Trap cultures reveal higher species richness of arbuscular mycorrhizal fungi in comparison to soil samples in the Phoenix metropolitan area.

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INTRODUCTION

Arbuscular mycorrhizal fungi (AMF) form a symbiotic relationship with plant roots and are a key functional group found in desert soils. Benefits of the symbiosis are conferred to the host plant and soil and may include improved drought tolerance and nutrition as well as soil aggregate stability. AMF have traditionally been studied in natural environments, but little is known about AMF diversity in urban ecosystems. Urbanization processes and environmental changes due to urban development have been shown to affect ectomycorrhizal communities (Baxter *et al.*, 1999; Danielson and Pruden, 1989) and may influence AMF diversity as well.

Studies of AMF species diversity rely on the extraction and identification of fungal spores because they are quantifiable and identifiable to a species level (Morton *et al.,* 1995). AMF diversity based on soil samples alone may be underestimated for two reasons: 1/spores extracted from soil are often impossible to identify due to parasitism and 2/not all AMF species may be sporulating at the time the soil sample is collected. The establishment of successive trap cultures have been successful in detecting non-sporulating AMF in desert ecosystems and can provide more complete representations of species present in the soil (Stutz and Morton, 1996).

This research represents the second phase of a project assessing AMF diversity at sites in the CAP-LTER Survey 200 pilot study. Soil samples were collected from twenty sites located in the Phoenix valley in May of 1999 and AMF spores were extracted and identified. Successive trap cultures were established using the soil samples to stimulate sporulation and to determine if additional AMF species could be detected.

METHODS

Establishment of Trap Cultures

Soil samples were collected from twenty sites in the Phoenix Valley as part of the Central Arizona Phoenix-Long Term Ecological Research Project (CAP-LTER) Survey 200 Pilot Study performed in May, 1999. Three soil samples were collected from each site from the rhizosphere of trees, shrubs or other vegetation. In the absence of vegetation, samples were collected from one meter north of the center of the plot, one meter south of center and two meters south of center. In order to stimulate sporulation and determine if additional AMF species could be detected from each soil sample, two generations of successive trap cultures were established in the greenhouse following a modification of the protocol used by Stutz and Morton (1996). *Sorghum sudanese* was grown in 656 mL Deepots, in a planting media consisting of a 1:1:1 mixture of sample soil and steam-sterilized #20 and #12 grades silica sand. Second generation trap cultures were established using planting mix and fungal propagules from the first generation trap in place of field sample soil. Plants were watered every one to three days over the course of four months. Upon flowering (three to four months), water was removed, and the plants were allowed to dry down. Samples were placed in one-gallon self-sealing plastic bags and stored at 4ºC until analysis.

Spore Extraction and Identification

Spores were extracted from a subsample (50-100 cm^3) of each soil sample and a 100 cm^3 subsample from each second generation trap culture sample by wet sieving and sucrose density gradient centrifugation (Daniels and Skipper, 1982). Soil was washed through a 500 µm sieve, collected in a 45 µm sieve, poured onto a 20/60% sucrose density gradient, and centrifuged at 900 x *g* for three minutes. The supernatant was collected in a 45 µm sieve and washed into a petri dish. Collected spores were observed under a dissecting microscope, and a representation of each spore morphotype (as distinguished by color or size) was mounted on slides in polyvinyl alcohol-lactic acid-glycerol (PVLG) and PVLG mixed 1:1 (v/v) with Melter's reagent (Koske and Tessier, 1983). Identification to a species level was made based on characteristics of spore cell walls and comparison to voucher specimens. Species richness was determined as a count of the different AMF species detected at each site.

RESULTS AND DISCUSSION

Species richness increased from 0-6 species to 2-10 species per site after analyzing trap cultures (Figure 1).
The number of AMF species that were detected in trap cultures but not in soil samples ranged from 1 to 6 species/sampling site with a mean of 2.9 AMF species/sampling site. At about half of the sites, spores of some AMF species were present in soils but were not detected in pot cultures grown in the greenhouse. If trap cultures alone were used to assess AMF biodiversity, overall mean species richness would decrease 1.3 species/sampling site. In their study of AMF populations associated with turf grasses, Koske and Gemma (1997) also found that the AMF community detected in soil samples was highly dissimilar to that estimated by trap cultures.

The number of species detected across the study area increased from 14 to 18 species after analyzing trap cultures (Figure 2).
Additional species detected in the study area included *Glomus claroidium*, *Glomus luteum*, *Acaulospora delicata* and an undescribed Acaulospora species. Two species, *Entrophospora infrequens* and *Acaulospora scrobiculata*, were found in the soil but not in the trap cultures. This is most likely because they were present in low numbers in the soil (data not shown).
In a study of AMF biodiversity in the Sonoran Desert by Stutz *et al.* (1999), fewer species were detected in trap cultures but there was an overlap in AMF species composition with those detected in this study.

The frequency with which most species were detected increased from 0-65% in the soil to 5-80%, after analyzing soil and trap culture samples (Figure 2).
In the soil samples, *Glomus eburneum* was found to predominate and was detected 15% more frequently than any other species. After analyzing the second generation trap cultures, four species (*G. eburneum*, *G. microaggregatum*, *G. spurcum*, and *G. intraradices*) were detected in nearly all of the sites.

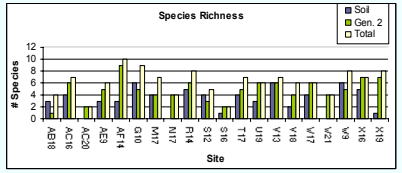

Figure 1. The species richness (calculated as a count of the number of species detected at each site) detected in the soil samples (blue) and second generation trap cultures (green). The total species richness detected from soil and trap cultures is shown in yellow.

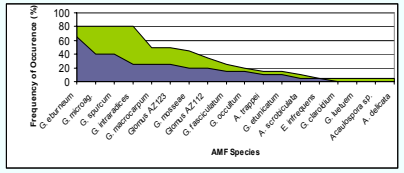

Figure 2. The frequency of occurrence (as determined by the percent of sites the species were detected) for each species detected in the study area. The blue area indicates results obtained from soil samples only and the green area indicates the results after analyzing both soil and second generation trap cultures.

Trap cultures grown in the greenhouse, using *Sorgum sudanese* as a host.

CONCLUSION

Many difficulties have been encountered when attempting to characterize AMF diversity from soil, and species richness may be underestimated when only soil samples are used. Results from this study show that species richness per site as well as the number of species detected in the study area increased after analyzing trap cultures in addition to soil samples. Moreover, a greater number of species were detected more frequently in the sites. Species richness between the soil and trap culture samples was dissimilar in many of the sites. There would be some underestimation of species richness if pot cultures alone were used to assess AMF biodiversity in future studies, but underestimation would not be as great as that if only soil samples were used. As urban areas increase in size and number, there is a growing need for research to understand AMF diversity and functioning in these systems. Future work will include analyzing species richness at the sites with respect to urban variables such as soil chemistry, land use and land use history, and vegetation cover.

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