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Arbuscular mycorrhizal colonization of *Larrea tridentata* and *Ambrosia dumosa* roots varies with precipitation and season in the Mojave Desert

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Abstract
We investigated seasonal dynamics of mycorrhizal colonization in response to precipitation in a Mojave Desert *Larrea tridentata-Ambrosia dumosa* shrub community as part of the overall Nevada Desert FACE (Free-Air CO_2 Enrichment) Facility (NDFF) with the goal to understand carbon flow through desert ecosystems in the context of increased carbon availability associated with climate change. Arbuscular mycorrhizal (AM) fungal colonization of fine roots varied with season and with species in the co-dominant shrubs *L. tridentata* and *A. dumosa* at a site adjacent to the NDFF. We collected fine roots (<1.0 mm diameter) at monthly intervals throughout 2001 and from October 2002 to September 2003 to quantify percent colonization via the line intercept method in cleared roots stained with trypan blue to visualize fungi. Colonization was highest in fall, increased throughout spring, and decreased during summer drought periods. Increases in colonization during summer and fall reflected increases in precipitation. Although peak precipitation occurred in spring, mycorrhizal colonization was not correspondingly high, suggesting that fine root initiation and growth, early season shoot growth, and flowering may have reduced carbon availability to the fungus.

Keywords: Arbuscular mycorrhizae, Mojave Desert, creosote bush, root colonization

1. Introduction

The Mojave Desert of the southwestern United States can be characterized by episodic precipitation and a scarcity of water. The average annual precipitation is only 130 mm and temperature ranges from −10°C in winter to 48°C in summer (Morgan et al., 2004; Jordan et al., 1999). *Larrea tridentata*, Zygophyllaceae (the Creosote Bush, which is evergreen), and *Ambrosia dumosa*, Asteraceae, (the White Bursage, which is deciduous) are two notably hardy and co-dominant shrubs found in the Mojave Desert along with *Ephedra nevadensis* (Ephedraceae), *Opuntia* sp. (Cactaceae), *Lycium* sp. (Solanaceae), *Eriogonum inflatum* (Polygonaceae), *Achnatherum hymenoides* (Gramineae), and *Pleuraphis rigida* (Gramineae).

Plants have an increased likelihood of survival in this harsh environment when arbuscular mycorrhizal (VAM) fungi form associations with their roots (St. John, 2000; Stutz et al., 2000) because hyphal filaments effectively increase the surface area of root systems, leading to increased water and nutrient uptake (Mather and Vyas, 2000).

The *Larrea tridentata-Ambrosia dumosa* shrub community of the Mojave Desert has heterogenous nutrient distribution with soils around perennial shrubs having higher nutrient levels (Titus et al., 2002a) that may be maintained by the soil-root conduit influence of mycorrhizal fungi on nutrient distribution. Hyphal quantity varied between spring and fall and mycorrhizae were more prevalent in perennial than in annual species in the Mojave Desert (Titus et al., 2002b).

The Nevada Desert FACE (Free Air Carbon Enrichment) Facility, or NDFF, is a long-term global climate change research station located in the midst of a *Larrea tridentata-*

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Ambrosia dumosa shrub community in the Mojave Desert of Nevada. We investigated seasonal dynamics of mycorrhizal colonization in response to precipitation in this shrub community as part of the overall FACE site goal of understanding carbon flow through desert ecosystems in the context of the increased carbon availability associated with climate change.

2. Materials and Methods

Study site location

The field site was situated on an alluvial fan at an elevation of 970 m in an undisturbed Mojave Desert ecosystem, where the co-dominant shrubs in the vegetation are the evergreen *Larrea tridentata* (DC.) Cov., (Zygophyllaceae) that reaches over 1 m in height, and the deciduous and smaller but abundant *Ambrosia dumosa* (A. Gray) Payne (Asteraceae). Other plants in the community include *Ephedra nevadensis* (Ephedraceae), *Opuntia* sp. (Cactaceae), *Lycium* sp. (Solanaceae), *Eriogonum inflatum* (Polygonaceae), *Achnatherum hymenoides* (Gramineae), and *Pleuraphis rigida* (Gramineae).

Precipitation averages ~135 mm annually, and temperatures range from a winter minimum of –10°C to a summer maximum of 48°C. This site was approximately 100 m away from the Nevada Desert FACE Facility (NDFF) on the Nevada Test Site (Jordan et al., 1999) in order to avoid destructive sampling within the NDFF. Precipitation was measured at the NDFF.

Samples

Fifty-four randomly selected shrubs per species had nine plants randomly assigned to one of six groups. Roots were collected from one group each month from January 2001 – December 2001 and from October 2002 – November 2003 by excavating outward 25 cm from the bases of *L. tridentata* and 12.5 cm from the bases of *A. dumosa* (generally the smaller species), to a depth of 10–15 cm for a soil/root sample volume of approximately 750 cc, a sample size that almost always contained fine roots (<1 mm diameter). Only 6 of the 432 samples did not contain fine roots.

Roots were washed from their surrounding soil and cleared for 4–6 h in 10% KOH. Fungal structures were stained with 0.01% trypan blue (Giovannetti and Mosse, 1980; Brundrett et al., 1996). Approximately five 1–4 cm long segments of fine roots (<1 mm diameter) were mounted on 2 mm gridded slides (Electron Microscopy Supply, Inc., Ft. Washington, PA). Percent mycorrhizal colonization was quantified via the line intercept method (Tenant, 1975) with a compound light microscope (American Optical Co., New Haven, CT) at 100× magnification.

Statistical analyses

Data were analyzed using Proc Mixed in SAS (SAS Institute, Inc., Cary, NC). A repeated measures ANOVA experimental design was used, with year and species (*Larrea* or *Ambrosia*) as fixed factors and month as the repeated measures factor. In order to normalize the data, we removed two *Ambrosia* data points from the total of 426 data points that appeared to be statistical outliers and used a square root transformation. $P<0.05$ was considered significant, but we also indicate results where $0.05<P<0.10$.

3. Results

Arbuscular mycorrhizal colonization was not exceptionally high at any time throughout the study in either the evergreen *Larrea* or the deciduous *Ambrosia*, co-dominant Mojave Desert shrubs with different life cycle strategies. Average monthly maximum values reached only into the 30–40% range following precipitation events in late summer and fall and remained below 20% for most of both years (Figs. 1A–B). Arbuscular mycorrhizal fungi were present at every sampling interval.

Arbuscular mycorrhizal colonization varied significantly both in percentage of roots colonized and in seasonality for both *Larrea* and *Ambrosia* (Figs. 1A–B). These variations occurred in 2001 and again in 2002–03, two years with different seasonal patterns of precipitation. During the first year, a number of precipitation events occurred from January through mid-April 2001 (Fig. 1E), and only small precipitation events occurred after mid-April until a 16 mm event in late-October 2001. Thus in 2000–2001, most of the precipitation (Fig. 1G) occurred before or during the early portion of the time period when these two species typically grow roots (Figs. 1C–D).

During the second year, the first major precipitation event occurred about a month later than in 2000–2001 (Fig. 1F), and precipitation continued to accumulate into May 2003 (Fig. 1H), which is near the middle of the time period that these species typically produce new roots (Figs. 1C–D). By mid-May, total cumulative precipitation in 2002-2003 was approximately 10% greater than in 2000–2001. In addition, two large precipitation events occurred in summer 2003.

Averaged over all months, AM colonization of *Ambrosia* (16%) was significantly greater than that of *Larrea* (12%) (Table 1).

However, this difference was significant only in certain parts of the growing season, as indicated by the significant interaction between species and month (Table 1, Figs. 1A–B). The difference between species was significant at $P<0.05$ at midgrowing season (May) and in autumn (September and October) and at $0.05<P<0.01$ in winter (January $P=0.08$ and February $P=0.06$).

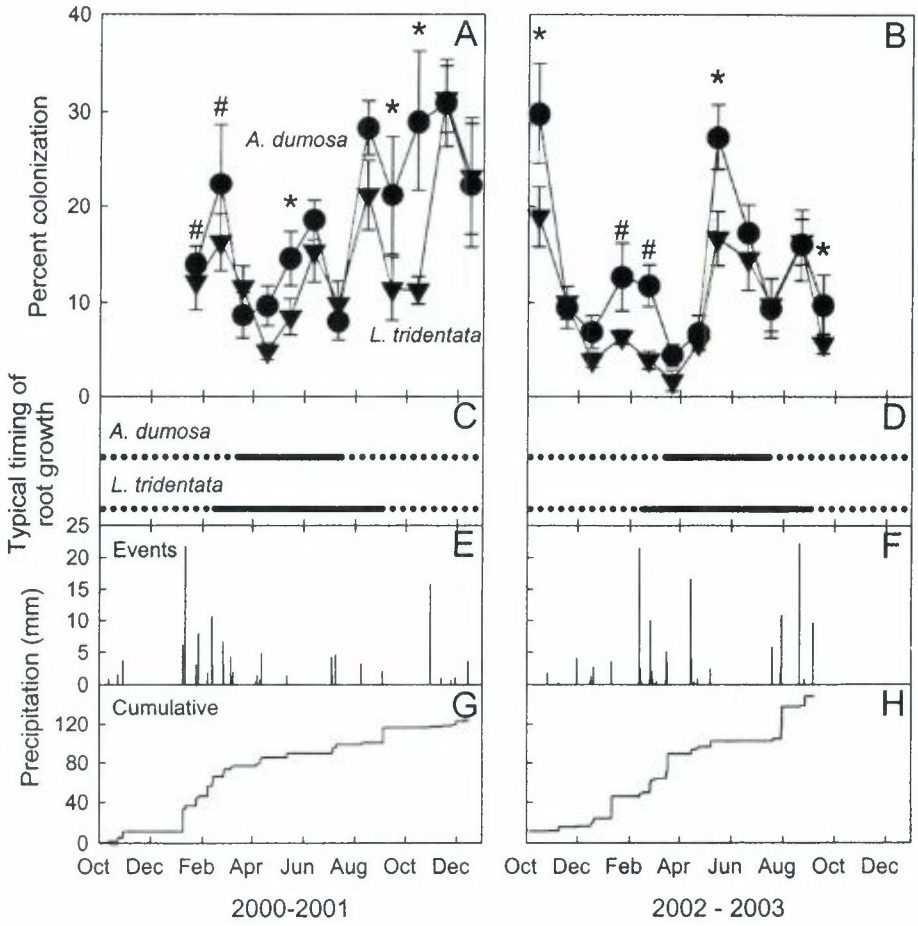

Figure 1. A, B. Percent mycorrhizal colonization of *Ambrosia dumosa* (circles) and *Larrea tridentata* (inverted triangles), averaged across January–December, 2001 (A) and October 2002 – September 2003 (B). Asterisks (*) indicate significantly ($P<0.05$) greater percent colonization in *Ambrosia* than in *Larrea* based on the Species × Month interaction term; pound signs (#) indicate $0.05<P<0.10$. Error bars are standard errors. C, D. Typical period of root growth for *Larrea* and *Ambrosia*, based on minirhizotron observations near the area (Wilcox et al., 2004). E, F, G, H. Individual precipitation events during October 2000 – December 2001 (E) and October 2002 – December 2003 (F) and cumulative precipitation over those same time periods (G and H, respectively).

Table 1. Results from the repeated measures ANOVA of root colonization by arbuscular mycorrhizae for *Larrea tridentata* and *Ambrosia dumosa*. In order to normalize the data, two statistical outliers, the highest value and one of the zero values for *A. dumosa*, were removed from the total data set of 424 data points, and data were transformed with a square root transformation.

Factor	Numerator d.f.	Denominator d.f.	F value	P value
Species	1	284	22.70	<0.001
Year	1	284	42.81	<0.001
Species × Year	1	284	0.05	0.820
Month	11	97	11.82	<0.001
Species × Month	11	284	1.91	0.038
Year × Month	11	284	8.26	<0.001
Species × Year × Month	11	284	1.21	0.277

In 2000–2001, both species had low levels of colonization during April (Fig. 1A), which corresponded to the beginning of new root growth for these two species (Figs. 1C–D).

Mycorrhizal colonization increased during the rest of the growing season for both species, followed by a small decline during the summer drought period. Mycorrhizal colonization increased during August for both species in response to several small rain events and was highest in November, following the October precipitation event. In 2002–03 as in 2000–2001, the highest levels of colonization were in autumn and lowest in spring, and colonization gradually increased during the growing season and was followed by a decline during the summer drought. Colonization increased for both species following the summer precipitation in 2003. Although these general seasonal patterns were similar between years, the exact timing of increases, decreases, and peaks in colonization differed between the two years, as indicated by the significant Year X Month interaction term (Table 1). Averaged across both species, colonization in early spring (February and March), late summer (August and September) and late fall (November and December) of 2000–2001 was significantly greater than that of 2002–2003, whereas 2002–2003 was significantly greater than 2000-2001 only in May.

All roots examined exhibited the Arum type of arbuscular mycorrhizal fungi (Gallaud, 1905). Colonization was not especially intense and generally consisted of 3–4 extraradical

hyphae loosely entwined around the outer surface of the roots with an average of 1–5 interior hyphae visible in fields of view that contained fungi. Ungerminated spores and spores with subtending hyphae were present in approximately 70% of the root segments examined. Colonized root segments contained arbuscules and vesicles but did not contain hyphal coils.

4. Discussion

Arbuscular mycorrhizal colonization varied significantly with both season and species in *L. tridentata* and *A. dumosa* in the Mojave Desert. These results are in accord with those of Titus et al. (2002b), who found that perennial plant species of the Mojave Desert were colonized by arbuscular mycorrhizal fungi, and that the proportions of hyphae, arbuscules, and vesicles changed between spring and autumn in the Mojave Desert. Seasonal and specific change in AM colonization is not limited to the desert and occurs in other ecosystems (Sanders and Fitter, 1992; Mullen and Schmidt, 1993; Titus, 2002a,b; Klironomos, 2003).

Seasonal patterns of AM colonization in *L. tridentata* and *A. dumosa* were similar but not identical for both shrub species. Both species had their highest percent AM colonization in autumn, had low colonization rates when root growth began in the spring, and had declines during summer drought. Colonization increased in both species after summer rains, but only *Ambrosia* maintained high colonization rates through late summer and early fall in 2001.

Seasonal changes in AM colonization were likely influenced by rainfall. Summer rainfall in both years may have contributed to the subsequent August spikes in colonization. Similarly, autumn peaks in colonization followed large rain events for both years. Because of the time interval between precipitation events and root sampling dates, we could not determine whether a spike in colonization occurred immediately after precipitation or whether there was a delay of several weeks. Although fungi may have responded quickly to rainfall, their increased presence would not have been detected until the next sampling date.

During spring, colonization declined with the onset of root growth in both species, despite the greater abundance of precipitation and peaks in soil water availability (Nowak et al., 2004). We suspect that soil temperature does not limit AM growth during the spring, as daily mean soil temperatures at 10 cm soil depth increase from ~13°C in March to ~30°C in June (unpublished data). Allocation of carbon to shoot growth and flowering along with the associated major flush of root growth may reduce carbon availability to mycorrhizae (Gianinazzi, 1991; Brundrett, 2002), or the rate of root growth may exceed the rate of AM colonization (Bruce et al., 1994). The generally low rates of colonization suggest that AM fungi did not have a large influence on carbon sequestration.

Mojave Desert AM spore densities were generally low (0–0.2 spores g^{-1} soil, Titus et al., 2002a). If we assume that spores alone colonized new roots produced after precipitation or soil warming in the spring, then a sufficient quantity of spores must have been in place or possibly concentrated near the roots by earthworm casts (Lee et al., 1996). Colonization rates in inoculated oak seedlings were 4–12% (Tateishi et al., 2003), similar to those of uninoculated *Larrea* and *Ambrosia*. However, colonization can occur very rapidly even when spores are infrequently present (McGee et al., 1999), suggesting that colonization may not have been initiated solely by spores (McGee, 1987, 1989; Nehl et al., 1999). Hyphae may have spread along previously colonized roots. Hyphae, root fragments with hyphae, as well as hyphal bridges between species may have been important contributors to mycorrhizal colonization in these desert shrubs.

Knowledge of seasonal changes in mycorrhizal associations can be linked with investigations of the physiology of the initiation of mycorrhizal colonization with respect to precipitation and soil moisture. Because increases in CO_2 associated with climate change indirectly influence mycorrhizal fungi, and because mycorrhizal fungi respond to increases in soil temperature (Fitter et al., 2000, 2004; Staddon et al., 1998, 1999, 2002), an understanding of mycorrhizal dynamics in desert ecosystems will be useful in tracking the movement of carbon from the host plants to the rhizosphere and outward throughout the ecosystem.

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