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ROOT COLONIZATION BY MYCORRHIZAL FUNGI IN DIFFERENT SOIL MANAGEMENT SYSTEMS: AFSs AND IN THE MANAUS REFINERY

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ABSTRACT

Arbuscular Mycorrhizal Fungi (AMF) form symbiotic associations with the roots of most vascular plants, helping to absorb water and nutrients. The objective of this work was to evaluate the occurrence of these fungi in three agroforestry systems and in an area of the Manaus Oil Refinery. Root samples were collected from 21 host species with five replications for AMF colonization assessments. Soil samples were collected to evaluate chemical characteristics. From the higher pH values and Ca and Mg contents in the soils of some areas, it can be inferred that there was an application of dolomitic limestone in the three Agroforestry Systems (AFSs) and, in the REMAN sites with mimosa and kikuyu. Root colonization with fungal hyphae was high in all areas, indicating that they contribute, at least partially, to plant nutrition and increased soil water absorption capacity.

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INTRODUCTION

The practice of agriculture in tropical regions, such as the Amazon, becomes a delicate activity due to the natural characteristics of the region, high temperatures and humidity, soils with low natural fertility and high acidity (Moreira and Fageria, 2009). The latter is considered the main limiting factor for the activity, as it hinders the development of species of agricultural importance. Coupled with this, the high cost of acquiring agricultural inputs, such as fertilizers and correctives, makes this practice a challenge to be carried out in the tropics. In this sense, it is necessary to look for alternatives of cultivation consistent with the reality of the region in an accessible way to be used by the farmer. These should provide improvements for the development of plants of agricultural interest and, at the same time, favor the development of beneficial soil microbiota to plant development, especially the microorganisms present in the root system that contribute to a more efficient absorption of soil nutrients. Studies point to Agroforestry Systems (AFSs) as one of the most suitable crop models for the Amazon (Loss *et al*., 2009; Pinho *et al*., 2012; Lima *et al*., 2020).

This is due to its similarity with natural forest systems where occur a combination of species of multiple uses that contribute, through the deposition of organic matter to the maintenance of fertility, as well as improvements in the physicochemical and biological characteristics of the soil. According to Santos *et al*. (2013), Oliveira *et al*. (2021), to achieve the sustainability of an ecosystem, strategies that maximize the use of microorganisms and beneficial biological processes in the soil must be used. Among the microorganisms of importance for plant nutrition, emphasis is given to Arbuscular Mycorrhizal Fungi (AMF's), which form a mutualistic symbiotic association with the roots of host plants, called arbuscular mycorrhiza. For Fitter *et al*. (2011), these microorganisms are one of the most ecologically important for the world. This association (Oliveira *et al*., 2003; Oliveira and Oliveira, 2003, 2004, 2005) provides improvements in the absorption of water and nutrients, especially those with low mobility in the soil, such as P, Cu and Zn, in addition to improvements in water absorption. , resistance to attack by pests and diseases, resistance to drought and environmental stresses and tolerance of plants to environments contaminated with heavy metals. These benefits occur due to the presence of hyphae, which function as an extension of the plant's root system.

These fungi are also considered important components in the recovery and restoration of vegetation in degraded areas (Moreira *et al*., 2019 a,b). Therefore, knowledge of the diversity and dynamics of these organisms in the soil is of fundamental importance for the development of more efficient management systems. In the Amazon region, several studies show the importance of mycorrhizal associations with plant roots (Bovi *et al*., 2000; Oliveira and Oliveira, 2004). In agroforestry systems, however, few studies on these associations are reported in the literature (Rocha *et al*., 2020; Oliveira *et al*., 2021). Thus, studying the AMF associated with host plants in these systems is of fundamental importance, as they serve as an indicator of soil sustainability in these areas.

METHODOLOGY

Description of areas: The study was carried out in the State of Amazonas in four management systems, three characterized as Agroforestry Systems with fruit species and forest essences, and one located within the Manaus Refinery, Isaac Sabbá (REMAN). The agroforestry properties are located on the Janauary branch, in the Municipality of Iranduba, at geographic coordinates 03°14. 906'S and 060°08.848'W, 03°13.559'S and 060° 07.457'W and 03°12.148'S and 060°08.339'W. AFS 1- Information on the history of use and fertilization of the area was not provided. The property has an area of 120 m in front by 60 m in depth, with the cultivation part intended for fruit species, especially orange and papaya, maintained with exposed soil, through weeding, without any cover. AFS 2- It has an area of 92m in the front by 180m in the back. According to information from the owner, the last fertilization (NPK) and liming were carried out in 2011. After this period, only cultural practices, such as weeding and mowing, were carried out in the area. The agroforestry system was implemented in lines with the consortium of fruit species. AFS 3- It has 25 ha, its main species is the orange tree, which stands out as the main source of income for the producer. Among the orange trees, species of cupuaçu and palm trees, such as açaí and peach palm, were inserted. This area is maintained with cover promoted by grass. The fourth area is in Manaus at the geographic coordinates 03° 08.390' S and 059° 57.466' W, in the Manaus Refinery of Isaac Sabbá (REMAN). The area has undergrowth and ten years ago regional species were introduced to recover the area. There are no records of cultural practices, providing that along with the introduced species, there was regeneration of the area by native species.

Collection of root and soil samples: Root and soil samples were collected from the rhizospheres of each species that compose the AFS's and from the REMAN area in April 2014. A completely randomized design (DIC) was adopted with five replications in a 4x5 factorial scheme, being four the factors referring to the management systems, and five the factors referring to the forest species collected in each system. Root samples of 20 species identified in Table 1 were collected. In each AFS, roots of five host plant species were collected. Five replicates (plant) were performed for each species and a sample composed of five subsamples was formed for each replicate, totaling 25 samples per management system. Approximately 200 mg of fine roots (<2mm in diameter) were removed from each plant to assess root colonization by arbuscular mycorrhizal fungi. Soil samples were submitted to chemical analysis (EMBRAPA, 1997) at the Thematic Laboratory of Soil and Plant Analysis of the National Institute for Research in the Amazon (INPA).

Root colonization assessment: The roots were washed, cut and clarified with 10% KOH, using the methodology of Kormanik *et al*. (1980). To quantify the percentage of root colonization, the method described by Giovannetti and Mosse (1980) was adopted, using 50 root segments of 1 cm in length per plant from each collected area. To visualize the colonization by hyphae, vesicles and arbuscules, the slides were observed with the aid of an optical microscope at 40x magnification.

Statistical analysis of data: The data were analyzed by the F test, and when significant, the means of the treatments were compared by the Skotty-Knott test at the 5% probability level. For data analysis, the Assistet version 7.7 Beta program was used.

RESULTS AND DISCUTION

Soil fertility analyzes: Table 2 shows the result of the soil fertility analysis in the three areas of AFS's and in the degraded area of REMAN. The interpretations of the soil analysis results of the samples were made according to Cochrane *et al*. (1985). Soil fertility levels varied depending on the place of collection and species sampled. In general, the best fertility levels were observed in the AFS's areas. Araújo and Collier (2006) evaluated soil fertility parameters in agroforestry systems in the state of Tocantins and associated that the maintenance of soil fertility occurred due to the greater diversity of plants, which favored greater deposition and accumulation of litter in the soil. According to Cunha *et al*. (2012), AFSs provide better conditions for the action of the organisms responsible for the fragmentation and cycling of nutrients, thus contributing to the greater amount of nutrients available to be absorbed by the roots of plants. The acidity ranged from 4.6 to 5.9. According to Malavolta (1981), there is an ideal pH range (6.0 to 6.5), which is considered an equilibrium point at which most nutrients remain available to be absorbed by plant roots. In this work, only the samples collected in soil with kikuyu (6.1) were in this range, the others were below these values, indicating high acidity of the samples. These results are consistent with those of Matos *et al*. (2012), which observed pH values that ranged from 4.40 to 4.63 in a farming property in the Benjamim Constant community, municipality of Bragança-PA.

Phosphorus (P) contents were considered high for the three AFS's; in the REMAN area, only the vismia showed a high value by the criteria of Cochrane *et al*. (1985). High levels of this nutrient indicate the application of phosphate fertilizer by farmers, due to the low natural fertility of regional soils (Magalhães *et al*., 2019). AFS 1 showed much higher P content than the other areas, suggesting that fertilization in this area was carried out without agronomic criteria. Phosphorus is considered a nutrient with low mobility in the soil, so plants that have a more developed root system have an advantage in capturing this element in the soil (Santos and Cury, 2011). According to Cochrane *et al*. (1985), the Ca values are considered medium for the areas of AFS 2 (2.57 cmolc kg-1), AFS 3 (2.52 cmolc kg-1) and for the mimosa species (0.83 cmolc kg-1). kg^{-1}) and kikuyu (2.57 cmol/kg-1). AFS 1 was the only one to present a content considered high (6.42 cmolc kg^{-1}). The Ca and Mg contents were higher in the soils of AFS's (Table 2). A similar result was verified by Menezes *et al*. (2008), who found higher pH, Ca and Mg values in AFS soils than in soils from adjacent remaining forests in the State of Rondônia. Similar results were also observed by Alfaia *et al*. (2007) in an area of AFS, pasture and primary forest in the municipality of Nova California-RO. They considered that the best fertility levels of AFS soils, especially for Ca and Mg, occurred as a result of the slash and burn of primary forest, which provided the maintenance of fertility levels for these systems, even after 10 years of slash and burn. They also observed that pH and Al levels were lower in soils under AFS. However, K and P were in lower concentration in the AFS than in the other areas, due to the export of these elements by the fruit species present in the systems, which need these nutrients in greater quantities, such as cupuaçu and açaí.

The pH values and higher levels of Ca, Mg and K in the soils of agroforestry systems suggest correction of acidity with dolomitic liming and fertilization with K and P. Para Silva *et al*. (2007), high values of pH and P can negatively influence the colonization of roots by some AMF species, but at the same time, select AMF species adapted to these conditions. As shown in Table 2, higher levels of organic matter (OM) were also observed in the AFSs. Similar data were obtained by Loss *et al*. (2009), who observed higher levels in agroforestry systems than in pastures. On the other hand, the soils in the REMAN area showed low levels of Ca and Mg, results similar to those obtained by Aguiar *et al*. (2013).

Tabela 1. Plant species evaluated for Arbuscular Mycorhizae Fungi (FMA's) root colonization in different managenment systems in the Amazonia

AFS 1 S 03° 14.906' e W 060° 08.848'	**AFS 2** S 03° 13.559' e W 060° 07.457'	**AFS 3** S 03° 12.148' e W 060 ° 08.339'	**REMAN** S 03° 08. 391' e W 059° 57.109'
Banana (*Musa* spp.)	Cupuaçu (*Theobroma grandiflorum*)	Cupuaçu (*Theobroma grandiflorum*)	Vismia (*Vismia guianensis* Aubl. Choisy)
Orange (*Citrus sinensis* L.)	Assai (*Euterpe precatoria)*	Orange (*Citrus sinensis* L.)	Bignoniaceae
Assai (*Euterpe oleraceae)*	Guava (*Psidium guajava* L.)	Palm tree (*Bactris gasipaes)*	Mess apple (*Bellucia grossularioides* L.)
Cupuaçu (*Theobroma grandiflorum)*	Crabwood (*Carapa guianensis)*	Guava (*Psidium guajava* L.)	Mimosa *(Mimosa spruceanum* Mart. Ex-Benth)
Blackberry (*Morus nigra)*	Lemon (*Citrus limon)*	Assai (*Euterpe precatoria)*	Kikuyu (*Brachiaria humidicula)*

Tabela 2. Chemical characteristics of the soils collected from three Agroforestry Systems (AFS's), and from a deforested area of Refinery of Manaus (REMAN)

	pH	**Al^{3+}**	**Ca^{2+}**	**Mg^{2+}**	**K^{+}**	P	**C**	**MO**	**N**	**Soma de bases (SB)**	**CTC efetiva**
Identification	H_2O	................$cmol_c\ kg^{-1}$..............				$mg\ kg^{-1}$	.............$g\ kg^{-1}$			$cmol_c\ kg^{-1}$	$cmol_c\ kg^{-1}$
REMAN											
Vismia	4.8 L	0.45 L	0.37 L	0.09 L	0.05 L	15.0 H	6.0	10.4	0.30	0.51	0.96
Bignoniacea	4.8 L	0.05 L	0.24 L	0.06 L	0.07 L	1.8 L	7.5	13.0	0.38	0.37	0.42
Mess apple	4.6 L	0.95 M	0.31 L	0.09 L	0.08 L	3.8 M	12.1	20.9	0.61	0.48	1.43
Mimosa	5.1 L	0.50 M	0.83 M	0.13 L	0.06 L	2.3 L	12.1	20.9	0.61	1.02	1.52
Kikuyu	6.1 M	0.00 L	2.57 M	0.16 L	0.05 L	3.2 M	9.1	15.6	0.46	2.78	2.78
AFSs											
AFS1	5.9 M	0.00 L	6.42 H	0.81 H	0.12 L	103.2 H	15.2	26.1	0.76	7.35	7.35
AFS2	4.9 L	0.85 M	2.57 M	0.53 M	0.05 L	12.6 H	21.3	36.6	1.07	3.15	4.00
AFS3	4.9 L	0.40 L	2.52 M	0.60 M	0.15 M	79.4 H	18.2	31.3	0.91	3.27	3.67

Classification as Cochrane *et al.* (1985) criteria: H= High; L = Low and M= Medium.

Tabela 3. Root colonization by Arbuscular Mycorhizae Fungi (FMA's) of different plant species presentes in Agroforestry Systems and in the Refinery of Manaus (REMAN), Amazonas

Areas evaluated	**Species**	**Hyphae**	**Vesicles**	**Arbuscules**
		------------------------%	------------------	
AFS 1	Banana	98,0 a	63,2 a	22,3 b
	Orange	70,0 b	17,2 c	21,8 b
	Assai	86,7 a	47,8 a	33,1 b
	Cupuaçu	85,1 a	43,3 b	5,5 c
	Blackberry	90,0 a	51,8 a	22,3b
	AAverages	85,9	44,6	21,0
AFS 2	Cupuaçu	74,6 b	46,4 a	12,0 c
	Assai	84,2 a	57,2 a	14,5 c
	Guava	85,0 a	28,9 b	14,1 c
	Crabwood	73,1 b	11,8 c	1,6 c
	Lemon	72,6 b	8,4 c	0,0
	Average	77,9	30,5	8,4
AFS 3	Cupuaçu	84,0 a	6,6 c	0,0
	Orange	88,0 a	5,8 c	0,0
	Peach palm	94,4 a	30,4 b	0,0
	Guava	63,1 b	7,4 c	0,0
	Assai	75,5 b	10,9 c	0,0
	Average	81,0	12,2	0,0
REMAN	Vismia	73,7 b	32,8 b	5,5 c
	Bignoniaceae	78,0 b	38,6 b	20,3 b
	Guava	90,2 a	39,3 b	33,4 b
	Mimosa	89,6 a	42,8 b	50,1 a
	Kikuyu	93,5 a	63,8 a	57,4 a
	Average of REMAN	85	43,46	33,34

Médias seguidas pela mesma letra nas colunas não diferem entre si pelo teste Skott-knott ao nível de 5% de probabilidade.

Root colonization by arbuscular mycorrhizal fungi: The results indicate that the means of root colonization by hyphae ranged from 77.9 to 85.9% in the studied areas (Table 3). The most colonized roots were in the AFS 1 area with 85.9%, followed by the area of REMAN with 85%, AFS 3 with 81.0% and AFS 2 with 77.9%. The means of colonization by vesicles ranged from 12.2 to 44.6%, being higher in AFS 1 with 44.6%, followed by the REMAN area with 43.46%, AFS 2 with 30.5% and AFS 3 with 12.2%. For the arbuscules present in the plant cells of the roots of the host plants, the area that presented the highest average of colonization was that of REMAN with 33.34%, followed by AFS 1 with 21% and AFS 2 with 8.4%. In the AFS 3 area, arbuscules were not found. The high colonization values may be associated with the time of collection. Some authors have already documented higher values of mycorrhizal colonization in the rainy season in crops in the Amazon region, as for example, Oliveira *et al*. (2003) in three banana cultivars in a plantation in Manaus (AM) in the months of December, January and February, months of high rainfall in the region. A similar result was also observed by Oliveira and Oliveira (2004) in cupuaçu and guarana trees in an Agroforestry System in the Amazon. These authors also observed that, in addition to the season, mycorrhizal colonization and sporulation were also influenced by the host species, soil moisture and soil chemistry. These factors, which are also possibly influencing the high values of mycorrhizal colonization in this research. Oliveira and Oliveira (2010) and Souza *et al*. (2013) also evaluated the influence of soil-climatic factors on sporulation and colonization by arbuscular mycorrhizal fungi in native Amazonian species, finding greater mycorrhizal colonization in the rainy season. According to Oliveira and Oliveira (2004), greater colonization in this season is related to the emission of new fine roots in the root system of the host plant species. High colonization values were also found in the REMAN area. In addition to the factors already mentioned, kikuyu, a grass, could be influencing the high colonization values in this area. According to Miranda *et al*. (2005), legumes and grasses are highly mycotrophic, which could be contributing to greater colonization of this species. Another important factor to be considered is the concentration of phosphorus in the area (Table 1). According to Moreira and Siqueira (2006), the inhibition of mycorrhizal symbiosis starts with P concentrations above 50 mg kg^{-1} in the soil, which did not occur in this work and could be contributing to a greater dependence on the association. When individually analyzing the presence of hyphae in each plant species, it was observed that colonization varied from a minimum of 63.1% in the roots of guava trees in AFS 3, to a maximum of 98.0% in the roots of banana trees in AFS 1. This fact may indicate that these high incidences of vesicular-arbuscular mycorrhizae in plants may, in some way, be contributing to a better use of water and nutrients in the soil by these plant species, as already proven by other studies carried out in the region (Oliveira *et al*. 2003; Oliveira and Oliveira, 2003, 2004, 2005).

Banana was the species with the highest abundance of hyphae, with 98% in AFS 1, followed by peach palm with 94.4% in AFS 3; however, there was no statistically significant difference between these species. Some authors evaluated the mycorrhizal association in the banana root system. Jeffwa *et al*. (2012) studied several cropping systems with banana trees in Kenya, and observed root colonization by AMF, around 36.8 to 56.9%. In the Amazon region, similar results were obtained by Oliveira *et al*. (2003) which evaluated mycorrhizal colonization in three banana cultivars (apple, pacovan and silver), observing colonization from 40.3 to 75.3%. A similar result was obtained by Oliveira and Oliveira (2005), who found mycorrhizal colonizations ranging from 44.7% to 54.9%, both lower than the results obtained in this work. According to Aguiar *et al*. (2013) the banana tree is a highly mycotrophic species, with high mycorrhizal dependence, justifying the high colonization values obtained. In peach palms collected in AFS 3, colonization was superior to the records made in the literature. Silva Junior and Cardoso (2006) found lower results than those observed in this work. These authors evaluated the mycorrhizal colonization in peach palm and cupuaçu in two management systems (monoculture and AFS's) and concluded that the mycorrhizal root colonization in these species was affected by the cropping system and the season of the year, and that peach palm showed greater colonization in the monoculture during the dry season. Guava present in AFS 3 showed less colonization by hyphae (63.1%) followed by orange in AFS 1 with 70% and lemon with 72.6% in AFS 2, not differing statistically from each other. The fact that the guava tree presented lower colonization than the other species may be related to its root system. According to Fracaro and Pereira (2004), this species has highly lignified and thick roots. These authors concluded that from a total of 16.82 kg of roots collected in each volume of soil, 14.20 kg were thick roots and 2.62 kg were fine roots, corresponding respectively to 84.39% of thick and 15.61% of fine roots. The presence of vesicles ranged from 5.8% (AFS 3 orange) to 63.8% (REMAN kikuyu) and arbuscules from 1.6% (AFS 2 crabwood) to 57.4% (REMAN kikuyu). Vesicle colonization was significantly higher in banana trees present in AFS 1, followed by Amazonian kikuyu in REMAN and assai in AFS 2 with 63.2%, 63.8% and 57.2% respectively, with no difference between them. Lower percentages of vesicles were observed in orange (5.8%), cupuaçu (6.6%) and guava (7.4%) in AFS 3. As for the presence of arbuscules, the highest values were observed in the REMAN area in the kikuyu species (57.4%), mimosa (50.1%) and guava (33.4%). Crabwood and seal showed the lowest values, with 1.6% and 5.5% respectively. In AFS 3, the presence of this structure was not observed. Arbuscules are branched structures that are established inside the host cells, being considered the main point of nutrient exchange between the fungus and the plant. It can be inferred that for those species that present colonization by arbuscules, the exchange of nutrients is more efficient. Still in Table 3, it is observed that the species collected in more than one area showed different colonization values. The orange trees, collected in AFS's 1 and 3, showed significantly higher colonization of hyphae in AFS 3 with 88%, but there was no difference in the presence of vesicles between the collected areas, while arbuscules were only present in samples from AFS 1. of citrus is highly dependent on the mycorrhizal association (Ortas *et al*., 2002). In a study carried out by Nunes *et al*. (2006) to evaluate mycorrhizal colonization in different rootstocks in the municipality of Cruz das Almas (BA), a high percentage of mycorrhizal colonization was observed, ranging from 42% to 83% in the dry season and from 58% to 83% in the dry and rainy seasons.

The same authors also report that mycorrhizal colonization remained high in areas with high (100 mg kg-1 of P) and low (5 mg kg-1 of P) phosphorus levels, ranging from 34% to 82% and 55 % to 77% respectively. A similar fact was observed in this work, indicating that this species, even being in areas with high phosphorus content (Table 1), presents high values of mycorrhizal colonization. In the açaí trees collected in the three AFS's, there was no difference in colonization by hyphae and vesicles between the areas of the AFS 1 and 2. In the AFS 3, however, there were lower colonization values and the presence of arbuscules was not observed. Medina *et al*. (2012) observed an abundant presence of hyphae, arbuscules and spores, above 70% in Euterpe edulis in Minas Gerais. The cupuaçuzeiro, present in the three AFS's, presented the highest percentage of hyphae in the AFS 1; however, it did not differ from AFS 3, only from AFS 2. For vesicles, there was a significant difference between the three AFS, being more representative in AFS 2. As for the arbuscules, there was no difference between AFS's 1 and 2 and this structure was not observed in AFS 3. It can be inferred that the differences in colonization found in this study, for the species collected in the AFS's and in the REMAN area, may be associated with the management system adopted in each area.

CONCLUSION

Higher values of pH and Ca and Mg contents in the soils of some areas suggest that there was application of dolomitic limestone in the three AFS's and, in the REMAN sites with chameleon's tail, kikuyu. Root colonizations with fungal hyphae were high in all sampled areas and were influenced by the interaction between management system and host species present in each area.

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