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INTERFACES CIENTÍFICAS

ASPECTS OF MYCORRHIZAL ASSOCIATION WITH THREE PLANT SPECIES IN AGROFORESTRY SYSTEMS IN THE AMAZON

ASPECTOS DA ASSOCIAÇÃO MICORRÍZICA COM TRÊS ESPÉCIES VEGETAIS EM SISTEMAS AGROFLORESTAIS NA AMAZÔNIA

ASPECTOS DE LA ASOCIACIÓN MICORRÍZICA CON TRES ESPECIES VEGETALES EN SISTEMAS AGROFORESTALES EN LA AMAZONÍA

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ABSTRACT

Agroforestry Systems (Portuguese acronym, SAF) encompass diverse plant species coexisting within the same space, influenced by factors such as fertilization and water availability, shaping the occurrence and diversity of soil microorganisms, including Arbuscular Mycorrhizal Fungi (AMF). This study aimed to assess the diversity, colonization, and density of AMF in the roots of plant species from two SAF areas: one subjected to green manure, biofertilizers, compost fertilizer, limestone application, and artificial irrigation (SAF-1), and the other without such interventions (SAF-2). Both SAFs harbored the species Mariri (*Banisteriopsis caapi*), Castanheira (*Bertholletia excelsa*), and Tapiririca (*Tapiririca guianensis*). Samples of rhizospheric soil and roots were collected for physical-chemical analysis, isolation, and identification of AMF, as well as quantification of spore density and percentage of colonization. Significant differences were observed in organic matter content, being high in SAF-1 and medium in SAF-2, while phosphorus content was high in SAF-1 and low in SAF-2. The occurrence of four distinct orders of AMF, Archaeosporales, Glomerales, Diversisporales, and Gigasporales, was noted, albeit with varying richness. Spore density was significantly higher in SAF-1 with intervention, attributed to elevated phosphorus and organic matter content. Conversely, mycorrhizal colonization was greater in SAF-2 without intervention, owing to phosphorus deficiency favoring colonization formation.

KEYWORDS

Mycorrhizal fungi; Spore density; colonization.

RESUMO

Os Sistemas Agroflorestais (SAF) são configurados por diferentes espécies vegetais no mesmo espaço. Essa formação, atrelada a outros fatores como adubação e disponibilidade de água influência a ocorrência e diversidade dos microrganismos do solo, como os Fungos Micorrízicos Arbusculares (FMA) mudando a dinâmica da riqueza e densidade de esporos. Dessa forma, objetivo desse estudo foi avaliar a diversidade, colonização e densidade de FMA nas raízes de espécies vegetais de duas áreas de SAFs, uma com adubação verde, biofertilizantes, adubo de composteira, calcário na cova e irrigação artificial (SAF-1) e a outra sem adubação e irrigação (SAF-2). Em ambos os SAFs são encontrados as espécies Mariri (*Banisteriopsis caapi* (Spruce ex Griseb) Morton), Castanheira (*Bertholletia excelsa* H.B.K) e Tapiririca (*Tapirica guianensis* Aubl), dessas foram coletadas amostras de solo rizosférico e raízes para análise físico-química, isolamento e identificação, assim como quantificar a densidade e a porcentagem de colonização das espécies de FMA encontradas. Ambos os SAF apresentaram diferenças significativas no teor de matéria orgânica, sendo alta no SAF-1 e médio no SAF-2, já o teor de P, foi alto no SAF-1 e baixo no SAF-2, e as demais características semelhantes. Em virtude disso, observou-se ocorrência de quarto ordens distintas de FM, Archaeosporales, Glomerales, Diversisporales e Gigasporales, porém em riqueza diferente. A densidade de esporos foi significativamente maior em SAF-1 com intervenção, devido ao alto teor de fósforo e matéria orgânica. A colonização micorrízica foi maior em SAF sem intervenção devido à deficiência de fósforo favorecer a formação da colonização.

PALAVRAS-CHAVE

Fungos micorrízicos; Densidade de esporos; Colonização.

RESUMEN

Los Sistemas Agroforestales (SAF) abarcan diversas especies de plantas que coexisten en un mismo espacio, influenciadas por factores como la fertilización y la disponibilidad de agua, configurando la ocurrencia y diversidad de los microorganismos del suelo, incluidos los Hongos Micorrízicos Arbusculares (HMA). Este estudio tuvo como objetivo evaluar la diversidad, colonización y densidad de HMA en las raíces de especies vegetales de dos zonas SAF: una sometida a abono verde, biofertilizantes, fertilizante compost, aplicación de caliza y riego artificial (SAF-1), y otra sin tales intervenciones (SAF-2). Ambos SAF albergaron las especies Mariri (*Banisteriopsis caapi*), Castanheira (*Bertholletia excelsa*) y Tapiririca (*Tapiririca guianensis*). Se recolectaron muestras de suelo rizosférico y raíces para análisis físico-químico, aislamiento e identificación de HMA, así como cuantificación de densidad de esporas y porcentaje de colonización. Se observaron diferencias significativas en el contenido de materia orgánica, siendo alto en SAF-1 y medio en SAF-2, mientras que el contenido de fósforo fue alto en SAF-1 y bajo en SAF-2. Se observó la presencia de cuatro órdenes distintos de HMA: Archaeosporales, Glomerales, Diversisporales y Gigasporales, aunque con riqueza variable. La densidad de esporas fue significativamente mayor en SAF-1 con la intervención, atribuida al elevado contenido de fósforo y materia orgánica. Por el contrario, la colonización de micorrizas fue mayor en SAF-2 sin intervención, debido a que la deficiencia de fósforo favoreció la formación de colonización.

PALABRAS CLAVE

Hongos micorrízicos; Densidad de esporas; Colonización

1 INTRODUCTION

Approximately 30% of the world´s soils suffer from some degree of degradation, resulting from poor agricultural land use, disorderly urban expansion, or other anthropogenic actions exploiting natural resources (EMBRAPA, 2016). This scenario necessitates the use of technologies that promote the recovery of environmental functionality, including soil. In the Brazilian Amazon, for example, Agroforestry Systems (known as SAF from their Portuguese acronym) stand out in this regard. These systems involve the use and management of natural resources by associating plant species with agricultural crops or animals, thus improving plant productivity through the enhancement of the biological, physical, and chemical properties of the soil (CAMARGO *et al*., 2013).

The use of Agroforestry Systems (SAF) as a management technique can favor environmental recovery, particularly benefiting soil microbiota like Arbuscular Mycorrhizal Fungi (AMF), which, in turn, support plant development (CORREIA *et al*., 2022). This symbiosis enhances the absorption and mobilization of nutrients and provides benefits to both plants and soil, making it a potential indicator of soil quality (ASSIS *et al*., 2014). However, soil characteristics such as water and nutrient content, particularly phosphorus levels, as well as the plant species present at the site, influence the effectiveness of mycorrhizal symbiosis (KIVLIN *et al*., 2011).

In SAF, soil fertilization can reduce AMF species richness compared to similar conditions without fertilization (DANTAS *et al*., 2015; PRATES *et al*., 2021). Therefore, it is understood that the management of SAF, particularly with forest species, can influence the diversity of AMF, as well as the number of spores and the extent of root colonization (OLIVEIRA *et al*., 2021; MAIA *et al*., 2023). Maia *et al*. (2023) and Oliveira *et al*. (2021) observed an increase in root colonization in forest species cultivated in SAF areas. However, soil fertilization in SAF can reduce species richness and spore density compared to SAF without fertilization, as noted by Dantas *et al*. (2015). Prates *et al*. (2021), studying the diversity of AMF, observed the lowest AMF richness in areas with fertilization.

In addition to the influence of cultural practices, the host plant species can also affect the AMF community (KIVLIN *et al*., 2011). Among the species with adaptive characteristics to the Amazon climate and cultivated in SAF are *Banisteriopsis caapi* (Spruce ex Griseb.) Morton, commonly known as Mariri; *Bertholletia excelsa* H.B.K, known as Castanha do Pará or Brazil nut; and *Tapirira guianensis* Aubl., known as Tapiririca.

Given the scarcity of scientific research on the interaction of AMF with the mentioned above plant species in SAF cultivation, this study aimed to: (i) evaluate the diversity and density of AMF spores in the rhizospheric soils of *Banisteriopsis caapi*, *Bertholletia excelsa*, and *Tapirira guianensis*, cultivated in two agroforestry system areas with different fertilization and irrigation interventions. (ii) assess root colonization by AMF and (iii) Comparison of agroforestry systems with and without intervention

2. MATERIAL AND METHODS

2.1 COLLECTION AREA

The SAF are located in the Eixo-Forte region in Santarém, in the western part of the state of Pará (coordinates: 02º28'68.37" S, 54º47'23.32" W). These SAF are managed by the Centro Espírita Beneficente União do Vegetal (CEBUDV), Núcleo Castelo de Marfim. The cultivation has been ongoing for approximately 20 years. The collections were conducted in two SAF areas: one with interventions such as green manure, biofertilizers, compost, limestone in pits, and irrigation (SAF-1), and the other without direct intervention in terms of fertilization and irrigation (SAF-2).

2.2 SOIL AND ROOT COLLECTION

The collection of rhizospheric soil and roots was conducted during the period of low rainfall intensity in the Amazon forest. Samples were collected from the three plant species in both SAF. In each SAF, nine composite samples of 1,000 grams of rhizospheric soil were collected, each composed of four sub-samples of 250 grams collected around each plant species, forming a square. The same methodology was used for root collection, with approximately 10 grams of roots collected per sub-sample, totaling 40 grams for each composite sample. The collected soil was analyzed for its physical-chemical properties by the Safrar Análises Agrícolas Laboratory, accredited by Embrapa Solo, and used for spore extraction. The soil was classified according to Sobral *et al*. (2015).

2.2.1 SPORE EXTRACTION AND AMF IDENTIFICATION

The extraction of AMF spores was carried out from 100 g of soil from the composite sample, which were dissociated using the wet sieving method with centrifugation (GERDEMANN; NICOLSON, 1963) in 50% sucrose (JENKINS, 1964). The spores extracted from the soil were transferred to Petri dishes (90 mm diameter) lined with a layer of absorbent paper and then quantified under a stereoscopic microscope. After this step, they were separated by morphotypes based on morphological characteristics and stored in 1.5 mL Eppendorf-type tubes containing 1 mL of distilled water. Slides were mounted with a solution of Polyvinyl Alcohol and Lactoglycerol (PVLG) and Melzer reagent in a 1:1 ratio. For each sample, 10 slides were prepared with 10 spores each. The slides were covered with a coverslip at a 45° angle, and after 48 hours, photographic recording was carried out using an optical microscope. Identification was performed using the descriptions provided on the Glomeromycota virtual data page (https://biologiademicorrizas.wixsite.com/glomeromycota)

2.2.2 MYCORRHIZAL COLONIZATION

To evaluate the percentage of mycorrhizal colonization (%C), 5 g of roots from each plant species were clarified in 10% KOH for 40 minutes and then stained with Trypan Blue (PHILLIPS; HAYMAN, 1970). The percentage of colonization was calculated by placing the roots in a Petri dish (90 mm in diameter) and examining them under a stereoscopic microscope using the quadrant intersection technique (GIOVANETTI; MOSSE, 1980).

2.2.3 DATA ANALYSIS

The analyses were carried out using the statistical program Sisvar® (FERREIRA, 2011) version 5.8.92. The normality of the data was verified by the Shapiro-Wilk test ($p > 0.05$), and the means were compared by the Tukey test at 5% significance.

3. RESULTS

SAF-1, which underwent fertilization and irrigation intervention, presented higher levels of nutrients P (phosphorus), K (potassium), Ca (calcium), and Mg (magnesium), in addition to organic matter (OM) when compared to SAF-2, without intervention, pH and aluminum (Al) remained similar between the SAF (Table 1). Based on the classification by Sobral *et al*. (2015), the rhizospheric soil of both SAF are acidic soils with a high level of aluminum (Al) saturation, low levels of potassium (K), calcium (Ca), and magnesium (Mg). In both SAF, the soil of the Mariri species had a high OM content, while the Castanheira and Tapiririca species had a medium content (Table 1).

Table 1 - Rhizospheric soil analysis in the area with fertilization and irrigation intervention (SAF-1) and area without fertilization and irrigation intervention (SAF-2).

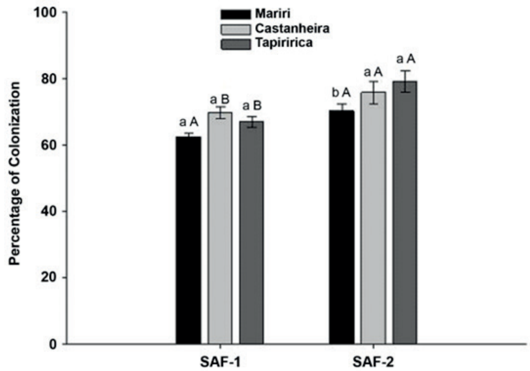
Cultivation		pH	pH	OM dag	P meh-¹	K	Ca	Mg	Al
		H_2O	$CaCl_2$	kg^{-1}	dm^{-3}		cmolc dm^{-3}		
SAF-1	Mariri	4.9	4.6	3.5	20.1	20	0.95	0.28	1.5
	Castanheira	5.3	4.8	2.8	20.2	20	0.84	0.27	1.3
	Tapiririca	5	4.6	2.9	15	33	0.48	0.18	1.5

Cultivation		pH	pH	OM dag	P meh-¹	K	Ca	Mg	Al
		H_2O	$CaCl_2$	kg-¹	dm-³		cmolc dm-³		
SAF-2	Mariri	5.3	4.9	3.2	7.9	19	0.66	0.17	1.6
	Castanheira	4.8	4.5	2.7	5.9	20	0.67	0.13	1.1
	Tapiririca	5.3	4.8	2.8	6.5	23	0.56	0.13	1.2

pH - hydrogen potential; OM - Organic Matter; P - phosphorus; K- potassium; Ca - calcium; Mg - magnesium; Al - aluminum.
Source: Research data

The percentage of colonization (%C) between SAF-1 species did not show significant differences ($p \leq 0.05$). In SAF-2, the Mariri species presented a %C approximately 10% lower in relation to the other species. Between the Castanheira and Tapiririca species, no significant difference was observed ($p \leq 0.05$) (Figure 1). On the other hand, when comparing the SAF, a higher %C was observed in SAF-2, where the Mariri and Tapiririca species presented higher %C by around 12% and 19%, respectively, when compared to the same species in SAF-1 (Figure 1).

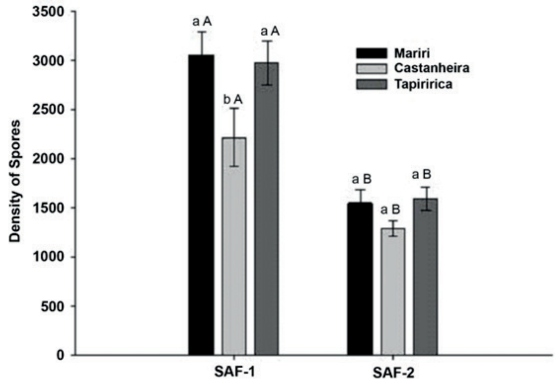
Figure 1 - Percentage of colonization (%C) of mycorrhizal fungi present in the substrate of Mariri, Castanheira and Tapiririca plants in two areas of the Agroforestry System (SAF-1 and SAF-2). The mean with the same lowercase letter within the SAF and the uppercase letter between the SAF do not differ statistically by the Tukey test ≤ 5%.



Source: Research data

Regarding spore density (SD) in SAF-1, Mariri and Tapiririca differed significantly from Castanheira, being on average 36% higher. In SAF-2, no significant difference was observed between the means ($p \leq 0.05$). However, when comparing the spore densities (SD) between SAFs, SAF-1 provided higher spore densities than SAF-2, being 97%, 72%, and 86% for Mariri, Castanheira, and Tapiririca, respectively (Figure 2).

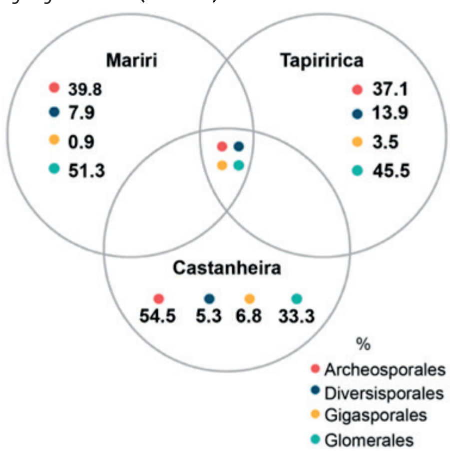
Figure 2 - Density of spores of arbuscular mycorrhizal fungi present in the substrate of Mariri, Castanheira and Tapiririca plants in two areas of the Agroforestry System (SAF-1 and SAF-2). The mean with the same lowercase letter within the SAF and the uppercase letter between the SAF do not differ statistically by the Tukey test ≤ 5%.



Source: Research data

In the SAF-1 area, the AMF orders Archaeosporales, Diversisporales, Gigasporales, and Glomerales were found in all plant species. Mariri and Tapiririca exhibited a higher number of spores belonging to the Glomerales order and a lower number of spores belonging to the Gigasporales order. Conversely, Castanheira showed a higher number of spores from the Archaeosporales order and a lower number from the Diversisporales order (Figure 3).

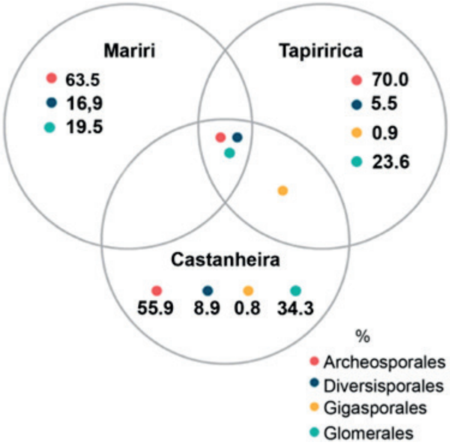
Figure 3 - Representation of the shared richness of AMF taxa in the rhizosphere of Mariri, Tapiririca and Castanheira of Agroforestry System 1 (SAF-1).



Source: Research data

In the SAF-2 area, the orders Archaeosporales, Glomerales, and Diversisporales were found in all three plant species, while the order Gigasporales was absent only in Mariri. Castanheira, Tapiririca, and Mariri exhibited a higher number of spores from the Archaeosporales order, whereas Castanheira and Tapiririca species had a lower number of spores from the Gigasporales order. In Mariri, the smallest number of spores was observed from the Diversisporales order (Figure 4).

Figure 4 - Representation of the exclusive and shared richness of AMF taxa in the rhizosphere of Mariri, Tapiririca and Castanheira of Agroforestry System 2 (SAF-2).



Source: Research data

4 DISCUSSION

The percentage of colonization (%C) and spore density (SD) behaved antagonistically in SAF, with a higher %C being observed in SAF-2, while a higher SD was observed in SAF-1. Recent research by Santos *et al.* (2020) and Junior *et al.* (2021) corroborates our findings, as there is no direct relationship in which a higher %C will provide greater SD.

Root colonization is characterized as a sensitive stage in the biological activity of AMF, influenced by factors such as the type of host, soil physical-chemical attributes (MAIA *et al.*, 2023), and the availability of phosphorus (P) and water (FALL *et al.*, 2023). In our study, the %C of AMF in SAF-1 did not differ significantly between species but was, on average, 13% lower than the %C observed in SAF-2. We attribute this result to the higher availability of P in the soil, as elevated P levels can decrease colonization (TREJO *et al.*, 2020). Similar findings were reported by Silva *et al.* (2021), who observed reduced AMF colonization in soils with higher P content. Conversely, the percentage of colonization in SAF-2 species was higher. We attribute this result to lower P availability, as plants under P limitation often produce signaling molecules in their roots, thereby stimulating symbiosis (NAGAHASHI; DOUDS JR., 2000).

The rhizospheric soil of Mariri in both SAF-1 and SAF-2, classified according to the scheme proposed by Sobral *et al*. (2015), exhibited a high organic matter (OM) content, with values ranging between 3.2 and 3.5 kg. This phenomenon can be attributed to the species' characteristics, as Mariri is a vine with a profusion of adventitious and fine roots, consequently leading to elevated OM levels (SALTON; TOMAZI, 2014). Despite the expectation of a higher percentage of colonization (%C) due to the abundance of fine roots compared to tree species like Tapiririca and Castanheira, this behavior was not observed. Ramana *et al*. (2023) proposed that root diameter correlates strongly with the composition of the arbuscular mycorrhizal fungal community, with plants possessing thicker roots exhibiting lower diversity of mycorrhizal fungi compared to those with thinner roots. However, our observations indicated that the %C of Mariri was lower compared to other species in both SAF-1 and SAF-2. Consequently, these results are attributed to the high levels of organic matter present in the rhizosphere. The OM content can act as a mediator in arbuscular mycorrhizal fungi (AMF) colonization, releasing excess nutrients into the soil and thereby minimizing the necessity for colonization (MOREIRA; SIQUEIRA, 2006; OLIVEIRA *et al*., 2013).

Conversely, SAF-1 exhibited higher average OM and nutritional contents, including phosphorus (P), thereby enabling greater spore production.

Sporulation can increase in the presence of P and organic matter (BEZERRA *et al*., 2022). Previous studies, such as Sale *et al*. (2015), observed this increase in sporulation in soils with high P and OM content, which corroborates the present work. It is important to note that this area included legumes, which, according to the study by Maia *et al*. (2023), acted as spore multipliers in in the soil. According to Miranda and Miranda (2003, 2007), spore density tends to progressively increase with plant cultivation combined with the application of fertilizers. Sporulation is a survival mechanism for AMF (MAIA *et al*., 2023), providing them with the ability to withstand soil disturbances (JAMIOŁKOWSKA *et al*., 2018), although it is not always correlated with colonization, since an excess of spores can lead to a decline in germination (MELLO *et al*., 2008).

However, we emphasize that in our study, mycorrhizal colonization is the process of greatest interest. Although the conditions in the agroforestry system (SAF) favor sporulation, colonization is the process that determines the success of mycorrhizal interactions in the soil, and consequently, the ecological and agronomic benefits of AMF. Although sporulation is necessary for the survival of AMF, there is no direct correlation between spore density and successful mycorrhizal colonization of the soil. The relationship between sporulation and colonization is complex and depends on several factors, such as nutrient availability, competition with other microorganisms, and soil dynamics. Future research is needed to further investigate these factors and how they affect mycorrhizal colonization.

The AMF community is primarily influenced by soil chemical and physical properties, such as soil pH, soil organic carbon, and available phosphorus (P), along with host species (SILVA *et al*., 2021). Representatives of the orders Archaeosporales, Glomerales, Diversisporales, and Gigasporales were found in both SAF. The acidic characteristics of the soil and high aluminum concentration, observed in both SAF, may favor the prevalence of certain orders, such as Glomerales (SÁNCHEZ-CASTRO *et al*., 2017). Conversely, acidic soils can reduce the abundance of AMF species with large spores, such as Gigaspora species (WANG *et al*., 1993), as observed in the present study, where this order had the lowest number of specimens.

Soils characterized by low organic matter content, high degrees of deterioration, and natural stress are often associated with a higher occurrence of AMF species from the order Gigasporales (STÜRMER *et al*., 2013; BERUDE *et al*., 2015). In the present study, the observed high and medium organic matter (OM) content in both SAF may have inhibited the establishment of this order. Additionally, environmental characteristics are known to influence the plant community and, consequently, the selection of different AMF species. The lower occurrence of the order Gigasporales in both AMF communities may also suggest local competition between AMF species (LEAL *et al*., 2013).

Furthermore, Donn *et al*. (2017) demonstrated that similar root types of *Brachypodium distachyon* were colonized by slightly more similar AMF communities than when comparing the AMF community on different roots. Thus, chemical and morphological traits of the root may play a role in the spatial structure of the AMF community (DEVEAUTOUR *et al*., 2021). In the Mariri root system, the orders Archaeosporales and Glomerales predominated in both SAF. Conversely, the order Gigasporales exhibited the lowest value in SAF-1, at 0.89%, and was non-existent in SAF-2 (Figure 4).

The orders Glomerales and Archaeosporales are considered generalists with a high capacity for sporulation and adaptation to agricultural soils (PALENZUELA *et al*., 2011; KIM *et al*., 2022). Dantas *et al*. (2015) indicated the remarkable ability of the order Glomerales to adapt to environments subjected to fertilization and cultivation practices. This same behavior was evident in the present study; the order Glomerales was present in greater numbers in two species, Mariri and Tapiririca, in SAF-1, which underwent fertilization, whereas this trend was not observed in SAF-2, which was not fertilized. Regarding the order Archaeosporales, Kim *et al*. (2022) observed a significant representation of this order in forest soil, consistent with the findings of the present study.

In SAF areas, the occurrence of the order Diversisporales has also been described. According to the literature, this group is among the most widely distributed in the world, with several species described in recent years (VIEIRA *et al*., 2020).

5 CONCLUSION

Intentional fertilization and irrigation resulted in the highest levels of organic matter and P in the soil of SAF-1, but did not influence other soil chemical variables. The observed physical-chemical characteristics in the soils did not affect the diversity of AMF, with the orders Archaeosporales, Glomerales, Diversisporales, and Gigasporales being found in both AMF communities. However, the order Gigasporales exhibited low presence or was absent in the Mariri root system.

Nevertheless, the cultivation conditions in the SAF contributed to higher spore density in SAF-1 and a higher percentage of mycorrhizal colonization in SAF-2, with these variables being inversely proportional. Our results indicate that fertilization and irrigation practices can promote AMF sporulation, but this does not result in greater colonization, which is essential to provide benefits to plants.

Therefore, in agroforestry systems, anthropogenic interventions can affect the soil microbiota and modify the dynamics of the symbiotic interaction between plants and AMF.

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