Is arbuscular mycorrhizal fungal species community affected by cotton growth management systems in the Brazilian Cerrado?

HELIAB B. NUNES1, BRUNO T. GOTO2, JOÃO LUIZ COIMBRA1, JAMILE S. OLIVEIRA1, DÉRICA G. TAVARES1, MARCELO S. ROCHA1, FABIANE L. SILVA3 and ANA CRISTINA F. SOARES3

1Universidade do Estado da Bahia. Br 242, Km 4, Loteamento Flamengo, 47802-682 Barreiras, BA Brazil
2Universidade Federal do Rio Grande do Norte, Departamento de Botânica e Zoologia, Programa de Pós-Graduação em Sistemática e Evolução, Campus Universitário, 59072-970 Natal, RN, Brazil
3Universidade Federal do Recôncavo da Bahia, Centro de Ciências Agrárias, Ambientais e Biológicas, Programa de Pós-Graduação em Microbiologia Agrícola, 44380-000 Cruz das Almas, BA, Brazil


Abstract: Conventional cotton production in western Bahia, Brazil, involves intensive use of agricultural inputs and mechanization, which may affect arbuscular mycorrhizal fungi (AMF). This work aimed at studying the impact of conventional and organic cotton production in the AMF of western Bahia. Soil samples were obtained from conventional white cotton and colored cotton organic production systems as well as from native Cerrado areas, close to the white cotton fields, and from the subcaducifolia vegetation, close to the organic colored cotton farms. The most frequent species in the conventional farming areas belonged to the genera Acaulospora (10 spp.), Glomus (8 spp.), Dentiscutata (3 spp.), Ambispora, Pacispora and Scutellospora (2 spp. each), as well as Claroideoglomus etunicatum, Diversispora sp., Entrophospora infrequens, Gigaspora sp., Orbispora pernambucana, Paradenticulata maritima, and Paraglomus occultum. Eighteen species were found in the organic farming areas, with the predominance of Glomus (5 spp.) and Acaulospora (5 spp.), and with Claroideoglomus, Dentiscutata, Gigaspora, Corymbiglomus, Orbispora, Paraglomus, Scutellospora, and Simiglomus (1 spp. each). Paraglomus bolivianum was first reported in Cerrado. In the native vegetation, nine species were found, with the predominance of Glomus and Acaulospora. The highest number of AMF species was found in the organic farming areas, which deserves further investigation.

Key words: Mycorrhiza, BRS Safira cotton variety, BRS 336 variety, Glomeromycota.

INTRODUCTION

Cotton represents one of the main commodities worldwide, with cotton fiber being used for making fabrics, and other products and cottonseed for oil extraction and production of biofuel (Onukwuli et al. 2017). The crop has a significant economic impact in the Brazilian economy since the country ranked as the fifth-highest cotton producer in the world in 2016/2017 (The Statistics Portal 2018). It is estimated that for the next ten years, the growth rate for cotton production in Brazil will surpass the main world producers such as China,
There are two systems of cotton production in Brazil: (i) the intensive conventional production of white cotton, with extensive use of agricultural chemical inputs and mechanization, and (ii) the family based organic production system for colored cotton, with a low level of technology and almost no use of agricultural inputs. Especially in the high agribusiness intensive production systems, cotton monoculture and its management may cause significant changes in soil characteristics, such as pH, fertility levels (Eskandari et al. 2017), and enzyme activity (Chen et al. 2017). Soil microbial community can also be affected, especially through the application of pesticides (Verdenelli et al. 2012) and herbicides (Kumar et al. 2017). In fact, Pereg and McMillan (2015) raised concerns with the high input agricultural systems for cotton production and reviewed the potential use of beneficial microorganisms. Arbuscular mycorrhizal fungi (AMF) are important soil microorganisms and play a major role in plant growth by several mechanisms such as improvement of plant nutrient absorption (Nadeem et al. 2014), water absorption (Bowles et al. 2016), tolerance to heavy metals (Lins et al. 2007) and protection against plant pathogens (Cofcewicz et al. 2001). The potential for cotton growth promotion of up to 300 % was reported for cotton plants with a commercial formulated AMF inoculum (Pereg and McMillan 2015).

Alterations in the diversity of AMF communities in soils under conventional and organic farming of different crops have been shown previously (Ramos et al. 2012, Johnson et al. 2013, Schneidera et al. 2015). AMF have positive effects on cotton growth and nutrition (Prince et al. 1989), but the communities of these fungi can be affected by farming systems (Oehl et al. 2009, Pereira et al. 2014). The diversity of AMF in cotton production areas was studied in the state of Pernambuco, Brazil (Maia and Trufem 1990). However, a study of the AMF in cotton areas with different crop management practices, compared to the native AMF population in areas with native vegetation has not been reported in Brazil.

Considering the importance of cotton for the Brazilian and global economies as well as the role of AMF in plant growth and nutrition, tolerance to biotic and abiotic stresses, and the sustainability of agricultural production and ecosystems, knowledge about the AMF community in cotton production areas is warranted. Herein we compared the fungal communities present in native soils and in soils with conventional and organic cotton production systems of the western region of the state of Bahia, Brazil. This region is part of the Cerrado biome known to present a highly diverse AMF population (Jobim et al. 2016). The aim of this study was to describe the occurrence of the AMF communities in conventional and organic cotton production systems in order to understand the impact of these cropping systems on the native AMF communities in the western region of the state of Bahia, Brazil.

MATERIALS AND METHODS

The areas studied for AMF community composition were from the western region of Bahia State, all with well-defined rainy periods, with droughts ranging from five to six months in the year, rainfall of 1200 to 1800 mm per year (Adámoli et al. 1986). The maximum, average, and minimum temperatures are 32.26 °C, 24.67 °C, and 18.68 °C, respectively (Soares Neto et al. 2011). Moreover, deep yellowish-red latosols predominate, and they were well drained for most of the year. These soils are also acid with aluminum toxicity and poor in essential nutrients such as calcium, magnesium, potassium, phosphorus, and some micronutrients (Andrade et al. 2002).

Ten areas were studied: three areas of cotton conventional production system (A1, A2 and A3); two areas of family based organic farming system (A7 and A8); three areas of native Cerrado biome
(Brazilian savanna, A4, A5 and A6), which were close to the conventional production fields, and two areas of native subcaducifolia forest (A9 and A10), which were close to the organic farming fields (Figure 1, Table I). The conventional cotton producing areas (A1 and A2) have been cultivated with white cotton, BRS 336 variety, for 10 years, while area A3 has been cultivated for only two years, all in rotation with maize and soybeans. All these three areas were managed with a fallow period, which started at the beginning of September and lasted up to November, as well as with intensive mechanization and received 20 to 25 applications of fungicides and pesticides per growing season. Before the introduction of cotton, these areas were planted with maize and soybeans. The small organic family-based production areas had been cultivating the colored cotton variety Safira for

Figure 1 - Map of the sampled areas. Areas 1, 2 and 3 are of conventional cotton production systems; areas 4, 5 and 6 are of native Cerrado biome vegetation; areas 7 and 8 are of colored cotton family-based organic farming, and areas 9 and 10 are of native subcaducifolia forest.
two years during the rainy period without the use of fungicides and pesticides. In these areas, crop management was manually done without rotation and, before cotton introduction, maize and beans were cultivated for subsistence.

Soil samples were randomly collected around the root zone of cotton plants, at 0 to 20 cm depth, at the end of the cotton cultivation season. In each area, forty sub-samples of soil were collected to form four combined soil samples, each composed of a mixture of ten sub-samples. The combined soil samples were used for extraction of glomerospores (AMF spores) right after being collected and for the growth of trap cultures for native AMF. Another part of the soil samples underwent chemical analysis at the Laboratory of Soil Analysis of the Department of Soils of the College of Agriculture Luiz de Queiroz, São Paulo State University (Table II).

To prepare the trap cultures, we first sterilized a mixture of field-sampled soil and sand (ratio 1:1, v/v) in an autoclave at 120 °C for 50 minutes, twice, with intervals of 24 hours between sterilizations. Then, in 3 L plastic pots, a layer of the field-sampled soil (inoculum soil) was sandwiched between two layers of the sterile mixture of soil and sand. Seeds of *Brachiaria decumbens* Stapf (used as the trap plant) were surface sterilized with sodium hypochlorite at 1 % for 1 min, rinsed for three times with sterile water, and were sowed in those plastic pots with soil. Four trap cultures were prepared for each area (Figure 2). The trap cultures were grown under greenhouse conditions, for one year, and were fertilized at every 20 days with 100 mL of a modified nutrient solution (Hoagland and Arnon 1950) with the following composition: KNO$_3$ (1Molar; 6mL/L); Ca(NO$_3$)$_2$ (1Molar; 4mL/L);
MgSO₄ (1Molar; 2mL/L); Micronutrients (1mL/L); Fe EDTA (1mL/L); and (NH₄)₂SO₄ (1Molar; 0.5 mL/L).

Aiming at the identification of AMF species that were not sporulating at the time of soil sampling, extraction of glomerospores occurred after one year of multiplication in trap cultures. Most studies with trap cultures for AMF are conducted for a period of only three to four months. However, a greater growth period for the culture traps can enrich the study by allowing for more AMF species to sporulate over this time, allowing for a more profound study of the AMF present in these agricultural fields and native vegetation areas (Souza et al. 2013).

Soil extraction of glomerospores followed the methodology of soil wet-sieving and decanting (50 g of soil in 1 L of water), described by Gerdemann and Nicolson (1963), followed by centrifugation in 45 % sucrose solution and rinsing with tap water (Jenkins 1964). After this process, glomerospores were counted in a Petri plate with 20 mL of the spore suspension, under a stereomicroscope with 50 X magnification. The number of glomerospores per gram of soil was calculated based on the total volume of the water suspension with glomerospores after the centrifugation process, and the initial soil weight of 50g. For AMF taxonomic identification, glomerospores were grouped by similarity according to size, shape, and color, and were transferred to a microscope slide using a 100-200uL micropipette. Similar glomerospores were placed in the two halves of microscope slides, and these were mounted with polyvinyl-lacto-glycerol alcohol (PVLG) in one half and with PVLG + Melzer (1:1; v/v) on the other half, and a coverslip (Morton et al. 1993). Slides were also prepared with glomerospores from all trap cultures. Species identification was carried out based on specialized literature (Schenck and Perez 1990) and articles describing AMF species (Oehl et al. 2011, Goto et al. 2012, 2013, Furrazola et al. 2013).

A correlation among the soil chemical and physical characteristics, the number of spores and the AMF species was determined by redundancy analysis (RDA) with the R statistical package. For the data related to AMF species identification in each area, there were no statistical analysis because sample replications per area were not considered during the preparation of the microscope slides for spore taxonomy.

**RESULTS**

The studied areas presented AMF species distributed in all orders described. A total of 34 AMF species were identified belonging to Acaulospora (10 spp.), Glomus (8 spp.), Dentiscutata (3 spp.); Ambispora, Pacispora and Scutellospora (2 spp. each) and the species Claroideoglomus,
Diversispora, Entrophospora, Gigaspora, Orbispora, Paradentiscutata and Paraglomus (1 sp. each) (Table III).

The areas A7, A8, and A10 exhibited the highest averages of glomerospores (AMF spores) density, with 539, 601, and 550 glomerospores per 50 g of soil, respectively. These areas were from colored cotton organic farming (A7 and A8) and native forest (A10). The areas A5 and A6, both with native Cerrado vegetation, revealed 464 and 390 glomerospores per 50 g of soil, respectively. The areas A1 and A2, A3 (conventional cotton areas), A4 (native Cerrado vegetation), and A9 (native subcaducifolia forest) presented 105, 214, 313, 238, and 292 glomerospores per 50 g of soil.

In the first conventional cotton production area (A1, Table III), six AMF species were identified, predominantly of the Acaulospora genus (3 spp.), followed by Glomus (2 spp.) and Dentiscutata sp. In the native Cerrado (A4, Table III), close to the area of conventional cotton production, 19 AMF species were recognized: Acaulospora (7 spp.), Glomus (5 spp.), and Dentiscutata cerradensis, Diversispora sp., Gigaspora sp., Orbispora pernambucana, Pacispora sp., Scutellospora sp., and Simiglomus sp. All these species were reported in the Cerrado biome by Jobim et al. (2016).

The second area with conventional cotton production (A2, Table III) presented eight species: Acaulospora excavata, A. herrerae, Ambispora callosa, Claroideoglomus etunicatum, Gigaspora sp., Glomus sp., Paraglomus occultum, and Scutellospora sp. The native Cerrado area (A5, Table III) presented 11 species: Acaulospora (3 spp.), Dentiscutata (2 spp.), Glomus (2 spp.), Ambispora appendicula, C. etunicatum, Orbispora

Figure 2 - Trap cultures of soil samples from areas of conventional cotton production system (A1, A2 and A3), native Cerrado biome vegetation (A4, A5 and A6), family-based organic farming system (A7 and A8) and native subcaducifolia forest (A9 and A10), with Brachiaria decumbens as the trap plant for AMF.
### TABLE III

Arbuscular mycorrhizal fungi in cotton growing areas under crop management systems, native biomes, and trap cultures.

<table>
<thead>
<tr>
<th>Areas</th>
<th>Conventional White Cotton</th>
<th>Native Cerrado Biome</th>
<th>Organic Colored cotton</th>
<th>Native Forest</th>
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<td>Species</td>
<td>A1</td>
<td>A2</td>
<td>A3</td>
<td>A4</td>
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<td><em>Acaulospora dilatata</em> J.B. Morton</td>
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<td><em>Acaulospora excavata</em> Ingleby &amp; C. Walker</td>
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<td><em>Acaulospora herreriae</em> Furrazola, B.T.Goto, G.A.Silva, Sieverd. &amp; Oehl</td>
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<td><em>Acaulospora mellea</em> Spain &amp; N.C. Schenck</td>
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<td><em>Acaulospora morrowiae</em> Spain &amp; N.C. Schenck</td>
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<td><em>Acaulospora scrobiculata</em> Trappe</td>
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<td><em>Acaulospora sp.</em> 1</td>
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<td><em>Acaulospora sp.</em> 2</td>
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<td><em>Acaulospora sp.</em> 3</td>
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<td><em>Ambispora appendicular</em> (Spain, Sieverd., N.C. Schenck) C. Walker</td>
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<td><em>Ambispora callosa</em> (Sieverd.) C. Walker, Vestberg &amp; A. Schüssler</td>
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<td><em>Claroideoglomus etunicatum</em> (W.N. Becker &amp; Gerd.) C. Walker &amp; A. Schüssler</td>
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<td><em>Dentiscutata cerradensis</em> (Spain &amp; J. Miranda) Sieverd., F.A. de Souza &amp; Oehl</td>
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<td><em>Dentiscutata scutata</em> (C. Walker &amp; Diefer.) Sieverd., F.A. de Souza &amp; Oehl</td>
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<td><em>Dentiscutata sp.</em></td>
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<td><em>Diversispora sp.</em></td>
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<td><em>Gigaspora sp.</em></td>
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<td><em>Glomus clavisporum</em> (Trappe) R.T. Almeida &amp; N.C. Schenck</td>
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<td><em>Glomus glomerulatum</em> Sieverd.</td>
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<td><em>Glomus macrocarpum</em> Tul. &amp; C. Tul.</td>
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<td><em>Glomus sp.</em> 1</td>
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<td><em>Glomus sp.</em> 2</td>
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<td><em>Glomus sp.</em> 3</td>
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<td><em>Corymbiglomus tortuosum</em> (N.C. Schenck et G.S. Sm.) Blaszk. &amp; Chwat</td>
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<td><em>Paraglomus bolivianum</em> (Sieverd. &amp; Oehl) Oehl &amp; G.A. Silva</td>
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<td><em>Pacispora sp.</em></td>
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<td><em>Paraglomus occultum</em> (C. Walker) J.B. Morton &amp; D. Redecker</td>
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<td><em>Rhizoglomus intraradices</em> (N.C. Schenck &amp; G.S. Sm.) Sieverd., G.A. Silva &amp; Oehl</td>
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<td><em>Scutellospora sp.</em></td>
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<td><em>Simiglomus sp.</em></td>
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pernambucana, and Scutellospora sp. Again, the soil with native vegetation of Cerrado biome presented a higher number of AMF species than the soil with conventional cotton production. The third area with conventional cotton (A3, Table III) and the adjacent native Cerrado area (A6, Table III) had in common the following species (and number of species): A. appendicula, Glomus sp. (1 spp.), Glomus sp. (2 spp.), and Gigaspora sp. The differences within these areas were C. etunicatum and E. infrequens, which were present only in the conventional cotton plantation, and Acaulospora sp., which was identified only in the native Cerrado. The area A3 had been cultivated for only two years, while the other conventional white cotton production areas had been cultivated for 10 years.

The area of organic colored cotton plantation, under family-based farming system, had 14 AMF species: Glomus (3 spp.), Acaulospora (3 spp.), Claroideoglomus etunicatum, Dentiscutata sp., Gigaspora sp., Orbispora pernambucana, Paraglomus bolivianum, Paraglomus occultum, Scutellospora sp., and Simiglomus sp. (A7, Table III). Among these, the fungus P. bolivianum was registered in Brazil for the first time in 2012 (Mello et al. 2013) and is rarely reported in studies with AMF. The second area of organic colored cotton, also under family-based farming system (A8, Table III) showed nine species: Acaulospora (4 spp.), followed by Glomus (2 spp.), Claroideoglomus etunicatum, Corymbiglomus tortuosum, and Paraglomus bolivianum. The samples from the native forest, which was close to the organic farming fields, showed eight species: Glomus (4 spp.), Acaulospora spinosa, Ambispora appendicula, Entrophospora infrequens, and Rhizoglomus intraradices (A9, Table III), and five species: Glomus (2 spp.), Claroideoglomus etunicatum, Dentiscutata sp., and Scutellospora sp. (A10, Table III). Compared to the areas with organic colored cotton production, these areas of native vegetation had a lower number of species and, the genus Glomus in common. A correlation among the soil chemical and physical characteristics, the number of AMF spores, and the AMF species for each studied area was not observed with redundancy analysis (RDA) (Figure 3).

**DISCUSSION**

Since soil management practices may favor the predominance of some AMF species, it is important to map the species that compose these soils under different management and compare with those present in soils with native vegetation. This study reports novel data regarding the AMF community composition in conventional and organic family-based cotton production systems as compared to the native AMF population of Cerrado and the transition area between the Cerrado and Caatinga biomes.

Acaulospora species were found in the native Cerrado areas, in two areas of conventional and all areas of organic cotton production sampled herein, suggesting a wide distribution of this genus with species adapted to different agricultural systems. Glomus was also well represented in all the areas studied. The preponderance of these two genera was reported previously for different agricultural management systems (Pereira et al. 2014). Sobrinha et al. (2000) observed the preponderance of Glomus and Acaulospora in the Cerrado biome in soil with Brachiaria grass. Ramos et al. (2012) also found that Glomus and Acaulospora dominated in areas with forage crops cultivated alone or combined with corn. The families Acaulosporaceae and Glomeraceae dominated in crop production areas in Switzerland with Acaulosporaceae showing the highest number of AMF species (Oehl et al. 2009).

However, the numbers of Acaulospora and Glomus species were lower in the first and second conventional cotton production areas (A1 and A2, Table III) than in the native Cerrado area (A4, A5, and A6, Table III). In the case of Acaulospora,
the underlying reason may be the soil pH as this genus is more frequent in acidic soils (Trufem 1990, Gomes and Trufem 1998). Indeed, the soils of the conventional cotton production areas A1 and A2 presented neutral pH (pH 6.59 and 6.77, respectively – see Tables I and II). These areas have been cultivated for many years with intensive mechanization and agricultural inputs. Trufem (1990) observed Acaulospora species in soils with pH ranging from 3.5 to 5.8 similar to the values also found in the Cerrado soils studied herein, where Acaulospora species predominated.

Interestingly, the conventional cotton production area A3, which was under cotton cultivation for only two years as opposed to the other areas that underwent cultivation for 10 years, showed similarities with the AMF community of the native Cerrado vegetation area. The similarities observed between the AMF species found in the native vegetation of the Cerrado areas and in the areas where conventional cotton production had been introduced recently as well as the disparities between the AMF species found in the areas under long-term cotton cultivation, suggest the possibility that the conventional cotton production systems may cause a reduction in the number of AMF species in these soils. Mechanisms of selection pressure and speciation of AMF in monoculture systems have been discussed by Vofrškova et al. (2016). These areas also had the lowest number of glomerospores in soil. According to Van der Heijden et al. (2015), plant roots are colonized by several

Figure 3 - Redundancy analysis (RDA) for the soil chemical and physical characteristics, the number of spores and the arbuscular mycorrhizal fungal species, of all studied areas. Areas 1, 2 and 3 are of conventional cotton production systems; areas 4, 5 and 6 are of native Cerrado biome vegetation; areas 7 and 8 are of colored cotton family organic farming systems, and areas 9 and 10 are of native subcaducifolia forest.
AMF, which are most of the time non-host specific, and can form a below-ground mycorrhizal network between plants. However, intensive farming systems can lead to the reduction and dominance of a few species (Voříšková et al. 2016). Furthermore, intensive mechanization favors some dominant species through fragmentation and spreading of the mycelium network (Verbruggen and Kiers 2010, Verzeaux et al. 2017). This lower number of AMF species may also be associated with the intensive use of agricultural inputs as correctives of soil acidity, fertilizers, and pesticides. Rivera-Becerril et al. (2017) found a reduced diversity of AMF in soils treated with pesticides, with fungi of the order Glomerales as the most tolerant to the effects of pesticides.

The areas with organic colored cotton cultivation studied herein are in the region of the Vale do rio grande, where the native vegetation differs from that of the Cerrado. Vegetation in the Vale do rio Grande is sub-deciduous, making a transition between Cerrado and Caatinga biomes in western Bahia. The predominance of the genera Acaulospora and Glomus was also observed in both areas of organically managed colored cotton production but was not observed in the areas with native forest, which had a much lower number of AMF species. The native forest areas with sub-deciduous vegetation of Vale do rio Grande presented the lowest number of AMF species. Up to seven species were found in both areas of native forest, with five species being from different genera. It is interesting to note that the organic cotton production areas, which had been cultivated with maize and beans as subsistence crops for the previous two years, had a much higher number, up to 14 AMF species, even when compared to the native vegetation areas. Interestingly, P. bolivianum, otherwise rarely reported in the literature, was found in both areas of organic cotton production but not in the areas of native forest vegetation. It appears that the organic production system brought an enrichment of AMF species. Souza et al. (2003) revealed Acaulosporaceae and Glomeraceae as the most representative among the 24 taxa of AMF they identified in the Caatinga biome of the Xingó area of the state of Alagoas, Brazil. Moreover, the areas with organic cotton production (A7 and A8), and one area of native forest (A10) presented the highest numbers of glomerospores.

Restrictions on the availability of soil nutrients induce root colonization and affect the increased sporulation of these fungi (Dantas et al. 2015). Phosphorus affects AMF sporulation by reducing spore density when present in higher levels (Nascimento et al. 2016). The diminished number of species in conventional cotton cultivation areas could be related to the increased soil fertilization with chemical inputs, which affects mycorrhizal colonization in several plant species (Diniz 2006) including cotton (Prince et al. 1989). However, the redundancy analysis (RDA) (Figure 3) indicates that soil chemical and physical characteristics do not explain the differences in AMF species nor the number of glomerospores in the studied areas. Schneidera et al. (2015) speculated that organically managed fields support AMF communities that promote better crop yield with more efficient use of phosphorus. Additionally, changes in the distribution of plant species can also affect the AMF communities (Kivlin et al. 2011).

Additional studies considering larger areas and distinct treatments are necessary to uncover the dynamics of the mycorrhizal community in the conventional and organic cotton production systems such as those studied herein. Furthermore, the functional diversity of the AMF species in cotton production areas, with different management practices, as well as the role of these fungi on plant productivity should be investigated for a better understanding of the complex plant – AMF interactions.

Altogether, our data suggest that cultivation of colored cotton under organic family-based
systems may have had positive effects on the AMF soil community structure. Indeed, these areas presented higher numbers of AMF species than the native forest areas and the conventional cotton production areas. In contrast, the conventional cotton production areas, which are managed with the intensive use of mechanization and agricultural inputs, showed lower numbers of AMF species and of glomerospores in soil than the areas with organic cotton production. These conventional cotton production areas also showed lower numbers of AMF species than the soils of the surrounding native vegetation Cerrado areas. This observation may reflect a selective pressure of cotton crop management practices on AMF community.

The knowledge presented herein may add to future research efforts about how to achieve an equilibrium between the use of agricultural inputs and the maintenance of AMF community in the cotton production areas in Brazil, which are increasingly important for the country’s economy both at the conventional and family-based farming systems.

CONCLUSIONS

Acaulosporaceae and Glomeraceae are the predominant AMF families in all areas studied in the western region of Bahia, Brazil. The highest number of AMF species is found in the soils of organic cotton family-based farming areas and the lowest in the soils of conventional cotton production systems. Organic cotton cultivation improved while conventional cotton production reduced the number of AMF species in relation to those found in the areas of Cerrado and in the Cerrado-Caatinga transition areas with native vegetation.

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AUTHOR CONTRIBUTIONS

Nunes HB, Soares ACF, Coimbra JL and Goto BT conceived and designed the experiments. Nunes HB, Tavares DG, Coimbra JL and Rocha MS performed the experiments. Goto BT performed the morphological identification of the AMF. Nunes HB, Oliveira J da S and Silva F de L analyzed the data. Nunes HB, Soares ACF, Goto BT and Oliveira J da S wrote the paper. All authors contributed to the manuscript.

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