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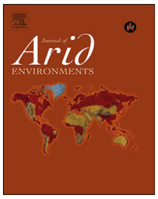

Spatial variation in edaphic characteristics is a stronger control than nitrogen inputs in regulating soil microbial effects on a desert grass

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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)

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