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Influence of Land Use Intensity and Management on Arbuscular Mycorrhizal Fungi-Avocado Symbiosis

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Abstract

This study was done to assess the effect of soil disturbance on arbuscular mycorrhizal fungi spore abundance and root colonization in avocado (*Persea americana* Mill.). Rhizosphere soil and root samples of avocado were collected from different farms in south Florida and analyzed for degree of mycorrhizal colonization in roots, spore density and diversity in soil along with soil characteristics. There was significant difference in the soil characteristics among the different farms. Similarly, there was a significant difference in the degree of mycorrhizal colonization in the roots and the arbuscular mycorrhizal fungi spore morphotypes among different land use and management practices. However, there was no significant difference in the total number of arbuscular mycorrhizal fungi spores among these sites. There was no correlation between the number of arbuscular mycorrhizal fungi spores and soil characteristics. However, arbuscular mycorrhizal fungi colonization in roots were strongly influenced by soil characteristics such as soil moisture, carbon, nitrogen, and organic matter. Further research is necessary to identify these AMF species and determine the role of in avocado growth tolerance to anthropogenic disturbance in highly disturbed urban soils.

Keywords: mycorrhizal fungi, soil management, soil use, organic farming, conventional farming

1. Introduction

After citrus, avocado (*Persea americana* Mill.) industry is the largest fruit industry in Florida. The avocado industry is an important part of the Florida agriculture economy and consists of about 7,400 acres of land area mainly in the Miami-Dade County (De Oleo et al., 2010). Avocado trees do not tolerate flooding and grow well in the well-drained sandy and limestone soils of south Florida. Tropical climate in south Florida provides an ideal climate for avocado and it is highly localized in the south-eastern Miami Dade County (Ploetz, 2007).

Avocado trees are shallow rooted with dense proliferation of fine roots close to the surface (Salazar-Garcia & Cortés-Flores, 1986). These roots are protected by the trees own natural much. Using mulch in avocadoes is reported to supress insect pests (Hoddle, Robinson, Brownbridge, & Ley, 2002), suppress white root rot (Bonilla et al., 2015), and increase root growth and fruit yield (Moore-Gordon, Cowan, & Wolstenholme, 1997). Past research has shown that avocado roots are colonized with arbuscular mycorrhizal fungi (AMF) (Menge, Davis, Johnson, & Zentmyer, 1978; Haas & Menge, 1990; Toprak, Soti, Jovel, Alverado, & Jayachandran, 2017; Viera et al., 2017) and AMF improve growth of avocado seedlings especially by increasing the availability of phosphorus (Menge et al., 1978; Menge, Larue, Labanauskas, & Johnson, 1980). Thus, soil disturbance in the root zone can not only reduce the feeder roots but can also influence the avocado-AMF symbiosis through the repeated damage to AMF hyphal matts (Kabir, 2005; Urcelay et al., 2009). Furthermore, changes in the soil environmental conditions such as soil compaction, nutrients, soil pH, light, and temperature resulting from soil disturbance can influence the mycorrhizal fungi colonization (Dumbrell et al., 2011; That & Sijam, 2012; Verbruggen, van der Heijden, Rillig, & Kiers, 2013; Soti, Jayachandran, Koptur, & Volin, 2015).

Studies investigating the influence of land use on AMF have reported that AMF are resilient to disturbance (Dai, Bainard, Hamel, Gan, & Lynch, 2013; Jansa, Erb, Oberholzer, Šmilauer, & Egli, 2014). Conversely, there are some studies that report land use practices causing negative impact on the AMF community (Douds & Millner, 1999; Manoharan, Rosenstock, Williams, & Hedlund, 2017; González-Cortés et al., 2012). It has also been reported that the influence of soil properties on AMF communities is site specific (Mangan, Eom, Adler, Yavitt, & Herre, 2004; Pereira, da Silva, de Almeida Ferreira, Goto, & Maia, 2014). For example, (Carrillo-Saucedo, Gavito, & Siddique, 2018) reported that diversity, evenness, and richness indices of AMF was lower in climax communities such as forests compared to continuously disturbed agricultural fields. However, Bainard et al. (2015) in their study in tropical dry forest ecosystem found that AMF fungal spore communities were resistant to land-use change and management.

While there are studies reporting AMF benefits on avocado seedlings, there is little information on the influence of anthropogenic soil disturbance on the AMF-avocado symbiosis in established trees. We conducted a preliminary survey to understand the impact of disturbance and organic vs conventional farm management practices on the AMF root colonization in avocado trees.

2. Materials and Methods

2.1 Study Sites

To include different land management practices, we selected two pairs of farms, organic and conventional, in both urban and peri-urban sites in Miami-Dade County Florida (Table 1). These agricultural growing areas of Miami-Dade County, situated between burgeoning urban and suburban metropolis of Miami, produce a wide variety of traditional and tropical vegetables, tropical fruits, and ornamental nursery and greenhouse products, along with smaller quantities of seed crops (Degner, Stevens, Mulkey, & Hodges, 2000).

Table 1. Information on different sampling sites, type, level of disturbance and location

Site	Farm management	Site description
Peri-urban (PRO)	Organic	Possum Trot is non-certified organic farm located in the Redlands Agriculture Area of Miami Dade County. The land has been under organic management for the past 25 years. The avocado trees in the farm have an average of 25-35 years and very low anthropogenic disturbance. Avocado leaf mulch not disturbed.
Peri-urban (PRC)	Conventional	This farm is also located in the Redlands Agricultural Area of Miami Dade county. This site has been managed conventionally (applications of pesticides). The avocado trees here are more than 10 years old and low anthropogenic disturbance. Avocado leaf mulch not disturbed.
Urban (URC)	Conventional	The site is located at the corner of 147th and 184th street in South West Miami Dade County. While historically this site had been occupied by a conventional avocado farm, recent urban expansion transformed it into an urban area surrounded by single family homes and shopping plaza. The age of avocado trees here is unknown and very high anthropogenic disturbance: soil disturbance, and compaction due to high human traffic). Natural avocado leaf mulch disturbed and mostly removed by human traffic.
Urban (URO)	Organic	Located at the Florida International University organic garden. The fruit forest has a wide variety of tropical fruit trees. The avocado trees here are more than 10 years old and very high anthropogenic disturbance: soil disturbance and compaction due to high human traffic). Natural avocado leaf mulch disturbed and mostly removed by human traffic.

2.2 Soil and Root Sample Collection

Six fully grown trees were selected in each of the four sites for sample collection, n = 24. Rhizosphere soil and root fragments of avocado plants were collected from the study sites during the summer of 2013. Three soil cores (depth between 0 and 12 cm) were collected around each selected tree in the four sites and mixed to prepare a composite soil sample. A portion of the soil sample was dried and stored in airtight containers to analyze the physical and chemical properties and the remaining portion was stored in a 4 °C refrigerator until biological analysis.

Soil samples were analyzed for moisture content by gravimetric method (Reynolds, 1970) and particle size distribution was analyzed using a hydrometer. Soil pH was measured using a glass probe (1:2 soil DI water solution). Total carbon and nitrogen in soil was measured using a TruSpec Carbon/Nitrogen Analyzer (Leco Corporation, St. Joseph, MI, USA). Total organic matter was measured based on the standard loss on ignition method (500 °C, 5 hours, (Storer, 1984)). For total P measurement, soil samples (0.25 g, finely ground) were ashed (500 °C), digested in 2 ml HCl (6N) and 10 ml HNO_3, and analyzed with an UV spectrophotometer (Shimadzu Scientific Instruments, Columbia, MD, USA) following the USEPA chemical analysis method (USEPA, 1983).

2.3 Arbuscular Mycorrhizal Fungal Spores Extraction and Identification

Arbuscular mycorrhizal fungal (AMF) spores were extracted and separated from the soil using wet sieving and decanting followed by sucrose density gradient technique (Gerdemann & Nicolson, 1963). Fifty-gram dry equivalent weight soil from each site was placed in a 250 ml beaker. One hundred ml DI water was added to the soil and mixed vigorously to separate the spores from soil aggregates. The slurry mixture was washed through a series of sieves (2 mm, 100 µm and 32 µm). This process was repeated several times until the water flowing through the sieves was clear. The sievate retained on each sieve was washed into a 50 ml centrifuge tube. The tubes containing sievate were balanced and centrifuged at 300 rpm for three minutes to remove floating organic debris. After centrifugation, the supernatant was discarded. The pellet in the bottom was re-suspended in a 50% sucrose solution and centrifuged at 2000 rpm for one minute to separate the AMF spores from denser sucrose solution. Immediately after centrifugation, AMF spores in the sucrose supernatant were rinsed in a fine 32 µm sieve to rinse off sucrose. The AMF spores were collected on a Whatman number 42 filter paper by vacuum filtration. The filter paper containing AMF spores was placed under a stereomicroscope at 100× and 400× magnification for observation. The AMF spores were separated based on color, size, type, and morphology of the subtending hypha attached at the spore wall. The AMF spores were mounted on microscopic slides for taxonomic identification based on the spore morphology and wall characteristics, using the descriptions by the International Culture Collection of Arbuscular and Vesicular-Arbuscular Mycorrhizal Fungi (http:// invam.caf.wvu.edu). Since the spores were field samples, some small and irregular shaped spores could not be identified at species level, they were grouped as unidentified.

2.4 Degree of Mycorrhizal Colonization in Roots

Twenty-five 1.5 cm root fragments were collected from the randomly selected four plants in each site, and the colonization of AMF was quantified by staining the roots with Trypan blue (McGonigle et al., 1990). Roots were cleared in 15% KOH at 70 °C for 4 hours, rinsed twice with tap water, bleached with ammoniated H_2O_2, and acidified with 1 N HCl. Root staining was done using Trypan blue in acidified glycerol at 80 °C for 20 minutes. The stained roots were then examined with a dissecting microscope at 30-60× magnification for the degree of mycorrhizal colonization; the portions that showed the presence of mycorrhizal fungi were mounted on slides and examined at 100-400× magnification to further analyze various mycorrhizal structures such as vesicles, arbuscules and hyphe structure. Percentage of mycorrhizal colonization in roots was estimated by:

$$\% \text{ Colonization} = \frac{\text{Total number of AMF root segments}}{\text{Total number of root segments observed}} \times 100 \qquad (1)$$

2.5 Statistical Analysis

Analysis of variance was done to determine the difference between the different soil characteristics, spore density and degree of mycorrhizal colonization in the roots of avocado among the different sites. Data was tested for normality, and when it failed to pass the normality assumption it was subjected to log transformation (soil moisture, Carbon%, and AMF% in roots). Two-way ANOVA was done to analyze the relationship between the two factors site and farm management and AMF root colonization. Means were separated by Fisher LSD and differences were considered significant at a P value of < 0.05. Relationship between root mycorrhizal colonization and rhizosphere spore density and soil parameters was evaluated using Pearson correlation analysis. All the data was analyzed using JMP Pro 13 software.

3. Results

In this study, we analyzed the farm soil status and mycorrhizal spore density and diversity in avocado growing in different land use types in south Florida. There was a significant difference in the soil status among the different farms (Table 2). Soil pH in the study sites was slightly alkaline with no significant difference among the farms. However, there was a significant difference in the soil moisture among the different sites ($P < 0.001$). The farms in peri-urban sites had significantly higher moisture compared to the urban site, and highest soil moisture was found in the conventional farm at the peri-urban site. Similarly, for total N %, conventional farm at the urban site had the lowest amount and there was no significant difference among the other farms ($P = 0.001$). Total C% was significantly higher in both the farms at the peri-urban site compared to the urban sites ($P < 0.001$). Soil organic matter % was also highest in the organic farm at the peri-urban site and lowest at the organic farm in the urban site ($P = 0.001$).

Table 2. Mean with standard error (SE) in parenthesis of soil characteristics and AMF in the study sites

Site	pH	Moisture %	Total N %	Total C%	OM%	No of Spores	Spore Morphotypes	AMF % in roots
PRO	7.83(0.06)	14.6(1)	0.54(0.07)	11.98(1.09)	19.95(1.12)	281.83(8.90)	9(0.86)	78(1.74)
PRC	7.63(0.15)	19.49(1.96)	0.57(0.08)	9.33(0.93)	15.42(1.35)	272.67(9.81)	7(0.37)	77.16(2.55)
URO	7.47(0.05)	4.04(0.44)	0.38(0.08)	3.56(0.59)	11.97(1.01)	275(18)	6.5(0.22)	50.33(2.10)
URC	7.68(0.02)	5.4(0.56)	0.15(0.02)	3.62(0.38)	13.73(1.16)	278(10.45)	5.1(0.47)	48(3.11)

Note. PRO: peri-urban organic; PRC: peri-urban conventional; URO: urban organic; and URC: urban conventional.

The rhizosphere soil sample from different locations harbored different spore types. There was no significant difference in the AMF spore numbers among the different sites (Table 2), however there was a difference in the AMF spore diversity in these sites. We found a total of 15 different AMF morphospecies in the four different study sites. Of the 15 morphospecies, spores of *Glomeraceae* species were found in all sites in high abundance. Spores of *Aculospora* spp. and *Scutellospora* spp. were found in the two peri-urban sites but not found in both the urban sites.

The roots of avocado from all the sampling locations showed signs of high degree of mycorrhizal colonization indicating avocado is highly mycorrhizal (Table 2). The degree of mycorrhizal colonization in the roots ranged from 40% to 77% with higher degree of colonization in the peri-urban sites and lower in the urban sites. While the AMF colonization in roots was not significantly different in the organic or conventional farm plants ($P = 0.105$), it was significantly higher in the peri-urban farms with low soil disturbance than the urban farms with high soil disturbance ($P < 0.001$). In addition, the effect of site on root AMF colonization was significant ($P < 0.001$). However, farm management and the interaction of farm management and site had no significant impact on root AMF colonization.

Correlation analysis indicated no significant relationship between the total number of AMF spores and soil characteristics. However, there was a strong correlation between the soil variables and AMF root colonization and the number of spore morphotypes (Table 3). Soil moisture, pH, and C% had a significant correlation with the AMF spore morphotypes ($p < 0.001$ and 0.0046 respectively).

Table 3. Correlations with correlation probability in parenthesis between soil variables and mycorrhizal spore density in soil and root colonization by AMF

	pH	Moisture	Nitrogen%	Carbon%	Organic Matter %	Number of spores
Number of spores	-0.187(0.38)	-0.544(0.65)	0.141(0.50)	0.085(0.32)	0.057(0.79)	
AMF % in roots	0.315(0.31)	0.810(<0.0001)	0.624(0.001)	0.821(0.0001)	0.424(0.039)	0.117(0.52)
Spore morphotypes	0.258(0.22)	0.385(0.06)	0.472(0.019)	0.468(0.021)	0.50(0.012)	-0.018(0.93)

4. Discussion

This study presents the results of a preliminary study of disturbance in avocado AMF symbiosis in South Florida farms. Our results show that anthropogenic soil disturbance did not have an influence on the avocado rhizosphere AMF spore abundance. However, the AMF root colonization and spore morphotypes were influenced by anthropogenic soil disturbance which potentially resulted in different soil edaphic conditions. These results are similar to previous studies that found a negative impact of land use intensity on the AMF diversity (Oehl et al., 2003, 2010; Schnoor, Lekberg, Rosendahl, & Olsson, 2011; Xiang et al., 2014). Major disturbance in our site was mechanical disturbance, leading to the disruption of hyphal network. While we did not identify the AMF spore species using molecular techniques, microscopic analysis of the spores showed the higher presence *Glomeraceae* species spores. These results are in line with previous studies (van der Heyde, Ohsowski, Abbott, & Hart, 2017; De León et al., 2018) demonstrating that species in the *Glomeraceae* were most tolerant to mechanical disturbance. Traits such as high growth rate (Hart & Reader, 2005), high rate of hyphal turnover (Staddon, Ramsey, Ostle, Ineson, & Fitter, 2003), and reproduction from both spores and hyphal fragments (Ohel et al., 2009), could potentially result in higher tolerance to disturbance in some AMF species.

High degree of AMF colonization was found in the peri- urban sites while urban site plants had the lower level of mycorrhizal colonization in roots, regardless of the farm management practices. Lower degree of AMF colonization in urban trees roots has been reported in previous studies (Bainard & Klironomos, 2011). Soil variables such as soil moisture were strongly associated with the root AMF colonization. Avocado plants are shallow rooted with most of the fine roots, which absorb water and nutrients, are in the greatest abundance at or near the surface (Coit, 1940). Both the peri-urban sites had the natural mulch which was important in protecting the roots and maintaining higher soil moisture and soil organic matter. Our results indicate that mulch cover not only maintained high soil moisture and organic matter but also potentially increased the AMF colonization in the roots. In addition, both the urban sites were highly disturbed with increased human activities around the trees. Intense anthropogenic activities such constant soil disturbance could lead to the disturbance in the AMF hyphal matts as reported previously (Urcelay et al., 2009).

Overall, while the total number of spores did not vary among the different farms there was significant difference in the AMF colonization in avocado roots and AMF spore morphospecies. The less disturbed peri-urban sites had significantly higher soil organic matter also had higher soil moisture. The natural much, which help to protect the roots from diseases and reduce evaporative water loss in avocadoes, was not existent in the peri-urban highly disturbed sites. Our results show that anthropogenic disturbance removing the natural avocado leaf mulch not only influence the root growth but also the avocado-AMF symbiosis. Further detailed analysis is necessary to determine the role of leaf mulch on promoting the AMF-avocado symbiosis. Also, if we are to use AMF in avocado for seedling establishment and yield increase in mature trees, proper identification the AMF species associated with avocado under field conditions is necessary.

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