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# Spatial variation in edaphic characteristics is a stronger control than nitrogen inputs in regulating soil microbial effects on a desert grass

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ABSTRACT

Increased atmospheric nitrogen (N) deposition can have wide-ranging effects on plant community structure and ecosystem function, some of which may be indirectly mediated by soil microbial responses to an altered biogeochemical environment. In this study, soils from a field N fertilization experiment that spanned a soil texture gradient were used as inocula in the greenhouse to assess the indirect effects of soil microbial communities on growth of a desert grass. Plant performance and interaction with soil microbiota were evaluated via plant above- and belowground biomass, leaf N concentration, and root fungal colonization. Nitrogen fertilization in the field increased the benefits of soil microbial inoculation to plant leaf N concentration, but did not alter the effect of soil microbes on plant growth. Plant-microbe interaction outcomes differed most strongly among sites with different soil textures, where the soil microbial community from the sandiest site was most beneficial to host plant growth. The findings of this study suggest that in a desert grassland, increases in atmospheric N deposition may exert a more subtle influence on plant-microbe interactions by altering plant nutrient status, whereas edaphic factors can alter the whole-plant growth response to soil microbial associates.



## 1. Introduction

Increased nitrogen (N) inputs from agricultural fertilizers and the combustion of fossil fuels have altered the biogeochemistry of terrestrial ecosystems, with important consequences for the ecological dynamics of terrestrial communities (Elser et al., 2007; Bobbink et al., 2010; Gerstner et al., 2014). For example, in temperate grasslands, N additions generally increase plant productivity and reduce diversity (Clark and Tilman, 2008; Fay et al., 2015). Nitrogen deposition can also alter soil microbial biomass, community composition, and enzymatic activity (e.g. Compton et al., 2004; Waldrop et al., 2004; Treseder, 2008; Leff et al., 2015; Mueller et al., 2015). These consequences of anthropogenic N deposition are especially important because soil microbial communities couple abiotic and biotic components of the ecosystem. Microbes mediate nutrient availability to plants through decomposition, mineralization, and mutualisms, and a disruption of these interactions could have cascading effects across multiple trophic levels (Wardle et al., 2004).

Our knowledge of direct plant and microbial community responses to N manipulations derives mostly from mesic ecosystems and fewer such studies have been conducted in arid ecosystems (but see Mueller et al., 2015; Sinsabaugh et al., 2015; McHugh et al., 2017). Responses to N inputs in arid ecosystems may differ from mesic ecosystems for several reasons. First, arid ecosystems, such as the Colorado Plateau, USA, historically derive much of their N from $N_2$-fixing components of biological soil crusts (biocrusts) (Belnap, 2003), which can be very sensitive to environmental changes (Belnap, 2001). In most areas, atmospheric N deposition equals or exceeds the magnitude of N inputs from microbial $N_2$ fixation in biocrusts (Belnap, 2003). Second, soil N content and ambient N deposition are generally low in US deserts compared to other ecosystems (Fenn et al., 2003), which may make these systems more sensitive to increases in N inputs. In addition, past work in deserts has shown increased primary productivity from N addition to be greater when precipitation was high (Ladwig et al., 2011;

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Yahdjian et al., 2011). This suggests that local edaphic characteristics that determine water availability, such as soil texture, could strongly mediate N deposition effects in arid ecosystems. Increased knowledge of how N inputs alter arid ecosystems is therefore crucial to a global understanding of the consequences of anthropogenic N deposition.

In addition to direct effects on plants and soil microbes, N deposition can have indirect effects on biological communities via interactions between plants and soil microbes (Classen et al., 2015). For example, in many systems, soil microbes can be strong drivers of plant diversity and productivity (reviewed by Van Der Heijden et al., 2008). Mutualistic interactions between plants and their root-associated microbes are often based on resource exchange (Reynolds et al., 2003; Kiers and Denison, 2008; Kiers et al., 2011). Changes in the resource context, such as with N deposition, could indirectly alter plant productivity and diversity via changes in symbiont resource strategy. For example, a meta-analysis confirmed that N-fertilization decreased the benefits of mycorrhizal fungi to host plants (Hoeksema et al., 2010). Another class of commonly-found root fungal symbionts in arid ecosystems, dark septate endophytes, has been shown to increase plant nutrient uptake only in the presence of additional organic N (Jumpponen et al., 1998). More broadly, N addition could directly alter the composition of the soil microbial community, thereby indirectly affecting plant performance via changes in pathogen or mutualist loads (Egerton-Warburton and Allen, 2000; Dean et al., 2014). To resolve the causality of these indirect effects, evidence is needed beyond documenting plant and microbe community responses separately. Microbial inoculation studies provide a powerful tool to experimentally separate the indirect effects of N deposition on plants via changes in microbial community composition and function.

In this study, we used *Achnatherum hymenoides* (Indian ricegrass), a dominant herbaceous $C_3$ grass on the Colorado Plateau, USA, to assess indirect effects of N addition on plant performance via alteration of natural dryland soil rhizosphere and biocrust microbial communities and their function. Soil samples from an ongoing field N addition experiment were used as inocula in a greenhouse experiment to isolate possible microbial effects on the performance of the plant, allowing us to ask the following questions: 1) What are the indirect effects of N addition on plant biomass via shifts in rhizosphere and biocrust soil microorganisms? Nitrogen addition in the field could result in rhizosphere and biocrust microbial communities that have different effects on the plant host, and this effect should be present only in plants inoculated with living microbes. 2) What are the indirect effects of N addition on interactions between plants and root-associated fungi? Past work has shown that N addition can directly decrease fungal colonization in plant roots (Treseder, 2008), which may decrease the colonization potential of soil inocula applied to plants. 3) What is the relative importance of experimental N addition versus among-site edaphic differences in driving microbial mediation of plant performance, and does natural spatial variation outweigh the local effects of N addition? Plant-microbe interaction outcomes are often context-dependent; therefore, we expect that environmental differences should interact with N addition to alter the effects of soil microbial inoculation on plant performance.

## 2. Methods and materials

### 2.1. Field site description

Soil samples were collected from an ongoing N deposition fertilization experiment in Arches National Park, Utah, USA (38° 47′ N, 109° 39′ W). The experiment includes three sites which are <5 km apart and have similar plant and biocrust communities, but vary in soil texture, ranging from sandy loam, to loamy sand, to sand (Table 1). Variation in soil texture derives from differences in aeolian depositional patterns influenced by the shape of the valley and the sites' proximity to bordering cliffs. All three sites are classified as aridisols according to the Natural Resources Conservation Service's (NRCS) survey and classification system. Due to their proximity to one another, the sites experience the same climate and have similar vegetation. Average annual precipitation is 229–279 mm, and mean annual temperature is 14.5 °C. Vegetation at all sites is a mix of shrubs, $C_3$ and $C_4$ bunch grasses, and annual grasses and forbs. Common species include the perennial grass *Achnatherum hymenoides* (Indian ricegrass; (Roem. & Schult.) Barkworth), as well as the shrubs *Atriplex canescens* (Fourwing saltbush; (Pursh) Nutt.) and *Ephedra torreyana* (Torrey's jointfir; S. Watson). Biocrust communities at all three sites are dominated by lightly pigmented cyanobacterial crusts (*Microcoleus vaginatus*), with some mosses (*Syntrichia caninervis*) present in the sandy loam and loamy sand sites.

### 2.2. N deposition experiment

A field experiment was established in March 2011 to assess the effects of N deposition on aridland ecosystem function, resilience to exotic plant invasion, and above- and belowground community composition. The experiment was replicated at three sites along a soil texture gradient (see above; Table 1), with five replicate blocks of three N addition levels and control in each site, totaling 60 experimental plots. The N addition levels were 2, 5, and 8 kg N $ha^{-1}$ annually, in addition to a de-ionized water control. The fertilizer was applied to 1 $m^2$ experimental plots with an additional 0.25 m of treated but unsampled plot buffer on each side, each centered on a mature *Achnatherum hymenoides* individual. The N addition levels are low in comparison to other similar studies (see references in Treseder, 2008; Sinsabaugh et al., 2015), but appropriate here due to the low ambient N deposition in this system (2–3 kg N $ha^{-1}$; Fenn et al., 2003). Each year the annual N treatment was split into two pulses, delivered in a 3 mm rainfall event in March and September, and applied in the form of dissolved ammonium nitrate (similar to Aber et al., 1993). Treatments had been applied for three years prior to soil collection for our greenhouse experiment.

### 2.3. Field collection methods

We collected ~40 ml of soil from each N deposition plot (N = 60 plots) in the field in August 2013, prior to the September N addition that year. In each plot, we collected four small samples (~5 ml each) from the rhizosphere of the central *Achnatherum hymenoides* by inserting a metal Scoopula® directly into the rooting zone of the plant to 10 cm depth. Another four small samples (~5 ml each) in each plot were carefully collected to a 5 mm depth from biocrusted

**Table 1**
Characteristics of the three sites along the soil texture gradient in Arches National Park near Moab, UT. All chemical data were collected at 0–10 cm depth from the plots just prior to the first fertilization event, which occurred in the spring of 2011. Soil texture values were collected from just outside of the plots and are 0–20 cm depth. Values are means and standard errors are provided in parentheses.

| Characteristic | Sandy loam | Loamy sand | Sand |
|---|---|---|---|
| Sand (%) | 71.5 (1.9) | 80.3 (1.2) | 87.9 (0.5) |
| Silt (%) | 15.1 (1.2) | 13.1 (1.2) | 7.6 (0.6) |
| Clay (%) | 13.4 (0.8) | 6.6 (0.8) | 4.5 (0.1) |
| Soil pH | 7.99 (0.02) | 7.72 (0.02) | 7.67 (0.01) |
| Soil organic C (%) | 0.40 (0.06) | 0.26 (0.02) | 0.20 (0.01) |
| Soil N (%) | 0.04 (0.002) | 0.03 (0.002) | 0.01 (0.001) |

interspaces showing signs of cyanobacterial colonization. The eight samples were homogenized per plot and stored at 4 °C.

### 2.4. Bioassay experiment

To isolate the effects of the microbial community, half of each soil sample was sterilized by autoclaving for 3 h at 121 °C. While this treatment may not kill every microbe, it resulted in suppression, including a four-fold decrease in root fungal colonization (see *Statistical analyses*). We filled 120 pots with spore-free river sand (checked for spores using light microscopy). We then added live or sterile soil inocula (10 ml) from each of the 60 field plots onto the soil surface of each pot, such that the inocula comprised 20% of total pot volume (50 ml). *Achnatherum hymenoides* seeds (var. Paloma, Southwest Seeds, CO, USA) were incubated at 10 °C in 500 ppm gibberellic acid for two weeks, and then germinated at 15 °C on sterile filter paper. Germinated seedlings were transplanted into prepared pots in November 2013 and maintained inside a greenhouse (20–23 °C). Throughout the growth phase, we recorded transplant mortality every two weeks. All pots were fertilized with a weak solution of commercial fertilizer (2.5 ml of FloraGro and FloraMicro at 650 ppm, General Hydroponics, Sebastopol, California, USA) twice throughout the duration of the experiment to sustain plant growth. The amount added was small (equivalent to 2.3 kg N $ha^{-1}$), applied equally across all pots, and did not result in soil N concentration beyond that recorded from the field experiment (0.017% ± 0.0006% SE in this experiment vs. 0.04–0.05% N as observed in the field, McHugh et al., 2017). In September 2014 (10 months after initiation of the greenhouse experiment), surviving plants were harvested for above- and belowground biomass. Harvested biomass was dried to constant mass at 60 °C, and weighed. A subsample of roots from each pot were stained and scored for fungal colonization with 100 views per sample (following McGonigle et al., 1990; Vierheilig et al., 1998). Dried leaf tissues and soils from each pot were combusted to determine N concentration on a Costech elemental analyzer (Costech Analytical Technologies, Valencia, CA, USA).

### 2.5. Statistical analysis

To compare the effects of microbial inoculation among sites and field N treatments, the effect size of microbial inoculation was calculated using the relative interaction index (*RII*, Armas et al., 2004). *RII*s were calculated for leaf N concentration, plant total biomass, aboveground biomass, and belowground biomass. This index ranges from −1 to 1, and compares the relative performance (biomass) of plants in paired control (sterile inoculation; 'sterile') and treatment (live inoculation: 'live') pots, where:

$$RII = \frac{\text{biomass(live)} - \text{biomass(sterile)}}{\text{biomass(live)} + \text{biomass(sterile)}}$$

.

An *RII* of zero indicates no effect of microbial inoculation, whereas a negative *RII* indicates a detrimental effect of microbial inoculation on plant performance and *vice versa*. At harvest, 78% of initial *Achnatherum* plants were still alive. Mortality was not significantly different among any treatment or site ($p > 0.05$), and only plants alive at harvest were used in *RII* calculations.

*RII*s of aboveground biomass, belowground biomass, total biomass, and leaf N concentration were analyzed individually using a two-way ANOVA with N treatment, site, and their interaction as fixed effects. Site was considered fixed because sites were chosen *a priori* to represent different soil types, rather than as a random subset of possible sites in the region. Mixed effects versions of ANOVA models that included spatial block as a random effect did not explain sufficient additional variation in the data to justify the additional parameter ($\Delta AICc > 2$ when compared to models with all fixed effects). Therefore, we dropped block as a variable and did not report on the mixed models in our results. *Post hoc* pairwise comparisons were conducted using Tukey's HSD test. To determine the effect of microbial inoculation on plant performance, least square mean *RII*s for plant aboveground, belowground, and total biomass at each site under each N treatment were tested for significant difference from zero using the test function in package lsmean in R (Lenth, 2016). The relative effect of microbial inoculation is known to depend on the absolute nutrient status of the plant (Johnson, 2010). Therefore, we also investigated the effects of site, N treatment, inoculation status (live/sterile), and all possible interactions on leaf N concentration directly using a 3 × 2 × 2 factorial ANOVA.

Root fungal colonization rates were first compared between live- and sterile-inoculated pots to ascertain treatment efficacy (ANOVA). Using only live-inoculated pots, we then compared arbuscular mycorrhizal fungi (AMF), dark septate endophyte (DSE), and total fungal colonization among sites and N treatments using ANOVA. We also determined if total fungal colonization in live-inoculated plants was driven by total root biomass using linear regression. We *ln*-transformed root fungal colonization data to fit analysis assumptions. All analyses were conducted in R version 3.1.2 (R Core Team, 2014).

## 3. Results

### 3.1. Indirect effects of N treatment and soil texture on plant performance via the microbial community

In general, microbial inoculation had weak effects on plant growth: most *RII*s of plant responses to microbial inoculation were not significantly different from zero (Fig. 1). The effects of microbial inoculation on *Achnatherum* plant performance differed strongly among sites, but not among N addition treatments within sites (Fig. 1). This pattern was consistent for all plant biomass responses (Table 2). Plant biomass responded positively to live soil microbes from the sand site ($RII > 0$), whereas the other two sites showed weak/neutral effects of live microbial inoculation. In contrast, within no sites were there significant differences in soil microbe effects on plant growth among N treatments (Table 2).

It is possible that treatments could influence plant nutritional status, but not overall growth. Similar to plant growth results, effects of live microbial inoculation on leaf N concentration were either neutral (*RII*s not significantly different from zero) or positive ($RII > 0$). However, in contrast to the growth response, plant leaf N concentration increased when inoculated with microbial communities that originated from plots fertilized with higher N concentrations ($RII > 0$, Fig. 2A), relative to controls, and there were no differences among sites in the microbial effect on leaf N (Table 2). When we considered leaf N concentration directly (Fig. 2B), we found that average greenhouse plant leaf N concentration decreased with the amount of N added to the original field plots from which the inocula were collected (Fig. 2B, $F_{3,69} = 2.80$, $p = 0.047$), and that this effect differed among sites ($F_{6,69} = 3.47$, $p = 0.005$). The main effect of field N in decreasing leaf N was stronger in sterile-inoculated plants (interaction $F_{3,69} = 3.79$, $p = 0.01$) than live-inoculated plants, thus driving the increase in the magnitude of the effect size of microbial inoculation across N treatments (Fig. 2A and B).

### 3.2. Changes in host plant root fungal associations

In live-inoculated pots, the mean proportion of roots colonized

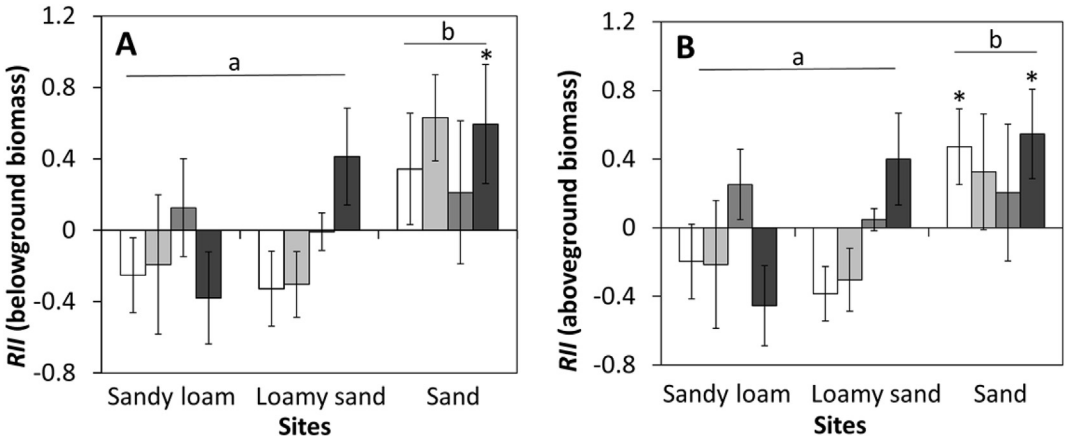


**Fig. 1.** *RII* of (A) belowground biomass and (B) aboveground biomass (±SE) across sites and N addition treatments. Sample size included in each bar ranged from n = 3–5. Shading from light to dark indicates increasing magnitude of N addition treatments at each site (white is control, light gray, dark gray, and black are 2, 5, and 8 kg N $ha^{-1}yr^{-1}$, respectively). Mean *RII*s of the sandy loam and loamy sand sites are significantly different from the *RII* of the sand site, but not from each other (indicated by letter groupings). No individual bars are significantly different from each other in a *post hoc* comparison. *RII*s that are significantly different from zero are marked with an asterisk.

**Table 2**
ANOVA table for the effect of N treatment and Site on the effects of microbial inoculation (*RII*) on plant performance metrics. Significant *p* values (<0.05) are bolded.

| | *df* | *RII* Aboveground biomass | | *RII* Belowground biomass | | *RII* Total biomass | | *RII* Leaf nitrogen | |
|---|---|---|---|---|---|---|---|---|---|
| | | *F* | *p* | *F* | *p* | *F* | *p* | *F* | *p* |
| N treatment | 3 | 0.93 | 0.44 | 0.61 | 0.61 | 0.76 | 0.52 | 3.04 | **0.047** |
| Site | 2 | 5.58 | **0.007** | 5.35 | **0.008** | 5.44 | **0.008** | 2.10 | 0.14 |
| Site:N | 6 | 1.63 | 0.16 | 1.06 | 0.40 | 1.37 | 0.25 | 1.77 | 0.14 |
| Residuals | 43 | | | | | | | | |

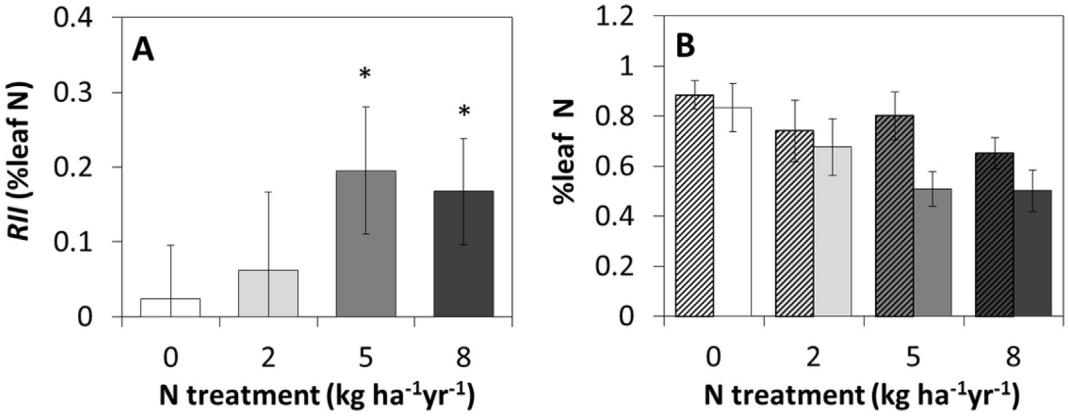


**Fig. 2.** (A) Mean *RII* of leaf N concentration (±SE) across N addition treatments. *RII*s that are significantly different from zero are marked with an asterisk. (B) Mean leaf N concentration (±SE) across N addition treatments. Hatched bars were inoculated with live microbial communities, while non-hatched bars were inoculated with sterilized soils.

by fungi was 0.20 ± 0.03 SE, five times that of sterile-inoculated pots (mean 0.04 ± 0.009 SE; 0.03 for AMF and 0.01 ± 0.004 SE for DSE) ($F_{1,86} = 26.44$, $p < 0.001$), confirming the efficacy of sterilization. Total root biomass did not significantly predict total fungal colonization ($F_{1,45} = 0.26$, $r^2 = 0$, $p = 0.62$). In live-inoculated pots, colonization of roots was predominately by arbuscular mycorrhizal fungi (AMF; mean proportion 0.19 ± 0.03 SE) and dark septate endophytes (DSE 0.013 ± 0.006 SE; Fig. 3). Dark septate endophyte colonization of plants inoculated with soil microbes from the sand site (0.035 ± 0.02 SE) was four times that of the plants inoculated with microbes from the sandy loam and >60 times that from the loamy sand site (site effect $F_{2,40} = 5.30$, $p = 0.009$). Arbuscular mycorrhizal fungi followed the opposite trend, where plants grown in soils with live inocula from the loamy sand and sandy loam sites had colonization rates twice that of plants with inocula from the sand site. However, these trends in AMF were not statistically significant ($p > 0.1$). There were no significant differences in fungal colonization among pots inoculated with soils from different N treatments.

## 4. Discussion

We hypothesized that the soil microbial community would regulate plant growth responses to increased N inputs, such that plants grown with soil microbial communities collected from a 3 year fertilization experiment would show differential growth

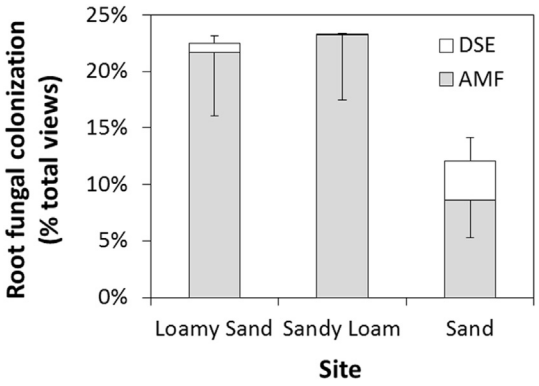


**Fig. 3.** Root fungal colonization of live-inoculated plants. Each bar consists of mean colonization by arbuscular mycorrhizal fungi (AMF) and dark septate endophytes (DSE) ± SE. Roots of plants inoculated with live soils from the sand site showed less total fungal colonization, and higher DSE colonization compared to plants from any other treatments.

among soils from different field-based applications of N additions. In particular, we predicted that plants receiving live microbial soil inocula would show larger indirect responses to N treatments than those receiving sterilized inocula. We found that plants grown in soil that had received N inputs ranging from 0 to 8 kg N $ha^{-1}yr^{-1}$ did not consistently show different soil microbial community effects on plant biomass, highlighting an overall absence of indirect responses to N via soil microbes. However, we found evidence of microbially-mediated effects of N fertilization on plant leaf N concentration (Fig. 2). Live soil microbes increased leaf N concentration compared to those receiving sterilized inocula in the more highly-fertilized field treatments, which suggested that live microbes could be compensating for declines in leaf N that occurred when soils were sterilized. Site differences, reflected largely in soil texture, were the stronger control in regulating the magnitude and sign of plant-microbe interactions outcomes for plant growth, as well as for fungal colonization of plant roots (Figs. 1 and 3).

### 4.1. Indirect effects of N inputs on plant performance via the soil microbial community

Nutrient supply conditions may alter effects of soil microbes on host plants via changes in soil microbial community species composition (e.g. Leff et al., 2015), or by shifting interactions between the same plant hosts and microbial partners along the mutualism-parasitism continuum (Johnson and Graham, 2013; Mandyam and Jumpponen, 2015). Recent direct sequencing of rhizosphere and biocrust soil microbial communities from one site (sandy loam) of the field experiment from which we sampled our soil microbial inocula found no differences in soil microbial abundance, diversity, or composition among N treatments (McHugh et al., 2017). This corroborates past work that has also shown aspects of soil microbial community composition to be resistant to N inputs in arid ecosystems (Mueller et al., 2015; Sinsabaugh et al., 2015; McHugh et al., 2017 and references therein). And while we did not directly measure microbial community composition in this study, together these results suggest the differences we observed in plant nutrient status were unlikely to be a result of microbial community composition change, but instead may reflect shifts in microbial expression, activity, or function.

Past syntheses have indicated that N fertilization generally decreased positive responses of plants to arbuscular mycorrhiza (AMF) and dark septate endophyte (DSE) inoculation (Hoeksema et al., 2010; Newsham, 2011). This is likely due to an increase in resource availability that decreases the net benefit to the plant host for engaging in resource mutualisms with microbial partners (Johnson, 2010). At first glance, our finding that plant N concentration was increased under live inoculation of soil microbial communities from the high N addition treatments seemed to indicate the opposite trend. However, it is possible that N addition in the field increased microbial activity in N cycling. And when inoculated in the greenhouse, these microbes continued to benefit plant N uptake and compensated for the lower leaf N concentrations in the plant host in a process analogous to "priming" effects (Kuzyakov et al., 2000). Our results suggest that indirect effects of N addition on plant nutrient content may have potential to cascade to higher levels of the food web. Further, higher N concentrations are critical in allowing plants to maximize photosynthetic and water use efficiency (e.g., Wright et al., 2004), which is particularly important in drylands, and thus such microbial effects on foliar N represent a fundamental connection to multiple plant functions.

Few studies have explored the effects of N fertilization on feedbacks between plant hosts and soil microbial communities (plant-soil feedbacks *sensu* Bever et al., 1997). Of these, two studies in mesic grassland communities did not find any significant effect of fertilization on plant-soil feedbacks as measured by plant growth (Harrison and Bardgett, 2010; Kos et al., 2015), and another (in southern Californian grassland communities) found species-specific effects of fertilization on plant-microbial interaction outcomes (Larios and Suding, 2015). Our results show that N fertilization has little effect on soil microbial effects on plant growth in an arid grassland. These data suggest that it may be fruitful to consider ecosystem-specific abiotic conditions (such as soil texture) to explain idiosyncratic effects of N inputs on plant-microbe interactions.

### 4.2. Effects of edaphic characteristics on plant-microbe interaction outcomes

The biggest differences in plant-soil microbe interaction affecting plant growth occurred *among sites*. These differences were likely driven by variation in soil properties, because sites were situated close together (<5 km) and experienced the same climatic conditions and have similar vegetation. High heterogeneity in soil microbial community composition at local scales is well-documented (Ettema and Wardle, 2002). For rhizospheric microbial communities, past work in a Californian grassland has suggested that the strongest driver of community composition was plant host identity, followed by edaphic properties, and then spatial location (Burns et al., 2015). In our study, plant hosts were all the same species, but soil inocula were sampled from sites with soil textures grading from sandy loam to sand, and differing in soil carbon (C) and N (Table 1). Our results suggest potential site-level controls on soil microbial community composition, and thus on plant-microbe interaction outcomes. Past work has documented differences in rhizospheric soil communities for a given plant species in varying soil textures. For example, Moebius-Clune et al. (2013) found that soil texture explained ~20% of the variation in AMF assemblages across maize fields in eastern New York, and Shakya et al. (2013) found that edaphic properties explained ~10% of the variation among *Populus* rhizosphere fungal communities in Tennessee and North Carolina. Neither study, however, linked the variation in microbial composition to positive or negative outcomes of microbial interactions for the host plant.

Our most novel result is that soil microbial communities from the three adjacent sites with different edaphic characteristics differed in their effects on the growth of the same host plant, Indian ricegrass. In particular, plant-microbial interactions were beneficial to plant biomass only at the sand site, whereas the effect of live

microbial inocula was not distinguishable from sterile controls at the other two sites. Root microscopy showed that it could be the relatively high abundance of DSE, unique to plants inoculated with soil from the sand site, which benefitted plant growth. However, we cannot rule out differences in bacterial or nematode assemblages. Dark septate endophytes are common root symbionts worldwide, and are frequent in environments with high abiotic stress (Porras-Alfaro and Bayman, 2011). While reported and hypothesized functions of DSE range from pathogenic to mutualistic (Mandyam and Jumpponen, 2005), a recent meta-analysis reported generally mutualistic interactions between DSE and plant hosts (Newsham, 2011), which is in line with our results. The hypothesized mechanisms through which DSE benefit plants include improved drought and heat tolerance, and improved nutrient use efficiency (Mandyam and Jumpponen, 2005). While we did not see significant differences in microbial effects on leaf N concentration among sites in this study, and all plants were well-watered in the greenhouse, there are potentially unexamined benefits of DSE symbiosis to plants. Future experimental manipulations and direct sequencing efforts to further investigate the connection between edaphic properties, rhizosphere fungal species pool composition, and their interactions with plants would shed light on how local factors mediate large scale global change effects on plant communities.

## 5. Conclusion

The results of this study suggest that an increase in N deposition, which can be a major driver of plant and microbial dynamics, could alter the effects of microbial symbionts on plant nutrient status. In addition, our results point to variability in edaphic properties as an important determinant of plant-soil microbial interaction outcomes and plant growth in drylands.

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