though plant–soil feedbacks (Bever et al. 1997). For example, Sigüenza et al. (2006a, b) found that growth of a native CSS species was inhibited by N-impacted soil communities, while an invasive annual grass, *Bromus madritensis*, responded positively.

The purpose of this study was to understand the effects of soil N availability and N-impacted soil communities on the performance of three invasive plant species of southern California and how plant responses to inoculation and N addition might change once plants have established and conditioned the soil, with a focus on soil fungi. This was completed in a two-phase factorial greenhouse experiment. In the first phase, we grew individuals of three invasive plant species in pots inoculated with soil from field plots subject to simulated N deposition, control field plots or sterile soil, and plants were grown with or without supplemental N addition. In the second phase, we grew the same species in soils conditioned in phase one, under the same conditions. We hypothesized that higher N availability would lead to increases in plant growth overall, but that responses would differ across plant species, due to species-specific responses to soil biota and N availability. We also hypothesized growth responses to live soil treatments would remain positive once plants had conditioned the soil, especially under high N availability and in soils previously impacted by N enrichment. An alternative hypothesis is that growth responses will be negative once plants have conditioned the soil, due to accumulation of soil pathogens.

Materials and methods

Study site

Soil to be used as whole-soil inoculum was collected from plots that are part of an experimental N addition gradient, located in the foothills of the Santa Monica Mountains, California (34.15°N, 118.96°W). The site receives approximately 8.8 kg N ha^{-1} yr^{-1} of background N deposition, most of which falls as dry deposition (Tonnesen et al. 2007). Typical of a Mediterranean climate, precipitation is seasonal, falling mostly during the cooler winter months, and summers are hot and dry. Average yearly rainfall for the site is 420 mm, but has been below average since 2012, with rainfall totals during the winter growing season, November to May, ranging from 33 to 44 % of 30-year normals. Vegetation at the site is mature CSS with *Artemisia californica* the dominant shrub species. Native and nonnative annuals and herbaceous perennials are present in shrub interspaces.

Study species

We selected three nonnative plant species for our study: *Bromus diandrus* Roth (Poaceae), *Centaurea melitensis* L. (Asteraceae) and *Hirschfeldia incana* L. (Brassicaceae). These species are native to the Mediterranean Basin and invasive in California. They are also three of the dominant nonnatives at our field site and represent a variety of plant functional groups. *Bromus* is an annual C_3 grass, *Centaurea* is an annual forb and *Hirschfeldia* is an annual to perennial forb. Seeds of each species were collected to be used in our growth experiment from invaded CSS adjacent to our experimental N addition plots during spring and summer 2013.

Soil inoculum

We collected soil from plots that receive supplemental N and unfertilized control as part of a multi-year N addition experiment. Fertilized plots have received 3 g N m^{-2} annually in the fall since 2011 to simulate the accumulation of dry N deposition during the summer dry period. This is equivalent to 30 kg N ha^{-1} yr^{-1}, which is the high range of modeled N deposition rates in the region (Fenn et al. 2010; Tonnesen et al. 2007). In December 2013, we collected five 10 cm deep soil cores from around the drip-line of mature *Artemisia* shrubs within 10 replicate plots for each treatment. We collected soil inoculum from the drip-line of shrubs, as this area is likely to have actively growing roots, and therefore presumably higher levels of microbial activity compared to shrub interspaces or soils with older, suberized roots. Soil was transported back to the lab where it was sieved through a 1 cm^2 stainless steel mesh. Root fragments were cut into 1–2 cm fragments and mixed back into the inoculum. We homogenized soil from each of the two plot types (N fertilized and unfertilized controls) to produce two whole-soil inocula with soil microbial communities characteristic of native CSS soils and N-impacted CSS soils. We also included a sterilized control treatment consisting of inocula from both sites which was steam-sterilized in a process including a 24 h steam-sterilization, followed by a 48 h incubation period and a second 24 h sterilization period. Total extractable N of the fertilized and unfertilized soils was 58.6 μg N g^{-1} (SE ± 8.9) and 19.8 μg N g^{-1} (SE ± 1.4). Sterilization did not result in a significant increase in total extractable N in either fertilized (t test; $t_6 = -0.003$, $P > 0.05$) or unfertilized inoculum (t test; $t_6 = 0.001$, $P > 0.05$). To account for differences in available N in the two live inocula, when adding these we also included the same amount of sterilized inoculum from the other plot type, prepared as follows:

1. CSS Inoculum: 25 g live soil from unfertilized control plots and 25 g sterilized soil from N addition plots added to each pot.
2. N + CSS Inoculum: 25 g live soil from N addition plots and 25 g sterilized soil from unfertilized control plots added to each pot.
3. Sterilized Control: 25 g steam sterilized soil from unfertilized control plots and 25 g sterilized soil from N addition plots added to each pot.

Growth experiments

We used a 1:1 mixture of field-collected soil from undisturbed CSS adjacent to experimental plots and silica sand for potting, to which live soil inocula was added. We mixed sand with field soil using an electric cement mixer and steam-sterilized it as described above. Sand was added to improve water drainage and infiltration and to facilitate the recovery of root biomass when plants were harvested. Total KCl extractable N of our soil was 15.0 μg N g^{-1} (SE ± 0.7). Each pot (650 ml Deepots; Steuwe and Sons, Corvallis, Oregon, USA) was filled with 500 g of soil mix, with inoculum mixed in about 10 cm from the soil surface. Growth experiments were conducted from November 2013 to February 2014 in a greenhouse at the University of California, Riverside. High and low daily greenhouse temperatures during this time were approximately 20 and 16 °C.

Phase 1

In the first phase, we grew 20 replicates of each species (3) by inoculum (3) by N (2) treatment (total number of pots = 360). Seeds of each species were sown directly in pots and watered with distilled water until germination, about 5 days for all species. Once seedlings emerged, we thinned them to a density of one per pot. We then switched to watering as needed every 3–5 days with distilled water and initiated N treatments. Half of the pots in each soil inoculum

treatment received two applications of supplemental N to simulate high soil N availability as a result of elevated N deposition early in the growing season. The first N treatment was applied after plants had established in each pot and the second 10 days later. Each time we applied approximately 20 μg N g^{-1} of soil from ammonium nitrate (NH_4NO_3) in solution to the high N pots, for a total of 40 μg N g^{-1}. Low N pots received an equal amount of distilled water each time. Twice per week, we rotated pots within racks and randomly re-distributed pot racks on greenhouse benches to account for differences in microclimate. After 60 days of growth, we clipped plant shoots off at the soil surface from all pots. Ten replicates of each treatment were allocated for root and soil analyses and the other ten for Phase 2 of the experiment.

Phase 2

In the second part of the experiment, we examined the response of our study species to the soils conditioned in Phase 1. After plant shoots were harvested from Phase 1, the ten replicates of each treatment reserved for Phase 2 were allowed to air-dry on greenhouse benches for two weeks. Soils within pots were left intact, and thus contained the root systems of plants grown in Phase 1. Prior to planting, we added a CSS nutrient solution (Padgett and Allen 1999; Table 1), so nutrients other than N would not be limiting to plant growth. Mean total extractable N in pots at the end of Phase 1 ranged from 2.4 to 5.2 μg N g^{-1} and did not differ significantly among species or treatments (ANOVA; $F_{17,72} = 1.33$, $P = 0.2753$). All pots also received approximately 10 μg N g^{-1} in solution from NH_4NO_3, which restored available N to levels comparable to the beginning of Phase 1. We then re-seeded conspecifics into pots and maintained the same watering and N fertilization regime as in Phase 1under identical greenhouse conditions. We again grew plants for 60 days before harvesting.

Table 1 Modified coastal sage scrub nutrient solution from Padgett and Allen (1999)

Element	Nutrient solution (mM)	Specific compound
Cl	3	HCl
Ca	1.2	$CaCO_3$
S	1.2	$MgSO_4$
Na	1	NaOH
Mg	0.6	MgO
P	0.16	KH_2PO_4
K	0.14	KCl
B	0.003	H_3BO_3
Zn	0.001	$ZnCl_2$
Mn	0.0001	$MnSO_4$
Cu	0.0001	$CuSO_4$
Fe	5 mg l^{-1}	Fe EDTA

Harvests

At each harvest, we separated plants into roots and shoots. Roots were carefully removed from the soil and washed with distilled water. Roots and shoots were dried at 60° C and weighed. We estimated root biomass in the pots at the end of Phase 1 that were reserved for Phase 2 using regressions of root and shoot biomass of fully harvested plants. Dried leaf tissue from was ground and analyzed for percent C and N using a Thermo-Finnigan FlashEA 1112 Nitrogen and Carbon Analyzer (Thermo Fisher Scientific, Waltham, Massachusetts, USA) at the University of California, Riverside, Environmental Science Research Laboratory.

Root colonization

After drying and weighing roots, we rehydrated them in distilled water and stained them with trypan blue (Koske and Gemma 1989). For each plant, we mounted ten randomly selected fine root fragments on slides and assessed percent colonization of mycorrhizal and other non-mycorrhizal fungi in a procedure based on McGonigle et al. (1990). For each fragment, we made ten observations and noted presence or absence of fungi within roots. We distinguished between coarse AM fungi, fine AM endophyte and other fungi based on staining, hyphal diameter and morphology and the presence or absence of septa. Percent colonization of all fungi was low (<2.5 %) in roots of plants grown in sterile soil at each harvest and these were not included in our statistical analyses of percent colonization.

Calculation of inoculum response

We calculated relative inoculum responses in order to better illustrate the response of our study species to the different live soil inoculum treatments and understand

how these relationships vary with N availability. Relative inoculum response was calculated as the difference in total biomass of plants grown in live soil inoculum relative to the mean total biomass of those grown in sterilized soil under the same N availability:

$$\textit{Relative inoculum response} = \frac{(\textit{Biomass}_{\textit{Live}} - \textit{Mean Biomass}_{\textit{Sterile}})}{\textit{Mean Biomass}_{\textit{Sterile}}}$$

Using this calculation, a value greater than zero indicates a positive response to soil inoculation relative to sterile controls, while a value less than zero indicates a negative response.

Statistical analysis

Our experimental design included three species, *Bromus*, *Centaurea*, and *Hirschfeldia*, grown in each of the three soil inoculum treatments (CSS Inoculum, N + CSS Inoculum and Sterile) and with or without supplemental N (High N and Low N) in a full factorial design. For each harvest, we analyzed data for the three species separately, performing two-way ANOVA with inoculum, N and the interaction between inoculum and N as factors. Relative inoculum responses for each harvest were also analyzed using two-way ANOVA, with species included as a factor along with inoculum type and N treatment. Prior to analysis, data were tested for the assumptions of ANOVA and transformed as necessary (log, arcsine, square root). Following ANOVA, we performed Tukey's honest significant difference test (HSD) to compare means and assign significance at $P < 0.05$. We also ran regressions to compare biomass from Phase 1 with biomass in Phase 2. Analyses were performed using RStudio Version 0.98.57, RStudio, Inc.

Results

Phase 1

During Phase 1 of the experiment, growth of *Bromus* was significantly affected by inoculum treatment ($P < 0.0001$) but not by N availability ($P = 0.11$) or the interaction of inoculum and N ($P = 0.83$; Table 2; Fig. 1a). Plants grown in the CSS inoculum had the greatest aboveground biomass and were on average 21 % larger than those inoculated with N + CSS soil inoculum and 49 % larger than plant grown in sterile soil (Fig. 1a). Belowground biomass was significantly influenced by inoculum type ($P < 0.0001$), with plants grown in CSS inoculum having higher mean root biomass ($F_{5,149} = 18.24$, $P < 0.0001$; Table 3). Percent N content of leaves was not significantly affected by inoculum or N individually ($P > 0.05$), but was influenced by their interaction ($P = 0.0067$), with N addition leading to a significant increase in tissue N only in sterile soil ($F_{5,23} = 3.71$, $P = 0.0175$; Fig. 2a). We observed very low levels of root colonization by AM fungi (<2.2 %) and 5.2–10.6 % colonization by fine AM endophytic fungi, with no significant differences among treatments (Table 4). Colonization by nonmycorrhizal fungi was significantly affected by inoculum type, with plants grown in N + CSS inoculum on average 45 % more colonized (Table 4).

Shoot biomass of *Centaurea* was significantly affected by both inoculum type ($P < 0.0001$) and N treatment ($P = 0.0088$; Table 2). Relative to plants grown in sterile soil, shoot biomass was on average 84 % higher in N + CSS inoculated plants and 79 % higher in CSS inoculum, indicating a positive growth response in both live soil inoculation treatments (Figs. 1b, 3a), with more positive responses observed under low N availability. Belowground biomass was significantly affected by inoculum type ($P < 0.0001$) and N treatment ($P = 0.0012$), but not the interaction ($P = 0.1662$), and followed a similar trend as aboveground biomass ($F_{5,149} = 34.56$, $P < 0.0001$; Table 3). Percent N of leaf tissue was generally higher in N fertilized plants, but this difference was only significant in the CSS inoculum treatment ($F_{5,23} = 5.70$, $P = 0.0025$; Fig. 2b). *Centaurea* had the highest levels of colonization by AM fungi of the three species, but there were no significant differences among treatments within *Centaurea* (Table 4). Colonization by fine AM endophyte was significantly higher in plants inoculated with N + CSS soil, with the highest percent colonization occurring in plants also receiving supplemental N (Table 4).

Both inoculum ($P = 0.0003$) and N ($P = 0.0156$) significantly influenced growth of *Hirschfeldia* (Table 2; Fig. 1c). The highest shoot biomass was observed in plants inoculated with CSS soil and grown under high N (Fig. 1c), which were on average 46 %

Table 2 Results from two-way ANOVA tests of inoculum (I), nitrogen (N) and the interaction of inoculum and nitrogen (I × N) on aboveground plant biomass for each species in Phase 1 and Phase 2 of the experiment

Plant species	Source	Phase 1			Phase 2		
		df	*F*	*P*	*df*	*F*	*P*
Bromus diandrus	I	2	13.475	**<0.0001**	2	5.024	**0.0081**
	N	1	2.642	0.1069	1	238.084	**<0.0001**
	I × N	2	0.182	0.8342	2	38.108	**<0.0001**
Centaurea melitensis	I	2	117.166	**<0.0001**	2	28.619	**<0.0001**
	N	1	7.113	**0.0088**	1	44.102	**<0.0001**
	I × N	2	0.098	0.9072	2	2.623	0.0772
Hirschfeldia incana	I	2	8.907	**0.0003**	2	2.891	0.0599
	N	1	6.021	**0.0156**	1	152.242	**<0.0001**
	I × N	2	1.546	0.2176	2	14.552	**<0.0001**

Bolded values indicate statistical significance ($\alpha = 0.05$)

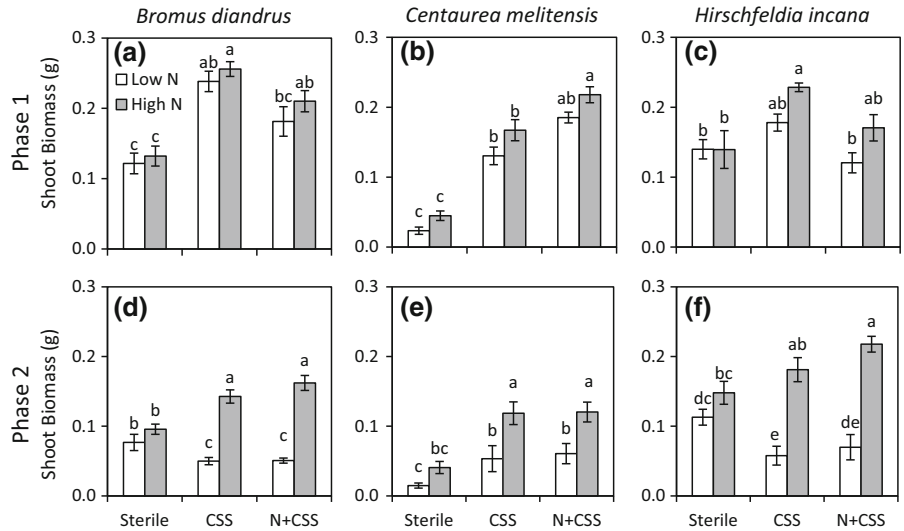

Fig. 1 Mean dry aboveground biomass of plants for each species grown in each inoculum type, native CSS soils (CSS), native soils subject to experimental N deposition (N + CSS) and sterile controls (Sterile) and with (*shaded bars* High N) or without (*white bars* Low N) supplemental N for Phase 1 (**a**–**c**) and Phase 2 (**d**–**f**). *Error bars* represent standard errors of the mean. *Letters* represent significant differences within species for each phase at $\alpha = 0.05$ from ANOVA

larger than those grown in sterile soil. There were no other significant differences in shoot growth among treatments (Fig. 1c). There were also no significant differences in belowground biomass across treatments ($F_{5,133} = 2.14$, $P = 0.0646$; Table 3). Percent N of leaves was significantly affected by the interaction of inoculum type and N ($P = 0.0174$), with the highest N content found in plants grown in sterile soil with supplemental N ($F_{5,23} = 3.08$, $P = 0.0349$; Fig. 2c). *Hirschfeldia* had very low levels (<0.6 %) of AM and fine endophytic fungi. There was a significant inoculum by N effect on the percent colonization of nonmycorrhizal fungi, with low N plants grown in CSS soil having the highest colonization (Table 4).

Across all species and treatments in Phase 1, relative inoculum responses were significantly ($F_{11,239} = 85.98$, $P < 0.0001$) affected by species identity ($P < 0.0001$), N ($P = 0.0005$) and the interaction of species and inoculum ($P < 0.0001$), species and N ($P < 0.0001$) and species, inoculum and N ($P = 0.0459$). *Bromus* exhibited a positive growth response to N + CSS inoculum only when N fertilized, but had a positive response to the native CSS inoculum regardless of N availability (Fig. 3a). *Centaurea* plants had a positive growth

Table 3 Belowground biomass (g) for each species (n = 20) grown in three inoculum types, native CSS soils (CSS), native soils subject to experimental N deposition (N + CSS) and a sterile control (Sterile) and under high and low N availability (Low N, High N) in Phase 1 of the experiment

Plant species	Inoculum treatment					
	CSS		N + CSS		Sterile	
	Low N	High N	Low N	High N	Low N	High N
Bromus diandrus	**0.23a** ± 0.01	**0.26a** ± 0.02	**0.17b** ± 0.02	**0.15b** ± 0.01	**0.10b** ± 0.01	**0.11b** ± 0.01
Centaurea melitensis	**0.14b** ± 0.01	**0.20ab** ± 0.03	**0.20ab** ± 0.01	**0.27a** ± 0.01	**0.02c** ± 0.01	**0.03c** ± 0.01
Hirschfeldia incana	0.35 ± 0.02	0.39 ± 0.01	0.42 ± 0.03	0.29 ± 0.05	0.41 ± 0.04	0.41 ± 0.08

Bolded values indicate statistical significance ($\alpha = 0.05$)

Letters within rows represent significant differences at $\alpha = 0.05$ from ANOVA

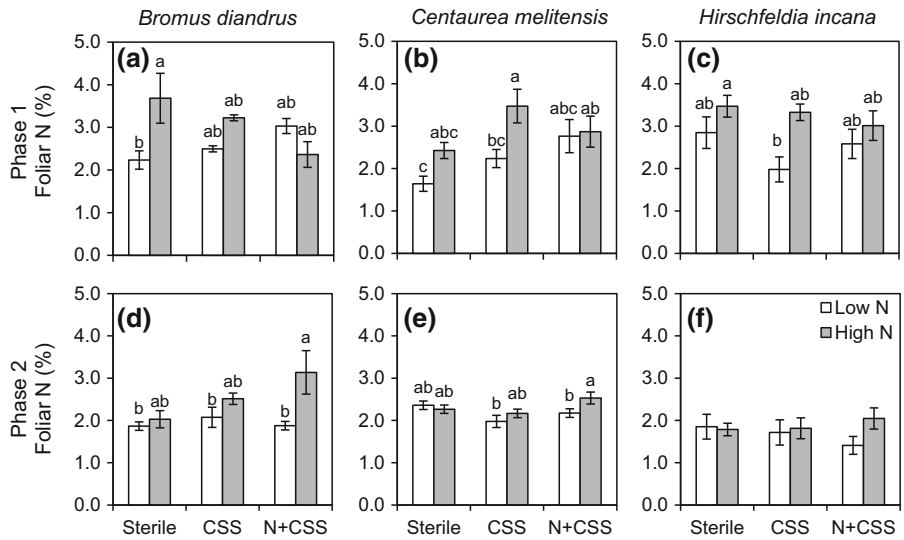

Fig. 2 Percent foliar N of leaf tissue for each species (n = 5) grown in three inoculum types, native CSS soils (CSS), native soils subject to experimental N deposition (N + CSS) and a sterile control (Sterile) and under high and low N availability (*shaded bars* High N; *white bars* Low N) for Phase 1 (**a**–**c**) and Phase 2 (**d**–**f**). *Error bars* represent standard errors of the mean. *Letters* represent significant differences within species for each phase at $\alpha = 0.05$ from ANOVA

response to both soil inoculation types, but this was reduced in plants receiving supplemental N. *Hirshcfeldia* plants grown in CSS inoculum under high N exhibited a positive inoculum response, but in all other live inoculum treatments plants showed a neutral response to inoculation.

Phase 2

In the second phase of the experiment, inoculum ($P = 0.0081$), N ($P < 0.0001$) and the interaction of these two factors ($P < 0.0001$) had significant effects on growth of *Bromus* (Table 2; Fig. 1d). The highest shoot biomass was observed in the two live inoculum treatments under high N, where biomass was 65 and 69 % higher in plants receiving supplemental N in the CSS and N + CSS inoculated plants respectively (Fig. 1d). Plants receiving supplemental N had higher percent N content in leaf tissue in the N + CSS inoculum, but not in any other soil treatment ($F_{5,23} = 3.77$, $P = 0.0164$; Fig. 2d). In this phase, plants grown in the N + CSS soil under high N availability had significantly higher colonization of fine endophyte compared to low N plants (Table 4). Plants

Table 4 Mean (n = 10) percent colonization of roots by arbuscular mycorrhizal (AM) fungi, fine endophytic (FE) fungi and nonmycorrhizal (NM) fungi for each species inoculated with native coastal sage scrub soil (CSS) or native soils impacted by simulated nitrogen deposition (N + CSS) and grown with (High N) or without (Low N) supplemental nitrogen for Phase 1 and Phase 2 of the experiment

Plant species	Phase 1							Phase 2						
	CSS		N + CSS		I	N	I × N	CSS		N + CSS		I	N	I × N
	Low N	High N	Low N	High N				Low N	High N	Low N	High N			
Bromus diandrus														
AM fungi	2.0 ± 0.5	2.2 ± 0.7	1.6 ± 0.4	2.0 ± 1.1	–	–	–	0.6 ± 0.2	0.8 ± 0.6	0.6 ± 0.2	0.4 ± 0.2	–	–	–
FE fungi	7.0 ± 0.9	5.2 ± 2.4	6.2 ± 1.2	10.6 ± 2.0	–	–	–	6.2ab ± 0.8	5.6ab ± 0.8	5.0b ± 1.4	10.2a ± 1.8	–	–	*
NM fungi	10.0bc ± 1.3	8.0c ± 1.1	15.0ab ± 1.3	18.0a ± 2.3	***	–	–	50.4a ± 3.7	36.6ab ± 2.2	31.2b ± 4.0	34.8ab ± 7.3	*	–	–
Centaurea melitensis														
AM fungi	23.6 ± 2.7	33.2 ± 7.2	18.4 ± 3.9	20.4 ± 3.5	–	–	–	7.6ab ± 0.1	4.2ab ± 1.3	7.8a ± 2.1	1.8b ± 0.1	–	*	–
FE fungi	1.6c ± 0.2	2.2c ± 0.8	5.6b ± 0.7	10.2a ± 1.0	***	**	*	6.0b ± 2.1	18.6a ± 3.3	4.6b ± 1.4	12.4ab ± 1.4	–	*	–
NM fungi	12.8 ± 2.4	6.2 ± 1.7	10.2 ± 2.1	6.4 ± 1.1	–	–	–	12.6a ± 2.9	2.2b ± 0.1	7.0ab ± 1.1	9.6ab ± 1.6	–	*	**
Hirschfeldia incana														
AM fungi	0.6 ± 0.6	0.0	0.0	0.4 ± 0.2	–	–	–	0.2 ± 0.1	0.2 ± 0.1	0.4 ± 0.2	0.0	–	–	–
FE fungi	0.6 ± 0.4	0.2 ± 0.1	0.0	0.2 ± 0.1	–	–	–	0.2 ± 0.1	0.0	0.2 ± 0.1	0.2 ± 0.1	–	–	–
NM fungi	17.6a ± 4.0	4.0b ± 1.0	8.2ab ± 1.6	13.8a ± 1.6	–	–	***	5.6 ± 1.0	6.6 ± 2.3	10.4 ± 1.1	6.4 ± 1.0	–	–	–

Significant sources of variation are shown from ANOVA, including inoculum (I), nitrogen (N) and the interaction (I × N). Different letters within species for each phase represent values were significantly different at $\alpha = 0.05$

*,**,*** F ratios were significant at $P \leq 0.05$, 0.01 and 0.001, respectively

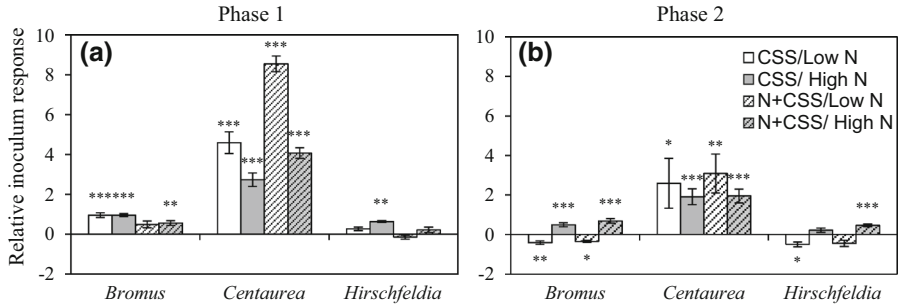

Fig. 3 Mean inoculum response for each species grown in each live inoculum type, native CSS soils (CSS = non-patterned bars) and native soils subject to experimental N deposition (N + CSS = patterned bars) and with (*shaded bars* High N) or without (*white bars* Low N) supplemental N for Phase 1 (**a**) and Phase 2 (**b**). *Error bars* represent standard errors of the mean. *Asterisks* indicate mean plant biomass was significantly different than that of plants grown in sterile controls under the same conditions (ANOVA, Tukey's HSD; $*P < 0.05$; $**P < 0.001$; $***P < 0.0001$)

grown in CSS soil without added N had the highest colonization of nonmycorrhizal fungi (Table 4).

Centaurea shoot biomass was again significantly affected by both inoculum ($P < 0.0001$) and N ($P < 0.0001$; Table 2; Fig. 1e). Effects of the two live inocula on shoot biomass were similar in both treatments, with N fertilized plants 50–55 % higher than low N plants in the CSS and N + CSS treatments respectively. Plants grown in sterile soil were again significantly smaller than those inoculated with live soil (Fig. 1e). Percent leaf N was highest in plants inoculated with N + CSS soil under high N availability ($F_{5,23} = 4.57$, $P = 0.0073$; Fig. 2e). Plants receiving supplemental N generally had lower colonization of AM fungi, but higher colonization by fine endophyte. In plants grown in CSS inoculum, colonization by nonmycorrhizal fungi was significantly reduced by N addition (Table 4).

In this phase, shoot biomass of *Hirschfeldia* was not significantly affected by inoculum type ($P = 0.0599$), but was influenced by N ($P < 0.0001$) and the interaction of inoculum and N ($P < 0.0001$; Table 2; Fig. 1f). In the two live inocula, N fertilized plants were on average 68 % larger than low N plants (Fig. 1f), but N had no significant effect on plants grown in sterile soil (Fig. 1f). There were no significant differences in percent leaf N across treatments ($F_{5,23} = 0.83$, $P = 0.5479$; Fig. 2f), and there were no effects of inoculum or N on percent colonization of roots by soil fungi (Table 4).

Across all species and treatments in Phase 2, relative inoculum responses were significantly ($F_{11,107} = 22.53$, $P < 0.0001$) affected by species identity ($P < 0.0001$), N ($P < 0.0001$) and the interaction of species and N ($P = 0.0003$). In this phase, *Bromus* exhibited a positive growth response in both live inoculum treatments when N fertilized, but a negative response when N availability was low (Fig. 3b). *Centaurea* plants again showed the most positive inoculum response, regardless of inoculum type, but this effect was minimized in plants receiving supplemental N (Fig. 3b). In the CSS soil inoculum, *Hirschfeldia* plants exhibited a negative growth response when N availability was low, but a neutral response under high N, while in the N + CSS soil inoculum, plants showed a positive inoculum response under high N availability and no response when N was low (Fig. 3b).

Finally, we explored potential relationships between plant performance in each phase using regression. For each species, biomass in Phase 2 was not significantly correlated with biomass, percent colonization of roots or extractable N of soils from Phase 1 ($P > 0.05$ for all correlations). We also did not find any significant correlations between plant biomass and percent colonization of mycorrhizal or nonmycorrhizal fungi in either phase of the experiment ($P > 0.05$).

Discussion

The success of invasive plants is often enhanced by soil N addition (Brooks 2003; Cox et al. 2014; Davis et al. 2000; Huenneke et al. 1990; Weiss 1999) and positive responses to soil biota (Klironomos 2002; Pringle et al. 2009; Reinhart and Callaway 2006; Van der Putten et al. 2007). Here we show that these two

factors may increase growth of three nonnative invaders in California, which could potentially influence plant–soil feedbacks between native and invasive species under chronic N deposition. Initially in Phase 1, all species exhibited neutral to positive responses to native soil communities. Contrary to our hypotheses, in the first phase of the experiment N addition was less important than soil community in determining plant growth, and only one of our species, *Centaurea*, performed better in native soil communities previously impacted by simulated N deposition. However, once species had conditioned soils, all exhibited positive growth responses in N-impacted soil communities under high N conditions. While positive responses to soil biota in invasive plants are often predicted (Klironomos 2002; Pringle et al. 2009; Reinhart and Callaway 2006), we found that when soil N was limiting, two species, *Bromus* and *Hirschfeldia* experienced neutral to negative inoculum responses. This work highlights the importance of soil N in determining plant responses to soil microbial communities and has important implications for plant invasion under anthropogenic N deposition.

Previous work has shown that the response of invasive species to N addition can be strongly influenced by the soil microbial community (Bozzolo and Lipson 2013; Sigüenza et al. 2006a; Yoshida and Allen 2001), and invasives may benefit from changes in the soil fungal community resulting from N deposition (Sigüenza et al. 2006a). However, plant responses may change once plants have conditioned soil communities, and this is the first study of which we are aware to utilize a two-phase approach to explore the effects of N enrichment on the response of invasive plant species to soil microbial communities. There is limited evidence that N-induced changes to plant–soil feedbacks may alter plant community structure (Manning et al. 2008), and the positive inoculum responses we observed under N enrichment may contribute to the success of these species in the field. In southern California, N deposition appears to facilitate the invasion of native CSS shrublands by nonnative grasses and forbs (Allen et al. 1996; Cox et al. 2014; Kimball et al. 2014), including the species investigated here, but the underlying mechanisms are not fully understood. This study provides evidence that N enrichment may enhance the growth of invasive plant species due to both elevated soil N availability and through plant-mediated changes to soil microbial communities.

Our results illustrate how the interacting dynamics of N deposition and plant responses to soil microbial communities might operate through multiple stages of the invasion process (Theoharides and Dukes 2007). The first phase of the experiment assessed the response of plants to soil communities of native CSS soils and native soils impacted by simulated N deposition. All species exhibited neutral to positive inoculum responses, showing soil communities of native CSS vegetation may not provide any "biotic resistance" against arriving invaders (Levine et al. 2004). However, the direction and magnitude of responses changed once plants had conditioned soils. Results from the second phase of the experiment show that once plants have established they may influence the subsequent performance of conspecifics through changes to the soil community, and N enrichment has the potential to alter these relationships. The negative inoculum responses observed in Phase 2 in the absence of N enrichment are particularly revealing. This shows that while invading species may initially respond positively to soil communities of a novel environment, as is often predicted (Klironomos 2002; Reinhart and Callaway 2006), they may limit the success of subsequent generations through feedbacks with soil biota. When N availability is high, however, these negative effects appear to weaken, indicating that in addition to enhancing soil N availability, N deposition may promote the success of invasives through changes to soil communities.

There are several strong lines of evidence that suggest observed inoculum responses are due to effects of soil biota on plant performance. First, while many studies take a "black box" approach to plant–soil interaction experiments (Ehrenfeld et al. 2005; van der Putten 2010), we found clear differences in mycorrhizal and nonmycorrhizal fungal colonization of plant roots that may partially explain growth responses to live soil inocula. Second, while plant biomass differed among treatments in the initial conditioning phase, the possibility that responses in Phase 2 are due to nutrient depletion can be excluded, as we found no negative correlations between biomass in Phase 2 with biomass in Phase 1 (Kardol et al. 2006; Pernilla Brinkman et al. 2010). Furthermore, nutrients (minus N) were added to simulate extractable levels of CSS soils (Padgett and Allen 1999). It also appears

unlikely that N accumulation in pots contributed to the greater N responses in Phase 2, as mean soil extractable N at the end of Phase 1 (beginning of Phase 2) was low and did not differ significantly among treatments. In addition to the effects of mycorrhizal and nonmycorrhizal fungi on inoculum responses, competition with soil microbes for soil N may have also played an important role (Kaye and Hart 1997). For example, in Phase 2, after two phases of N treatments, soil microbes may have been less N-limited, allowing greater for uptake by plants, resulting in great growth responses with N addition. It is interesting to note, however, that while growth responses were overall greater in high N inoculum in Phase 2, foliar N content was generally lower in all treatments. Finally, all species responded similarly to sterilized controls in both phases of the experiment, strongly suggesting that changes to inoculum responses were due to biotic factors, or interactions between soil biota and N availability.

Ecologists have long sought to identify generalizable mechanisms of plant invasion (Elton 2000; Levine et al. 2003), and the difficulty in doing so is likely due in part to species-specific variability in responses to biotic and abiotic factors. Species differ in effects on soil microbial communities and responses to soil biota, forming the basis of microbially mediated plant–soil feedbacks (Bever et al. 1997; Ehrenfeld et al. 2005). While feedbacks with soil biota may contribute to plant invasion (Klironomos 2002; van der Putten 2010), as is often predicted, the response of invasive species to soil microbial communities will depend on the identity of both plants and soil microbes (Klironomos 2003), as well as the biotic and abiotic context (Hoeksema et al. 2010), including nutrient availability (Johnson et al. 1997). Species exhibit various nutritional strategies and responses to soil nutrient availability (Chapin 1980), and these factors have the potential to interact (Bozzolo and Lipson 2013; Gustafson and Casper 2004; Innes et al. 2004; Sigüenza et al. 2006a). For example, Gustafson and Casper (2004) found that N addition negated negative feedback in one grass species, but had variable effects on another. In another experiment using *Centaurea* and a grass and mustard species closely related to the ones used here, *Brassica nigra* and *Bromus rubens*, Bozzolo and Lipson (2013) found *Brassica* and *Bromus* grew equally well in live and sterile soil, while *Centaurea* grew best in sterile soil. Further, only *Bromus* and *Centaurea* were responsive to N addition (Bozzolo and Lipson 2013). This stands in contrast to our results, where we observed positive inoculum responses in all species, especially under N addition. However this previous study only used soil collected from a low N deposition site, and did not include a soil conditioning phase (Bozzolo and Lipson 2013). While N enrichment appears to contribute to positive responses to soil biota in all three species studied here, species differed in their relationships with soil fungi. For example *Bromus* associated mostly with fine AM fungi, *Centaurea* formed mycorrhizae with both coarse and fine AM fungi and *Hirschfeldia* was found to be nonmycorrhizal. Such differences in microbial associations may be responsible for the variable growth responses observed among plant species.

The response of *Bromus* to live inoculum treatments appears to have been mediated by both mycorrhizal and nonmycorrhizal fungi. Initially, plants responded less positively to the N-impacted soil community relative to the unfertilized CSS soil community, and these plants also had significantly higher colonization of potentially pathogenic fungi. Then in Phase 2, plants exhibited a positive inoculum response in N-impacted soils under high N and had higher colonization of fine AM fungi and lower colonization of nonmycorrhizal fungi. Plants grown in CSS soils under low N availability showed a negative inoculum response and higher colonization of fungal pathogens. Hilbig and Allen (2015) also found that fine AM fungi contribute to positive inoculum responses in this species in soils from a high N deposition site, and this may counteract potential negative impacts of fungal pathogens. Others have reported higher mycorrhizal colonization of *Bromus* in N rich soils (Parker and D'antonio 2008), and this may be partially responsible for increased invasion of this species under N deposition (Going et al. 2009). Typically a reduction in mycorrhizal colonization is expected with N fertilization, as plants are able to directly take up sufficient N without the added carbon cost of mycorrhizal association (Smith and Read 2010). However, chronic N addition, may select for more aggressive AMF fungi that maintain high rates of colonization even when soil N availability is high (Corkidi et al. 2002; Johnson et al. 1997). While natives in this system may be negatively impacted by these AM fungal strains (Sigüenza et al. 2006a), invasives, such as *Bromus* may benefit, as

demonstrated here. Previous accounts regarding the response of this species to soil microbial inoculation is mixed, with some reporting little or no response to microbial inoculation (Vogelsang 2004) and others reporting a positive growth response relative to growth in sterile soil (Bennett and Strauss 2013; Hilbig and Allen 2015). Our results suggest that one possible cause of this variability in responses is differences in soil N availability or historical N inputs, with N-impacted soil communities promoting more positive plant growth responses. Plant soil feedbacks involving native species and invasive annual grasses could promote invasion of this ecosystem if invasive respond positively to soil communities. For example, Sigüenza et al. (2006a) found native species respond negatively to N-impacted soil communities, while an invasive grass, *B. madritensis* (L.), responded positively.

Previous studies have found *C. melitensis* to be highly mycorrhizal (Callaway et al. 2001, 2003). However, these two studies reported negative responses to colonization by AM fungi when plants were grown individually; responses were only positive when plants were grown in competition (Callaway et al. 2001, 2003). Bozzolo and Lipson (2013) found a different result, where plants performed best in sterile soil relative to those grown in live soil. Our results stand in contrast to this work and clearly demonstrate that this species can exhibit positive inoculum responses when grown individually, and these positive responses are likely due to colonization by mycorrhizal fungi. Initially, *Centaurea* was highly colonized by coarse AM fungi, however after soil conditioning, there was a shift to higher colonization of fine AM fungi, particularly when N availability was high. This shows that while this species may associate with species of AM fungi that predominate in native CSS soils (Egerton-Warburton and Allen 2000; Sigüenza et al. 2006a, b), it may select for species of fine AM fungi, especially under high N conditions. It is also possible that this species is a better competitor for N than soil microbes, and this could have been partially responsible for the observed increase in growth and foliar N in *Centaurea* plants receiving supplemental N. Regardless of differences among treatment, we observed a highly positive response to live soil inoculation across treatments and in both phases of the experiment. This clearly demonstrates that performance of this species may be enhanced by soil biota, which could generate positive feedbacks with fine AM fungi under some conditions.

One of the more surprising results of this study was the positive inoculum response observed in *Hirschfeldia* in high N treatments. Like many annual species in the Brassicaceae, *Hirschfeldia* does not form mycorrhizae (Gerdemann 1968; Oliveira et al. 2005), and our methods are unable to explain these positive inoculum responses. There are other accounts of this species responding positively to soil biota; Bonanomi et al. (2008) found that removal of the soil microbial community through soil solarization and chemical fumigation inhibited the growth of *Hirschfeldia* seedlings relative to untreated control plots in the field, but it is unknown what soil microorganisms were involved. The positive growth responses we observed only occurred in plants receiving supplemental N, and it is conceivable that growth promoting bacteria or other soil biota involved in nutrient cycling may have facilitated these growth responses. For example, rhizobacterium associating with a related species, *Brassica napus*, have been found to enhance N uptake and promote higher biomass (Bertrand et al. 2000). *Hirschfeldia* has been shown to be highly responsive to simulated N deposition in the field (Allen et al. 1996), and is a frequently observed nonnative in high N-polluted sites in southern California (Cione et al. 2002; Wood et al. 2006). While we are unable to explain the positive growth response of *Hirschfeldia* plants grown in N-impacted soil communities with the methods employed for this study, this could contribute to the success of this species under elevated N deposition.

While the positive inoculum responses we observed in these species could influence plant–soil feedbacks in the field, favoring invasion, a true test of plant–soil feedback requires pair-wise comparisons with native species, or at the very least, responses of species to soils conditioned by heterospecifics (Bever et al. 1997; Kulmatiski et al. 2008). Our experiment did not close this feedback loop, as we did not include native species in our design. There are also further limitations inherent to our experimental approach. We homogenized our field-collected inocula within treatments prior to addition to pots in Phase 1, which could confound results due to non-independence amongst replicates. While we focused on fungi colonizing plant roots, it is likely other soil organisms played an

important role. For example, competition between soil microbes and plants for N played a role in generating observed inoculum responses (Kaye and Hart 1997; Schimel et al. 1989), and this could explain the greater growth responses in high N treatments in Phase 2, if competition for N from microbes was less intense. However, we did not measure microbial N of soils and could not test this directly. Our approach also did not incorporate other factors that could influence plant–soil interactions, such as competition between plants or litter inputs, and further research is needed to determine the importance of these processes in the field.

Conclusions

Global change factors such as N deposition may increase the success of biological invasions (Dukes and Mooney 1999), but effects of N enrichment on plant performance is often mediated by soil biota. The results of this study clearly demonstrate N enrichment can alter interactions between three invasive plant species and soil microbial communities. These relationships have important implications for community invasibility and invasion success under N deposition. Invasive plants benefitting from increased soil N availability and positive feedbacks with soil biota may be more difficult to control or spread more quickly. With rates of anthropogenic N deposition expected to continue to rise globally in the near future (Galloway et al. 2004; Vitousek et al. 1997a), a better understanding of how N enrichment influences both aboveground and belowground aspects of plant invasions is needed.

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References

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, Padgett PE, Bytnerowicz A, Minnich R (1996) Nitrogen deposition effects on coastal sage vegetation of southern California. In: Bytnerowicz A, Arbaugh MJ, Schilling S (tech. coords) Proceedings of the international symposium on air pollution and climate change effects on forest ecosystems, Riverside, California. General Technical Report PSW-GTR

Belnap J, Phillips SL, Sherrod SK, Moldenke A (2005) Soil biota can change after exotic plant invasion: does this affect ecosystem processes? Ecology 86:3007–3017

Bennett AE, Strauss SY (2013) Response to soil biota by native, introduced non-pest, and pest grass species: is responsiveness a mechanism for invasion? Biol Invasions 15:1343–1353

Bertrand H, Plassard C, Pinochet X, Touraine B, Normand P, Cleyet-Marel J (2000) Stimulation of the ionic transport system in *Brassica napus* by a plant growth-promoting rhizobacterium (*Achromobacter* sp.). Can J Microbiol 46:229–236

Bever JD (2002) Negative feedback within a mutualism: host-specific growth of mycorrhizal fungi reduces plant benefit. Proc R Soc Lond B Biol Sci 269:2595–2601

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

Bonanomi G, Chiurazzi M, Caporaso S, Del Sorbo G, Moschetti G, Felice S (2008) Soil solarization with biodegradable materials and its impact on soil microbial communities. Soil Biol Biochem 40:1989–1998

Bozzolo FH, Lipson DA (2013) Differential responses of native and exotic coastal sage scrub plant species to N additions and the soil microbial community. Plant Soil 371:37–51

Bradley BA, Blumenthal DM, Wilcove DS, Ziska LH (2010) Predicting plant invasions in an era of global change. Trends Ecol Evol 25:310–318

Brooks ML (2003) Effects of increased soil nitrogen on the dominance of alien annual plants in the Mojave Desert. J Appl Ecol 40:344–353

Burke MJ, Grime J (1996) An experimental study of plant community invasibility. Ecology 77:776–790

Callaway R, Newingham B, Zabinski CA, Mahall BE (2001) Compensatory growth and competitive ability of an invasive weed are enhanced by soil fungi and native neighbours. Ecol Lett 4:429–433

Callaway RM, Mahall BE, Wicks C, Pankey J, Zabinski C (2003) Soil fungi and the effects of an invasive forb on grasses: neighbor identity matters. Ecology 84:129–135

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

Callaway RM et al (2008) Novel weapons: invasive plant suppresses fungal mutualists in America but not in its native Europe. Ecology 89:1043–1055

Chapin FS (1980) The mineral nutrition of wild plants. Annu Rev Ecol Syst 11:233–260

Cione NK, Padgett PE, Allen EB (2002) Restoration of a native shrubland impacted by exotic grasses, frequent fire, and nitrogen deposition in southern California. Restor Ecol 10:376–384. doi:10.1046/j.1526-100X.2002.02038.x

Corkidi L, Rowland DL, Johnson NC, Allen EB (2002) Nitrogen fertilization alters the functioning of arbuscular mycorrhizas at two semiarid grasslands. Plant Soil 240:299–310. doi:10.1023/A:1015792204633

Cox RD, Preston KL, Johnson RF, Minnich RA, Allen EB (2014) Influence of landscape-scale variables on vegetation conversion to exotic annual grassland in southern California, USA. Glob Ecol Conserv 2:190–203

Davis MA, Grime JP, Thompson K (2000) Fluctuating resources in plant communities: a general theory of invasibility. J Ecol 88:528–534

de la Pena E, De Clercq N, Bonte D, Roiloa S, Rodríguez-Echeverría S, Freitas H (2010) Plant–soil feedback as a mechanism of invasion by *Carpobrotus edulis*. Biol Invasions 12:3637–3648

Dukes JS, Mooney HA (1999) Does global change increase the success of biological invaders? Trends Ecol Evol 14:135–139

Egerton-Warburton LM, Allen EB (2000) Shifts in arbuscular mycorrhizal communities along an anthropogenic nitrogen deposition gradient. Ecol Appl 10:484–496

Egerton-Warburton LM, Graham RC, Allen EB, Allen MF (2001) Reconstruction of the historical changes in mycorrhizal fungal communities under anthropogenic nitrogen deposition. Proc R Soc Lond B Biol Sci 268:2479–2484

Ehrenfeld JG, Ravit B, Elgersma K (2005) Feedback in the plant–soil system. Annu Rev Environ Resour 30:75–115

Elton CS (2000) The ecology of invasions by animals and plants. University of Chicago Press, Chicago

Fenn ME et al (2003) Ecological effects of nitrogen deposition in the western United States. Bioscience 53:404–420

Fenn ME et al (2010) Nitrogen critical loads and management alternatives for N-impacted ecosystems in California. J Environ Manag 91:2404–2423. doi:10.1016/j.jenvman.2010.07.034

Galloway JN et al (2004) Nitrogen cycles: past, present, and future. Biogeochemistry 70:153–226

Gerdemann J (1968) Vesicular–arbuscular mycorrhiza and plant growth. Annu Rev Phytopathol 6:397–418

Going BM, Hillerislambers J, Levine JM (2009) Abiotic and biotic resistance to grass invasion in serpentine annual plant communities. Oecologia 159:839–847

Goldstein LJ, Suding KN (2014) Applying competition theory to invasion: resource impacts indicate invasion mechanisms in California shrublands. Biol Invasions 16:191–203

Gustafson DJ, Casper BB (2004) Nutrient addition affects AM fungal performance and expression of plant/fungal feedback in three serpentine grasses. Plant Soil 259:9–17

Hawkes CV, Wren IF, Herman DJ, Firestone MK (2005) Plant invasion alters nitrogen cycling by modifying the soil nitrifying community. Ecol Lett 8:976–985

Hilbig BE, Allen EB (2015) Plant–soil feedbacks and competitive interactions between invasive *Bromus diandrus* and native forb species. Plant Soil 392:1–13

Hoeksema JD et al (2010) A meta-analysis of context-dependency in plant response to inoculation with mycorrhizal fungi. Ecol Lett 13:394–407

Huenneke LF, Hamburg SP, Koide R, Mooney HA, Vitousek PM (1990) Effects of soil resources on plant invasion and community structure in Californian serpentine grassland. Ecology 71:478–491

Innes L, Hobbs PJ, Bardgett RD (2004) The impacts of individual plant species on rhizosphere microbial communities in soils of different fertility. Biol Fert Soils 40:7–13

Johnson N, Graham JH, Smith F (1997) Functioning of mycorrhizal associations along the mutualism–parasitism continuum*. New Phytol 135:575–585

Johnson NC, Wilson GW, Bowker MA, Wilson JA, Miller RM (2010) Resource limitation is a driver of local adaptation in mycorrhizal symbioses. Proc Natl Acad Sci 107:2093–2098

Kardol P, Martijn Bezemer T, van der Putten WH (2006) Temporal variation in plant–soil feedback controls succession. Ecol Lett 9:1080–1088

Kaye JP, Hart SC (1997) Competition for nitrogen between plants and soil microorganisms. Trends Ecol Evol 12:139–143

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

Kimball S, Goulden ML, Suding KN, Parker S (2014) Altered water and nitrogen input shifts succession in a Southern California coastal sage community. Ecol Appl 24:1390–1404

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

Klironomos JN (2003) Variation in plant response to native and exotic arbuscular mycorrhizal fungi. Ecology 84:2292–2301

Koske R, Gemma J (1989) A modified procedure for staining roots to detect VA mycorrhizas. Mycol Res 92:486–488

Kourtev PS, Ehrenfeld JG, Häggblom M (2002) Exotic plant species alter the microbial community structure and function in the soil. Ecology 83:3152–3166

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

Levine JM, Vila M, Antonio CM, Dukes JS, Grigulis K, Lavorel S (2003) Mechanisms underlying the impacts of exotic plant invasions. Proc R Soc Lond B Biol Sci 270:775–781

Levine JM, Adler PB, Yelenik SG (2004) A meta-analysis of biotic resistance to exotic plant invasions. Ecol Lett 7:975–989

Levine JM, Pachepsky E, Kendall BE, Yelenik SG, Lambers JHR (2006) Plant–soil feedbacks and invasive spread. Ecol Lett 9:1005–1014

Manning P, Morrison S, Bonkowski M, Bardgett RD (2008) Nitrogen enrichment modifies plant community structure via changes to plant–soil feedback. Oecologia 157:661–673

McGonigle T, Miller M, Evans D, Fairchild G, Swan J (1990) A new method which gives an objective measure of colonization of roots by vesicular–arbuscular mycorrhizal fungi. New Phytol 115:495–501

Mills KE, Bever JD (1998) Maintenance of diversity within plant communities: soil pathogens as agents of negative feedback. Ecology 79:1595–1601

Mitchell CE, Power AG (2003) Release of invasive plants from fungal and viral pathogens. Nature 421:625–627

Oliveira R, Vosátka M, Dodd J, Castro P (2005) Studies on the diversity of arbuscular mycorrhizal fungi and the efficacy of two native isolates in a highly alkaline anthropogenic sediment. Mycorrhiza 16:23–31

Owen SM, Sieg CH, Johnson NC, Gehring CA (2013) Exotic cheatgrass and loss of soil biota decrease the performance of a native grass. Biol Invasions 15:2503–2517

Padgett PE, Allen EB (1999) Differential responses to nitrogen fertilization in native shrubs and exotic annuals common to Mediterranean coastal sage scrub of California. Plant Ecol 144:93–101

Parker SS, D'antonio CM (2008) The role of mycorrhizal fungi in promoting exotic grass persistence. Paper presented at the Ecological Society of America 93rd annual meeting, Milwaukee, Wisconsin

Pernilla Brinkman E, Van der Putten WH, Bakker EJ, Verhoeven KJ (2010) Plant–soil feedback: experimental approaches, statistical analyses and ecological interpretations. J Ecol 98:1063–1073

Pimentel D, Lach L, Zuniga R, Morrison D (2000) Environmental and economic costs of nonindigenous species in the United States. Bioscience 50:53–65

Pringle A, Bever JD, Gardes M, Parrent JL, Rillig MC, Klironomos JN (2009) Mycorrhizal symbioses and plant invasions. Annu Rev Ecol Evol Syst 40:699–715

Rao LE, Allen EB (2010) Combined effects of precipitation and nitrogen deposition on native and invasive winter annual production in California deserts. Oecologia 162:1035–1046

Reinhart KO, Callaway RM (2006) Soil biota and invasive plants. New Phytol 170:445–457

Schimel JP, Jackson LE, Firestone MK (1989) Spatial and temporal effects on plant-microbial competition for inorganic nitrogen in a California annual grassland. Soil Biol Biochem 21:1059–1066

Sigüenza C, Corkidi L, Allen EB (2006a) 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

Sigüenza C, Crowley DE, Allen EB (2006b) Soil microorganisms of a native shrub and exotic grasses along a nitrogen deposition gradient in southern California. Appl Soil Ecol 32:13–26

Smith SE, Read DJ (2010) Mycorrhizal symbiosis. Academic press, Cambridge

Stinson KA et al (2006) Invasive plant suppresses the growth of native tree seedlings by disrupting belowground mutualisms. PLoS Biol 4:727

Talluto MV, Suding KN (2008) Historical change in coastal sage scrub in southern California, USA in relation to fire frequency and air pollution. Landsc Ecol 23:803–815

Theoharides KA, Dukes JS (2007) Plant invasion across space and time: factors affecting nonindigenous species success during four stages of invasion. New Phytol 176:256–273

Tonnesen GS, Wang ZW, Omary M, Vhien CJ (2007) Assessment of nitrogen deposition: modeling and habitat assessment. California Energy Commission, Sacramento

van der Heijden MG et al (1998) Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. Nature 396:69–72

van der Putten WH (2010) Impacts of soil microbial communities on exotic plant invasions. Trends Ecol Evol 25:512–519

Van der Putten WH, Klironomos JN, Wardle DA (2007) Microbial ecology of biological invasions. ISME J 1:28–37

Vitousek PM, Walker LR (1989) Biological invasion by *Myrica faya* in Hawai'i: plant demography, nitrogen fixation, ecosystem effects. Ecol Monogr 59:247–265

Vitousek PM et al (1997a) Human alteration of the global nitrogen cycle: sources and consequences. Ecol Appl 7:737–750

Vitousek PM, D'antonio CM, Loope LL, Rejmanek M, Westbrooks R (1997b) Introduced species: a significant component of human-caused global change. N Z J Ecol 21:1–16

Vogelsang KM (2004) The use of mycorrhizal fungi in erosion control applications. California Department of Transportation Sacramento, California

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

Wedin DA, Tilman D (1996) Influence of nitrogen loading and species composition on the carbon balance of grasslands. Science 274:1720–1723

Weiss SB (1999) Cars, cows, and checkerspot butterflies: nitrogen deposition and management of nutrient-poor grasslands for a threatened species. Conserv Biol 13:1476–1486

Wood YA, Meixner T, Shouse PJ, Allen EB (2006) Altered ecohydrologic response drives native shrub loss under conditions of elevated nitrogen deposition. J Environ Qual 35:76–92. doi:10.2134/jecl2004.0465

Yoshida LC, Allen EB (2001) Response to ammonium and nitrate by a mycorrhizal annual invasive grass and native shrub in southern California. Am J Bot 88:1430–1436

Zhang Q, Yang R, Tang J, Yang H, Hu S, Chen X (2010) Positive feedback between mycorrhizal fungi and plants influences plant invasion success and resistance to invasion. PLoS ONE 5:e12380