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Authors

Valliere, Justin M
Allen, Edith B

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Interactive effects of nitrogen deposition and drought-stress on plant-soil feedbacks of *Artemisia californica* seedlings

Justin M. Valliere · Edith B. Allen

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Abstract

Aims Nitrogen (N) deposition and drought are major drivers of global change that will influence plant-soil feedbacks. We investigated how N availability, N-impacted soil communities and drought affect feedback in seedlings of a drought-deciduous mycorrhizal shrub, *Artemisia californica*.

Methods Seedlings were inoculated with soil from either a high or low deposition site or sterilized inoculum and grown with or without supplemental N and under well-watered or drought-stressed conditions.

Results Inoculum, N and water had interactive effects on feedbacks. Seedlings grown in low deposition inoculum exhibited a neutral to positive feedback under drought and had the highest root to shoot ratios and mycorrhizal colonization. Seedlings inoculated with high N-deposition soil experienced a positive feedback when N fertilized and well-watered, but plants allocated large amounts of biomass to shoots and had a negative response to drought.

Conclusions The soil community mediates plant response to varying belowground resource availability. We found N-impacted communities may reduce

mycorrhizal colonization and allocation to roots and provide less protection against drought. Our results highlight the context dependency of plant-soil feedbacks and the potential for climate change and N deposition to have interactive effects on these relationships.

Keywords Arbuscular mycorrhizae · Drought · Global change · Nitrogen deposition · Plant-soil feedback

Introduction

Atmospheric nitrogen (N) deposition and drought are two major components of global change likely to have substantial effects on terrestrial plant communities, both above- and belowground, that will influence plant-soil feedbacks (Bardgett and Wardle 2010; Tylisanakis et al. 2008; Van der Putten et al. 2013). Interactions between plants and the soil microbial community are increasingly recognized as important drivers of large-scale vegetation patterns and processes (Bever et al. 1997; Klironomos 2002; Van der Putten et al. 1993) and these aboveground-belowground linkages may play a critical role in mediating ecosystem response to environmental change (Mohan et al. 2014; Schlesinger et al. 1990; Wolters et al. 2000). Many single factor studies have established the response of plants to soil microorganisms is strongly influenced by both soil N (Corkidi et al. 2002; Johnson et al. 2003; Manning et al. 2008) and water availability (Meijer et al. 2011; Meisner et al. 2013), but given the strong potential for these two factors to have complex and interactive effects on

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J. M. Valliere (✉) · E. B. Allen
Department of Botany and Plant Sciences, University of California, Riverside, CA 92521, USA
e-mail: jvall007@ucr.edu

E. B. Allen
Center for Conservation Biology, University of California, Riverside, CA 92521, USA

ecosystems (De Marco et al. 2014; Kimball et al. 2014; Southon et al. 2012; Zavaleta et al. 2003), multi-factor studies are needed to elucidate potential impacts of co-occurring global change drivers on these relationships.

Nitrogen deposition, the input of reactive N from the atmosphere, is increasing globally due to anthropogenic emissions (Galloway 2005; Galloway et al. 2008; Vitousek et al. 1997), with the potential to negatively impact terrestrial plant diversity and community composition (Bobbink et al. 2010). In southern California, which receives high levels of N deposition due to air pollution (Fenn et al. 2003), N deposition has been implicated in the widespread conversion of coastal sage scrub (CSS) to exotic annual-dominated grasslands (Allen et al. 1998; Cox et al. 2014; Fenn et al. 2003). Coastal sage scrub is a low growing, drought-deciduous shrub-land plant community, which has been reduced to a fraction of its historic range as a result of land-use change, invasion and increased fire frequency (Minnich and Dezzani 1998; Talluto and Suding 2008; Westman 1981). High soil N may favor exotic annual grasses over native shrub seedlings (Rao and Allen 2010; Wood et al. 2006) reducing native establishment, but there may be other direct and indirect effects of increased N inputs that affect native seedling survival and performance.

Nitrogen deposition can have a number of impacts belowground that might influence plant-soil feedbacks, such as reduced microbial biomass (Johnson et al. 1998; Treseder 2008; Wallenstein et al. 2006), altered microbial enzyme activity (Carreiro et al. 2000; Saiya-Cork et al. 2002) and changes to soil microbial community composition and diversity (Eisenhauer et al. 2012; Wolters et al. 2000). Increased soil N due to anthropogenic N deposition has resulted in a decline of arbuscular mycorrhizal fungal (AMF) diversity in areas of high pollution in southern California, characterized by a loss of large-spored species within the Gigasporaceae and an increase in abundance of small-spored *Glomus* species (Egerton-Warburton and Allen 2000; Egerton-Warburton et al. 2001; Sigüenza et al. 2006b). These N-impacted mycorrhizal communities may be less mutualistic, especially when soil N availability is high, resulting in reduced growth of natives in these soils (Johnson et al. 1997; Johnson 1993; Sigüenza et al. 2006a). In addition to providing plants with nutrients in exchange for photosynthate, AMF also supply plants with water and improve plant resistance to drought (Allen 2007; Augé 2001). It remains unknown how N deposition influences plant-water relations

through changes to the soil microbial community, but less effective mutualists could increase host susceptibility to drought (Bobbink et al. 2010; Smith and Read 2008).

Drought is a second factor that has been cited as a possible cause of conversion of CSS to exotic annual grasslands (Cox and Allen 2008; Kimball et al. 2014). Typical of a Mediterranean climate, precipitation in this region is highly variable year to year (Jones 2000; Pratt and Mooney 2013) and CSS species are subject to seasonal and long-term drought, with extreme drought events expected to increase in the near future due to climate change (Bell et al. 2004; Griffin and Anchukaitis 2014; Jones 2000). Many CSS shrub species are drought-deciduous, avoiding water deficit by shedding their leaves during the summer dry period (Harrison et al. 1971). However, during the winter growing season, these species exhibit high rates of transpiration (Harrison et al. 1971; Jacobsen et al. 2007) and may be quite sensitive to short-term water-stress (Kolb and Davis 1994). Seedling recruitment of CSS shrub species is highly dependent on precipitation (Cox and Allen 2008; Keeley et al. 2006) and plants are particularly vulnerable to drought during seedling stage (Fenner 1987; Moles and Westoby 2004).

Nitrogen deposition can increase plant susceptibility to drought (Friedrich et al. 2012; Meyer-Grünefeldt et al. 2013; Wu et al. 2008), and one possible explanation for poor CSS seedling establishment under N deposition is that high N availability alters morphological and physiologically plant traits in a way that makes them less able to withstand short- and long-term drought stress. Enhanced soil N availability may reduce biomass allocation to roots, thereby increasing evapotranspirative demand and decreasing root:shoot ratios (Aerts et al. 1991; Reynolds and D'antonio 1996). High soil N can also decrease mycorrhizal colonization of roots (Egerton-Warburton and Allen 2000), and altered AMF diversity and functioning due to N eutrophication could conceivably influence plant-fungal water relations. These effects may negatively impact water uptake and drought tolerance of plants (Bobbink et al. 2010) and be partially responsible for the observed decline of CSS under simultaneous drought and high N conditions (Kimball et al. 2014). Hence, there is a strong need to better understand the combined effects of N deposition and drought on plant growth responses as mediated by the soil microbial community.

In this study we evaluated effects of inoculation with soils from low and high N deposition sites and differential N and water availability on growth and biomass allocation of seedlings of the dominant CSS shrub species, *Artemisia californica* Less. (Asteraceae). This species is highly mycorrhizal (Sigüenza et al. 2006a) and plastic in its growth response to both N (Padgett and Allen 1999; Sigüenza et al. 2006a; Yoshida and Allen 2001) and water (Pratt and Mooney 2013). Sigüenza et al. (2006a) found a strong negative feedback of N-impacted mycorrhizal communities on growth of *A. californica* seedlings, likely due to the selection of inferior mutualists, but this experiment was conducted under well-watered conditions and it is unknown how drought might change these dynamics.

The purpose of this study was to investigate the effects of N deposition on growth and biomass allocation of *A. californica* seedlings due to increased N availability and N-impacted soil microbial communities, and to understand how these factors influence the response of seedlings to drought. We hypothesized: (1) plant growth would differ in low and high deposition soil communities, resulting in different plant-soil feedbacks; (2) seedlings would experience a more negative feedback in N-impacted soil communities when soil N was high (Johnson et al. 1997; Sigüenza et al. 2006a); (3) drought would reduce plant growth, negatively impacting feedbacks, especially in plants grown in N-impacted soils and under high N availability; and (4) belowground resource availability would alter biomass partitioning, with N addition favoring allocation to shoots and drought favoring allocation to roots.

Materials and methods

Study sites

We collected soil for plant inoculations from two sites (Table 1) receiving different levels of N deposition in the Santa Monica Mountains National Recreation Area, California. Rancho Sierra Vista is located in eastern Ventura County on the western end of the mountain range (34.15°N, 118.96°W) and receives low levels of N deposition, modeled at about 8.8 kg N ha⁻¹ yr⁻¹ (Fenn et al. 2010; Tonnesen et al. 2007). Franklin Canyon is located near the geographic center of the City of Los Angeles (34.12°N, 118.41°W) on the eastern end of the Santa Monica Mountains. The modeled rate of N deposition

for Franklin Canyon is about 20.1 kg N ha⁻¹ yr⁻¹ (Tonnesen et al. 2007), and the area has experienced high levels of N deposition for decades (Egerton-Warburton et al. 2001). Both sites are similar in elevation and, typical of a Mediterranean climate, experience hot dry summers and cooler winters with variable precipitation falling between November and April. Rancho Sierra Vista receives approximately 420 mm rainfall annually, while Franklin Canyon receives 379 mm. The soils at each site are loamy and similar in texture and soil P (Table 1). While other factors that influence soil microbial communities besides N deposition may differ between sites, our goal was to evaluate the response of seedlings to soil communities representative of sites along the anthropogenic N deposition gradient of the Santa Monica Mountains. Seeds of *A. californica* for our growth experiment were collected from Zuma Canyon (34°04'N, 118°82'W), a site located roughly between the two inoculum collection sites that receives approximately 12.3 kg N ha⁻¹ yr⁻¹ (Fenn et al. 2010; Tonnesen et al. 2007).

Soil inoculum

Soil was collected from both sites in December 2012 to be used as whole-soil inoculum. At each site we collected soil cores 0–10 cm in depth beneath the outer canopy of mature *A. californica* shrubs. We collected five cores from 20 individual shrubs over an area of approximately one hectare at each site. Soil was sieved through a 1 cm² stainless steel mesh and transported to the laboratory where it was refrigerated until potting. We homogenized the whole-soil inoculum for each site to ensure plants in each treatment were inoculated with a complete soil microbial community representative of each site. Spore density of AMF was similar in the high and low deposition inocula, 136.7 spores g⁻¹ (SE ± 8.6) and 156.8 spores g⁻¹ (SE ± 21.4) respectively, but the high deposition inoculum was dominated by a few species of small-spored *Glomus*, especially *G. clarum* and *G. deserticola* (Schenck and Perez 1990). We included a sterile control as our third soil treatment for comparison in order to better understand the response of seedlings to the two live soil communities. The different soil inoculation treatments were prepared as follows:

1. Low deposition: 25 g of whole-soil inoculum from the low N deposition site and 25 g of steam-sterilized whole-soil inoculum from the high N deposition site added to each pot.

Table 1 Sites used for soil inoculum collection and their geographic locations, elevation, average annual rainfall, modeled N deposition, and soil P and N concentrations

Site	Latitude (°N)	Longitude (°E)	Elevation (m)	Rainfall (mm)	N Dep. ^a (kg ha ⁻¹ yr ⁻¹)	Soil P ^b (ppm)	Soil N ^c (μg N/g)
Satwiwa	35°15'	118°96'	271	420	8.8	18.8 (±2.9)	13.9 (±3.0)
Franklin Canyon	34°12'	118°41'	275	379	20.1	15.5 (±2.3)	37.4 (±6.1)

^a Modeled rates of atmospheric nitrogen deposition from Tonnesen et al. (2007)

^b Mean soil phosphorus (Olsen-P) concentrations (0–10 cm depth) and SE

^c Mean KCl extractable soil nitrogen concentration (0–10 cm depth) and SE

- High deposition: 25 g of whole-soil inoculum from the high N deposition site, and 25 g of steam-sterilized whole-soil inoculum from the low N deposition site added to each pot.
- Sterilized control: 25 g of steam-sterilized whole-soil inoculum from the low N deposition site, and 25 g of steam sterilized whole-soil inoculum from the high N deposition site added to each pot.

Each pot that received live inoculum also had steam-sterilized soil from the other site added to account for any differences in nutrient availability, while the sterilized control received steam-sterilized soil from both sites. Total KCl extractable N of the low and high deposition inoculum was 13.9 μg N g⁻¹ soil (SE ± 3.0) and 37.4 μg N g⁻¹ soil (SE ± 6.1) respectively. Steam sterilization did not result in a significant increase of extractable N.

Growth experiment

We conducted the growth experiment from January to March 2013 in a greenhouse at the University of California, Riverside set at 21/16° day/night temperature to simulate winter growing season conditions for this Mediterranean-type climate. The potting media, to which inoculum was added, was a 1:1 mix of field soil to silica sand. Sand was added to promote water infiltration and drainage. We collected field soil for potting at our low deposition site, Rancho Sierra Vista, from a mature stand of CSS. Prior to potting, this soil mixture was steam sterilized for 24 h, followed by a 48-h incubation period, and then an additional 24-h steam treatment. We filled 650 ml plastic pots (Deepots; Steuwe and Sons, Corvallis, Oregon, USA) with approximately 500 g of our potting soil mixture. The appropriate inoculum was added to each pot about 10 cm from the surface and mixed into the soil. Extractable N in the potting soil after

sterilization and dilution with sand was 12.5 μg N g⁻¹ soil (S.E. ± 2.1), which was well below our high N treatment. Prior to planting we leached all pots with 250 ml distilled water. Seeds of *A. californica* were sown into individual pots and watered daily with distilled water until seedlings germinated and established in each pot, at which point seedlings were thinned to a density of one per pot. We rotated pots within racks and randomly rearranged racks on greenhouse benches each week.

Nitrogen fertilization

Seedlings were grown in one of two N treatments. Half of the pots in each inoculum treatment received supplemental N (high N treatment) while the other half did not (low N treatment). In order to simulate the high amounts of soil N that would be available early in the growing season at high N deposition sites, we fertilized each of the high N pots twice early in the experiment, first after seedlings were established in each pot and again 1 week later. When fertilized, each high N pot received approximately 22.5 μg N g⁻¹ soil from ammonium nitrate (NH₄NO₃) in solution for a total of 45 μg N g⁻¹ soil total, while the low N plants received an equal volume of distilled water.

Watering treatments

We initiated watering treatments after 2 weeks of growth. Half of the pots in each soil treatment were maintained at 60 % water holding capacity by weight (well-watered treatment), while the other half were maintained at 20 % water holding capacity (drought treatment) for the duration of the experiment. Every 3–5 days depending on greenhouse conditions, individual pots were weighed and watered with distilled water to the desired water holding capacity according to the two treatments. Drought-stressed seedlings were often visibly wilted prior to watering indicating plants in this

treatment were consistently water-limited throughout the duration of the experiment.

Harvest

We harvested plants ($n = 10$) after 10 weeks of growth. Seedlings were separated into roots and shoots, dried to constant mass at 60 °C and weighed. Subsamples of roots were weighed and measured to determine root length for each plant. Dry leaf tissue was analyzed for percent N content 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

We quantified the colonization of mycorrhizal and non-mycorrhizal fungi in plant roots as percent root length colonized (PRLC). Once roots were dried and weighed, we rehydrated them in distilled water overnight and cleared and stained them with trypan blue (Koske and Gemma 1989). Previous work in our lab has demonstrated drying does not affect microscopic recognition of fungal structures within roots. We employed a modified line-intercept method based on the procedure of McGonigle et al. (1990) to assess percent colonization of mycorrhizal and other non-mycorrhizal fungi within roots and from these measurements calculated PRLC. For each plant, we examined ten randomly selected fine root fragments under the microscope, making observations on presence or absence within ten fields along transects for each fragment. We distinguished between mycorrhizal and other fungi based on visual appearance, including hyphal morphology, size and presence or absence of septa. We found no mycorrhizal colonization and very low levels (< 1 %) of other fungi in the roots of plants grown in sterilized control inoculum, and these were not included in analyses of PRLC.

Calculation of feedback

We calculated plant-soil feedback as the difference in total biomass of seedlings grown in live soil relative to the mean total biomass of those grown in sterilized controls under the same resource conditions:

$$\text{Relative feedback} = \frac{(\text{Biomass}_{\text{Live}} - \text{Mean Biomass}_{\text{Sterile}})}{\text{Mean Biomass}_{\text{Sterile}}}$$

Using this calculation of feedback, a value less than zero indicates a negative feedback, likely due to below-ground pathogens or possibly antagonistic mutualists (e.g. Johnson 1993), while a value greater than zero indicates a positive feedback, likely due to beneficial organisms such as AMF or other soil biota involved in nutrient cycling.

Statistical analyses

We grew seedlings in three soil inoculum treatments (low deposition, high deposition and sterile control), with or without supplemental N and under well-watered or drought conditions in a full factorial experimental design ($3 \times 2 \times 2$). We performed analysis of variance (ANOVA) with inoculum, N, water and the interactions between these as factors on plant response variables measured including root, shoot and total dry mass, relative feedback, root:shoot ratio, PRLC of mycorrhizal and non-mycorrhizal fungi and leaf percent N content. Prior to statistical analysis, we tested all data for normality and homogeneity of variance, transforming data when necessary to meet the assumptions of ANOVA. Following ANOVA, we performed Tukey's honest significant difference test (HSD) to compare means and assign significance at $p < 0.05$. We used linear regression to compare rates of mycorrhizal and non-mycorrhizal colonization with plant biomass. Finally, we used polynomial regression to compare root:shoot ratios with the natural logarithm of total biomass to determine the influence of plant size on biomass partitioning (Gedroc et al. 1996), and completed allometric analysis using linear regressions of log-transformed shoot and root biomass (Farrar and Williams 1991; Staddon and Fitter 1998). Statistical analyses were performed using RStudio Version 0.98.57, RStudio, Inc.

Results

Plant growth response

Inoculum ($p < 0.0001$), N ($p < 0.0001$), and water ($p < 0.0001$) and the interactions between these treatments significantly influenced plant biomass (Table 2, Fig. 1). In the low deposition inoculum, mean total biomass did not significantly differ between N and water

Table 2 *F* ratios from two-way ANOVA tests of inoculum (I), nitrogen (N) and water (W) on plant responses

Response	Source of variance						
	Main effects			Interactions			
	Inoculum	Nitrogen	Water	I × N	I × W	N × W	I × N × W
Shoot mass	44.35***	88.64***	310.45***	54.78***	41.49***	49.54***	45.40***
Root mass	15.95***	21.36***	66.45***	17.36***	19.48***	10.21**	15.80***
Total mass	20.99***	69.59***	237.42***	45.65***	38.63***	37.59***	38.89***
Root:shoot	81.36***	0.15	12.62***	0.16	9.06***	0.20	0.89

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

treatments (Fig. 1). Under low N conditions, seedlings grown in low deposition inoculum had significantly higher biomass than those grown in high deposition inoculum (Fig. 1). Plants grown in high deposition inoculum had very low biomass when grown under low N availability or drought conditions (Fig. 1). However, when plants inoculated with high deposition soil were grown under high N and well-watered conditions, they accrued the highest biomass of any treatment (Fig. 1). In the sterile controls, seedlings were significantly larger in the well-watered treatments relative to drought-stressed plants, but N

addition did not significantly affect total biomass in this treatment (Fig. 1).

Plant-soil feedbacks

Relative plant-soil feedbacks differed by inoculum type and with resource availability (Fig. 3). Feedbacks were significantly affected by inoculum type ($p = 0.0005$) and the interaction of inoculum with N ($p = 0.0004$) and water ($p < 0.0001$), indicating plants responded differently to soil communities from low and high N deposition sites. Water availability significantly influenced feedbacks ($p < 0.0001$), as did the interaction of water and N ($p < 0.0001$). Nitrogen alone had no significant effect ($p = 0.2756$). There was also a significant three-way interaction of treatments ($p < 0.0001$). In the low deposition inoculum, seedlings exhibited a negative feedback when well-watered, regardless of N availability (Fig. 3). However, in the drought treatment, seedlings showed a very positive feedback when soil N was low, and a neutral response when soil N was high. Conversely, seedlings grown in high deposition inoculum exhibited a negative feedback when soil N was low, but a positive to neutral feedback under high N availability (Fig. 3).

Drought influenced feedbacks differently depending on inoculum type and N availability. Biomass of seedlings grown in low deposition inoculum was unaffected by drought, and drought positively influenced feedbacks in this inoculum (Fig. 3). A similar pattern was observed in seedlings grown in high deposition inoculum under low N, where well-watered plants exhibited a negative feedback, and drought-stressed plants showed a neutral feedback. However, in high deposition inoculum under high N, drought diminished the positive feedback

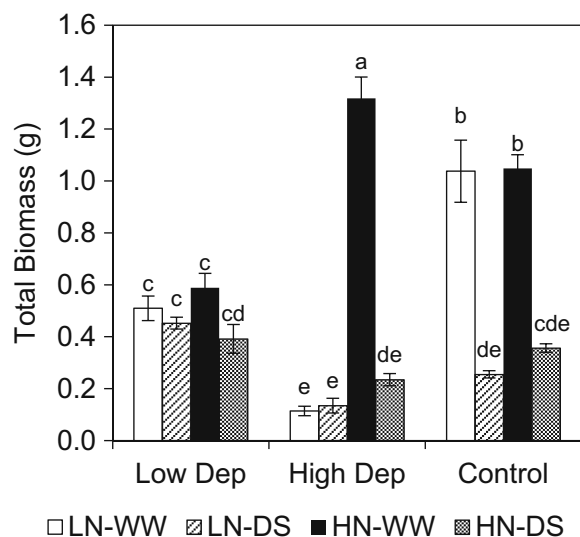


Fig. 1 Total plant biomass of seedlings after 10 weeks \pm SE. Data represent average values of ten plants. LN = low nitrogen, HN = high nitrogen, WW = well-watered, DS = drought-stressed, Low Dep = inoculum from a low deposition site, High Dep = inoculum from a high deposition site, Control = sterilized control. Different letters above bars indicate significant differences (Tukey's HSD test, $P < 0.05$)

observed under well-watered conditions, resulting in a neutral feedback (Fig. 3).

Biomass allocation

We also assessed effects of inoculum source, soil N and water on root:shoot ratios of plants, which is instructive in understanding how plants allocate biomass under varying belowground resource availability. Root:shoot ratios of seedlings were significantly affected by inoculum ($p < 0.0001$), water ($p < 0.0001$) and the interaction between inoculum and water ($p < 0.0001$; Table 2). Nitrogen had no significant effect ($p = 0.15$; Table 2). Average root:shoot ratios were highest in seedlings grown in low deposition inoculum compared to seedlings from other soil treatments, ranging from 0.57 to 0.81 (Fig. 2). In this inoculum, drought resulted in higher allocation to roots, but this difference was only statistically significant under low N (Fig. 2). In high deposition inoculum, mean root:shoot ratios ranged from 0.32 to 0.47 and were not significantly affected by N or water availability (Fig. 2). The lowest root:shoot ratios were observed in seedlings grown in sterile soil, which averaged 0.28 to 0.33, with no significant differences between N and water treatments (Fig. 2). The results of a third-order polynomial regression of

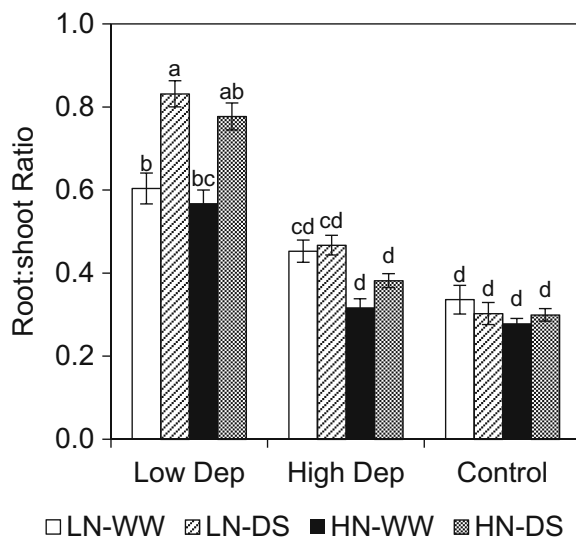


Fig. 2 Root:shoot ratios of seedlings after 10 weeks \pm SE. Data represent average values of ten plants. LN = low nitrogen, HN = high nitrogen, WW = well-watered, DS = drought-stressed, Low Dep = inoculum from a low deposition site, High Dep = inoculum from a high deposition site, Control = sterilized control. Different letters above bars indicate significant differences (Tukey's HSD test, $P < 0.05$)

root:shoot ratios vs. the natural logarithm of total biomass across treatments (Gedroc et al. 1996) indicates that allocation patterns were not correlated with plant size ($p = 0.316$). Furthermore, correlations between log-transformed shoot and root biomass were different in seedlings grown in low deposition ($r = 0.35$, $p = 0.004$) and high deposition ($r = 0.83$, $p < 0.0001$) soils, showing relative growth rates of root vs. shoots differed in low and high deposition soils (Farrar and Williams 1991).

Plant nitrogen

We analyzed effects of the different soil, N and water treatments on leaf tissue N of seedlings and found a significant influence of inoculum ($p < 0.0001$) and water ($p < 0.0001$), as well as an interactive effect of inoculum, N and water ($p = 0.015$; Table 3). Seedlings grown in the two live inocula tended to have lower percent leaf N than in sterile controls and exhibited a similar response to N and water availability, except percent leaf N was significantly higher in the high deposition plants in the high N, drought treatment. Across soil and N treatments, drought-stressed plants generally had higher percent leaf N than well-watered counterparts.

Root colonization

We observed mycorrhizal and non-mycorrhizal fungal structures in roots of plants grown in live soil inocula (Table 4). Percent root length colonized by AMF was significantly affected by inoculum ($p < 0.0001$) the interaction of N and water ($p = 0.007$) and the interaction of inoculum, N and water ($p = 0.0316$). The highest levels of PRLC by mycorrhizal fungi were observed in the roots of plants grown in low deposition inoculum, especially when N and water were limiting (Table 4). Seedlings grown in high deposition inoculum showed a different pattern, where PRLC was reduced under low N or drought conditions (Table 4). There were no significant correlations between PRLC by AMF and plant biomass within or among treatments ($p > 0.05$ for all correlations).

Percent root length colonized by non-mycorrhizal fungi was significantly affected by inoculum type ($p < 0.0001$), N ($p = 0.002$) and the interaction of inoculum and N ($p = 0.025$) and inoculum and water ($p = 0.017$). Mean PRLC by non-mycorrhizal fungi ranged from 22.5 % to 34.5 % in seedlings grown in

Table 3 Percent leaf N of seedlings grown in high and low N deposition inoculum and in a sterilized control under differential N and water availability after 10 weeks

Soil inoculum	Nitrogen Treatment			
	Low N		High N	
	Well-watered	Drought-stressed	Well-watered	Drought-stressed
Low deposition	2.53de	3.52abc	2.49e	3.03cde
High deposition	2.94cde	3.34ab	2.43e	3.97ab
Control	3.33bcd	4.13ab	3.41bcd	4.13a

Values followed by different letters are significantly different at $P < 0.05$

low deposition inoculum, and did not differ significantly across resource treatments (Table 4). Seedlings grown in the high deposition inoculum had a similar PRLC to those grown in low deposition inoculum under high N and water availability, but significantly lower colonization under low N and drought treatments (Table 4). There were no significant correlations between PRLC of non-mycorrhizal fungi within or across treatments ($p > 0.05$ for all correlations).

Discussion

Effects of nitrogen deposition

We found that plant-soil feedbacks of *A. californica* seedlings differed in low and high deposition soils, and N and water availability influenced feedbacks differently in the two soil communities (Fig. 3). While soil N availability had significant effects on plant growth and feedbacks (Table 2, Fig. 3), the results of this study suggest that N deposition may also exert strong indirect

effects on native seedling performance through changes to soil biota. Moreover, we observed significant interactions of soil inoculum, N and water on plant performance (Table 2), suggesting seedlings at high N deposition sites may respond differently to drought. These results highlight the critical role of the soil microbial community in mediating plant performance in response to varying environmental conditions, which could have important implications for seedling establishment under global change. Reestablishment or restoration of this species is often limited by seedling recruitment (Allen et al. 2000; Cox and Allen 2008; Eliason and Allen 1997), and the different growth strategies observed might differ in benefits conferred to seedlings in a realistic field setting. For example, native shrub seedlings often face intense competition from invasive grasses and forbs, and increased growth could enhance their competitive ability. Reduced growth of native shrub seedlings in N-impacted soils under drought could be partially responsible for loss of CSS observed under high N deposition (Fenn et al. 2010; Kimball et al. 2014; Talluto and Suding 2008).

Table 4 Mean percent root length colonization (PRLC) of roots by mycorrhizal (AMF) and other non-mycorrhizal (NMF) fungi of seedlings grown in high and low N deposition inoculum and under

differential N (Low N or High N) and water (Well-watered or Drought-stressed) availability

	Inoculum Treatment							
	Low deposition				High deposition			
	Well-watered		Drought-stressed		Well-watered		Drought-stressed	
	Low N	High N	Low N	High N	Low N	High N	Low N	High N
AMF	6.5a	5.6a	14.4a	5.3a	0.7c	4.9ab	1.3bc	0.2c
NMF	22.2a	32.0a	34.5a	31.4a	6.9b	32.6a	5.3b	7.5b

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

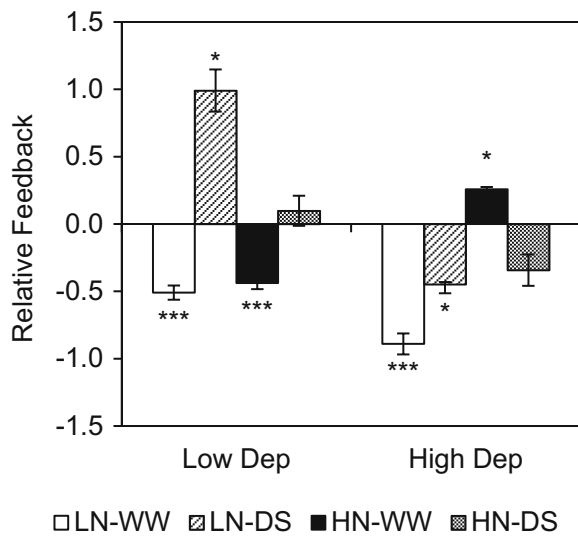


Fig. 3 Relative feedback calculated as the difference in biomass relative to sterilized controls at 10 weeks \pm SE. LN = low nitrogen, HN = high nitrogen, WW = well-watered, DS = drought-stressed, Low Dep = inoculum from a low deposition site, High Dep = inoculum from a high deposition site. ***, **, * indicate significance relative to sterilized controls at $P \leq 0.05$, 0.01 and 0.001, respectively (ANOVA, Tukey's HSD test)

While plant-soil feedbacks were altered in N-impacted soil communities, unlike previous studies (Corkidi et al. 2002; Johnson 1993; Sigüenza et al. 2006a), we found no evidence of N deposition leading to an antagonistic mycorrhizal relationship. In fact seedlings grown in high N deposition inoculum under high N and water availability had the greatest biomass of any treatment (Fig. 1). However, our results suggest N deposition could negatively impact mycorrhizal relationships and plant-soil feedbacks in several ways. The high deposition soil community only led to a positive growth response under high resource availability; under drought conditions, seedlings exhibited a neutral feedback, and under well-watered and low N conditions, seedlings grown in high deposition soils experienced a very negative feedback (Fig. 3). This suggests the benefits of this N-impacted soil community are diminished when N or water are limiting. Furthermore, in high deposition inoculum under low N or drought, AMF colonization was much lower than in seedlings grown in low deposition inoculum (Table 4), indicating that under some conditions, N deposition may severely reduce mycorrhizal activity. This could negatively impact native seedling performance and establishment in the field, given the important role AMF can play in increasing plant nutrition and tolerance to biotic and abiotic stressors (Smith

and Read 2008), including drought (Augé 2001; Birhane et al. 2012).

Effects of drought

Our results are particularly informative in the context of global climate change, and effects of drought on seedlings grown in the two soil communities under varying N availability may further illustrate the potential negative impacts of N deposition on dryland species. Contrary to our initial hypothesis, drought only negatively impacted plant growth and feedbacks in high deposition inoculum (Figs. 1 and 3). Seedlings grown in N-impacted soils with supplemental N had the most negative response to drought, while biomass of plants inoculated with low deposition soil was unaffected by water-deficit (Fig. 1). Furthermore, when both N and water supply was low, seedlings grown in low deposition inoculum exhibited a positive feedback (Fig. 3). Species from arid and semi-arid environments may respond more positively to AMF under low amounts of pulse-driven precipitation (Birhane et al. 2012), and our results indicate N deposition could minimize the benefit of these mutualistic relationships when water is limiting. This shows native soil communities may help protect against drought-stress and supports the hypothesis proposed by others, that N-impacted AMF communities may increase susceptibility to drought (Bobbink et al. 2010; Smith and Read 2008).

Biomass allocation

Effects of inoculation, N and water on biomass allocation may help further explain how N deposition will influence plant performance in the field under variable environmental conditions. Plants are often able to adjust biomass partitioning in response to resource availability, increasing allocation to structures that acquire a limiting resource (Chapin et al. 1987; Poorter and Nagel 2000). After 10 weeks of growth, we found no significant effects of N availability on root:shoot ratios, although patterns of allocation differed in low and high deposition soils (Fig. 2), suggesting N deposition may influence plant allocation indirectly via changes to the soil community. Seedlings grown in low deposition inoculum had the highest root:shoot ratios and exhibited a significant increase in root:shoot ratios under drought (Fig. 2), consistent with the theory of optimal partitioning. However, seedlings grown in high deposition soil allocated

significantly less biomass to roots, and did not adjust root:shoot ratios in response to N or water availability (Fig. 2). Thus, it appears this species is able increase allocation to roots in response to drought under some conditions, but this effect may be diminished in N-impacted soils. While plant size is often a key determinant of biomass allocation (Gedroc et al. 1996), with plants tending to have higher allocation to roots early in development, we found no significant relationship between plant size and root:shoot ratio within or across treatments, and allometric relationships of shoot and root biomass differed in the two soil communities. Furthermore, seedlings with the highest root:shoot ratios, those grown in low deposition soil, had significantly higher mean biomass than several other treatments. Altogether, this indicates that differences in patterns of allocation are due to treatment effects, and not ontogenetic drift (Farrar and Williams 1991; Gedroc et al. 1996).

Shifts in allocation due to N deposition could have important implications for seedling establishment and large-scale vegetation patterns. Mediterranean plant species typically have higher root:shoot ratios than plants from more mesic biomes (Hilbert and Canadell 1995), possibly as an adaptation to seasonal drought (Lloret et al. 1999). In addition, seedling survival is positively correlated with root:shoot ratio (Lloret et al. 1999) and rooting depth (Padilla and Pugnaire 2007) in a number of Mediterranean shrub species. Increased allocation to roots, as observed in seedlings inoculated with low deposition soil (Fig. 3), could promote survival in this semi-arid ecosystem. Conversely, low root:shoot ratios of seedlings grown in high deposition inoculum (Fig. 3) would be expected to increase susceptibility to drought, and lower allocation to roots could be partially responsible for the significantly lower biomass in drought-stressed plants grown in this inoculum under high N (Fig. 1).

Pathogens and other biota

While AMF are known to be important in shaping feedback in this system (Bozzolo and Lipson 2013; Hilbig and Allen 2015; Sigüenza et al. 2006a), pathogens, including both fungi and bacteria, probably also play a significant role. However, our methods only allowed for an assessment of potential fungal pathogens in plant roots. In several treatments, colonization by non-mycorrhizal fungi was higher in roots of seedlings grown in low N deposition inoculum vs. high deposition inoculum (Table 4), even though biomass was

significantly lower in the latter (Fig. 1). The majority of these fungi were ascomycetous and probably facultative pathogens. We did not observe any symptoms of disease in plant roots or shoots, but pathogenic activity likely contributed to the negative feedbacks observed under some conditions. It is possible that the high density of *A. californica* shrubs at the low N deposition site promoted a higher pathogen load (Packer and Clay 2000; Van der Putten et al. 2001) relative to the high N deposition site where shrub density is lower, resulting in higher levels of colonization. Root pathogens are also strongly influenced by soil moisture (Cook and Papendick 1972) and increased pathogenic activity could be responsible for the negative feedbacks observed under well-watered conditions (Fig. 3).

It is also possible plant-soil feedbacks were influenced by non-symbiotic organisms in our soil inocula through competition for resources and changes in nutrient cycling. Growth depressions in inoculated plants may be partially attributable to immobilization of nutrients by the microbial community (Barber 1978; Lekberg and Koide 2013). Plants grown in sterile inoculum generally had higher percent leaf N than plants inoculated with live soil communities (Table 3), which could be evidence plants grown in live soil were competing with soil microbes for N (Kaye and Hart 1997). High levels of N deposition may also increase rates of N mineralization in CSS soils (Vourlitis et al. 2007), and similar changes in microbially-mediated nutrient cycling could have also contributed to the positive feedback observed in high deposition inoculum under high resource availability (Fig. 3).

Methodological considerations

We used a whole-soil inoculation approach similar to previous studies aimed at understanding feedbacks in this system (Bozzolo and Lipson 2013; Sigüenza et al. 2006a). As such, our inocula likely contained a range of soil microorganisms. While AMF and other fungi likely played an important role in shaping plant responses, other organisms, both free-living and symbiotic, undoubtedly were also involved. However, we did not observe any micro-fauna in spore extractions, nor did we find any evidence of belowground herbivory when examining roots under the microscope. It is important to note soils at our collection sites may differ in other factors besides N inputs, but despite these and other limitations inherent to this experimental approach, our study successfully

demonstrates how seedlings of this species would respond to actual soil communities at low and high N deposition sites within our study area. This work also calls into question the ecological relevance of similar experiments conducted under well-watered conditions using drought-adapted species. We observed dramatic differences in the direction and magnitude of feedback with watering regime, and water significantly affected a number of parameters. In dryland ecosystems, plants and microbes are frequently water-limited, and attempts to characterize feedbacks in these systems under a single, often artificially high, watering regime may be misleading.

Conclusions

Our understanding of the ecological impacts of N deposition comes largely from temperate biomes (Bobbink et al. 2010). However, there is also evidence from dryland ecosystems other than CSS that N addition can result in plant community shifts, including deserts (Báez et al. 2007; Brooks 2003; Rao and Allen 2010), semi-arid shrublands (Ochoa-Hueso and Manrique 2010; Ochoa-Hueso et al. 2013) and arid and semi-arid grasslands (Bonanomi et al. 2006; Schwinning et al. 2005; Zeng et al. 2010). With N deposition (Galloway et al. 2008) and extreme drought events (Sheffield and Wood 2008) expected to increase globally in the near future, there is a strong need to understand potential interactive effects of these co-occurring global change drivers on both plants and soils. This is the first study we are aware of to explicitly consider the role of the soil microbial community in shaping plant responses to these two factors simultaneously. We found native soil communities promote higher mycorrhizal activity and allocation to roots and may protect against drought, but N deposition can diminish these potentially beneficial effects through elevated N availability and changes to the soil community. In addition to other stressors, interactive effects of N and drought on plants and soil communities could contribute to vegetation shifts in arid and semi-arid ecosystems under chronic N deposition.

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