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SOIL MICROBIOLOGICAL PROPERTIES AND ENZYME ACTIVITY IN AGROFORESTRY SYSTEMS COMPARED WITH MONOCULTURE, NATURAL REGENERATION, AND NATIVE CAATINGA

PROPRIEDADES MICROBIOLÓGICAS E ATIVIDADE ENZIMÁTICA DO SOLO SOB SISTEMAS AGROFLORESTAIS COMPARADO COM MONOCULTURA, REGENERAÇÃO NATURAL E CAATINGA NATIVA

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ABSTRACT: The objective of this study was to evaluate the influence of agroforestry systems of different ages (AFS1: one-year old; AFS5: five-years old) on the biological attributes of soil; the following systems were used for comparison: a slash-and-burn (SBF) farming area, Caatinga which has been undergoing regeneration for 6 years (CaR6), and native Caatinga (NCa) in Brazil. Enzyme activity, abundance and composition of arbuscular mycorrhizal fungi (AMF), and production of glomalin-related soil proteins (GRSP) were evaluated at soil depths of 0–0.05 m. AMF species composition in the AFS was more similar to that in the NCa than in the SBF and CaR6 systems. In the rainy season, sporulation was most abundant in the AFS-1, CaR6, and SBF systems, whereas GRSP concentrations were highest in the AFS5 during the dry season. Acid phosphatase and arylsulfatase enzyme activity was lower in the AFS1 soils than in the NCa and SBF soils (rainy period), and levels of β-glucosidase and fluorescein diacetate hydrolysis in the AFS were equal to or higher than those in the NCa in the dry season but lower in the rainy season. AFS thus appear to promote the maintenance of soil biological quality, and may be more sustainable than SBF farming systems in the Brazilian Caatinga over the long term.

KEYWORDS: Acid phosphatase. β-glucosidase. FDA. Arylsulfatase. Soil management. Arbuscular mycorrhizal fungi spores.

INTRODUCTION

Expansion of agriculture and livestock in Brazil can be traced back to the initiation of slash-and-burn systems that are still common in northern and northeast Brazil, and which have considerable environmental effects. In the municipality of Pedro II (Central-Northern region of the Piauí), an area dominated by the Caatinga biome, agriculture is almost exclusively based on the slash-and-burn approach. The continuous use of this cultivation system has resulted in depletion of natural soil fertility, leading to increased farmer dependency on chemical fertilizers and pesticides, accelerated soil degradation, and loss of soil biodiversity (LIMA et al., 2010, 2011).

In view of growing concerns about the dominant model of current agricultural production being both unsustainable and environmentally harmful, there is a need to look for alternatives that can maintain agricultural productivity without dramatically affecting terrestrial ecosystems. Agroforestry systems (AFS), in which agricultural crops are intercropped with forest species, have been shown to increase soil sustainability (CEZAR et al., 2015; DOLLINGER; JOSE, 2018). Forest arboreal components, together with the great biodiversity of species, promote a continuous supply of organic detritus that enhances soil organic matter content, which directly benefits soil physical, chemical, and biological attributes (YADAV et al., 2011; CEZAR et al., 2015; WEERASEKARA et al., 2016; DOLLINGER; JOSE, 2018; YENGWE et al., 2018).

Numerous previous studies (SILVA et al., 2011, 2016; SOUZA et al., 2018) have examined the effects of AFS on soil chemical and biological attributes in several distinct biomes, such as the Atlantic Forest, but research on the biological attributes of AFS soils in the Caatinga is scarce (LIMA et al., 2010), and especially so regarding soil enzyme activity and arbuscular mycorrhizal fungi (AMF) in this biome.

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Soil microorganisms are the main sources of soil enzymes, and thus soil enzyme activity can be used as an indicator of alterations in soil microbial activity; however, this is largely restricted to processes in which enzymes are involved, such as the formation and degradation of organic matter or nitrogen mineralization (VIDICAN; STOIN 2015; BALOTA et al., 2013). Evaluations of enzyme activity are known to be efficient indicators that reflect changes that improve soil quality, as well as the decomposition of organic matter and nutrient availability due to cultivation or natural processes (SILVA et al., 2012b; TIAN et al., 2013; WEERASEKARA et al., 2016). Cultivation systems that minimize soil disturbance, maximize organic residue contributions, and incorporate crop rotation practices usually have higher levels of soil quality and microbiological activity, which is reflected in higher levels of enzyme production and, over time, enzyme accumulation in the soil matrix (PEIXOTO et al., 2010; BALOTA et al., 2013; FERREIRA et al., 2017).

In addition to soil enzymes, AMF are also considered to be important indicators of soil quality (DOBO et al., 2018; POSADA et al., 2018), largely due to the diverse soil processes in which these microorganisms are involved (FOLLI-PEREIRA et al., 2012). In addition to influencing plant nutrition, AMF, through their hyphae and the production of glomalin (a glycoprotein that amalgamates soil particles), are also directly involved in soil aggregation (WU et al., 2014; WRIGHT et al., 2007). Because of its composition (36–59% carbon) and its role in the stability of soil aggregates, glomalin contributes both directly and indirectly to soil carbon accumulation (KOIDE; PEOPLES, 2013; SINGH et al., 2013; WU et al., 2014). Similar to AMF, this protein (operationally defined as glomalin-related soil protein, GRSP) responds to changes in land-use and soil cultivation practices, and is often incorporated into environmental monitoring protocols because it is considered a good indicator of soil quality and AMF activity (SILVA et al., 2014a; ISLAS et al., 2016).

Quantification and evaluation of the beneficial effects of agroforestry practices are important for scientists, policymakers, and landowners for making significant decisions about land-use practices while at the same time diversifying farm income (KUMAR et al., 2010; WEERASEKARA et al., 2016). Our primary objective was thus to learn more about soil microbiological dynamics in agroforestry systems of different ages. We examined the abundance and diversity of soil AMF, GRSP production, and soil enzymatic activity in five ecosystems: 1- and 5-year-old agroforestry systems, native forest, naturally regenerated forestlands, and slash-and-burn farming, in the municipality of Pedro II, Piauí, Brazil. Our primary working hypothesis was that AMF, GRSP, and soil enzyme activity would be similar between the soils of AFS and native Caatinga ecosystems. Therefore, this study aimed to evaluate the influence of agroforestry systems of different ages (AFS1: one-year old; AFS5: five-years old) on the biological attributes of soil; the following systems were used for comparison: a slash-and-burn (SBF) farming area, Caatinga which has been undergoing regeneration for 6 years (CaR6), and native Caatinga (NCa) in Brazil.

MATERIAL AND METHODS

Our study was conducted in the municipality of Pedro II, Piauí State (04°25′30″ S, 41°27′32″ W; 630 meters above sea level), in a region dominated by Caatinga. According to the Köppen-Geiger classification system, the municipality of Pedro II fits into the climate type Aw (hot and humid). Rainfall occurs between November and March, and averages 900 mm annually (mostly concentrated in December–February). The driest months are August through October; however, dry spells lasting for 4–6 consecutive months are not uncommon. Average annual temperatures range from 18–30°C.

Five distinct areas were evaluated, consisting of 1-year-old agroforestry systems (AFS1), 5-year-old agroforestry systems (AFS5), Caatinga vegetation under regeneration for 6 years (CaR6), slash-and-burn systems under continuous cultivation with annual cycle monocultures (SBF), and areas of native Caatinga vegetation (NCa) (Table 1). With the exception of AFS1, all areas consisted primarily of desert coverage, which is characteristic of soils in semi-arid regions; this layer hinders cultivation with tools or agricultural machinery under animal traction, which are common in small-scale farming. Each field or area was approximately 1 ha in size.

Sampling was performed in October 2009 (dry season) and April 2010 (rainy season). A 400 m^2 area was selected in each management system from which soil samples were taken; collection points were equidistant and a back-and-forth route was followed to ensure coverage of the entire area.

Samples were collected from a 0–0.05 m deep layer, with every three individual samples combined to form a composite sample, including three repetitions within each unit of study; thus, a total of nine samples were collected in each area. Soil chemical attributes were evaluated following the procedures described by Donagemma et al. (2011) whereas total organic carbon (TOC) was determined following the procedures described by Yeomans & Bremner (1988) (Table 2).

Table 1. Description of the areas under study in the Caatinga biome, Brazil.

Areas	Description
AFS1	1-year-old agroforestry system: Cultivation of corn (*Zea mays* L.), cowpea (*Vigna unguiculata* L.), black mucuna (*Mucuna aterrima* L.), and cashew (*Anacardium occidentale* L.); presence of chalk-browed mockingbird (*Mimus saturninus arenaceus* Chapman) and *mororó* (*Bauhinia cheilantha* (Bong.) Steud.), and other shrubs and local species affecting the formation of the sparse brush called *capoeira rala*.
AFS5	5-year-old agroforestry system: Cultivation of maize, cowpea, mango (*Mangifera indica* L.), and cashew; and the presence of chalk-browed mockingbird, *mororó*, and *catingueira* (*Caesalpinia pyramidalis* Caesalpiniaceae Tul.), and other local shrubs and species that result in the formation of sparse *capoeira*.
SBF	Slash-and-burn farming with continuous cultivation of annual cycle monocultures: Cultivation of rice (*Oryza sativa* L.), and grazing of sheep and goats when the cultivated area/pasture is developed. The annual burn produces residues that are used for cultivation the following year. After the removal of livestock, grass production is favored.
CaR6	Caatinga vegetation under regeneration for 6 years: Land is cultivated for 6–8 years (sometimes less), following which it is left to regenerate for a period of 9–12 years (sometimes more). This use pattern is typical of itinerant agriculture, in which farmers migrate to different areas within the same region.
NCa	Native Caatinga: Preserved to maintain native vegetation.

Table 2. Soil chemical attributes and total organic carbon in soils under different cropping systems in the Caatinga biome, Brazil.

Areas	pH in water		P		K		Ca		Mg		TOC	
			mg dm^{-3}		----------$cmol_c$ dm^{-3}----------							
	D	R	D	R	D	R	D	R	D	R	D	R
AFS1	5.91b	5.52b	0.37a	4.57b	0.16b	0.11b	2.57c	2.50d	1.47c	1.03b	11,9d	10,5e
AFS5	6.39a	6.48a	0.72a	8.42 a	0.38a	0.23a	6.30b	6.30b	2.63b	2.00b	17,4c	12,6d
NCa	5.92b	5.89b	0.25a	2.63d	0.16b	0.15b	7.38a	8.00a	4.30a	4.60a	20,3b	22,5a
CaR6	5.71b	5.97b	0.35a	3.16d	0.11c	0.11b	1.73c	2.30d	1.27c	1.37b	9,5a	14,0c
SBF	5.85b	6.00b	0.59 a	3.73c	0.43a	0.15b	5.07c	5.00c	3.67a	4.03a	21,8a	17,3b

Averages followed by the same lowercase letter in a column are not statistically different by the Scott-Knott test at 5%; AFS1 and AFS5: Agroforestry systems with one and five years old, respectively; CaR6: Caatinga vegetation under regeneration for 6 years; NCa: Native caatinga; SBF: Slash-and-burn farming with continuous cultivation of annual cycle monocultures; D: Dry period; R: rainy period.

To assess spore abundance and composition of the AMF community, 50 cm^3 of soil was separated from each sample. Spores were extracted by wet sieving (GERDERMANN; NICOLSON, 1963) followed by centrifugation in water and sucrose (JENKINS, 1964), and counted using a magnifying glass and a canelet plate. Spores were then transferred to a Petri dish and divided into two groups, one of which was placed on a slide with polyvinyl alcohol in lactoglycerol (PVLG) under a cover slip and the other placed on the same slide with Melzer's reagent under a second cover slip. Identification of AMF species was performed with the aid of an optical microscope, and followed the descriptions outlined in the International Culture Collection of (Vesicular) Mycorrhizal Fungi (INVAM; http://invam.caf.wvu.edu/) and the Arbuscular Mycorrhizal Fungi Phylogeny (http://www.amf-phylogeny.com/index.html).

Glomalin in the soil was quantified as GRSP. Two fractions of GRSP (easily extractable glomalin, EEG; and total glomalin, TG) were differentiated as per the extraction conditions. The

easily extractable glomalin-related soil protein (GRSP-EE) was obtained by autoclaving, using 1 g of soil in 8 mL of sodium citrate at 20 mmoL L^{-1} (pH 7.4) and 121°C for 30 min, and the total glomalin-related soil protein (GRSP-T) was obtained using 1 g of soil in 8 mL of sodium citrate at 50 mmoL L^{-1} (pH 8.0), at 121°C for 60 min. More than one autoclaving cycle was necessary for the extraction of this fraction to produce a light-yellow colored sample. After autoclaving, both fractions were centrifuged at 5,000 *g* for 20 min, and the supernatant was removed for subsequent protein quantification (Bradford method; WRIGHT et al., 1996).

Enzymatic analysis was performed for β-glucosidase, acid phosphatase, and arylsulfatase based on the colorimetric reading of p-nitrophenol, which varies according to the level of enzyme activity (TABATABAI, 1994). For each sample, 1 g of soil was incubated for 1 h in a buffer solution containing one of the substrates p-nitrophenyl-glycoside (PNG), p-nitrophenyl-phosphate (PNF), or p-nitrophenyl-sulfate (PNS). Total enzyme activity was also evaluated through the hydrolysis of fluorescein diacetate (FDA) (DICK et al., 1996), whereby the colorless FDA solution changes to a bright yellowish-green when hydrolysis occurs, allowing its spectrophotometric quantification at 490 nm. The standard curve of fluorescein was established from standard solutions at different concentrations of fluorescein.

Data were evaluated for homoscedasticity by Cochran's test and for normal distribution of residues by the Lilliefors test, and results were subjected to ANOVA and the Scott-Knott test, at 5%. All statistical analyses were carried out with SAEG v5.0 (System of Statistical and Genetic Analysis - Federal University of Viçosa) and SISVAR software, with the exception of analysis of cluster grouping and relationships between edaphic attributes (Pearson's correlation analysis), for which the statistical program PAST was used.

RESULTS AND DISCUSSION

Spore abundance and AMF community composition

No significant differences in spore abundance were observed between the study areas in the dry season (Table 3), whereas in the rainy season, the most abundant sporulation was observed in the AFS-1, CaR6, and SBF sites (Table 3). Lower sporulation in the AFS5 may be due to higher phosphorus (P, 8.42 mg dm^{-3}) concentrations in this soil (Table 1); for instance, Dobo et al. (2018), in an evaluation of AMF spore abundance in the rhizospheric soils of different plants in Sidama, Ethiopia, reported that spore density was higher when concentrations of P and N were relatively low.

Table 3. Spore abundance and AMF species richness in soils under different cropping systems in the Caatinga biome, Brazil.

Areas	Spore abundance		Species total richness	
	D	R	D	R
	----Spore 50 g^{-1} $soil^{-1}$----			
AFS1	901a	2603a*	9	12
AFS5	1304a	852b	5	12
NCa	1251a	1184b	11	11
CaR6	1820a	2569a	4	7
SBF	1084a	2163a*	10	7

Averages followed by the same lowercase letter in a column are not statistically different by the Scott-Knott test at 5%; AFS1 and AFS5: Agroforestry systems with one and five years old, respectively; CaR6: Caatinga vegetation under regeneration for 6 years; NCa: Native caatinga; SBF: Slash-and-burn farming with continuous cultivation of annual cycle monocultures; D: Dry period; R: rainy period.

Low sporulation also occurred in the NCa, a result similar to that of Piotrowski et al. (2008), who suggested that low sporulation in areas with native vegetation compared to agricultural systems was indicative of greater AMF activity in soils that are still undergoing constructive processes and have not yet attained stability.

Significant differences in spore abundance between seasons were only observed for AFS1 and SBF, with the highest values occurring in the rainy season (Table 3). According to Bonfim et al. (2010), seasonal influence on the occurrence of AFM spores is not yet well understood, and many contradictory results can be found in the literature.

Explanations for the occurrence of higher sporulation, whether in the rainy or the dry season, can differ greatly, and can include factors such as the moisture level in each soil type, the characteristics of host plants, and the genetic tendency of a species for sporulation, among others (MOREIRA et al., 2009; OEHL et al., 2009). Furthermore, according to Gehring et al. (2003), higher light intensity during the rainy season promotes root exudation and carbohydrate concentrations in the roots, both of which contribute to higher rates of sporulation.

In the dry season, AMF species richness was highest in the NCa, with that in the SBF almost reaching equivalent levels (Table 3), whereas in the rainy season, AMF species richness was almost as high in both the AFS1 and AFS5 as in the NCa (Table 3). Total AMF richness increased in the AFS5, AFS1, and CaR6 areas from the dry season to the rainy season (Table 3).. The difficulty in establishing AMF distribution patterns may be due to various biotic and abiotic factors, as well as to the various survival strategies of these fungi (SOUZA et al., 2003). According to Posada et al. (2018), the spatial heterogeneity found in soil parameters may influence the rates and stage of succession by native AMF species, rendering uneven AMF species distribution on a local scale. Hazard et al. (2013) demonstrated the influence of the local environment in determining AMF composition, whereas Silva et al. (2014b) found that soil characteristics in semi-arid regions play a major role in the determination of AMF community composition.

Souza et al. (2003) suggested that soil pH and P content are important factors for some species of AMF, and it was observed in this study that *Entrophospora infrequens*, *Acaulospora spinosa*, and *Racocetra fulgida* were observed only in the NCa area, which had soil P contents of 0.22 and 2.63 mg dm^{-3} in the dry season and the rainy season, respectively. Moreover, *Racocetra undulata* and *Acaulospora cavernata* were identified only in the AFS5 area, in which soil pH values were 6.39 and 6.48 in the dry and rainy seasons, respectively, and soil P content was 0.72 and 8.42 mg dm^{-3} in the dry and rainy seasons, respectively.

A total of 30 morphotypes of AMF spores were identified in different areas and in both seasons (Table 4), which were determined to belong to eight genera and five families (Glomeraceae, Acaulosporaceae, Gigasporaceae, Claroideoglomeraceae, and Ambisporaceae). Souza et al. (2003) and Silva et al. (2014b), in a study on the diversity of AMF in Caatinga ecosystems, identified 24 and 50 AMF taxa, respectively, with greater representation of Acaulosporaceae and Glomeraceae.

Of the total number of AMF species observed in this study, 22 were sporulating in the dry season and 23 in the rainy season, with 15 species common to both seasons. Spores of *Claroideoglomus etunicatum*, *Glomus* sp2, *Scutellospora calospora*, *Racocetra fulgida*, *Acaulospora* sp1, *Acaulospora* sp3, and *Acaulospora spinosa* were observed only in the dry season, whereas spores of *Acaulospora* sp2, *Acaulospora bireticulata*, *Entrophospora infrequens*, *Acaulospora tuberculata*, *Scutellospora* sp2, *Scutellospora cerradensis*, *Racocetra undulata*, and *Scutellospora heterogama* were observed only during the rainy season (Table 4). The largest number of AMF species identified in this study belonged to the genus *Acaulospora* (14 species), followed by the genus *Glomus* (four species), *Scutellospora* (four species) *Claroideoglomus* (two species), *Racocetra* (two species), *Dentiscutata* (one species), *Entrophospora* (one species), *Ambispora* (one species), and *Gigaspora* (one species) (Table 4), representing 47%, 13%, 13%, 7%, 7%, 3%, 3%, 3%, and 3% of the total species identified in the survey, respectively.

Members of the genera *Acaulospora* and *Glomus* were found in all areas and in both seasons, with members of *Acaulospora* most often representing the highest percentage of species compared to other genera (Table 4). Sousa et al. (2014) reported that species of *Acaulospora* and *Glomus* were most common in Caatinga systems at different stages of succession. Species in these genera typically exhibit greater adaptability to soils that differ in organic matter levels, lime, and texture, among other factors, suggesting that these species are particularly resistant to adverse environmental conditions (WU et al., 2011; FOKOM et al., 2012).

Table 4. AMF species composition in soils under different cropping systems in the Caatinga biome, Brazil.

AMF species	AFS1		AFS5		NCa		CaR6		SBF	
	D	R	D	R	D	R	D	R	D	R
Acaulospora foveata Janos & Trappe	+	+	-	+	+	+	-	-	+	-
Acaulospora scrobiculata Trappe	+	+	+	+	+	-	+	-	+	-
Acaulospora tuberculata Janos & Trappe	-	+	-	-	-	+	-	-	-	-
Acaulospora mellea Spain & N.C. Schenck	+	-	+	+	-	+	-	-	+	-
Acaulospora spinosa C. Walker & Trappe	-	-	-	-	+	-	-	-	-	-
Acaulospora laevis Gerd. & Trappe	-	+	-	-	+	+	-	-	-	-
Acaulospora sp1	-	-	-	-	+	-	-	-	+	-
Acaulospora sp2	-	-	-	-	-	-	-	-	-	+
Acaulospora sp3	-	-	-	-	-	-	-	-	+	-
Acaulospora sp4	-	+	-	-	-	-	-	-	+	-
Acaulospora rehmii Sieverd. & S. Toro	+	+	+	+	+	+	-	-	-	-
Acaulospora denticulata Sieverd. & S. Toro	-	-	-	-	+	-	-	-	-	+
Acaulospora bireticulata F.M. Rothwell & Trappe	-	-	-	-	-	-	-	-	-	+
Acaulospora cavernata Blaszk.	-	-	+	+	-	-	-	-	-	-
Ambispora leptoticha N.C. Schenck & G.S. Sm. C. Walker, Vestberg & A. Schüssler	-	+	-	+	-	+	+	+	-	+
Claroideoglomus lamellosum Dalpé, Koske & Tews, C. Walker & A. Schüßler	-	-	-	+	-	+	-	-	+	+
Claroideoglomus etunicatum W.N. Becker & Gerd. C. Walker & A. Schüßler	-	-	-	-	-	-	-	-	+	-
Dentiscutata heterogama T.H. Nicolson & Gerd. Sieverd., F.A. Souza & Oehl	-	-	-	-	-	-	-	+	-	-
Entrophospora infrequens Hall, Ames & Schneider	-	-	-	-	-	+	-	-	-	-
Glomus macrocarpum Tul. & Tul.	+	+	-	+	+	+	+	+	+	+
Glomus glomerulatum Sieverd.	+	+	+	+	+	+	-	+	-	+
Glomus sp 1	+	+	-	-	-	-	-	+	-	-
Glomus sp 2	+	-	-	-	-	-	-	-	-	-
Gigaspora sp	-	-	-	-	-	-	+	+	-	-
Racocetra fulgida Oehl, F.A. Souza & Sieverd. (2008)	-	-	-	-	+	-	-	-	-	-
Racocetra undulata Sieverd., T.C. Lin & C.H. Yen	-	-	-	+	-	-	-	-	-	-
Scutellospora cerradensis Spain & Miranda	-	+	-	+	-	-	-	+	-	-
Scutellospora sp1	+	+	-	+	-	-	-	-	-	-
Scutellospora sp2	-	-	-	-	-	+	-	-	-	-
Scutellospora calospora T.H. Nicolson & Gerd., C. Walker & F.E. Sanders	-	-	-	-	+	-	-	-	+	-

AFS1 and AFS5: Agroforestry systems with one and five years old, respectively; CaR6: Caatinga vegetation under regeneration for 6 years; NCa: Native caatinga; SBF: Slash-and-burn farming with continuous cultivation of annual cycle monocultures; D: Dry period; R: rainy period.

G. macrocarpum was found to occur in all areas except AFS5 in the dry season (Table 4). Sousa et al. (2014) observed the presence of this species in areas of pasture and plant succession in early, middle, and late stages in the Caatinga. Species identified in this study, such as *Ambispora leptoticha, Claroideoglomus etunicatum, Acaulospora scrobiculata, Glomus macrocarpum*, and *Acaulospora rehmii*, have been previously reported to occur in the Caatinga (YANO-MELO, 2002; SOUSA et al., 2014).

Figures 1A and 1B show the degrees of similarity between the systems studied, with respect to the occurrence of AMF species in the dry season and the rainy season, respectively. AMF composition varies in both seasons when NCa is converted to other systems of land usage; for example, in the dry season, conversion of NCa to AFS results in a 60% change in AMF species composition, and a 70% change with conversion to SBF systems; diversity of AMF was highest in the CaR6 area (90%) compared to the NCa in the dry period. The areas that showed the greatest similarity in both periods were AFS1 and AFS5. In the rainy season, AMF composition in both AFS5 and AFS1 (35% and 45% in the dry and rainy

periods, respectively) were more similar to that of the NCa than any of the other systems.

The results of the groupings show that the degrees of similarity between the areas vary with the season, as was previously noted by Miranda et al. (2010), who proposed that this variation may be related to factors that affect mycorrhizal colonization, such as climatic variations that modify the hydrous relationships in the study areas, depending on the season. In addition, factors relating to the host plant in its different phenological phases, and AMF species themselves, which may have different survival strategies in each season, may also contribute to the variation between seasons.

Glomalin-related soil protein

Differences in glomalin levels between the study areas were only observed in the dry season, when AFS5 contained about 40% more GRSP-EE than did AFS1 and NCa, whereas GRSP-EE concentrations were approximately 30% lower in SBF and CaR6 systems than in NCa (Table 5). Fokom et al. (2013) reported that concentrations of GRSP-EE decreased when native forest was converted to agricultural cultivation, but no differences were observed between cultivated areas and an area under regeneration for 5 years. Previous studies have shown that soil disturbances reduce AMF populations (DODD et al., 2000; HAMEL et al., 1994) due primarily to disruptions of the mycelia networks, which may directly result in the inhibition of glomalin synthesis and deposition in the soil (FOKOM et al., 2013).

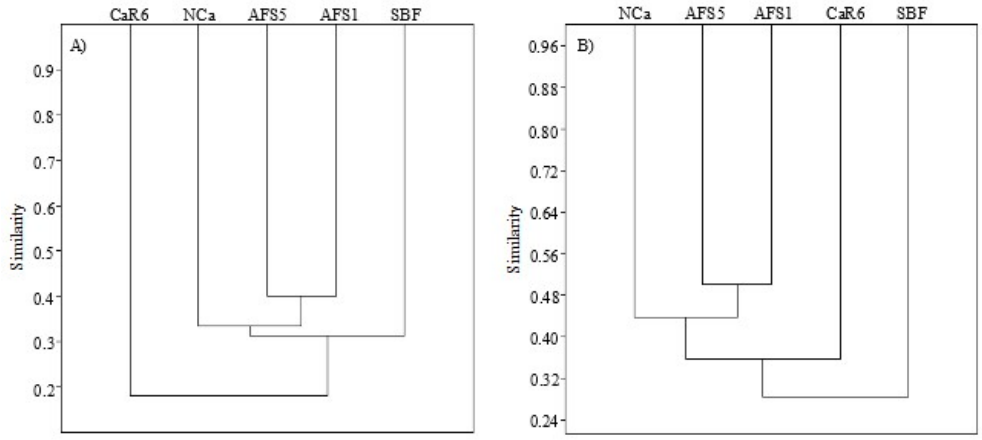

Figure 1. Dendrogram (simple Jaccard connection) of the occurrence of AMF species in the soils (0–0.05 m depth) of different areas. AFS1 and AFS5: Agroforestry systems of 1 yr and 5 yr of age, respectively; CaR6: Caatinga vegetation under regeneration for 6 years; NCa: Native Caatinga; SBF: Slash-and-burn farming with continuous cultivation of annual cycle monocultures; D: Dry period; R: rainy period.

GRSP-EE concentrations were significantly higher in AFS1 and AFS5 than in SBF (Table 5). Slash-and-burn agriculture can suppress production of the external mycelium, which is responsible for the synthesis of glomalin, in many types of AMF. Glomalin levels correlate positively with the production (CURAQUEO et al., 2011) and length (ZOU et al., 2016) of hyphae. However, agroforestry systems, in which farming practices tend to be less intensive and plant diversity is higher, possibly create a more conducive environment for the production of the external mycelium, and thus higher rates of GRSP deposition, after the hyphae have been decomposed by microorganisms in the soil (DRIVER et al., 2005). The accumulation of GRSP in soil is dependent on numerous factors including plant community composition, AM fungi richness, type of land-use system, and soil properties (TRESEDER; TURNER, 2007; SINGH et al., 2016).

Table 5. Total glomalin-related soil protein (GRSP-T) and easily extractable glomalin-related soil protein (GRSP-EE) in soils under different cropping in the Caatinga biome, Brazil.

Areas	GRSP-T		GRSP-EE	
	D	R	D	R
	---------- mg g^{-1} soil ----------			
AFS1	7,52b	5,09a	2,18b*	0,91a
AFS5	10,34a*	6,25a	2,99a*	1,03a
CaN	7,34b	8,49a	2,18b*	1,32a
CaR6	4,59b	7,15a*	1,18c	1,21a
SBF	5,74b	7,56a	1,55c	1,19a

Averages followed by the same lowercase letter in a column are not statistically different by the Scott-Knott test at 5%; * Indicates a significant difference between seasons by the Scott-Knott test at 5%; AFS1 and AFS5: Agroforestry systems with one and five years old, respectively; CaR6: Caatinga vegetation under regeneration for 6 years; NCa: Native caatinga; SBF: Slash-and-burn farming with continuous cultivation of annual cycle monocultures; D: Dry period; R: rainy period.

Similar to GRSP-EE, a statistically significant difference in GRSP-T between the areas was detected only in the dry season (Table 5). Concentrations of this fraction were 80% and 40% higher in AFS5 than in SBF and NCa, respectively. Higher GRSP levels may contribute to higher C and N uptake and enhance soil aggregation, given the high positive correlations between these soil properties and GRSP that have been reported by several authors (KOIDE; PEOPLES, 2013; SINGH et al., 2013; WU et al., 2014). Correlations between GRSP-T/GRSP-EE and TOC were highly positive ($r = 0.94$, $p = 0.02$, $r = 0.84$, $P = 0.04$, respectively) in the rainy season.

The absence of any significant differences between the areas during the rainy season may be due to improved growth conditions for roots due to the higher soil moisture levels; more extensive root systems may result in higher rates of AMF colonization, thereby increasing the production of hyphae (WU et al., 2014) and resulting in a more homogeneous pattern of GRSP production among the different areas.

Concentrations of GRSP-T did not vary between the sampling seasons in most areas (Table 5). GRSP-T is considered the most stable fraction of GRSP, and is deposited in soil over a longer period of time, and thus experiences more biochemical transformations and greater soil particle adherence (NICHOLS; WRIGHT, 2005). As such, variations in this fraction may be smaller over short time periods. On the other hand, concentrations of GRSP-EE, the most labile fraction and which is deposited in soil over shorter time periods (RILLIG, 2004; WU et al., 2014), were higher in AFS-5, AFS-1, and NCa in the dry season than in the rainy season. There is evidence that AMF hyphae produce more glomalin per unit weight or length of hyphae under conditions of stress (e.g., drought, salinity) (HAMMER; RILLIG, 2011); several studies have also shown that concentrations of EE-GRSP increased in the rhizosphere of *Trifoliate orange* seedlings under drought stress (WU et al., 2008; ZOU et al., 2014) because the drought-stress-induced death/senescence of mycorrhizal hyphae resulted in the release of more GRSP into the soil (DRIVER et al., 2005). Thus, it can be inferred that the higher levels of GRSP-EE in the dry season may be due to greater production of this protein by hyphae and/or more of this protein being released as a result of hyphae death/senescence.

Higher GRSP concentrations in the dry season may indirectly favor the development of plant species, given that its role in enhancing the stability of soil aggregates increases soil moisture retention (WU et al., 2008, 2014) and, more directly, the physiological effects the protein has on plants (CHI et al., 2018). In an evaluation of the effect of exogenous GRSP-EE on *T. orange* seedlings, Chi et al. (2018) observed that application of exogenous GRSP-EE improves the drought tolerance of this species, thereby significantly enhancing leaf water potential, net photosynthetic rate, transpiration rate, stomatal conductance, and intercellular CO_2 concentrations, while at the same time drastically reducing leaf temperature regardless of soil water status.

Enzyme activity

Total enzyme activity, as assessed by FDA hydrolysis, was higher in AFS1, CaR6, and SBF than in the other areas during the dry season, whereas total enzyme activity levels were similar in AFS5 and NCa (Table 6). The AFS1 area had been deforested just prior to the onset of the dry season, resulting in large amounts of vegetative

residues on the soil surface, and the increased availability of organic matter to primary soil decomposers may account for the high FDA activity observed in this area. Previous research has shown that inputs of organic materials, such as plant litter, stimulate soil FDA (LOPES et al., 2010; RODRIGUES et al., 2015).

Soil TOC content (Table 2) was highest in the SBF, which may explain why this area also had one of the highest levels of FDA activity. During the rainy season, FDA activity was greater in areas where TOC levels were the highest, specifically in areas of lower cultivation intensity (SBF) and greater conservation (NCa). On the other hand, FDA activity values were also high in CaR6 in the dry season, for which TOC content was the lowest. Thus, it is important to note that the enzymes may be stimulated according to the role they play in the processes that occur in the soil, i.e., enzymes involved in the degradation of a given substrate increase activity when the availability of that substrate in the environment also increases. In the same manner, the activity levels of enzymes associated with stress increase under conditions of environmental stress (WALLENSTEIN; WEINTRAUB, 2008).

In the dry period, β-glucosidase activity was higher in SBF and AFS5 than in NCa, AFS1, and CaR6, although activity levels in the latter two did not differ from that in NCa. In the rainy season, on the other hand, higher levels of β-glucosidase activity were observed in NCa than in AFS1, AFS5, CaR6, and SBF, with no differences observed among the latter four at any time. Variations in the activity of this enzyme between the areas may be due to differences in organic matter inputs to the soil, because β-glucosidase is directly related to the C cycle and it is primarily active in the final stages of cellulose decomposition, in which it hydrolyzes the residues from cellobiose to β-D-glucose (TABATABAI, 1994). Moreover, large quantities of highly complex residues may promote lower activity of this enzyme (MATSUOKA et al., 2003). For example, in this study, we observed a high correlation between β-glucosidase and soil TOC (r = 0.93; P = 0.02) in the rainy season.

Table 6. *β*-glucosidase, acid phosphatase, arylsulfatase, and fluorescein diacetate hydrolysis (FDA) activity in the soils of the different cropping systems in the Caatinga biome, Brazil.

Areas	FDA		β-glucosidase		Acid phosphatase		Arylsulfatase	
	D	R	D	R	D	R	D	R
	μg fluorescein g^{-1} soil h^{-1}		------- μg ρ-nitrophenol g^{-1} soil h^{-1} -----					
AFS1	148a	223b*	58b	130b*	261a	595d*	28a	50c*
AFS5	122b	223b*	80a	133b*	266a	574d*	32a	73b*
NCa	110b	315a*	68b	250a*	272a	907a*	44a	142a*
CaR6	143a	240b*	60b	148b*	268a	676c*	23a	59c*
SBF	134a	333a*	101a	153b*	270a	773b*	33a	79b*

Averages followed by the same lowercase letter in a column are not statistically different by the Scott-Knott test at 5%; * Indicates a significant difference between seasons by the Scott-Knott test at 5%; AFS1 and AFS5: Agroforestry systems with one and five years old, respectively; CaR6: Caatinga vegetation under regeneration for 6 years; NCa: Native caatinga; SBF: Slash-and-burn farming with continuous cultivation of annual cycle monocultures; D: Dry period; R: rainy period.

β-glucosidase activity levels were higher in the dry season than in the rainy season. Differences in acid phosphatase activity between the systems of land usage occurred only during the rainy season, with activity levels higher in NCa than all other areas (Table 6), possibly due to the lower concentrations of soil P in that system (Table 2); this may also explain why the lowest activity levels of this enzyme were observed in AFS5, given that P concentrations were higher in this area than in the others. In general, this pattern was observed when comparing the other areas evaluated. Silva et al. (2018), in an assessment of the activity levels of acid phosphatase in soils under different stages of forest regeneration in the Caatinga, observed that acid phosphatase activity was always higher in soils with lower P concentrations.

Balota et al. (2013) proposed that, in some cases, enzymatic activity can increase due to a deficit of, or the form (organic or mineral) in which, certain nutrients appear in the soil, using P and sulfur (S) as specific examples. Activity levels were higher in SBF and CaR6, whereas activity levels in AFS1 and AFS5 were lower than in SBF and CaR6 but similar to one another. The variation in acid phosphatase activity between the areas is most likely due to the concentration of TOC in the soil, as a high positive correlation (r = 0.97, P = 0.005) between acid phosphatase activity and TOC

was detected at this time. In addition, several studies have demonstrated that phosphatase activity is positively correlated with soil TOC content (CARNEIRO et al., 2008, JAKELAITIS et al., 2008).

In the dry season, arylsulfatase activity exhibited a pattern similar to that of phosphatase, that is, that water deficit reduces enzymatic activity to the point where activity does not reflect the characteristics and properties of the different land-use systems. Conversely, in the rainy season, increased activity of this enzyme was observed in NCa, to a lesser degree in SBF and AFS5, and even less so in CaR6 and AFS1. This variation may be related to soil TOC levels (ACOSTA-MARTINEZ et al., 2018), given that this enzyme is positively correlated ($r = 0.93$; $P = 0.02$) with soil TOC. According to Nogueira & Melo (2003), arilsulfatase activity in soils decreases with reductions in organic matter, because organic matter is the main source of sulfate esters, which are substrates of this enzyme; in contrast, however, McGill & Colle (1981) proposed that a considerable proportion of arylsulfatase found in soils is secreted by bacteria as a result of limited organic material. Under normal conditions, the occurrence of this enzyme in soils is related to microbial biomass and the level of immobilization (BALOTA et al., 2013).

Enzymatic activity was about 1.5–3 times greater in the rainy season than in the dry season for β-glucosidase, arylsulfatase, and FDA, and, in the case of acid phosphatase activity in NCa, as much as 3.5 times greater; as such, the time of year appears to determine the level of enzymatic activity in the soil, considering that the results were higher in the rainy period than in the dry period for all enzymes included in our study (Table 6). These seasonal differences are likely due to variations in soil moisture and temperature (ARAÚJO et al., 2013; RODRIGUES et al., 2015). In general, lower enzymatic activity in the dry season was attributed to water deficits, whereas in the rainy season, when water was not a limiting factor, enzymes were more readily able to hydrolyze decomposable compounds (ARAÚJO, 2013; 2014). Rodrigues et al. (1985) argued that soil microbial community structure tends to vary between seasons and responds differently to dry and humid conditions, as Araújo et al. (2013; 2014) observed in the tropical soils of northeastern Brazil. In addition, Caatinga vegetation regeneration during the rainy season likely promotes the addition of easily decomposable residues, which would promote higher levels of soil enzymatic activity.

CONCLUSIONS

Agroforestry systems AFS1 and AFS5 were found to maintain or increase the sporulation of AMF and the production of GRSP compared to native Caatinga. Although AFS operations altered AMF composition, AMF communities in these areas most closely resembled AMF communities in native Caatinga soils compared to slash-and-burn systems and Caatinga under regeneration.

AFS helps to maintain or even promote enzymatic activity (FDA, acid phosphatase, β-glucosidase, and arylsulfatase) in the dry season, although activity levels of these enzymes are lower in AFS soils than in native Caatinga during the rainy season. Based on most of the biological attributes evaluated in our study, we conclude that agroforestry systems promote the maintenance of, or can even improve, soil biological quality, and may be more sustainable than the slash-and-burn farming systems in Caatinga ecosystems over the long term.

RESUMO: O objetivo do estudo foi avaliar a influência de sistemas agroflorestais (AFS1: um ano de idade; AFS5: cinco anos de idade), nos atributos biológicos do solo usando como referência, uma área de agricultura de corte e queima (SBF), Caatinga em regeneração há 6 anos (CaR6), e Caatinga nativa (NCa), in Brasil. A atividade enzimática, a abundância e composição dos fungos micorrízicos arbusculares (AMF), e a produção de proteína do solo relacionada à glomalina (GRSP) foram avaliados, na profundidade de 0-5 cm do solo. A composição das espécies de AMF nos AFS foi mais semelhante a observada na NCa, do que os sistemas SBF e CaR6. Na estação chuvosa, a esporulação foi mais abundante em AFS-1, CaR6 and SBF quando comparada as outras áreas, enquanto a GRSP apresentou maiores teores no AFS5 no período seco. AFS1 apresentou atividade da fosfatase ácida e arilsulfatase inferiores tanto a NCa quanto a SBF, no período chuvoso. No período seco, a atividade de β-glicosidase e a hidrólise do diacetato de fluoresceína (FDA) na AFS foram iguais ou superiores a Nca, mas menor no período chuvoso. Verifica-se que os AFS são

potenciais para a manutenção da qualidade biológica do solo, podendo, em longo prazo, serem mais sustentáveis que a SBF, em ambiente de Caatinga.

PALAVRAS-CHAVE: Fosfatase ácida. β-glicosidase. FDA. Arylsulfatase. Manejo do solo. Esporos de fungos micorrízicos arbusculares.

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