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Structure of AMF Community in an Agroforestry System of Coffee and Macauba Palm

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Abstract

Coffee crop in Brazil is typically grown as a monoculture. However, we hypothesized that agroforestry system is favorable association for arbuscular mycorrhizal fungi (AMF), affecting its community structure and potentially impacting crop productivity and agroecosystem health. This study evaluated how the microclimate, soil depth, macauba field spacing and distance between coffee plants and palms affect the structure of the AMF community. The structure of the AMF community was influenced by the soil depth, microclimate features, soil moisture, maximum air temperature, and photosynthetically active radiation (PAR). The distance at which coffee-macauba influences ecological diversity indices of AMF, and higher diversity are related to the proximity between plants. AMF diversity (Richness and Shannon) in the agroforestry system exceeded that observed in the full-sun coffee in the 0-20 soil depth layer. Our results showed that the microclimate, soil depth, plant density, and distance between coffee from macauba affected the AMF community structure.

Keywords: Arbuscular mycorrhizal fungi, *Acrocomia aculeata*, Soil Quality, Microclime, *Coffea arabica*.

1. INTRODUCTION AND OBJECTIVES

Coffee plants show a high degree of dependence on arbuscular mycorrhizal fungi (AMF, Glomeromycota), especially in weathered soils with low natural fertility (Siqueira et al., 1998; Cardoso and Kuyper, 2006; Prates Júnior et al., 2020). These fungi are widely found in association with adult coffee plants in the field (Cardoso et al., 2003; Prates Júnior et al., 2019) and have high incidence and species richness in coffee crops (Dobo et al., 2018; Posada et al., 2018).

The AMF carry out key ecological activities for the operation and management of natural and agricultural systems (Davison et al., 2020; Belay et al., 2020), contributing to plant development, nutrition, and health (Ismail et al. 2013; Giovannini et al., 2020; Jaitieng et al., 2020). The best performance of mycorrhizal plants is related to its higher efficiency in water and nutrient absorption (Andrade et al., 2009; Püschel et al. 2020) and to improvements in this association in soil quality (Zhang et al., 2017). These fungi stand out on account of their increased absorption of water and low mobility nutrients in the soil, such as P and Zn (Gianinazzi et al., 2010; Díaz-Ariza et al., 2021) The hyphae network increase the volume of soil explored by the root system of plants, accessing ions located

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far from the root surface (Smith & Smith, 2011). Thus, the AMF association favors both growth, coffee productivity (Siqueira et al., 1998; Bhattacharya & Bagyaraj, 2002) and decreased phosphate fertilization (Perea Rojas et al., 2019; Araújo et al., 2020).

Prolonged monocultures, including coffee, decrease diversity and select AMF species that are generally less efficient in promoting the benefits of mycorrhization (Fernandes et al., 2016; Prates Júnior et al., 2019), interfering in the structure of the AMF community. However, intercropping with other species in agroforestry systems can improve coffee production and stimulates the AMF community, increasing the potential for inoculum in the cultivated and neighboring areas (Muleta et al., 2008; Sánchez et al., 2009; Arias et al., 2012; Prates Júnior et al., 2020).

The choice of the tree component in the coffee to be used in the agroforestry with coffee plays an important role. Tree species and density may affect coffee crop growth and yield, due to microclimate, including availability of water and photosynthetically active radiation (Jaramillo-Botero et al., 2010; Santos et al., 2012) and the microbial community (Veloso et al., 2020; Mukhtar et al., 2021). The use of palm trees (Arecaceae) can be an interesting strategy, given the monopodial canopy architecture, which facilitates partial blocking of solar radiation, and at the same time, allows the passage of sufficient radiation to maintain coffee productivity and farming incomes. The use of macauba (*Acrocomia aculeata* (Jacq.) Lodd. Ex Mart.) in agroforestry system has been highlighted in the literature (Dias et al., 2011; Viana et al., 2011; Moreira et al., 2018). This is a native palm from tropical regions in the American continent (Dransfield et al., 2008), which produces rich oils from fruit, and can be used in the biodiesel industry (Dias et al., 2011) and in biokerosene for aviation (Boeing et al., 2013), as well exhibit recognized potential for use in land reclamation (Mota et al., 2011) and in carbon credits projects (Moreira et al., 2020).

The most diversified management of coffee plants in agroforestry systems and native forests increases the diversity of AMF (Dobo et al., 2018; Prates Júnior et al., 2019; Belay et al., 2020). This is related, among others, to the AMF enhancing the soil physical-chemical and biological properties, with better exploitation of ecological interactions. The agroforestry system of coffee with macauba results in significant improvements in the edaphoclimatic conditions, including reductions in maximum air temperature and

photosynthetic active radiation, culminating in gains in coffee productivity (Moreira et al, 2018). In addition, the root system of macauba palm is extensive, reaching more than one meter in depth and occupies all the area under the palm canopy projection (Moreira et al., 2019), which favors the storage of soil organic carbon.

It has been recognized that agroforestry coffee systems have a higher number of spores in the deep layers of the soil, due to the greater abundance of roots in these layers (Cardoso et al., 2003; Muleta et al., 2008; Arias et al., 2012), shading (Aldrich-Wolfe et al. 2020) and temperature (Davison et al., 2021) are environmental factors of great influence in the AMF community. However, there is little information about the influence of distance, plant density and soil layers on the microclimate and soil physical-chemical factors of the soil and AMF community. In this context, we tested the hypothesis that the density, microclimate, soil depth and distance between coffee and macauba palm affect AMF community structure.

2. MATERIALS AND METHODS

2.1. Site location

The study was carried out in the Viçosa, located in the Atlantic Rainforest Brazilian biome, in Minas Gerais State, Brazil. The GPS coordinates of the location are 20°45'24.7" S and 42°50'33.5" W, at an altitude of 675 m. The annual average temperature is 19 ºC, with an average annual precipitation of 1,340 mm. The experiment was carried out in a clayed Red Yellow Latosol (Hapludox), with a 17 % slope and northwest facing the sun and its radiation (Moreira et al., 2018).

In the area, arabica coffee plants (*Coffea arabica* cv. Oeiras) are full-sun cropped and in agroforestry system with macauba palm (*Acrocomia aculeata*) at 2.80 x 0.75, (4,762 plants ha^{-1}). Macauba palms are grown with a high (11.20 x 2.80 m, 318 trees ha^{-1}) or low (11.20 x 4.40 m, 203 trees ha^{-1}) row density (Figure 1).

Aiming to evaluate the edaphoclimatic data and coffee productivity, the experimental area was divided into five treatments: full-sun coffee - control (C), coffee in agroforestry system with macauba palm planted at 1.4 m (HL1) and 4.2 m (HL2) distant from high-density macauba palms, and 1.4 m (LL1) and 4.2 m (LL2) distant from low-density macauba palms (Figure 1).
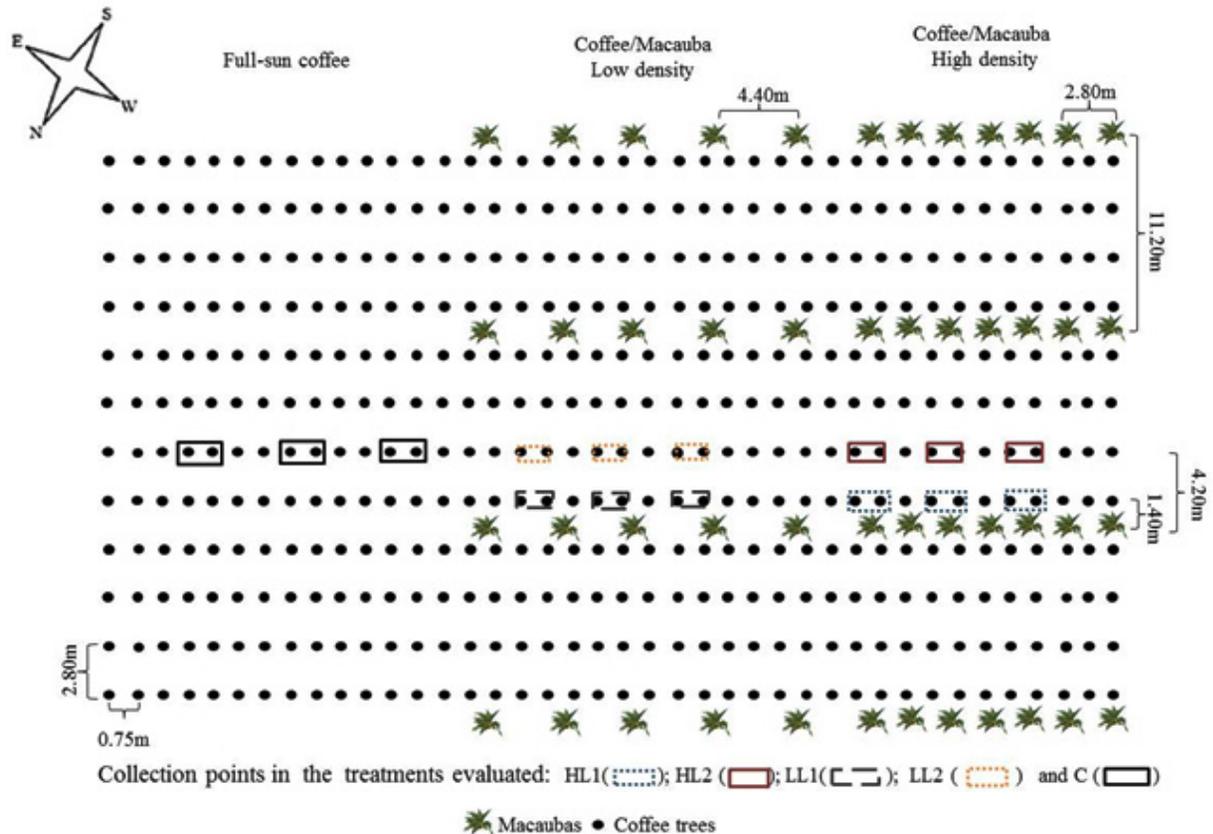
Figure 1. Sketch of the experimental area, indicating the treatments, with macauba with high (H) and, low density (L) and coffee near (L1) or distant (L2) from macauba and in full-sun (control = C).

2.2. History of the experimental area

The planting stage of the experiment was in November 2007, and the entire area received the same management until 2014 when the study was carried out. Coffee plants received annual mineral fertilization according to official recommendations for the crop in Minas Gerais State (Ribeiro et al., 1999), distributed in three applications during the rainy season. Fertilizer doses were applied to the coffee crop corresponding to 100 g (2013) and 150 g (2014) of 20-5-20 ($N-P_2O_5-K_2O$) per plant and application. Mechanical control of spontaneous plants was carried out periodically in all treatments, and the residues were left on the soil.

2.3. Soil temperature and moisture assessment

Soil temperature and moisture were monitored in 2014, coinciding with the dry season characterized by low rainfall rates and temperatures in the southeast region of Brazil.

Soil moisture and temperature were monitored using sensors (Decagon® Em50) installed in the center of two soil layers (0-20 and 20-40 cm) in the coffee row of all treatments. The sensors were coupled with a data logger (Decagon® ECH2O Logger) with data collection scheduled for every 60 min. Moisture sensors used were previously calibrated in the laboratory using soil samples collected in the same areas by the thermogravimetric method.

2.4. Chemical soil characterization

Soil samples were collected from the layers at depths of 0-20 and 20-40 cm in triplicate to form a composite sample in each treatment, near the location points of the soil temperature and moisture sensors across the coffee rows and sent for chemical routine analysis (Table 1).

Table 1. Soil chemical characterization from agroforestry system coffee with macauba planted in high (H) and low (L) densities, close (L1) and distant (L2) from the palms, and from full-sun cultivated coffee (control = C).

	pH (H2O)	P	K	Ca²⁺	Mg²⁺	Al³⁺	H +Al	SB	CEC (t)	CEC (T)	V	m	OM	P-rem
		mg dm⁻³					cmol$_c$ dm⁻³				%		dag/kg	mg/L
Treat							**0-20 cm**							
C	4.65	1.10	85.00	0.74	0.35	0.49	4.20	1.31	1.80	5.51	23.80	27.20	1.39	22.40
HL1	4.75	2.30	47.00	2.76	0.73	0.10	5.20	3.61	3.71	8.81	41.00	2.70	2.41	26.90
HL2	5.79	1.30	142.50	2.32	0.76	0.00	4.10	3.44	3.44	7.54	45.70	0.00	1.71	26.15
LL1	5.28	1.50	55.00	2.35	0.79	0.10	3.70	3.28	3.38	6.98	47.00	3.00	1.90	26.80
LL2	5.39	1.40	146.50	2.11	0.64	0.15	4.70	3.07	3.22	7.77	39.30	5.00	2.16	26.60
							20-40 cm							
C	5.19	0.50	21.00	0.69	0.31	0.39	3.20	1.05	1.44	4.25	24.70	27.10	1.14	27.70
HL1	5.60	0.90	31.00	2.12	0.59	0.00	3.70	2.79	2.79	6.49	43.00	0.00	1.65	26.20
HL2	5.73	0.95	35.00	1.83	0.55	0.00	2.70	2.47	2.47	5.17	47.75	0.00	1.20	21.20
LL1	5.75	1.00	45.00	1.96	0.63	0.00	3.90	2.71	2.71	6.61	41.00	0.00	1.39	27.30
LL2	5.64	0.45	32.00	1.49	0.53	0.05	2.35	2.10	2.15	4.45	47.15	2.55	0.83	24.05

pH in water-ratio of 1:2.5; P and K using Mehlich-1 extractor; Ca²⁺ Mg²⁺ Al³⁺ with KCl 1 mol/L; H+Al with calcium acetate 0.5 mol/L, pH 7.0; SB = sum of bases; CEC = cation exchange capability at original pH (t) and at pH 7.0 (T); V = base saturation; m = aluminum saturation; OM = organic matter (Org. C x 1.724), Walkley-Black method; P-rem. = remain P.

2.5. Microclimate characterization

The minimum and maximum air temperature in the coffee canopy were monitored by chapel-type thermometers. Thermometers were installed inside the coffee canopy at a height of 1.5 m above the soil surface to avoid direct sunlight. Readings were taken daily in the afternoon (16:30 h).

The percentage of shading was obtained through hemispherical photos (Schleppi et al., 2007), which were taken at five points in each treatment in the coffee plant rows. A Canon T2i 18-megapixel camera and a "fisheye" lens, mounted on a bubble level tripod, were used, with the images taken at 1.5 m above the soil surface with the camera pointing in a northward direction. The images were taken before sunrise and under diffuse light exposure, with the aim of obtaining the maximum possible contrast between the leaves and the sky (Whitford et al., 1995). Next, the photos were computer-processed using the GLS (Gap Light Analyzer 2.0) software program to estimate the average percentage of shading in each treatment.

The photosynthetically active radiation (PAR) was evaluated above the coffee canopy using an AccuPar Ceptometer®. Readings were taken in triplicate in each treatment.

2.6. AMF sampling and molecular analysis

Three composite soil samples (each composed of four sub-samples) from the coffee rows of all treatments were collected at a depths of 0-20 and 20-40 cm near the humidity and temperature sensors do solo and processed to AMF spore extraction using the wet sifting technique (Gerdemann & Nicolson, 1963). The total DNA of the AMF community was extracted with the Power Soil DNA kit, MoBio (MoBio Laboratories Carslbad, CA, USA)

according to the manufacturer's guidelines and minor adaptations in protocol steps (Prates Júnior et al., 2019). The PCR analysis used the GoTaq® Flex DNA Polymerase enzyme (Promega, Madison, USA) in 50 μL of buffer (20 mM Tris-HCl; 50 mM KCl; pH 8.4). The samples consisted of 3 μL of the DNA with 200 μM of the four triphosphate deoxynucleosides; 1.5 mM MgCl$_2$; 0.2 μM of each primer; 1.25 U of the GoTaq Flex DNA polymerase enzyme and 0.8 μL (0.8 μg μL⁻¹) of acetylated bovine albumin (BSA, Promega). The first amplification corresponded to the 18S rDNA of AMF, with the AM1 primers (5'- GTTTCCCGTAAGGCGCCGAA - 3') (Helgason et al., 1998) combined with the universal primer for eukaryotes NS31 (5'-TTGGGGGCAAGTCT - 3') with 580 bp fragments (Simon et al., 1992).

The PCR analysis was run in a thermocycler (Mastercycle epgradient, Eppendorf): i) a first cycle of 1 min at 94 ºC, 1 min at 66 ºC and 1.5 min at 72 ºC; ii) 30 cycles of 30 s at 94 ºC, 1 min at 66 ºC and 30 s at 72 ºC, and iii) a final extension period of 10 min at 72 ºC. The products of the PCR reactions were submitted to electrophoresis in 0.8 % agarose gel (p:v), stained with ethidium bromide and visualized under UV light in the Molecular Imaging photo documentation (Loccus Biotecnologic L-Pix Chemi). The second amplification (Nested PCR) used a 1 μL aliquot of the reaction product of the first PCR, with the NS31-GC primers (5'GGGGCGCGCCCCGGG CGGGGCGGGGGGCACGGGGGGTTGGAGGGA GTCTGGTGCC-3') (Kowalchuk et al., 2002) and Glo1 (5'GCCTGCTTTAAACACTCTA-3') (Cornejo et al., 2004), the same mixture as described for the first PCR reaction.

The amplifications were generated in a thermocycler in the following steps: i) initial DNA denaturation for 5 min at 94 ºC; ii) 35 cycles with denaturation for 45 s at 94 ºC, and iii) pairing for 45 s at 52 ºC and extension for 1 min at 72 ºC. The

products were checked by electrophoresis on an agarose gel (1.5 % w:v) stained with ethidium bromide and photographed under ultraviolet light. The products obtained from the second amplification were used for DGGE analysis (DCode™ System Model - BIO-Rad California USA), according to the methodology described by Liang et al. (2008), with minor modifications (Prates Júnior et al., 2019). The images were analyzed using the Bionumerics® program, which allowed for the construction of dendrograms for assessing the similarity between AMF communities in terms of the distance and the pattern of bands corresponding to the AMF 18S rDNA gene, according to the presence or absence of the amplified regions.

2.7. Evaluation of coffee productivity

Coffee yield was assessed, evaluating eight coffee plants per treatment obtained from four replicates each one being composed of two plants. After harvesting, fruit production was recorded, and subsamples were dried until reaching moisture between 12 and 13 %. These subsamples were processed to obtain productivity in kg of processed grains per plant.

2.8. Statistical analysis

The pattern of similarity and intensity of the bands present in the DGGE gels was evaluated by the Cosine coefficient index, followed by the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) cluster analysis for the construction of dendrograms, with the aid of the Bionumerics 5.1 program. The similarity matrix obtained was used with the soil chemical, thermal and moisture attributes, and microclimate variables in the principal components analysis (PCA) through the Euclidean distance and logarithmic transformation of the data (log. $(X + 1)$) by the Canoco 4.5 (Biometris) program.

The ecological diversity indices for OTU (operational taxonomic unit) of AMF obtained by the PCR-DGGE was submitted to the Kolmogorov-Smirnov normality test, followed by ANOVA. The Dunnett's test ($p < 0.05$) was used to compare the means of the macauba shaded treatments relative to the full-sun coffee (C). The mean values inside the shaded areas were compared using Tukey's test ($p < 0.05$). Coffee yield was analyzed by Tukey's test ($p < 0.05$). All statistical analyses were carried out using the R statistical software, version 3.4 (R Core Team, 2017).

3. RESULTS AND DISCUSSION

Bands obtained by PCR-DGGE indicated similarity of AMF community among treatments (Figure 2). These findings can be related to the dominant presence of the roots of macaubas, and, mainly, of coffee plants. Superficial layers (0-40 cm) represent the region with the highest concentration of absorbent coffee roots (Rena & Guimarães, 2000), while on the adult macauba, more than 80 % of the root system reaches down to a depth of one-meter (Moreira et al., 2019). The presence of AMF in depth (20-40 cm) may also be related to the fact that some species occupy preferential niches and sporulate in the deeper layers of the soil (Cardoso et al., 2003; Oehl et al., 2005), contributing to the soil biodiversity in intercropped systems (Arias et al., 2012; Dobo et al., 2018; Prates Júnior et al., 2019).
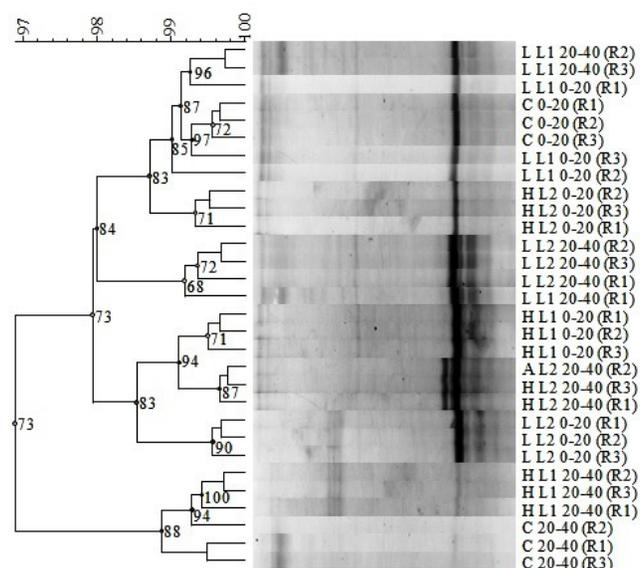
Figure 2. Dendrogram of AMF community obtained by the Cosine coefficient-UPGMA in the bands obtained by PCR-DGGE to evaluate the similarity of agroforestry system of coffee considering coffee planted close to (L1) and distant (L2) from macauba palm plants in high (H) and low (L) density, and with full-sun coffee crop (control = C), in the 0-20 cm and 20-40 cm layers of soil from macauba. R = repetition.

The PCA showed differences in the composition of the AMF community in the plots, with 23.1% of the variance explained by ordination from the first (PC1, 13.6%) and second (PC2, 9.5 %) axis (Figure 3). PCA revealed that microclimate features affect AMF community structure more than soil chemical attributes, mainly soil moisture, photosynthetically active radiation (PAR), and maximum and average air temperature (Figure 3). In our case, the soil chemical attributes do not play a critical role in determining AMF composition because our study site is homogeneous and received the same crop management practices, including fertilization and control of spontaneous herbs. On the other hand, microclimate conditions can influence soil AMF community dynamics. Temperature, shading, light intensity, and wavelength can stimulate or inhibit the formation of AMF spores, mycorrhizal colonization, and taxonomic composition of the AMF community (Heinemeyer & Fitter, 2004; Konvalinková & Jansa, 2016; Freire-Cruz, 2016; Aldrich-Wolfe et al., 2020).

Furthermore, temperature acts how an important abiotic environmental and spatial abiotic driver, defining the realized niche space of AMF (Davison et al., 2021).

Agroforestry system of coffee and macauba palm changed the microclimate of the environment and reduced the maximum air temperature and the PAR in the coffee plant canopy (Table 2). These changes can modify the soil water regime (Moreira et al., 2018) and promote physiological changes in the coffee plants, such as an increase in chlorophyll content and leaf water potential (Bonfim et al., 2010). The consequence can be a change in photoassimilate status in the soil, which impacts the AMF community, because the availability of shade, light and temperature has a great influence on the composition of the AMF community (Aldrich-Wolfe et al., 2020; Davison et al., 2021). However, information about the direct effects of microclimate factors on AMF dynamics is scarce (Bennett & Classen, 2020), since the result depends on the species of plants associated with AMF species.
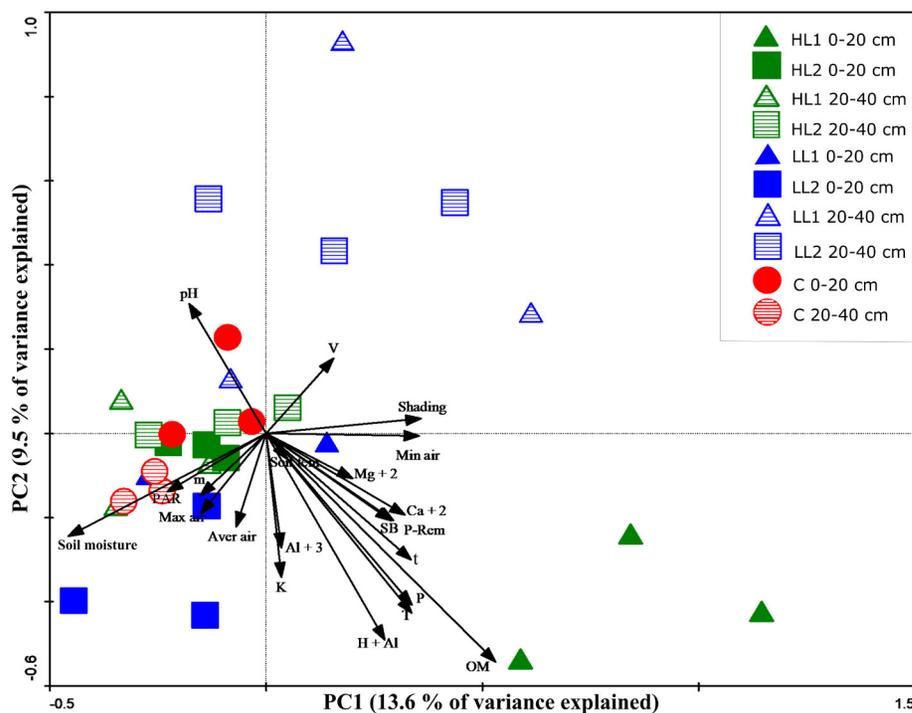
Figure 3. Principal Component Analysis (PCA) based on PCR-DGGE profiles for the AMF community, soil chemical attributes and microclimate variations in agroforestry system of coffee with macauba in high (green), in low (blue) macauba plant densities and in full-sun coffee (control = red), in two different soil layers: 0-20 cm (empty color) and 20-40 cm (cross-line). Distances from coffee plants to macauba represent the close to (L1 - triangle), and far from (L2 - square) distance; and control treatment (circle). SB = Sum of exchangeable bases; T = cation exchange capacity at pH 7.0; t = cation exchange capacity; V = bases saturation index; m = aluminum saturation index; OM = organic matter; P-rem = P-remaining; soil moisture; Soil temp = soil temperature; Max air = maximum air temperature; Min air = minimum air temperature; Aver air = average air temperature; Shading = percentage of shading; PAR = photosynthetically active radiation.

Table 2. Edaphoclimatic attributes evaluated for coffee agroforestry system with macauba palm in two different soil layers (0-20 and 20-40 cm), in high (H) or low (L) macauba densities, and coffee in full-sun (control = C), and grown close to (L1) and far (L2) from palms.

	Soil moisture (m³ m⁻³)		Soil temperature (°C)		Air temperature (°C)			Shading %	PAR μmolm⁻² s⁻¹
Treatments	0-20 cm	20-40 cm	0-20 cm	20-40 cm	Max	Min	Mean		
C	0.214	0.158	17.20	18.03	31.51	9.77	20.64	2.10	1,760.33
H L1	0.159	0.153	18.27	18.93	27.02	10.36	18.69	55.90	543.33
H L2	0.198	0.234	18.10	18.65	28.67	10.58	19.63	30.10	1,693.00
L L1	0.196	0.134	18.36	18.50	26.55	10.90	18.73	57.62	439.33
L L2	0.219	0.186	18.38	18.64	29.65	10.94	20.30	30.10	1,724.33

Soil moisture and temperature were measured using a sensor based on Time Domain Reflectometry (TDR). Data on soil moisture and temperature represent the average value recorded from June to August 2014. Air temperature data refers to the same period. Shading and PAR were evaluated once at the end of August 2014.

Soil chemical characteristics (P, Ca²⁺, Mg²⁺, H+Al, V e OM, SB, cation exchange capacity) were related to differences in the AMF community in HL1 agroforestry system of coffee and macauba palm at the 0-20 cm depth. The AMF community of the agroforestry system of coffee grown in LL2 at a depth of 20-40 cm was less influenced by microclimate features and soil characteristics (Figure 3). The high density of macauba and the position near coffee plants in HL1 may favor higher organic carbon contents due to the root turnover providing nutrients to the AFM community.

Although usually the soil fertility explains variations in species richness and diversity of AMF community in coffee plantations, especially in the surface layer (Siqueira et al., 1998; Posada et al., 2018), our study shows that microclimate attributes can play an important role in the AMF community, with potential impacts on crop productivity as observed by Moreira et al. (2018).

AFM diversity received less influence from the macauba planting density, and the distance of 1.4 m (HL1) exhibited a higher reading on the Chao richness estimator index than the 4.2 m (HL2) and the higher indices of richness, Shannon and Chao, than control (C) in the 0-20 cm soil layer (Table 3).

The high density and proximity of coffee and macauba favor AMF diversity (Table 3), probably due to the greater availability of organic matter and nutrients (Figure 3 and Table 1), characteristics which may be the consequence of the higher root concentration and the presence of a milder microclimate (Moreira et al., 2018). Functionally distinct plants such as coffee and macauba can recruit distinct fungal communities because they contribute to functional diversity (Davison et al., 2020). These integration effects were also reported in coffee plantations intercropped with green manure or perennial crops, where the improvements in availability of plant hosts and soil exudates contribute to the increasing number of propagules and AMF diversity (Sánchez et al., 2009; Dobo et al., 2018; Prates Júnior, 2019; Díaz-Ariza et al., 2021).

Table 3. Diversity indexes for OTU of arbuscular mycorrhizal fungi (AMF) obtained by the PCR-DGGE in agroforestry system of coffee and macauba palm, in high (H) or low (L) macauba densities, and coffee in full-sun (control = C), grown close to (L1) and far (L2) from palms.

	Richness	Dominance	Simpson	Shannon	Chao
Soil depth (0-20 cm)					
Treatment	Richness	Dominance	Simpson	Shannon	Chao
C	2.66	0.41	0.58	0.92	5.33
HL1	10.00 a*	0.10	0.90	2.30*	55.00 a*
HL2	3.33 b	0.36	0.63	1.06	6.33 b
LL1	5.33 ab	0.27	0.72	1.50	21.00 ab
LL2	6.00 ab	0.20	0.79	1.71	23.33 ab
CV % (Tukey' test)	35.96	57.21	17.38	30.11	50.85
CV % (Dunnett's test)	39.47	52.44	19.36	33.46	57.61
Soil depth (20-40 cm)					
Treatment	Richness	Dominance	Simpson	Shannon	Chao
C	5.33	0.25	0.74	1.50	22.33
HL1	4.66	0.22	0.77	1.52	13.66
HL2	5.66	0.21	0.78	1.63	22.00
LL1	6.66	0.16	0.83	1.84	27.66
LL2	3.33	0.34	0.65	1.13	8.00
CV % (Tukey' test)	43.25	43.53	13.55	28.05	79.43
CV % (Dunnett's test)	53.44	48.24	15.32	33.86	97.22

Means followed by the same letter in each column were not different by Tukey's test (p < 0.05) at the same soil depth and for shaded treatments (HL1, HL2, LL1, and LL2). Means followed by an asterisk (*) were significantly different from the full-sun coffee (C= control) by Dunnett's test (p < 0.05) at the same soil depth.

The biennial pattern of coffee production in Brazil was confirmed in our study. In a year characterized by low productivity (2013), the productivity of full-sun coffee (C) was like shaded coffee by macaubas, except for the LL2 treatment, which generated lower productivity compared to the control (Table 4). In the following year of higher production (2014), the shaded coffee grown farthest from macaubas (L2) presented higher productivity compared to the full-sun coffee (C) and the shaded coffee closest to the macaubas (L1). The biennium average productivity (2013-2014) shows that agroforestry system of coffee and macauba palm improves productivity only if coffee plants are cultivated farthest from the macaubas (L2). This can be related to the microclimate conditions more favorable to the coffee productivity and lower nutrient competition with macauba when the distance is 4.20 m (L2), which is three times more than in L1. The L2 position was associated with higher soil moisture, air temperature, sun radiation (PAR) and lower shading rates (Table 2). These microclimate conditions are related to improved coffee plant productivity (Jaramillo-Botero et al., 2010; Santos et al., 2012; Rigal et al., 2020) and the associated microbiota, that includes bacteria, fungi, and the AMF (Steidinger et al., 2019; Aldrich-Wolfe et al., 2020; Rao et al., 2020; Veloso et al., 2020; Mukhtar et al., 2021).

Table 4. Productivity of coffee plants in the agroforestry system with macaubas and in full-sun cultivation (C).

Treatment	Coffee yield (kg/plant)		
	2013	2014	Mean 2013/2014
C	0.185 a	0.299 b	0.242 c
HL1	0.178 a	0.277 b	0.228 c
HL2	0.159 ab	0.837 a	0.498 a
LL1	0.183 a	0.221 b	0.202 c
LL2	0.087 b	0.707 a	0.397 b
CV %	24.01	13.63	6.88

Coffee plants and macauba palm in high (H) and low (L) planting density and 1.4 m close to (L1) and 4.2 m (L2) distant from palms. Means followed by the same letter in each column are not different by Tukey's test (p < 0.05).

The literature relates that AMF can provide early development and yield increases in coffee (Siqueira et al., 1998) because it promotes increases in nutrient absorption and protects plants against several pathogens (Andrade et al., 2009; Ismail et al. 2013; Jaitieng et al., 2020). The same microorganisms improve soil structure and aggregation by extra-radical hyphae and/or glycoprotein exudation (Banks et al., 2011; Zhang et al., 2017). All these benefits are dependent on the ecological context, which includes both microclimatic and edaphic aspects (Aldrich-Wolfe et al., 2020; Cruz et al., 2020; Araújo et al., 2020). In intercropped systems, this concern needs to be considered, to manage the microclimate conditions (Arias et al., 2012; Dobo et al., 2018; Aldrich-Wolfe et al. 2020), as determined by the density planting and distance to the tree, aiming to maintain or reach optimum AMF diversity. Thus, further studies are required to find the best spatially and temporally organization of intercropped systems to balance the benefits of the agroforestry system on coffee and macauba yields with the diversity in AMF.

This study contributes to increasing the understanding of the AMF community distribution in agroforestry system of coffee and macauba palm, supporting decision-making, and aiming at sustainable and productive agricultural practices related to the microclimate and plant spacing variables. In addition to bringing benefits in microclimatic and ecological features shown in the present study, a coffee and macauba combination can provide economic benefits. While coffee is an international and consolidated agricultural commodity (Lewin et al., 2004), macauba is a relatively new crop in farming systems around the world (Motoike et al., 2013), experimenting with an expanding crop area where the fruit is rich in oils that can be used in several industrial sectors. A recent study highlighted the potential of the macauba as regards carbon sequestration (Moreira et al., 2020), and cultured agroforestry systems can mitigate change and maintain areas favorable to coffee production (Gomes et al., 2020), minimizing the effects of climate change and generating potential income from the carbon credits market. The benefits of macaubas on the microclimate can also be relevant to the AMF community because it is not yet understood how these microorganisms respond to global climate change (Bennett & Classen, 2020). However, it is already recognized that the AMF community responds strongly to shade and temperature gradients (Aldrich-Wolfe et al., 2020; Davison, et al., 2021), which will allow us to manipulate microclimate conditions to favor the multifunctional role of these fungi. The microclimate conditions provided by macaubas can probably minimize the impacts of climate change and favor mycorrhizal symbiosis in productive systems.

4. CONCLUSIONS

The structure of the AMF community in the agroforestry system of coffee and macauba palm is affected by microclimate, soil depth, palm field spacing, and the distance between coffee plants and the macaubas. The highest ecological diversity in this agroforestry system is reached when palms are located closer to the coffee plants.

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