Plant Soil (2006) 286:153–165
DOI 10.1007/s11104-006-9034-2

ORIGINAL PAPER

Feedbacks of soil inoculum of mycorrhizal fungi altered by N deposition on the growth of a native shrub and an invasive annual grass

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Received: 21 July 2004 / Accepted: 2 March 2005 / Published online: 11 August 2006

Abstract Anthropogenic nitrogen (N) deposition causes shifts in vegetation types as well as species composition of arbuscular mycorrhizal (AM) fungi and other soil microorganisms. A greenhouse experiment was done to determine whether there are feedbacks between N-altered soil inoculum and growth of a dominant native shrub and an invasive grass species in southern California. The region is experiencing large-scale loss of *Artemisia californica* shrublands and replacement by invasive annual grasses under N deposition. *Artemisia californica* and *Bromus madritensis* ssp. *rubens* were grown with soil inoculum from experimental plots in a low N deposition site that had (1) N-fertilized and (2) unfertilized soil used for inoculum, as well as (3) high-N soil inoculum from a site exposed to atmospheric N deposition for four decades. All treatments plus a nonmycorrhizal control were given two levels of N fertilizer solution. *A. californica* biomass was reduced by each of the three inocula compared to uninoculated controls under at least one of the two N fertilizer solutions. The inoculum from the N-deposition site caused the greatest growth depressions. By contrast, *B. madritensis* biomass increased with each of the three inocula under at least one, or both, of the N solutions. The different growth responses of the two plant species may be related to the types of AM fungal colonization. *B. madritensis* was mainly colonized by a fine mycorrhizal endophyte, while *A. californica* had primarily coarse endophytes. Furthermore, *A. californica* had a high level of septate, nonmycorrhizal root endophytes, while *B. madritensis* overall had low levels of these endophytes. The negative biomass response of *A. californica* seedlings to high N-deposition inoculum may in part explain its decline; a microbially-mediated negative feedback may occur in this system that causes poor seedling growth and establishment of *A. californica* in sites subject to N deposition and *B. madritensis* invasion.

Keywords Arbuscular mycorrhizal fungi · *Artemisia californica* · *Bromus madritensis* ssp. *rubens* · California · Coastal sage scrub · Fine endophyte · Nonmycorrhizal fungi

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Introduction

Soil microbial communities may be altered by anthropogenic activities such as nitrogen (N) deposition (Egerton-Warburton and Allen 2000; Egerton-Warburton et al. 2001; Johnson 1993) and other pollutants (Megharaj et al. 2000).

Microbial communities may also change under different host plant species, as plants alter soil biological properties and promote differential colonization by microbial species (Bardgett et al. 1999; Ehrenfeld 2003, Johnson et al. 2004). For instance, adjacent plants of different species in a Minnesota, USA, grassland had different arbuscular mycorrhizal (AM) fungal communities (Johnson et al. 1992). Exotic grassland in southern California had a different species composition of AM fungal spores than native grassland, that was partially restored by planting native grasses (Nelson and Allen 1993). Soil organisms associated with plant roots, including both mycorrhizal fungi and root pathogens, have the potential to control aboveground composition and processes (Wardle 2002). However, the feedbacks of changed microbial communities on plant growth have seldom been studied. Increases in N availability may modify the AM fungal community by selecting inferior mutualistic symbionts that cause smaller or even negative plant growth responses (Corkidi et al. 2002; Johnson 1993). The feedbacks of the changed microbial communities may be negative when pathogens are involved or when a particular plant species promotes the growth of a less mutualistic AM fungal species more than that of others (Bever 1994; Bever et al. 1997, 2001; Holah and Alexander 1999; Westover and Bever 2001). The feedbacks may be positive when a plant species promotes growth and reproduction of an AM fungus that reciprocally promotes its fitness (Bever et al. 1996).

Nitrogen enrichment may cause a loss in plant species diversity with an increase in nitrophilous species (Stevens et al. 2004; Tilman 1993; Vitousek et al. 1997), including shifts from shrubland to grassland as reported in the Netherlands (Bobbink 1991). Rapid vegetation changes are also occurring in southern California shrublands, where N deposition up to 30 kg ha^{-1} yr^{-1} has been reported (Bytnerowicz et al. 1987; Fenn et al. 2003b). Coastal sage scrub (CSS) in this region is undergoing conversion to Mediterranean annual grassland, at least in part because of N deposition (Allen et al. 1998; Fenn et al. 2003a; Minnich and Dezzani 1998). One explanation for the conversion is the differential ability of the native and exotic plants to take up N. Both exotic annuals and shrubs of CSS had a high growth response to N fertilizer (Padgett and Allen 1999), although the exotic *Bromus madritensis* ssp. *rubens* (L.) Husnot had a greater rate of N uptake than the native shrub *Artemisia californica* Less., indicating it may be more competitive for N (Yoshida and Allen 2004).

A further explanation for plant species shifts under N deposition may lie in changed soil microbial communities, and their feedbacks on plant growth. Southern California soils have undergone changes in AM fungal species composition, with losses of large-spored genera (*Gigaspora, Scutellospora*) and greater dominance of species of *Glomus* (Egerton-Warburton and Allen 2000; Egerton-Warburton et al. 2001). These studies showed a reduced density as well as diversity of AM fungal spores along a previously described rural to urban N-deposition gradient (Padgett et al. 1999). AM fungal colonization of native shrub roots was lower in the high-N portion of the gradient, but the annual grasses were colonized by a fine endophyte that showed no reduction in colonization with elevated soil N (Sigüenza et al. 2006). The impacts of N deposition on mycorrhiza are likely widespread, as N-eutrophied sites in Europe have reduced production of ectomycorrhizal sporocarps, and reduced lengths of extraradical mycelia and ectomycorrhizal formation on roots (Wallenda and Kottke 1998; Wöllecke et al. 1999). Experimental N fertilization caused reduced AM fungal colonization at four of five grassland sites in the western USA, with soil N/P ratio determining whether the mycorrhizal response to N fertilization would be negative or positive (Johnson et al. 2003). However, only a few studies have examined the feedbacks that these mycorrhizal communities altered by soil N will have on the native plant community. Johnson (1993) showed that N fertilization of a Minnesota grassland created a less mutualistic AM fungal community, as the plants responded with reduced growth to this inoculum in a glasshouse study. Similarly, Corkidi et al. (2002) showed that native grass growth was reduced by AM inoculum collected from N-fertilized soils at two semi-arid grassland sites in the western USA.

Our objectives were to show how N-induced changes in the AM fungal community, as well as associated microorganisms, affected the growth of a dominant native and a dominant exotic of CSS. Based on prior studies (Corkidi et al. 2002; Johnson, 1993), we hypothesized that plants would have reduced growth response to N-impacted inoculum, but response would be host-plant specific. We compared growth responses and mycorrhizal colonization among inoculum treatments in the greenhouse to determine whether the feedbacks of the fungi on plants would demonstrate a loss of mutualistic response of plants to the mycorrhizal fungi, and whether these feedbacks might help explain poor growth and establishment of *A. californica* seedlings in shrublands invaded by the annual grass *B. madritensis*.

Materials and methods

Study sites

Soil to be used as inoculum was collected at two sites along a previously described N-deposition gradient in California (Padgett et al. 1999). One is a low N-deposition site near Lake Skinner in the Western Riverside County Multispecies Reserve (33°37′N, 117°02′W). The other is a high N-deposition site at the University of California, Riverside Botanic Gardens (33°54′N, 117°15′W) (Padgett et al. 1999). Both sites lie at about 450 m elevation, have sandy clay loam, decomposed-granite soils and receive 280 mm of precipitation annually, mainly between November and April. High levels of N deposition have been reported for more than 40 years in the Riverside area (up to 30 kg ha^{-1} yr^{-1}) (Bytnerowicz et al. 1987; Fenn et al. 2003b). Lake Skinner is surrounded by CSS vegetation. The Botanic Gardens include an extensive stand of natural vegetation that is contiguous with a system of wildland reserves that were originally dominated by CSS, but have largely been replaced by annual grassland with sparse shrubs (Allen et al. 1998; Minnich and Dezzani 1998).

Soil inoculum

Soil samples to be used as inoculum were collected in June 1998 at Lake Skinner and the Botanic Gardens. At Lake Skinner the source of inoculum was a set of 20 experimental plots. Ten plots received 60 kg N ha^{-1} yr^{-1} NH_4NO_3 since 1994, the remainder were unfertilized controls (Allen et al. 1998). Total KCl extractable N (NO_3 plus NH_4) at Lake Skinner N was 21.0 μg/g (S.E. ± 2.1) in unfertilized soils, and 97.0 μg/g (S.E. ± 30.0) in fertilized soils. In the Botanic Gardens the collected soil averaged 52.5 μg/g (S.E. ± 14.5, $n = 5$). The soils of this region are decomposed granites with relatively high bicarbonate-extractable P of 30 μg/g and are described in more detail elsewhere (Padgett et al. 1999).

Soils for the three inoculum types (fertilized [FERT] and unfertilized [UNFERT] at Lake Skinner, and high N deposition [NDEP] at the Botanic Gardens) were collected at the canopy edge of five *A. californica* shrubs each, including shrub and grass roots, and homogenized within each inoculum source. The sites chosen were previously analyzed for AM fungal species composition, and both richness and density of spores declined with increasing N fertilization or N deposition along the gradient (Egerton-Warburton and Allen 2000). According to these analyses, the June density of AM fungal spores in the three treatments was UNFERT = 58/g; FERT = 10/g; and NDEP = 38/g. The richness of AM spore species per site was UNFERT = 19 species; FERT = 9 species; and NDEP = 14 species (Egerton-Warburton and Allen 2000). The inoculum soils were sieved through 2 cm and the roots were cut in < 1 cm pieces and added back as part of the inoculum. The inoculum was stored at 4°C for 2–3 weeks until the pots were ready to be planted. Soils were steam-sterilized by maintaining temperature at 90°C for 2 h on each of two consecutive days. Preliminary analyses in these inorganic soils (organic matter = 1–2% by combustion) showed that steaming did not release statistically significant amounts of extractable N and P. Soil inoculum treatments (10 replicates for each of the two plant species and two harvests of each = 40 pots of each inoculum or control) were prepared as follows:

1) FERT inoculum: 30 g per pot of soil from the fertilized plots and 30 g of steam-sterilized soil from the unfertilized plots of the low N deposition site (Lake Skinner). Both sources of soil were added to balance the N level.
2) UNFERT inoculum: 30 g per pot of soil from unfertilized plots and 30 g of steam-sterilized soil from the fertilized plots at the low N deposition site (Lake Skinner).
3) NDEP inoculum: 30 g per pot of inoculum from the UCR Botanic Gardens, the high N deposition site. This inoculum had an intermediate level of extractable N compared to FERT and UNFERT inoculum, so no additional steam-sterilized inoculum was added.
4) NM control: 30 g per pot steam-sterilized soil from fertilized plots and 30 g sterilized soil from unfertilized plots at Lake Skinner.

Growth experiment

The growth experiment was conducted in a greenhouse at the University of California, Riverside from July to September 1998, when the mean daily high greenhouse temperature was 30°C, and the night temperature averaged 20°C. Soil from the Botanic Gardens was used as a growth medium. The soil was mixed 1:1 with silica sand and steam-sterilized at ca. 90°C for 2 h. Because the soil had high N, it was leached with distilled water prior to use to reduce extractable N to about 10 $\mu g^{-1}\ g^{-1}$, as well as other labile nutrients that might be released during sterilization. The sand was added to increase infiltration, as this sandy clay loam had poor drainage in greenhouse pots. Plastic containers of 650 ml (Deepots; Stueuwe and Sons, Corvallis, Oregon, USA) were filled with 500 g sterile soil, with inoculum placed 8 cm from the top. To provide comparable nonmycorrhizal microbial inputs, soil washings were added to the controls (Koide and Li 1989). These washings were prepared by filtering 30 g soil/pot each of FERT and UNFERT soil inoculum through a 20 μm sieve.

Seeds of *A. californica* and *B. madritensis* ssp. *rubens* were collected near Lake Skinner and were sown into trays containing sterilized vermiculite. These species were chosen because they are the most abundant native and exotic species in regional CSS vegetation. Five days after seedling emergence, seedlings were selected for uniformity of size and transplanted to inoculated pots. The few plants that died during the first week were replaced.

Plants in each of the soil inoculum treatments were watered twice a week alternating between distilled water and 50 ml of a HIGH N or LOW N nutrient solution minus P that was modified from Sylvia and Hubbel (1986). The HIGH N solution contained 433 mg l^{-1} KNO_3; 8.4 mg l^{-1} Ca $(NO_3)_2{\cdot}4H_2O$; 199 mg l^{-1} $CaSO_4\ 2H_2O$; 130 mg l^{-1} K_2SO_4; 72 mg l^{-1} $MgSO_4$; 0.86 mg l^{-1} H_3BO_3; 0.54 mg l^{-1} MnCl $4H_2O$; 0.07 mg l^{-1} $ZnSO_4$; 0.02 mg l^{-1} $CuSO_4\ 5H_2O$; and 0.03 mg l^{-1} NaCl. The LOW N solution was the same but without KNO_3. Phosphorus was not added because it is relatively high in the Botanic Garden soils (30 μg/g of bicarbonate extractable P prior to mixing with sand). Twenty replicates of each treatment were distributed at random in the greenhouse and were rotated every week.

Ten plants of each treatment were harvested 6 and 12 weeks after transplanting. Prior to harvest, shoot height and the number of vegetative and reproductive tillers in *B. madritensis* were recorded for each plant. Plants were separated into shoots and roots, dried at 60°C to constant mass and weighed. The dried roots were rehydrated and cleared and stained (Koske and Gemma 1989). Prior observations showed that drying did not affect the mycorrhizal structures within the roots. The procedure of McGonigle et al. (1990) was used to assess colonization of arbuscules, coils, vesicles, and hyphae, and the occurrence of other fungi was also assessed. Fine versus coarse endophytes were distinguished according to Thippayarugs et al. (1999). Shoots were ground and sent to the University of California, Davis, Analytical Laboratory to determine N concentration using a CNS analyzer.

Statistical analyses

Plants were grown with and without supplemental N (HIGH and LOW N solution) in three AM inoculum treatments (FERT, UNFERT and NDEP) and a nonmycorrhizal control (2×4

ANOVA factorial experimental design). A two-way ANOVA with nitrogen and inoculum as factors was performed on plant and fungal variables: shoot, root and total dry mass, root to shoot ratios, tissue N analysis, and AM fungal colonization percentage (arbuscules, vesicles, coils, hyphae, and fungi other than mycorrhizal). Prior to statistical analysis, data were tested for normality and homogeneity of variance; transformations (log, square root) were used as appropriate. The arcsine transformation was used to analyze AM fungal colonization percentage. Mean contrasts were performed using Fisher's protected least significant difference (PLSD) with $P < 0.05$.

Results

Plant growth response

Mycorrhizal inoculation had negative effects on the biomass of *A. californica* compared to uninoculated controls (Fig. 1). At the first harvest at 6 weeks there was a reduction in biomass of two of the three inoculum treatments under LOW N solution, the NDEP and UNFERT inocula. Two inoculum treatments also had reduced biomass under HIGH N solution, the NDEP and FERT inocula ($P < 0.001$ for effects of N solution, for inoculum source, and for N–X inoculum interaction). Overall the biomass of plants with NDEP inoculum was reduced by 53% under HIGH N and 74% under LOW N solution compared to controls. This indicates a severe negative impact of this inoculum, which has been subject to long-term anthropogenic N deposition, on the seedlings during their first 6 weeks of growth. By the second harvest at 12 weeks, there was no longer a significant effect of inoculum among any of the treatments, although there was a N fertilizer effect ($P < 0.001$), as HIGH N plants were larger than LOW N plants (Fig. 1).

By contrast, biomass of *B. madritensis* had positive responses to inoculum (Fig. 2). At the first harvest the plants that were smallest overall were the uninoculated plants under LOW N solution. Two of the inoculum treatments under LOW N promoted increased biomass compared to the controls, with the NDEP-inoculated plants intermediate in biomass to the controls and plants with the two other inocula. There were no significant effects of inoculum on *B. madritensis* biomass under the HIGH N solution at the first harvest, but by the second harvest one of the inoculum treatments, the plants receiving FERT inoculum, had higher biomass than the controls. The pattern in response under LOW N was similar in the first and second harvests (Fig. 2).

A comparison of biomass of the two plant species is instructive in understanding their growth rate relative to each other. Under the same treatments and at the same harvest date, *B. madritensis* was 40–100% larger than *A. californica*. For instance, the average mass of the four inoculum treatments of *A. californica* under HIGH N was 1.9 g/plant at the second harvest, while for *B. madritensis* it was 3.1 g/plant ($P < 0.001$). This indicates that *B. madritensis* seedlings have a faster growth rate regardless of inoculum or N fertility.

The impacts of inoculum and N solution were also assessed on root/shoot (R/S) ratio. R/S was significantly reduced by N solution by the second harvest, with little response to inoculum at either harvest. For *A. californica*, R/S values were 0.52 for LOW N solution plants and 0.32 for HIGH N solution at the second harvest. For *B. madritensis*, R/S was 0.54 for LOW N and 0.33 for HIGH N solution at the second harvest.

Plant nitrogen

N fertilization significantly increased leaf tissue N concentration in both *A. californica* and *B. madritensis* ($P < 0.001$). Tissue N concentration increased by approximately 25% in *A. californica* with HIGH N solution and almost doubled in *B. madritensis*. Tissue N concentration did not increase with mycorrhizal inoculum for either species; instead, tissue N was lower with inoculum, or did not change significantly (Table 1). For *A. californica* in both N fertilization levels, the NDEP inoculum treatment had the highest tissue N concentration overall, which was significantly higher than at least one of the two other inoculum treatments within a fertilizer level. By contrast, for *B. madritensis* the NM plants had the highest

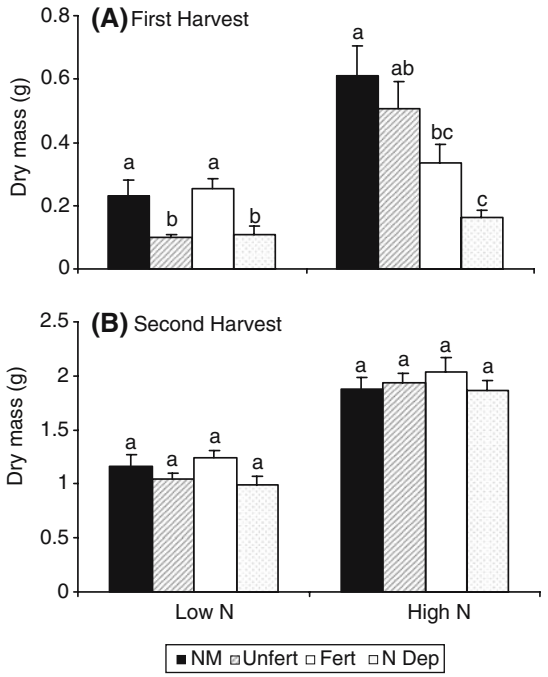

Fig. 1 Total biomass (root plus shoot) of *Artemisia californica* at (**A**) the first harvest (6 weeks) and (**B**) the second harvest (12 weeks) under two levels of N solution and three mycorrhizal treatments plus nonmycorrhizal control, means of 10 observations ± SEM. NM = nonmycorrhizal control, UNFERT = inoculum from unfertilized soil, FERT = inoculum from fertilized soil, NDEP = inoculum from a site with high levels of N deposition. LOW N and HIGH N indicate levels of fertilizer solution. Different letters above bars indicate significant differences within HIGH or LOW N solution treatments ($P \leq 0.05$)

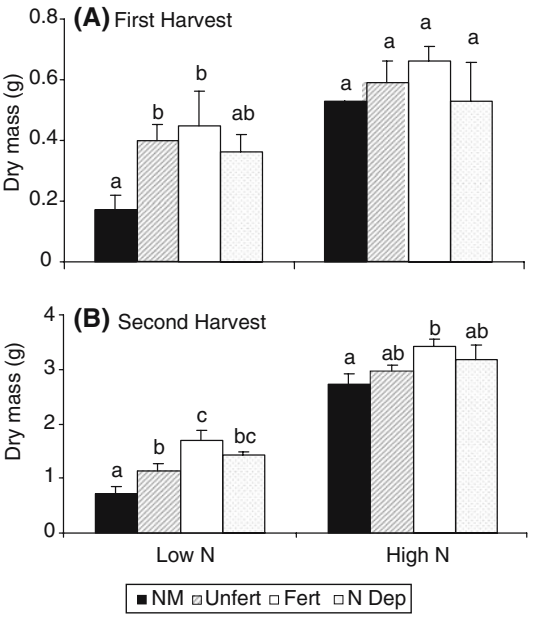

Fig. 2 Total biomass (root plus shoot) of *Bromus madritensis* at (**A**) the first harvest and (**B**) the second harvest under two levels of N solution and three mycorrhizal treatments plus nonmycorrhizal control, means of 10 observations ± SEM. Inoculum and fertilizer treatments as in Fig. 1. Different letters above bars indicate significant differences within HIGH or LOW N solution treatments ($P \leq 0.05$)

tissue N concentration, and they were also the smallest plants (Fig. 2).

Root colonization by AM fungi

Mycorrhizal colonization was greatly reduced in both *A. californica* and *B. madritensis* at the second harvest 12 weeks after transplanting, ranging from 1 to 3%. Therefore, only the data from the first harvest are shown.

Total AM fungal colonization in *A. californica* treatments averaged from 3 to 21% in different treatments at the first harvest. No AM fungal colonization was observed in the nonmycorrhizal controls (Fig. 3A). Overall response to inoculum type and N solution were highly variable, with significant inoculum XN solution interactions ($P < 0.001$). The NDEP treatment had significantly higher colonization under HIGH N solution than LOW N solution, but other treatments were not significantly different from each other. Arbuscular colonization ranged from 0 to 6%, and coils from 0 to 1% (data not shown; see Sigüenza 2000), with an absence of both arbuscules and coils only in the FERT, HIGH N treatment. This indicates that the two N-affected inocula were able to form arbuscules and coils, but the NDEP inoculum did so at both levels of N fertilizer solution. Vesicles were formed in all treatments, ranging from 0.2 to 3%, and were significantly lowest in the UNFERT, HIGH N treatment. Traces of fine endophyte (<1%) were observed in *A. californica* roots.

Unlike *A. californica, B. madritensis* formed primarily fine endophyte. Fine endophyte colonized up to 39% of inoculated *B. madritensis* roots, while coarse endophyte was 4–6% except with the UNFERT inoculum where it was 13–20% (Fig. 3B, C). The lowest levels of fine endophyte were found in plants inoculated with

Table 1 Percent leaf N in *Artemisia californica* and *Bromus madritensis* with four soil inoculum types at the second harvest after 12 weeks of growth

Plant species	N treatment	Inoculum treatment			
		NM	UNFERT	FERT	NDEP
Artemisia californica	High N	2.48ab	2.40b	2.44ab	2.62a
	Low N	2.01ab	2.03ab	1.91b	2.17a
Bromus madritensis	High N	3.69a	3.54a	3.17b	3.32b
	Low N	2.03a	1.74b	1.68b	1.56c

NM = nonmycorrhizal, FERT and UNFERT = inoculum from fertilized and unfertilized soils at the low N deposition site, NDEP = inoculum from the high N deposition site

Values in the same row followed by different letters are significantly different at $P < 0.05$

NDEP inoculum with HIGH N solution. The fine endophyte colonization included hyphae with intercalary vesicles, so vesicles were not evaluated separately. Other mycorrhizal structures of the coarse endophyte occurred in less than 1% of the observations. Nonmycorrhizal controls had 0–3% total colonization by mycorrhizal fungi.

Root colonization by nonmycorrhizal fungi

The highest level of nonmycorrhizal colonization was found at 6 weeks in plants inoculated with NDEP soil in the HIGH N solution treatment in both *A. californica* and *B. madritensis* (Fig. 4A, B). On average, nonmycorrhizal fungi were lower in *B. madritensis* than *A. californica* ($P < 0.001$), except in the NDEP inoculum treatment. The controls had very low incidence of nonmycorrhizal fungi, 0–1%. Most of the nonmycorrhizal fungi were septate fungi found inside or between the cortical cells, or external to the root. Disease symptoms of pathogens such as wilting or tissue necrosis were not observed. The incidence of nonmycorrhizal fungi was lower at 12 weeks than six weeks, with 14.2 (S.E. = 9.6)% in the NDEP, HIGH N treatment of *A. californica*, but all the other treatments of both species had virtually 0%.

Discussion

Inoculum and seedling biomass

The feedback responses to soil inoculum from N-eutrophied soils included increased seedling biomass in the invasive annual grass, *B. madritensis*, and decreased biomass of the native shrub, *Artemisia californica*, especially with inoculum from an area of high anthropogenic N deposition. These changes in seedling growth may have profound impacts on the ability of the two species to establish in coastal sage scrub (CSS) vegetation. CSS is a fire-driven vegetation type that reproduces mainly by seed of the dominant shrub species, including the abundant and widespread *A. californica* (Keeley and Keeley 1981). Shrub seedling establishment following fire may be impacted both by competition from the grasses directly, as shown by improved growth of *A. californica* following grass removal (Eliason and Allen 1997; Cione et al. 2002) and, according to our greenhouse experiment, indirectly because of reduced seedling growth from poor mycorrhizal mutualists in N-eutrophied soils. Exotic annual grasses have been in California for over 200 years (Heady 1977), but N deposition has been occurring for the past 40 years. This corresponds with the time since the start of conversion of CSS to annual grassland dominated by *B. madritensis* (Allen et al. 1998; Minnich and Dezzani 1998). The soil inoculum, as well as elevated N, may be responsible for the differential response of the two plant species.

Differential responses to the inocula

Negative growth responses of seedlings to mycorrhiza have often been observed when soil P is high (Bethlenfalvay et al. 1982), and the soils of this region have relatively high P values (Padgett et al. 1999). This may explain the negative response of *A. californica* biomass to inoculum, but not of *B. madritensis* which had a beneficial response. There is considerable evidence that

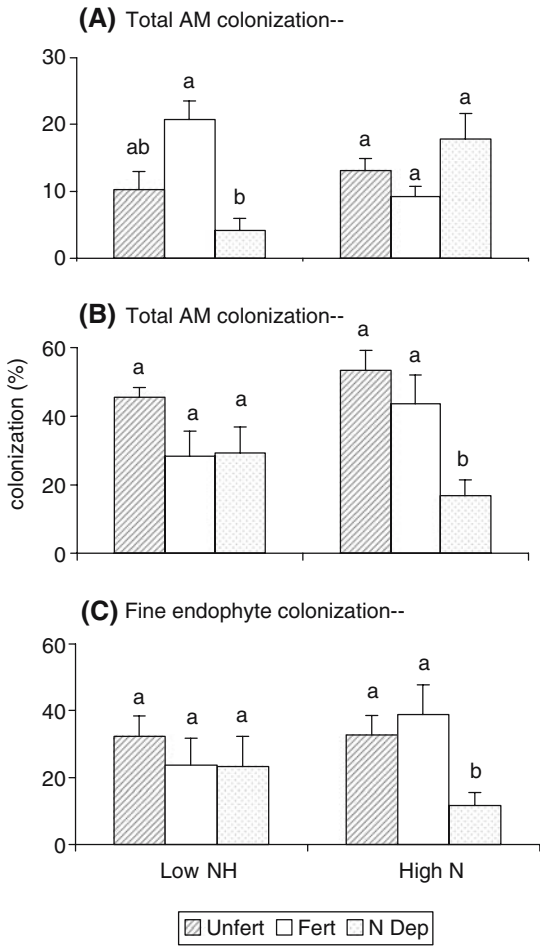

Fig. 3 Mycorrhizal colonization in 6-week-old *Artemisia californica* and *Bromus madritensis* under two levels of N solution and three mycorrhizal treatments: (**A**) total AM fungal colonization of *Artemisia*, (**B**) total AM fungal colonization of *Bromus*, (**C**) fine endophyte colonization of *Bromus*. *Artemisia* had only traces of fine endophyte (<1%), means of 10 observations ± SEM. The uninoculated plants of both species had 0–3% colonization. Inoculum and fertilizer treatments as in Fig. 1. Different letters above bars indicate significant differences within the HIGH or LOW N treatments ($P \leq 0.05$)

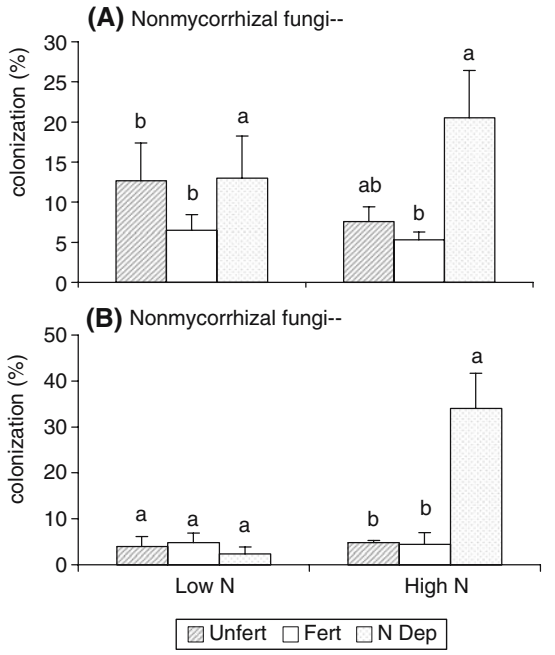

Fig. 4 Nonmycorrhizal colonization in 6-week-old (**A**) *Artemisia californica* and (**B**) *Bromus madritensis* under two levels of N fertilization and three mycorrhizal treatments, means of 10 observations ± SEM. Different letters above bars indicate significant differences among all treatments. The uninoculated controls had 0–1% colonization by nonmycorrhizal fungi. Inoculum and fertilizer treatments as in Fig. 1. Different letters above bars indicate significant differences within the HIGH or LOW N treatments ($P \leq 0.05$)

different species of AM fungi confer different growth responses on the same host plant species (e.g., Allen et al. 2003; Pearson et al. 1993; Roldán-Fajardo 1994; van der Heijden et al. 1998, 2003). These two species may also have had different responses to the inoculum, which had reduced AM spore density and species richness under elevated N (Egerton-Warburton and Allen 2000). Large-spored genera, particularly species of *Gigaspora* and *Scutellospora*, and the large sporocarpic *Sclerocystis*, dropped out along the N deposition gradient and in N-fertilized plots with an increased dominance of species of *Glomus*, especially *Gl. aggregatum*. Exactly what species were most abundant in the roots cannot be determined from our microscopic analyses, except for the difference between fine and coarse endophyte as described below. The results for *A. californica* support other studies that showed that N-eutrophication may select less mutualistic mycobionts, as the NDEP inoculum under HIGH N solution promoted the lowest biomass at the first harvest. For instance, several native perennial grass species grown with inoculum from experimentally N-fertilized soils had reduced growth compared to control inoculum (Corkidi et al. 2002; Johnson 1993). Thus the shift in AM fungal species in these soils due to N eutrophication may have promoted the growth of the invasive annual, but not of the native shrub.

Furthermore, *B. madritensis* formed an association preferentially with the fine endophyte, which may further explain its beneficial response to inoculum, as is discussed below.

Nonmycorrhizal fungi

Because whole-soil inoculum was used, the mycorrhizal response may have been confounded by the nonglomaceous, nonmycorrhizal fungi. Nonmycorrhizal fungal colonization was higher for *A. californica* than *B. madritensis*, and highest for both host plant species under the HIGH N solution with NDEP inoculum. In the case of *A. californica*, plants in this treatment were the smallest. It is not clear what the role of the nonmycorrhizal fungi was, especially considering that they did not have a negative effect on *B. madritensis* plants under the same inoculum treatment. Pathogenic fungi isolated from roots caused a more negative growth response of native rare plant species than exotic invasive species (Klironomos 2002). However in our study the septate fungi occurred only in the cortical cells with no indication of necrosis, unlike pathogens, and plants showed no disease symptoms. Root-inhabiting fungi with unknown identity may be ubiquitous and deserving of further study (Allen et al. 1993; Rillig et al. 1998; Jumpponen 2001; Vandenkoornhuyse et al. 2003).

The 20 μm mesh that was used to prepare the microbial filtrate for the controls apparently excluded virtually all of the nonmycorrhizal fungi, as the controls had only 0–1% nonmycorrhizal fungi. The small diameter mesh was used to reduce contamination by the small-spored fine endophyte, as explained below. The contribution of the nonmycorrhizal fungi is difficult to assess because true axenic controls that separate the AM fungi from other microorganisms are difficult to set up. Studies using the same two host plant species and soil inoculum from the low deposition site, but with a 40 μm mesh for the microbial filtrate, showed a neutral to positive response of both plant species to soil inoculum depending on N form (ammonium or nitrate; Yoshida and Allen 2001, 2004). Their experimental design is equivalent to the comparison of the NM and UNFERT treatments in the present experiment (N-impacted inoculum was not tested). The results of Yoshida and Allen (2001, 2004) suggest that even a 40 μm mesh did not allow passage of potentially detrimental, nonmycorrhizal fungi, or alternatively these nonmycorrhizal fungi are benign in their effect on the host plants.

Fine endophyte

A further difference between the two host plant species was the abundance of the fine endophyte in *B. madritensis*, but virtually none in *A. californica*. Fine endophyte also occurred primarily in grasses in the field from sites on a N deposition gradient that included the two sample sites (Sigüenza et al. 2006). Elevated N reduced colonization of fine endophyte only in the high N deposition inoculum, but this did not affect biomass of *B. madritensis*. The fine endophyte is believed to be *Glomus tenue*, which has been observed primarily in grasses (McGonigle and Fitter 1990; Rabatin 1979; Thomson et al. 1992; Thippayarugs et al. 1999). Tiny spores as small as 10 μm were noted from grass rhizospheres in the field, that may have been *Glomus tenue* (Sigüenza 2000). Some of these tiny spores likely passed through the 20 μm mesh with the microbial filtrate, as the control grasses with filtrate had 0–3% colonization by fine endophyte. These spores were not observed in the survey on spores in N-impacted soils in CSS (Egerton-Warburton and Allen 2000), but this study only examined shrub roots, not grasses. Growth response studies of fine endophyte have shown it to be an effective mutualist in promoting plant growth (Powell 1979; Powell and Daniel 1978). The different growth responses of the two host plants may be related to the abundance of the fine endophyte in the grass, but inoculum culturing studies are still needed to determine whether it is more effective in promoting growth than the coarse endophytes for these plant species.

Colonization levels

Although there was an inoculum response, the levels of AM fungal colonization detected were relatively low in this greenhouse study. However, the maximum levels of 21% for *A. californica* and

44% for *B. madritensis* were similar to those in another greenhouse study (Yoshida and Allen 2001). They are also similar to levels of 20% found in 2–3-month-old establishing *A. californica* seedlings in undisturbed soil at the low N-deposition field site (Sigüenza 2000). These are considerably lower than values of 99% for mature *A. californica* shrubs and 75% for *B. madritensis* at the field site. Thus the results of this study may be extrapolated to establishing seedlings of *A. californica* in a field setting rather than to a mature stand.

The levels of colonization of both mycorrhizal and nonmycorrhizal fungi were higher at 6 than 12 weeks, a pattern that we have observed in previous field and greenhouse experiments (Sigüenza 2000; Yoshida and Allen 2001). By 12 weeks there was no longer a response of *A. californica* to the initial inoculation, although *B. madritensis* responses were significant at this date. Mycorrhizal fungi and roots grow at different rates, and correlations between plant growth and percent AM fungal colonization will vary by species of plant and fungi (Allen 2001; Allen et al. 2003). In a realistic field setting establishing seedlings of *A. californica* would need to compete with annual grasses, so the first 6 weeks of poor growth with high N deposition inoculum could be important to their establishment, especially considering that *B. madritensis* seedlings are growing larger with this inoculum.

Plant N and P

Both plant species had a large growth response to N fertilizer as also shown in prior studies (Padgett and Allen 1999; Yoshida and Allen 2001), although ^{15}N studies have shown that the N uptake rate is 3–4 times greater for *B. madritensis* than *A. californica* (Yoshida and Allen 2004). N can be translocated to the plant via AM hyphae (Johansen et al. 1994), but there is no evidence for an increase in N nutrition by mycorrhiza in this experiment. Nitrate was used as the source of N in the nutrient solutions, because nitrate is the dominant form of soil N under N deposition in this region (Padgett et al. 1999). In experiments comparing form of N, inoculated plants responded with increased growth using ammonia but not with nitrate (Azcón et al. 1992; Johansen et al. 1994; Yoshida and Allen 2001, 2004).

We did not measure P concentrations in these plants, but in a prior experiment using the same high-P soil, Yoshida and Allen (2001) found that *B. madritensis* had 0.59% leaf P with or without N fertilization, and *A. californica* had 0.28% P when fertilized with N and 0.40% P when unfertilized. In addition, there were no increases in tissue P with inoculation. Thus results of the present study likely also cannot be explained by beneficial responses of mycorrhizal plants to P, most likely because the soil used was relatively high in P.

Conclusions

The feedback responses of soil inoculum altered by N deposition and N fertilization showed different patterns in a native and an invasive species. Invasive plants may be preferentially benefited by local mycorrhizal inoculum (Marler et al. 1999; Zabinski et al. 2002), and they may have a lower load of pathogens, also contributing to their ability to invade a system (Klironomos 2002). In our system *A. californica* had a high load of septate nonmycorrhizal fungi, although their role cannot be confirmed as pathogenic. More important was the negative response of *A. californica* seedlings to the coarse endophyte, especially from high N deposition soil, while the fine endophyte of *B. madritensis* may have promoted its growth. Vigorous seedling growth following fire is important to ensure reestablishment of shrubs in CSS (Keeley and Keeley 1981). Any factor that reduces shrub seedling growth, such as poor mycorrhizal mutualists, will put the seedlings at a disadvantage compared to their fast-growing grass neighbors (Padgett and Allen 1999; Yoshida and Allen 2001). Understanding the role of the different species of fungi will require further testing by culturing coarse and fine endophytes to examine their effects on plant growth. Additional work includes elucidating the role of root-inhabiting nonmycorrhizal fungi (Vandenkoornhuyse et al. 2003), which may also have different impacts on host plant species.

Acknowledgments We thank Louise Egerton-Warburton and Pamela Padgett for advice, Sheila Kee for logistic

support and editorial review, and Jenny Phui for laboratory assistance. This research was funded by the Mexican Council for Science and Technology (CONACYT) and the United States Department of Agriculture National Research Initiative Ecosystems Program (9503036).

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