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Occurrence of spores of arbuscular mycorrhizal fungi in Agroforestry Systems and at the Manaus refinery, Amazonas State

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***Abstract*—** *The Amazonian soils are characterized by being weathered and presenting low natural fertility and high acidity. With these conditions, the cultivation of species of economic interest becomes a challenge. Arbuscular Mycorrhizal Fungi (AMF) form symbiotic associations with the roots of most vascular plants, aiding in the absorption of water and nutrients. The objective of this work was to evaluate the occurrence of spores of these fungi in the soils of three agroforestry systems and in an altered area of the Oil Refinery of Manaus Isaac Sabbá-REMAN. Samples were collected from rhizosphere soils of 21 host species. Per area, five specimens of each species with five replications were sampled. The spore density was determined using 30 g of rhizospheric soil from each plant following the wet sieving extraction technique and counting with the aid of a magnifying glass. Chemical attributes for soil macronutrients were evaluated. High concentrations of spores of arbuscular mycorrhizal fungi found in soils indicate that they are not a factor that can influence root colonization. Spores' numbers in the rhizosphere soils of the studied host species ranged from 248 to 687 in 30 g of soil and were influenced by management systems. The number of spores was significantly higher in AFS 3 than in AFS 1, AFS 2 and in the REMAN area. The soil under peach palm trees in AFS 3 showed the highest numbers of spores. Mycorrhizal-arbuscular fungi of the genus Glomus predominated in the study areas, followed by Acaulospora and Gigaspora.*

I. INTRODUCTION

The soils of Terra Firme (upland) in the Amazon are acidic and very poor in nutrients, making the sustainability of productive systems with plant species of economic importance difficult. In this situation, a strategy to minimize the nutritional needs of plants is to create conditions to stimulate their associations with beneficial soil microorganisms.

Among these microorganisms, emphasis is given to Arbuscular Mycorrhizal Fungi (AMF's), which form a mutualistic symbiotic association with the roots of host plants, called arbuscular mycorrhiza. Several works have shown the occurrence of this fungi-plant association in the Amazon [1,2,3,4], known worldwide for providing improvements in the absorption of water and nutrients, especially those with little 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 stress and tolerance of plants to environments contaminated with

heavy metals. These benefits come from 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 reestablishment of vegetation in degraded áreas [5,6]. 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 [1,2,3,4,5, 6,7,8,9]. To occur, the arbuscular mycorrhizal fungi association must colonize the soil. Therefore, evaluating the presence of spores fungi in the soil is a factor to be considered in research.

The objective of this research was to evaluate the occurrence of spores of Arbuscular Mycorrhizal Fungi (AMF's) in the soils of three agroforestry systems and in an area of the Oil Refinery of Manaus Isaac Sabbá-REMAN.

II. METHODOLOGY

Area's description

The study was carried out in the State of Amazonas, Brazil, in four management systems, three of which are characterized as Agroforestry Systems with fruit species and forest essences on rural properties in Amazonas 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. A summary of each SAF is presented below:

SAF 1- Information on the history of use and fertilization of the area was not provided. The property has an area of 120m in front by 60m in depth, with the cultivation part destined to fruit species, with emphasis on orange and papaya trees, maintained with exposed soil, through weeding, without any cover.

SAF 2- Has an area of 92 m in front by 180 m in depth. 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- 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 cupuassu and palm trees, such as assai and peach palm were inserted. This area is maintained with grass coverage.

The Manaus Refinery area is located at geographic coordinates 03° 08.390' S and 059° 57.466' W and has low vegetation with regional species, introduced more than 10 years before the collections of the present study, for the recovery of 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 soil samples

Soils were collected from the rhizospheres of each species component of the AFS's and the REMAN area in April 2014. A completely randomized design was adopted with five replications in a 4x5 factorial scheme, with four factors referring to the soil systems. management, and five the factors referring to the forest species collected in each system. Samples of rhizosphere soils were obtained from 20 species identified in Table 1.

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

AFS 1	**AFS 2**	**AFS 3**	**REMAN**
S 03° 14.906' and W 060° 08.848'	S 03° 13.559' and W 060° 07.457'	S 03° 12.148' and W 060 ° 08.339'	S 03° 08. 391' and W 059° 57.109'
Banana (*Musa* spp.)	Cupuassu (*Theobroma grandiflorum)*	Cupuassu (*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.)
Cupuassu (*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)*

For each species, five replicates (plant) were performed and for each replicate, a sample was formed consisting of five subsamples, totaling 25 samples per management system. To quantify the density of arbuscular mycorrhizal fungi (AMF) spores, 30 g of soil collected from the rhizosphere of plants in a layer 10 cm deep were used.

The samples were stored in duly identified plastic bags, which were taken to the Laboratory of Ecology and Biotechnology of Microorganisms in the Amazon (LEBMAM) of the National Institute for Research in the Amazon (INPA). The samples remained in a refrigerator at 4 °C until the evaluations.

The soil samples were subjected to chemical analysis [10] at the Thematic Laboratory of Soil and Plant Analysis of the National Institute for Research in the Amazon (INPA) after being air-dried, homogenized, and passed through a 2 mm sieve, obtaining fine air-dried soil. The results of this analysis are shown in Table 2.

Table 2. Soil chemical characteristics of three Agroforestry Systems (SAF's), located in the municipality of Iranduba and of a component species in a degraded area of the Refinaria de Manaus Isaac Sabbá (REMAN), Amazonas.

	pH	Al $^{3+}$	Ca^{2+}	Mg $^{2+}$	K $^{+}$	P	C	MO	N	Bases Sat. (BS)	CEC efective
						mg kg^{-1}					
Identificação	H_2O	..... cmol$_c$ kg^{-1}					.....g kg^{-1}			cmol$_c$ kg^{-1}	cmol$_c$ kg^{-1}
REMAN											
Vismia sp.	4.8	0.45	0.37	0.09	0.05	15.0	6.0	10.4	0.30	0.51	0.96
Bignoniaceae	4.8	0.05	0.24	0.06	0.07	1.8	7.5	13.0	0.38	0.37	0.42
Mess apple	4.6	0.95	0.31	0.09	0.08	3.8	12.1	20.9	0.61	0.48	1.43
Mimosa	5.1	0.50	0.83	0.13	0.06	2.3	12.1	20.9	0.61	1.02	1.52
Kikuyu	6.1	0.00	2.57	0.16	0.05	3.2	9.1	15.6	0.46	2.78	2.78
SAFs											
SAF1	5.9	0.00	6.42	0.81	0.12	103.2	15.2	26.1	0.76	7.35	7.35
SAF2	4.9	0.85	2.57	0.53	0.05	12.6	21.3	36.6	1.07	3.15	4.00
SAF3	4.9	0.40	2.52	0.60	0.15	79.4	18.2	31.3	0.91	3.27	3.67

Extraction and quantification of spores

AMF spore density was determined using 30 g of soil from the rhizosphere of each plant using the wet sieving extraction technique [11], using 0.250 mm and 0.045 mm aperture sieves, followed by decantation in sucrose 40 % [12]. The spore count was performed with the aid of a stereoscopic microscope (magnifying glass), with magnification of 1.5 and 4x.

Statistical analysis of data

Data were analyzed using the F test, and when significant, the treatment means were compared using the Skotty-Knott test at a 5% probability level. For data analysis, the program Assistat version 7.7 Beta was used.

III. RESULTS AND DISCUSSION

Number of spores

Spores' numbers in the rhizosphere soils of the studied host species ranged from 248 to 687 in 30 g of soil and were influenced by management systems. The number of spores was significantly higher in AFS 3 ($p>0.05$) than in AFS 1 (401), AFS 2 (248) and in the REMAN area (393), which did not show statistical difference between them (Figure 1).

These densities indicate that there were between 8.3 and 22.9 spores per gram of soil, numbers that are sufficient to provide contact and colonization of plant roots, as shown in another complementary work to this one [13].

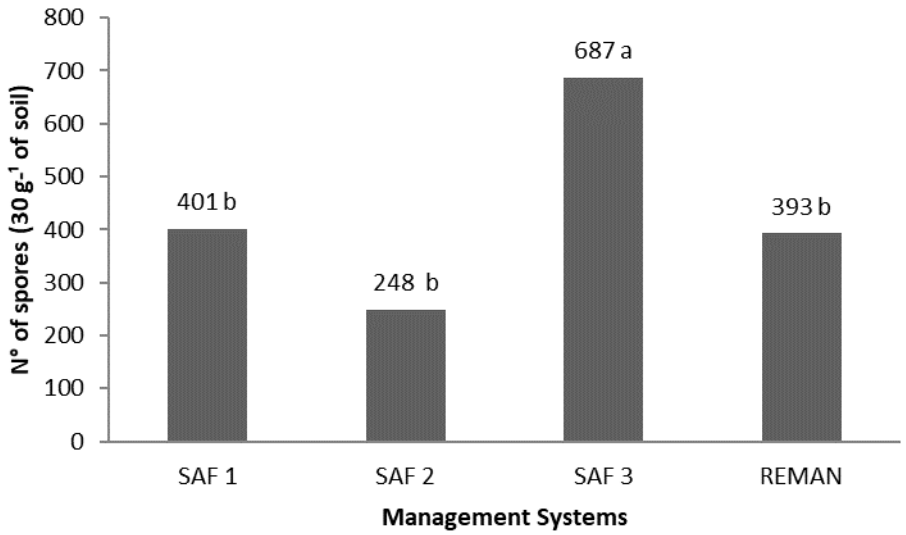

Fig.1. Number of mycorrhizal spores in three Agroforestry Systems and in a degraded area of Refinaria de Manaus-AM, Isaac Sabbá (REMAN).

There are several studies reporting variations in AMF spore densities because of the presence of host plant species. Souza et al. [14] evaluated the diversity of AMF spores in an agroforestry system, rubber plantation, pasture and primary forest and observed a higher density of spores in the pasture. Similar data were also obtained by Aguiar et al. [15], who observed a greater number of mycorrhizal spores, not only in an area with grasses, but also in an area under banana cultivation, both higher than the degraded area. Loss et al. [16] evaluated the density of mycorrhizal spores in agroforestry systems and pastures and obtained higher values in the agroforestry system. The same was observed by Silva Júnior et al. [17], when evaluating colonization by AMF in cupuassu and peach palm in AFS and monoculture, which showed a greater number of spores in agroforestry systems.

Several factors influence the presence of mycorrhizal spores in the soil. According to Moreira and Siqueira [18], Oliveira and Oliveira [19], management, as well as soil cover, can contribute to reducing the number of AMF spores. The very physiology of the plant, due to the production of root exudates, are important factors that favor or not mycorrhizal sporulation. According to Miranda et al. [20], knowledge of the diversity and dynamics of mycorrhizal fungi is important for the management of degraded soils, being important indicators of soil quality. According to Carrenho et al. [21], in agrosystems, the main factors that influence AMF's are agricultural practices, such as soil preparation, crop management and cultural practices.

In Figure 2, it can be seen more clearly that the distribution of AMF spores varied according to the host species. The species that presented the highest number of spores in 30g of soil are present in the AFS 3 area, with emphasis on peach palm (979), orange tree (805) and guava tree (752), which were significantly superior to the others; however, they did not differ from each other, and were statistically equal to the lemon, present in AFS 2. It should also be noted that in soils influenced by host species present in more than one system, differences in sporulation were observed, as is the case from guava and açai soils, which in AFS 2 contained low numbers of spores (80) and (17) respectively, and in AFS 3 showed high values, suggesting that not only the species, but also the management of the area influenced mycorrhizal sporulation.

Studies carried out by some authors have already verified the effect of crops and soil management on mycorrhizal sporulation. Angelini et al. [22] evaluated mycorrhizal colonization, spore density and diversity of AMF's in the rhizosphere of corn and soybean grown under no-tillage with different cover crops and compared it to an area of conventional tillage and fallow in Uberaba, MG. They observed a significant effect of cultures on mycorrhizal colonization and sporulation. A fact also observed by Santos et al. [23] evaluating different forest cover in Vitória da Conquista, BA.

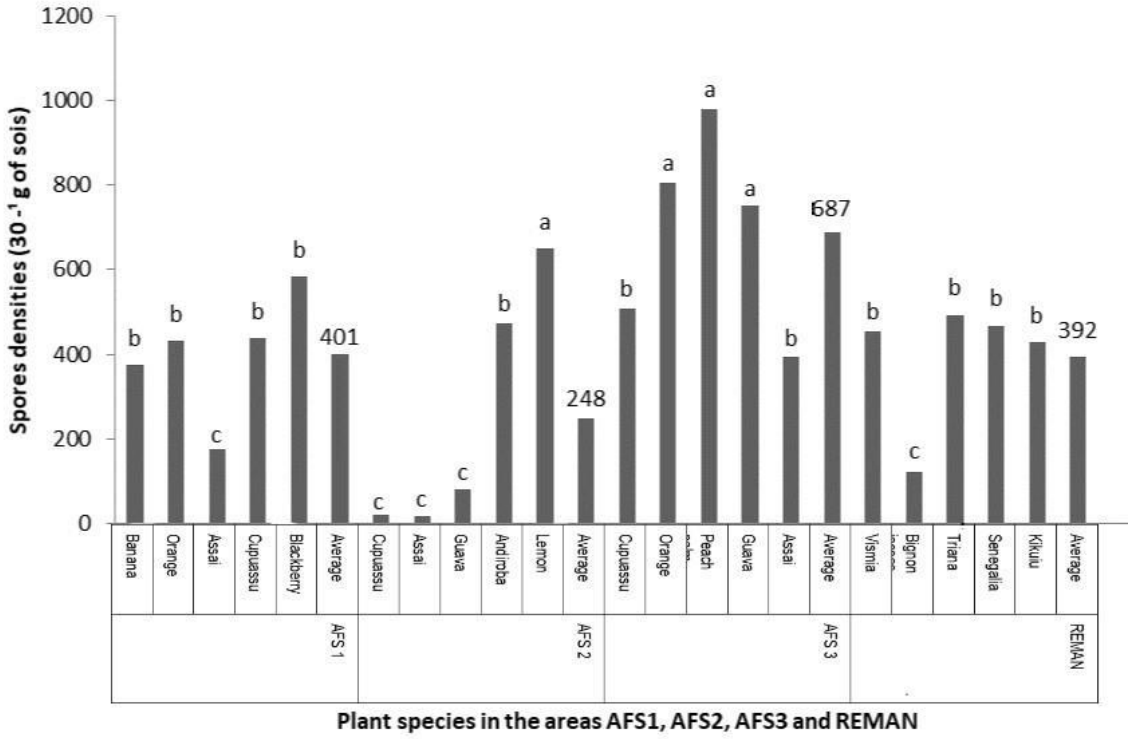

Fig.2. Density of spores per host species collected in the three Agroforestry Systems and in a degraded area of the Manaus Refinery, Isaac Sabbá (REMAN), in April 2014.

According to Moreira and Siqueira [18], Oliveira and Oliveira [2,3,4], Oliveira et al. [8], Carrenho et al. [24], plant species exhibit different susceptibilities to colonization, showing inter and intraspecific variations, and influencing the diversity and abundance of AMFs. Carrenho et al. [24] studied the effect of peanut (*Arachis hypogaea* L.), sorghum (*Sorghum bicolor* (L.) Moench) and maize (*Zea mays* L.) on the development and diversity of arbuscular mycorrhizal fungi (AMF). They found that peanuts were the species that showed the highest values of mycorrhizal colonization and sporulation.

Figure 3 shows the distribution of genders observed in the study areas. The presence of the genera *Acaulospora*, *Gigaspora* and *Glomus* were observed. The latter showed the highest density of spores in all evaluated areas.

According to Silva-Júnior [17], this genus is widely distributed in tropical zones, justifying its greater occurrence in the study areas. Some studies have already verified the predominance of these genera in the Amazon in different cultivation systems.

Correa et al. [25] evaluated cupuassu monoculture systems, SAF's, burnt capoeira, slash and burn in regeneration in the state of Pará, and observed the predominance of these genera. Occurrence of these genera was also recorded by Souza et al. [14] in areas under different systems of use, in the municipality of Rolim de Moura–RO. They registered a predominance of *Glomus macrocarpum* and *G. etunicatum* that presented higher spore density in soil under pasture cultivation system. Leal et al. [26] evaluated the occurrence of arbuscular mycorrhizal fungi (AMF) species in soil samples from the Amazon region under different land use systems using trap culture methods, and observed greater abundance of the genera *Acaulospora*, *Entrophosfora* and *Glomus* with higher spore density in young capoeira soil and pasture.

Other authors also observed this pattern of genres present in different regions. Loss et al. [16] evaluated the community of arbuscular mycorrhizae in pasture and agroforestry areas in the municipality of Seropédica-RJ and found a predominance of the genera *Glomus* and *Acaulospora*. The same reports were obtained by Zhao et al. [27] in forests in China, Mangan et al. [28] in Panama, Zangaro et al. [29] in Brazil, Sturmer and Siqueira [30] in the Amazon.

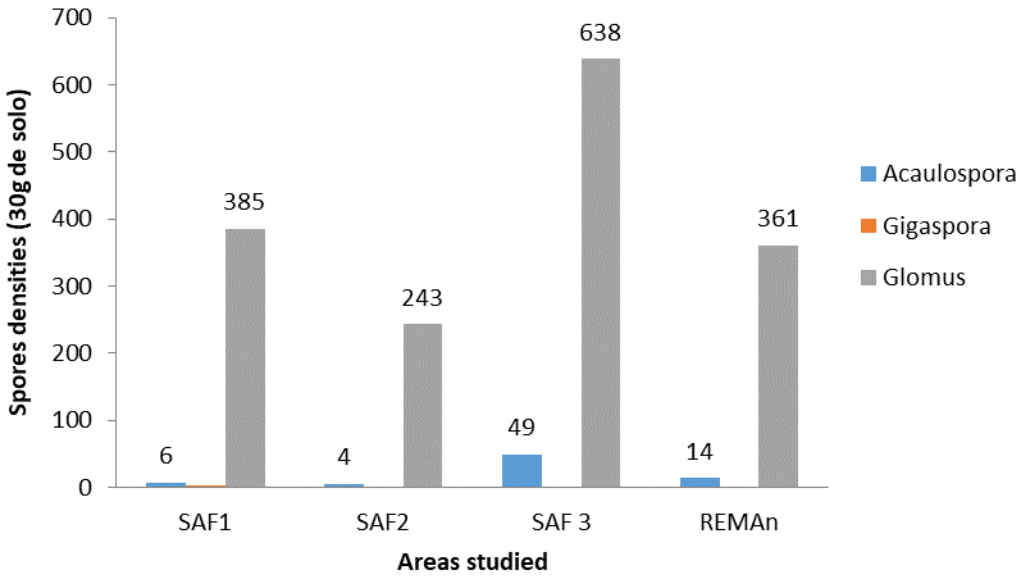

Fig.3. Genus of Arbuscular Mycorrhizal Fungi present in the SAF's areas and in the REMAN area.

IV. CONCLUSIONS

Spores' numbers in the rhizosphere soils of the studied host species ranged from 248 to 687 in 30 g of soil and were influenced by management systems.

The number of spores was significantly higher in AFS 3 than in AFS 1, AFS 2 and in the REMAN area.

The soil under peach palm trees in AFS 3 showed the highest numbers of spores.

Mycorrhizal-arbuscular fungi of the genus Glomus predominated in the study areas, followed by *Acaulospora* and *Gigaspora*.

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