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High diversity of arbuscular mycorrhizal fungi in two forestry ecosystems from central and northern Costa Rica

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FOREST ECOLOGY

ABSTRACT

Background: Arbuscular mycorrhizal fungi (AMF) generate a symbiotic relationship with most terrestrial plants, influencing the dynamics and functioning of ecosystems. There are few studies on the diversity of these fungi associated with forest species and in different types of ecosystems. The objective of this study was to characterize the diversity and structure of AMF communities associated with *Cordia alliodora* and *Swietenia macrophylla* in two forestry ecosystems with different types of management in Costa Rica. For this purpose, rhizosphere and soil samples were collected from 10 trees at random, spores and sporocarps were isolated and characterized, AMF abundance, richness and diversity were determined, and a physicochemical analysis of the soil was carried out.

Results: Fifty-seven AMF morphospecies belonging to five orders, 10 families and 15 genera were identified, with a predominance of Diversisporales and Glomerales; we report 15 new geographic records of AMF increasing the richness to 76 species and by 24% the Glomeromycota's richness in Costa Rica. There were no significant differences in total spore abundance between the two forest species, however, there were significant differences in the modes of formation and species composition.

Conclusions: The alpha diversity analysis showed that rare species largely explain the differences between the sites, and the AMF community structure was influenced by edaphic factors such as pH and available phosphorus content. These types of studies highlight the importance of considering the identity and diversity of AMF associated with forest species of commercial interest and ecological importance in different types of ecosystems.

Keywords: Agroforestry system, Santa Rosa National Park, Glomeromycota, Laurel, Mahogany

HIGHLIGHTS

57 AMF species were identified in two forest ecosystems of Costa Rica.
Fifteen new geographic records of AMF are reported for Costa Rica.
AMF communities differ between *S. macrophylla* and *C. alliodora*.
The pH and available phosphorus influence AMF community structure.

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INTRODUCTION

Arbuscular mycorrhizal fungi (AMF; Glomeromycota) inhabit the soil and form mutualistic symbiosis with about 80% of terrestrial vascular plants and an increasing number of aquatic plants (Li et al., 2024). This mutualistic association is ubiquitous (Enebe & Erasmus 2023), and it is considered one of the oldest existing interactions on Earth that is widely distributed in most continents (Antoine et al. 2021; Stürmer & Kemmelmeier 2021). Among many benefits this symbiosis provides, AMFs represent an important ecological role (Antoine et al. 2021; Li et al. 2024).

Some authors mention that AMF are key drivers of ecosystem processes by impacting plant-environment interaction, plant productivity, and ecosystem functions, as they establish a connection between plants' aerial and subterranean components (Edy et al. 2022). Since AMF facilitate the translocation and absorption of multiple nutrients and water, they also play a key role in plant establishment, function, and diversity, as well as their multitrophic interactions (Guzman et al. 2025)

However, it is important to consider the specific ecosystem where the AMF-plant symbiosis is established. When talking about soils with agricultural practices, for example, in agroforestry systems, agriculture or forestry activities can influence the availability of resources for AMF, which in turn can affect the composition of AMF communities (Antoine et al. 2021; Li et al. 2024). Likewise, activities such as agrochemical use and tillage often cause hyphal breakdown and generate physiological stress to AMF that can negatively affect their ecological functions and symbiotic services to plants (Enebe & Erasmus 2023).

Despite these conditions, AMF show diverse responses to different environmental conditions, so they can adapt and generate symbiont relationships with most agricultural and forest crops, where in previous studies a great diversity of AMF has been observed (Oehl et al., 2017; Polo-Marcial et al., 2022). As for ecosystems without any type of disturbance such as natural forests within national parks, they would be expected to be richer in organic matter and therefore may harbor a lower amount of AMF (Enebe & Erasmus 2023). However, AMF diversity will be influenced by the different vegetation types in the different periods of ecological succession of the forest (Li et al. 2024).

The study of AMF communities in the Americas has historically been very heterogeneous, as most studies have focused on some regions of North and South America (Stürmer & Kemmelmeier 2021; Vega-Herrera et al. 2023), however, the central part has been little explored in mycorrhizal terminus (Polo-Marcial et al. 2023; De Jesús Alarcón et al. 2025). Currently, 61 species have been reported from Costa Rica, mainly in Premontane wet forest and Tropical dry forest, with a dominance of Diversisporaceae and Glomeraceae, however, extensive areas of vegetation remain unexplored (Mardones et al. 2024; De Jesús Alarcón et al. 2025).

Costa Rica has led the way in forest management and forest conservation considering that 30% of the country is covered by forests (SINAC 2013). Under this approach, a special interest has been generated in understanding mycorrhizal interactions in these vegetation types and in forest species of economic or conservation importance (Aldrich-Wolfe et al. 2020). The forest species *Cordia alliodora*, commonly known as laurel, and *Swietenia macrophylla*, with its common name mahogany, are species of great forest importance, as well as of great economic value in Costa Rica and worldwide (Andrade et al. 2023). Both species are native to the country and are present in different eco-regions (Chinchilla-Mora et al. 2021; Valverde et al. 2021). Particularly for this study, laurel was found near organic banana plantations, which is considered an agroforestry system, in Turrialba, Cartago. In contrast, mahogany was found within the Santa Rosa National Park in Guanacaste, a conserved forest in a seasonally dry region.

Evolutionarily, the family Boraginaceae and Meliaceae form arbuscular mycorrhizal associations (Wang & Qiu 2006). Specifically in *C. alliodora* and *S. macrophylla* studies have reported considerable colonization and richness of AMF dominated by Glomeraceae, Diversisporaceae and Scutellosporaceae. However, these results are restricted to the Neotropical bioregion of Mexico (dos Santos et al. 2021; Sánchez-Reyes et al. 2023).

In Brazil, a study was conducted on the presence of HMA in mahogany established in an agroforestry system with different crops on a site with acidic soil and without any fertilizer application. Samples were collected at three different times of the year: during the dry season, at the beginning of the rainy season, and in the middle of the rainy season of the harvest in agroforestry systems, reflecting a high percentage of HMA in the rainy season despite being acidic soil with other agricultural crops nearby (dos Santos et al. 2021).

Regarding *C. alliodora*, Cuervo and Rivas (2007) quantified mycorrhizal fungi in soil samples from different plantations of *C. alliodora* in Costa Rica, once they determined the percentage of mycorrhization and which AMF species were present, including *Glomus* spp. and *Gigaspora* spp., they used the soil as a source of inoculum to evaluate the effect of AMF in the nursery stage. So far, there have been no reported studies of AMF diversity associated with laurel under natural growth conditions within agroforestry systems, nor of mahogany in natural forests in Costa Rica. The objective of this work was to characterize the diversity and community structure of AMF in the rhizosphere of two forest species from two contrasting life zones and forestry ecosystems with different management.

MATERIALS AND METHODS

Study site and sampling

Ten trees of *Cordia alliodora* and *Swietenia macrophylla* under natural growing conditions were randomly selected from two sites with different management, an agroforestry system for laurel and a conserved forest for mahogany. A minimum diameter of

500 m between each sampled tree was considered (Figure 1). Samples were collected in September and October 2022, during the rainy season in Costa Rica. A soil sample was collected from each tree, approximately 30 cm deep and at 1 m from the base of the trunk in the four cardinal directions (Polo-Marcial et al., 2023). Sample processing was carried out at the Laboratory of Pathology of the Forestry School of the Technological Institute of Costa Rica.

Samples of *S. macrophylla* were collected within Santa Rosa National Park, in areas considered secondary forest without anthropic intervention since its declaration as a National Park in 1971, located in the province of Guanacaste (10°53' N, 85°38' W). With an average elevation of 300 meters above sea level, the park is located between the Dry Tropical Forest (BsT) and Premontane Humid Transition to Basal Forest (BhPB) life zones. These life zones have an average temperature of 24.0 to 27.8 °C, annual precipitation varies between 1100 and 1500 mm, the climate has a dry season of 3.5 to 6.5 months between November and April, and the soils are predominantly Inceptisols, Alfisols, Vertisols, and Entisols (IMN, 2025a).

Samples of *C. alliodora* were collected in the canton of Turrialba, province of Cartago, specifically in the town of Platanillo (9°49' N, 83°42' W) in an agroforestry system with organic bananas, approximately four years old, where only organic fertilizer has been applied to the bananas and not to the laurel. Prior to organic banana cultivation, other agricultural crops were grown on the site using conventional management practices, with agrochemicals applied as needed. Regardless of the type of crop established, the laurel trees, approximately 12 years old, have not received any silvicultural management since their establishment. This site has an average elevation of 1,120 m above sea level. The canton of Turrialba is in the tropical rainforest zone, with an average temperature of 22 °C (72.2-63.7 °F) and an average annual rainfall of 2619 mm. The region is characterized by young soils, such as Inceptisols and Andisols (IMN, 2025b).

Soil analysis

Four soil and rhizosphere samples were collected for each of the selected trees in the different sampling sites, according to the methodology used by Polo-Marcial et al., (2023). The exact sampling point was geolocated. A composite 500g sample, formed by mixing the four samples of each tree was taken, and a complete chemical analysis was performed through the external service of the Soil and Foliar Laboratory of the Agronomic Research Center of the University of Costa Rica. Each variable analyzed was determined as follows: electrical conductivity (EC) was extracted with ultrapure water at a soil-extractant volumetric ratio of 1:2.5 and determined potentiometrically.

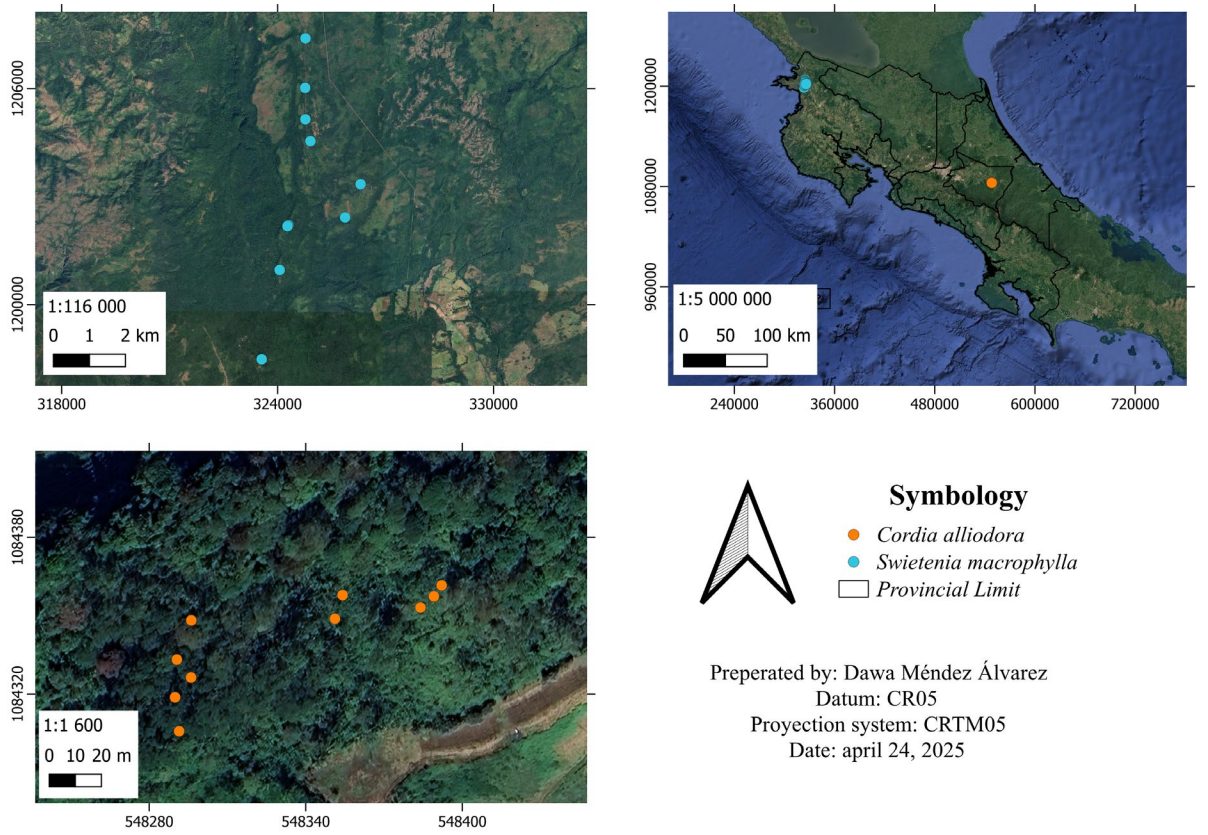

Figure 1: Location by GPS of sampling sites for *Cordia alliodora* and *Swietenia macrophylla* trees in Costa Rica.

The pH and exchangeable acidity were determined using a neutral salt, potassium chloride (KCl), with a displacement ion (K+) that causes the acid ions (aluminum (Al+) and hydrogen (H+)) to pass into the solution. This acidity is then titrated with a basic solution, so that the amount of acidity will be equal to the amount of base used between the neutralization points with phenolphthalein. To determine the amount of acidity corresponding to aluminum, a back titration is performed with an acid solution. In this case, 4% potassium fluoride (KF) was added to dissolve the previously formed aluminum hydroxides ($Al(OH)_3$), which, after the reaction, release hydroxyl ions (OH-) into the medium that are titrated with hydrochloric acid (HCl).

To determine acidity (CEC) and exchangeable aluminum, the soil was extracted in a potassium chloride (KCl 1M) solution, in a soil-extractant volumetric ratio of 1:10, and subsequently in a 10:10 dilution of ultrapure water filtrate. Acidity was determined by titration with sodium hydroxide (NaOH 0.01 M) and aluminum was determined by re-titration with potassium fluoride (KF 4%) and hydrochloric acid (HCl 0.01 M). For the elements Ca, Mg, K, P, Zn, Cu, Fe, Mn, the soil was extracted with Mehlich 3 solution (pH 2.5, HOAc 0.2 N, NH_4NO_3 0.25 N, NH_4F 0.015 N, HNO_3 0.013 N, EDTA 0.001 M), in a soil-extractant volumetric ratio of 1:10. The elements were determined using Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-OES). C and N were analyzed in an Elementar Vario Macro Cube autoanalyzer, whose determination is based on the Dumas dry combustion principle.

The acidity saturation percentage (SA%) is obtained by taking the exchangeable aluminum (Al) content and the sum of exchangeable bases (Ca, Mg, K, Na). To determine effective cation exchange capacity (ECEC), extraction was first performed in ammonium acetate (1.0 M; pH 7) and the bases present were determined by Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-OES). The remaining soil was washed with 95% alcohol, the supernatant obtained was discarded, and 25 mL of 10% KCl with a pH of 2.5 was added to the remaining soil. shaken for 10 minutes, centrifuged at 1600 rpm for 10 min, and the filtered supernatant was analyzed for CIC value by colorimetry in the Flow Injection Analyzer (FIA).

Isolation and taxonomic identification of AMF

Glomerospores and glomerocarps were isolated from 100 g of dry soil through sieving and decanting with 60% sucrose (Gerdemann and Nicolson 1963, Błaszkowski et al. 2012). Spores were separated and grouped into morphotypes according to their color, shape, and size. Permanent preparations were made with spores mounted on PVLG (polyvinyl alcohol acid glycerol) and a mixture of PVLG+Melzer 1:1 (v/v). Of the viable glomerospores, the number of layers and walls were determined, as well as their phenotype and histochemical characteristics, reaction to Melzer, color, and thickness of the layers (Medeiros et al. 2021). The terms glomerospore and glomerocarp, proposed by Goto and Maia (2006) and Jobim et al. (2019), respectively, were adopted. Morphospecies were identified with specialized literature and original species descriptions (Schenck and Perez 1990; Błaszkowski et al. 2012), taxonomically classified according to Oehl et al. (2008), Oehl et al. (2011), Błaszkowski (2012), Wijayawardene et al. (2020), and Tedersoo et al. (2024). Permanent slide vouchers are stored in the Forest Pathology laboratory of the School of Forest Engineering of the Instituto Tecnológico de Costa Rica.

Ecological and statistical analysis

The relative abundance (number of individuals of a species/total number of individuals of all species) *100) of AMF present for forest species was calculated per species and site, and based on these data, a Kruskal-Wallis non-parametric test and a Tukey ($p < 0.05$) significant difference test were performed. We estimated AMF diversity as qD in terms of effective number of species, where q represents the order of diversity (Jost 2006). To assess and compare sample completeness and diversity between *Cordia alliodora* and *Swietenia macrophylla*, we followed the protocol proposed by Chao et al. (2020), using the online tool iNext4steps (https://chao.shinyapps.io/iNEXT4steps/). Significant differences between plant species were evaluated by the overlap of the confidence intervals (Chao and Jost, 2012).

To analyze dissimilarities between the AMF communities of the two forest species, a non-metric multidimensional scaling (NMDS) was generated in conjunction with the Bray-Curtis similarity index, complemented by a one-way ANOSIM similarity test. Finally, an indicator species analysis was performed for order, family, genus, species, and mode of formation, and a taxon was considered as an indicator when its Indicator Value Index (IndVal) value was greater than or equal to 25% and $p < 0.05$ (Dufrêne and Legendre 1997). All analyses were performed in RStudio 4.4.1 software (R Core Team, 2019), with different packages: indicspecies (De Cáceres and Legendre, 2009), iNEXT.4steps (Chao et al., 2020), Rstaticx (Kassambara, 2023), Vegan (Oksanen et al., 2025).

RESULTS

Soil properties

Chemical analysis showed some differences between the sampled sites (Table 1). A more acid pH was obtained in the site sampled for *C. alliodora* (4.5) compared to the site sampled for *S. macrophylla* (5.9), this pattern was observed in the CEC values, where Platanillo (2.15 cmol(+)/L) had a higher value than Santa Rosa National Park (0.13 cmol(+)/L). According to Costa Rican reference ranges, the pH at Platanillo falls below the desirable threshold (5.5), with high saturation of acidity (22%), in contrast to Santa Rosa National Park, where soils

are classified as basic with minimal acidity saturation (1%) (Table 1). As for the available phosphorus (P) content, a low value was obtained for both sites (2 mg/L); below the national sufficiency range (10mg/L).

Likewise, as shown in Table 1, Santa Rosa National Park had higher values for effective cation exchange complex (CICE) (13.06 cmol(+)/L) compared to the Platanillo site (10.11 cmol(+)/L), suggesting greater retention capacity. On the other hand, the Platanillo site presented a higher value for organic carbon (C) with 4.84% content compared to the Santa Rosa National Park site (2.69%); despite these differences, the carbon-nitrogen (C/N) ratio, remained relatively stable between sites (9.2 in Platanillo and 10.9 in Santa Rosa National Partk). These results confirm the contrasting edaphic conditions of the two study sites.

Diversity, Richness, and abundance

A total of 382 glomerospores and glomerocarps were isolated, 252 from the rhizosphere of *C. alliodora* and 130 from *S. macrophylla*. No statistical differences in spore abundance were detected between the two forest species. However, according to the mode of formation, in *C. alliodora,* the acaulosporoid mode was significantly higher than in *S. macrophylla* ($p < 0.05$) (Figure 2a), but the glomoid and glomoid-radial modes were higher in *S. macrophylla* ($p < 0.05$) (Figure 2b and Figure 2c), and the gigasporoid mode showed no differences (Figure 2d).

Fifty-seven morphospecies belonging to five orders, 10 families, and 15 genera were identified. Diversisporales and Glomerales were dominant with 22 and 19 species, respectively (Table 2). *Acaulospora* was the dominant genus with 21 species. The highest AMF richness was found in *C. alliodora* (35 spp.), while 29 species were detected in *S. macrophylla*. As shown in Table 2, 15 new geographic records of AMF for Costa Rica were identified.

Sample coverage for *C. alliodora*, *S. macrophylla*, and the total sample was 65%, 74%, and 78%, respectively. The standardized coverage value (C_{max}) calculated for both species was 97.2%. Diversity estimates were as follows: for *C. alliodora*, q0 = 41.41, q1 (Shannon) = 11.99, and q2 (Simpson) = 4.98; for *S. macrophylla*, q0 = 35.66, q1 = 18.16, and q2 = 11.75 (Figure 3a). The estimated richness (q0) for the total sample was 61 species. There was no significant difference in species richness (q0) between the two tree species. The evenness profile further confirmed significantly higher evenness in the AMF community associated with *S. macrophylla* (Figure 3b).

InVal indicator species analysis (IV≥25%, $p < 0.05$) revealed that for *C. alliodora*, the acaulosporoid formation mode was significant ($p = 0.0007$), as well as the family Acaulosporaceae ($p= 0.0001$) and the genus *Acaulospora* ($p=0.0004$). Specifically, three species were identified as indicators (Figure 4): *Acaulospora reducta* ($p=0.0032$), *A. foveate* ($p=0.0046$), and *A. rehmii* ($p=0.0131$). In contrast, for *S. macrophylla*, the glomoid ($p=0.0075$) and radial-glomoid ($p=0.0031$) and the genera *Sclerocystis* ($p=0.0035$) and *Glomus* ($p=0.0411$). Five indicator species were detected (Figure 4): *Halonatospora* sp. 1 ($p=0.0008$), *Glomus spinuliferum* ($p=0.0120$), *Sclerocystis* sp. 1 ($p=0.0337$), *Sclerocystis taiwanensis* ($p=0.0425$), and *Glomus glomerulatum* ($p=0.0364$).

Table 1: Average values of soil chemical properties in the two sites sampled for *Cordia alliodora* and *Swietenia macrophylla*, in Costa Rica. CV coefficient of variation.

Soil chemical property	Reference for Costa Rica	*Cordia alliodora* (Platanillo, Turrialba, Cartago)	Standard deviation	CV(%)	*Swietenia macrophylla* (Santa Rosa National Park, Guanacaste)	Standard deviation	CV(%)
pH	5.5	4.5	0.07	1.51	5.9	0.16	2.81
CEC (cmol(+)/L)	0.5	2.15	0.29	13.66	0.13	0.01	7.47
Ca (cmol(+)/L)	4	5.02	1.15	22.86	9.13	0.66	7.24
Mg (cmol(+)/L)	1	2.80	0.55	19.90	3.35	0.56	16.79
K (cmol(+)/L)	0.2	0.14	0.02	14.76	0.45	0.06	14.09
ECEC (cmol(+)/L)	5	10.11	1.47	14.55	13.06	0.84	6.41
SA (%)		22	7.69	34.62	1	0.12	12.09
P (mg/L)	10	2	0.42	23.42	2	0.47	23.57
Zn (mg/L)	3	1.6	0.61	37.36	5.1	4.75	92.37
Cu (mg/L)	1	9	1.57	16.85	10	1.73	17.46
Fe (mg/L)	10	211	30.66	14.52	239	22.68	9.50
Mn (mg/L)	5	37	3.68	9.88	26	2.18	8.43
EC (mS/cm)	1.5	0.7	0.14	20.45	0.3	0.08	33.99
C (%)		4.84	0.55	11.33	2.69	0.31	11.44
N (%)		0.53	0.05	9.64	0.25	0.01	5.49
C/N		9.2	0.31	3.99	10.9	0.78	7.13

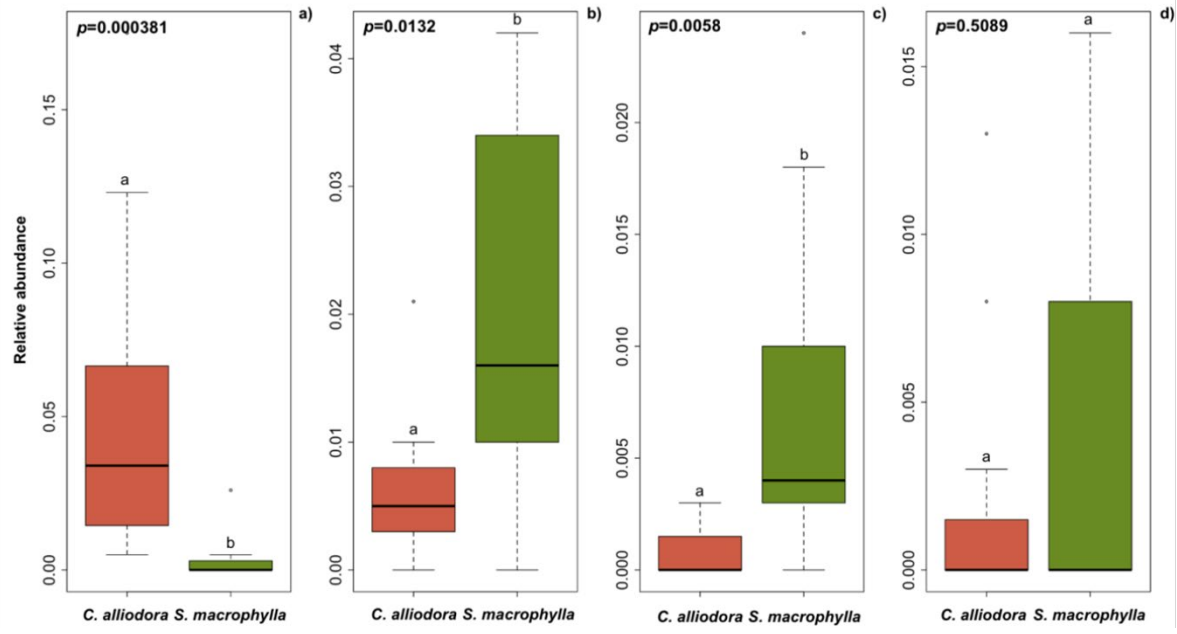

Figure 2: Relative abundance of AMF according to the mode of formation of glomerospores associated with two forest species in Costa Rica. a) acaulosporoid, b) glomoid, c) glomoid-radial and d) gigasporoid.

Table 2: Relative abundance of rhizospheric arbuscular mycorrhizal fungi of two forest species in Costa Rica. *glomerocarpic species. **New records for Costa Rica.

AMF species	Relative abundance	
	Swietenia macrophylla	Cordia alliodora
Archaeosporales C. Walker & A. Schüßler		
*Ambispora appendicula (*Spain, Sieverd. & N.C. Schenck) C. Walker	0.062	-
Ambispora reticulata Oehl & Sieverd. **	-	0.008
Ambispora sp. 1	0.008	-
Ambispora sp. 2	-	0.008
Diversisporales C. Walker & A. Schüßler		
Acaulospora aspera Corazon-Guivin, Oehl & G.A. Silva**	-	0.024
Acaulospora cavernata Blaszk.**	-	0.004
Acaulospora colossica P.A. Schultz, Bever & J.B. Morton	-	0.012
Acaulospora foveata Trappe & Janos	-	0.421
Acaulospora herrerae Furrazola, B.T. Goto, G.A. Silva, Sieverd. & Oehl **	-	0.020
Acaulospora lacunosa J.B. Morton **	-	0.040
Acualospora laevis Gerd. & Trappe	0.015	0.004
Acaulospora mellea Spain & N.C. Schenck	-	0.012
Acaulospora reducta Oehl, B.T. Goto & C.M.R. Pereira**	-	0.079
Acaulospora rehmii Sieverd. & S. Toro	-	0.079
Acaulospora scrobiculata Trappe	-	0.048
Acaulospora spinosa C. Walker & Trappe	-	0.024
Acaulospora spinossisima Oehl, Palenz., I.C. Sánchez, Tchabi, Hount. & G.A. Silva	-	0.028
Acualospora spinulifera Oehl, V.M. Santos, J.S. Pontes & G.A. Silva **	0.008	-
Acaulospora tuberculata Janos & Trappe	-	0.012
Acualospora sp. 1	0.008	-

Continue.

Table 2: Continuation.

AMF species	Relative abundance	
	Swietenia macrophylla	Cordia alliodora
Acaulospora sp. 2	-	0.016
Acaulospora sp. 3	-	0.004
Acaulospora sp. 4	-	0.004
Acaulospora sp. 5	-	0.004
Acaulospora sp. 6	-	0.004
Sacculospora sp. 1	0.008	-
Entrophosporales Błaszk., Sánchez-García, B.T. Goto, and Magurno		
Entrophospora sp. 1 (glomoid)	-	0.012
Glomerales J.B. Morton and Benny, emend. Błaszk., B.T. Goto, and Magurno		
Glomus glomerulatum Sieverd. *, **	0.123	-
Glomus spinuliferum Sieverd. & Oehl **	0.146	-
Glomus rubiforme (Gerd. & Trappe) R.T. Almeida & N.C. Schenck *	-	0.004
Glomus trufemii B.T. Goto, G.A. Silva & F. Oehl *, **	0.023	0.020
Glomus sp. 1 *	0.015	-
Glomus sp. 2	-	0.008
Glomus sp. 3	0.023	-
Glomus sp. 4 *	0.008	-
Glomus sp. 5 *	-	0.008
Halonatospora sp. 1	0.169	-
Rhizoglomus microaggregatum (Koske, Gemma & P.D. Olexia) Sieverd., G.A. Silva & Oehl *	0.008	0.032
Septoglomus aff. *constrictum*	0.015	0.004
Septoglomus sp. 1	-	0.004
Septoglomus sp. 2	0.031	-
Sclerocystis clavispora Trappe *	0.038	-
Sclerocystis coremioides Berk. & Broome *	0.008	0.008
Sclerocystis taiwanensis C.G. Wu & Z.C. Chen *	0.100	0.004
Sclerocystis sinuosa Gerd. & B.K. Bakshi *, **	0.023	-
Sclerocystis sp. 1 *	0.038	-
Gigasporales Sieverd., G.A. Silva, B.T. Goto & Oehl		
Gigaspora decipiens I.R. Hall & L.K. Abbott**	-	0.016
Gigaspora sp. 1	0.015	-
Scutellospora calospora (T.H. Nicolson & Gerd.) C. Walker & F.E. Sanders	0.046	-
Scutellospora sp. 1	0.008	-
Scutellospora sp. 2	0.008	-
Dentiscutata scutata (C. Walker & Dieder.) Sieverd., F.A. Souza & Oehl	0.008	-
Fuscutata heterogama Oehl, F.A. Souza, L.C. Maia & Sieverd.	0.015	-
Racocetra gregaria (N.C. Schenck & T.H. Nicolson) Oehl, F.A. Souza & Sieverd. **	-	0.020
Racoceta verrucosa (Koske & C. Walker) Oehl, F.A. Souza & Sieverd. **	0.015	-
Paraglomerales C. Walker & A. Schüßler		
Paraglomus occultum (C. Walker) J.B. Morton & D. Redecker	0.008	0.004
Paraglomus bolivianum (Sieverd. & Oehl) Oehl & G.A. Silva **		0.004

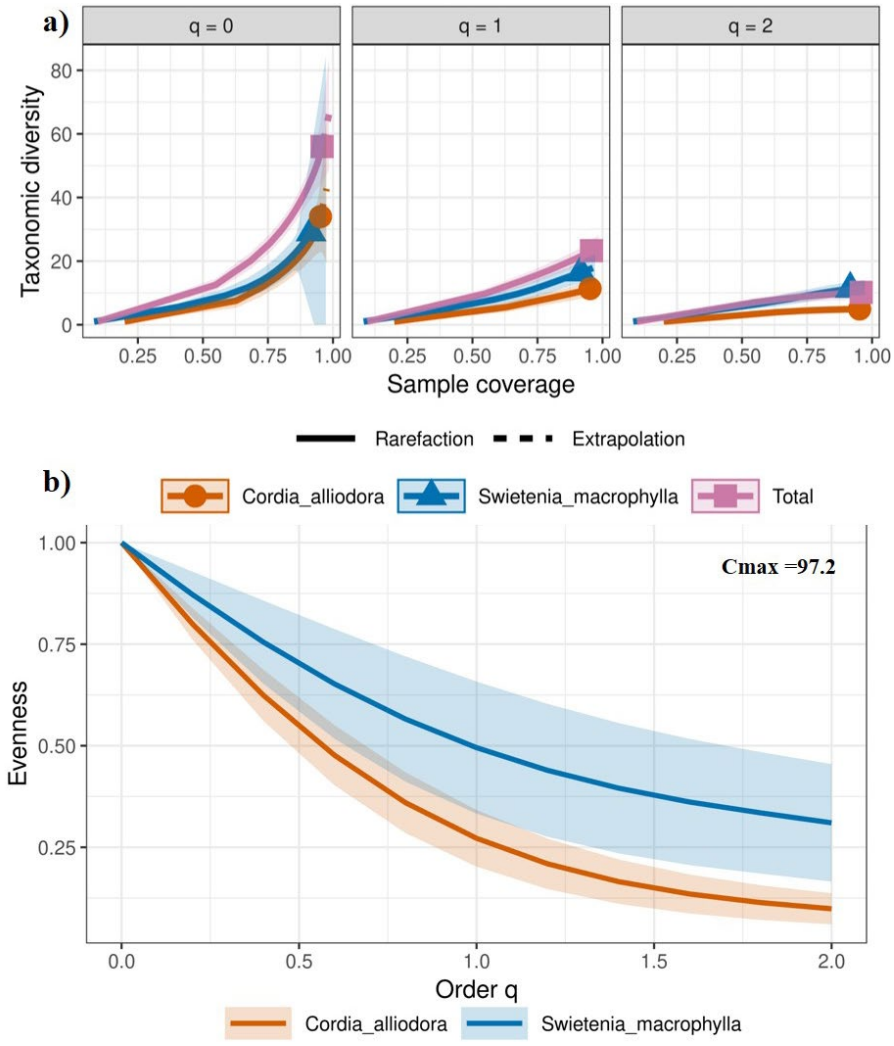

Figure 3: Taxonomic diversity and evenness **a)** Non-asymptotic coverage-based diversity estimates of orders q = 0, 1, and 2 at the standardized coverage value of Cmax 97.2 % for arbuscular mycorrhizal fungi associated with the rhizosphere of *Cordia alliodora* and *Swietenia macrophylla* and the total samples. **b)** Evenness profile as a function of order q, for Pielou J, q = 1 and q = 2, based on the normalized slope of Hill numbers for arbuscular mycorrhizal fungi associated with the rhizosphere of *Cordia alliodora* and *Swietenia macrophylla*. Shadow areas represent 95 % confidence intervals with a bootstrap method with 250 replications.

AMF community structure

Non-metric multidimensional scaling in conjunction with Permanova analysis evidenced that *C. alliodora* and *S. macrophylla* AMF communities are different (F= 5.088, p < 0.0001) (Figure 5). SIMPER analysis indicated that the AMF species contributing most to dissimilarity are *Acaulospora foveata* (20.9%), *Halonatospora* sp. 1 (8.8%), *A. reducta* (7.6%), *Glomus spinuliferum* (5.2%), *G. glomerulatum* (5.0%) and *Sclerocystis taiwanensis* (4.0%). Only seven species (*Acaulospora laevis*, *Glomus trufemii*, *Rhizoglomus microaggregatum*, *Septoglomus* aff. *constrictum*, *Sclerocystis coremioides*, *S. taiwanensis*, and *Paraglomus occultum*) are shared between the two forest species, 28 species are exclusive to *C. alliodora* and 22 species to *S. macrophylla*.

DISCUSSION

Despite the crucial role of arbuscular mycorrhizal fungi (AMF) in plant growth, and nutrient cycling, studies on AMF diversity in Costa Rica remain scarce (Mardones et al., 2024). Understanding species richness and distribution across different environments and vegetation types is essential for sustainable agroecosystems, forestry practices, and conservation programs (Liu et al., 2024). In this study, we detected contrasting AMF communities in terms of richness, dominance and formation modes in the two forest species (Figure 2, Table 2).

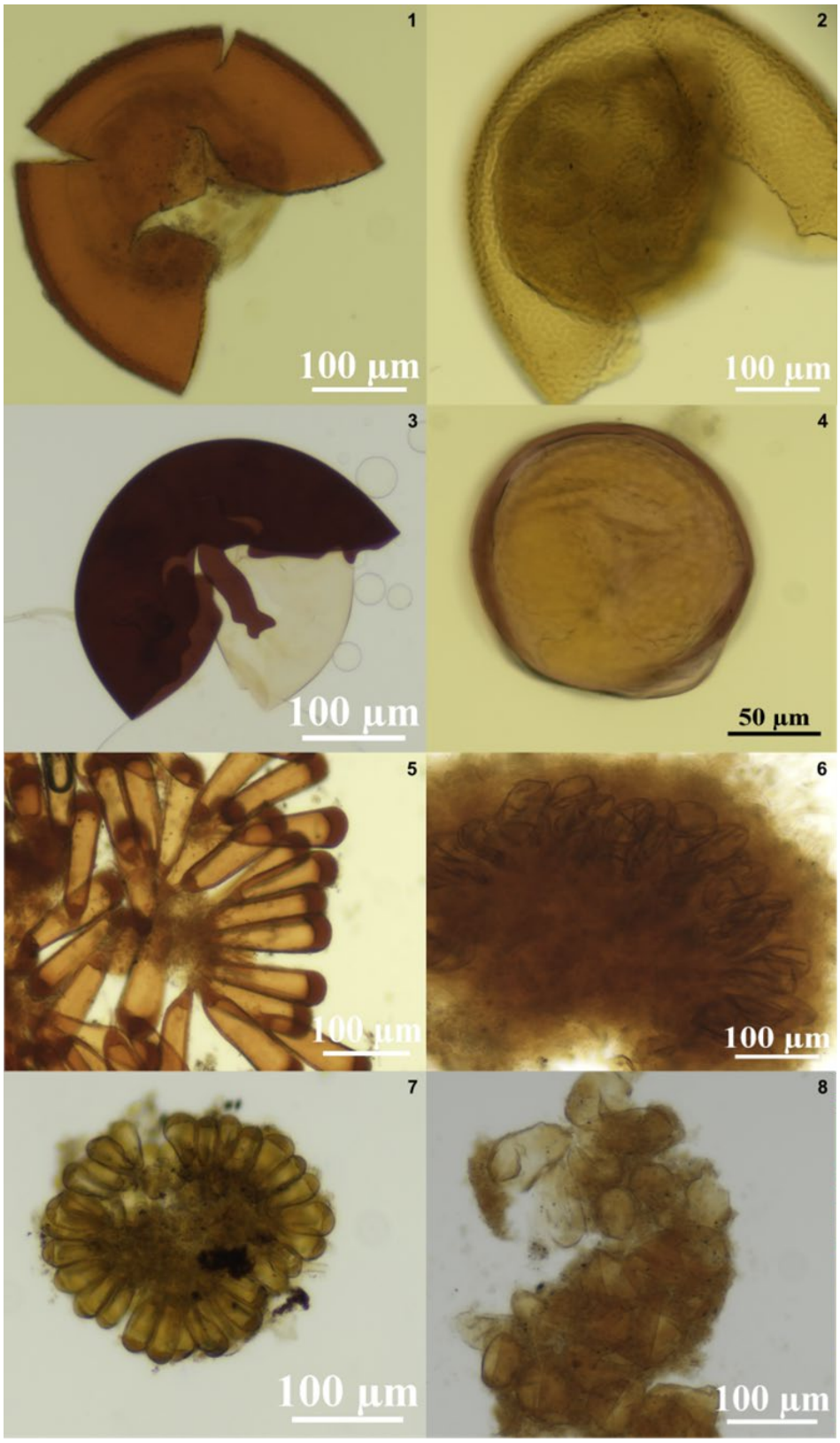

Figure 4: AMF richeness, 1) *Acaulospora foveata*, 2) *A. rehmii*, 3) *Fuscutata heterogama*, 4) *Ambispora reticulata*, 5) *Sclerocystis clavispora*, 6) *Sclerocystis coremioides*, 7) *Sclerocystis taiwanense*, 8) *Sclerocystis sinuosa*.

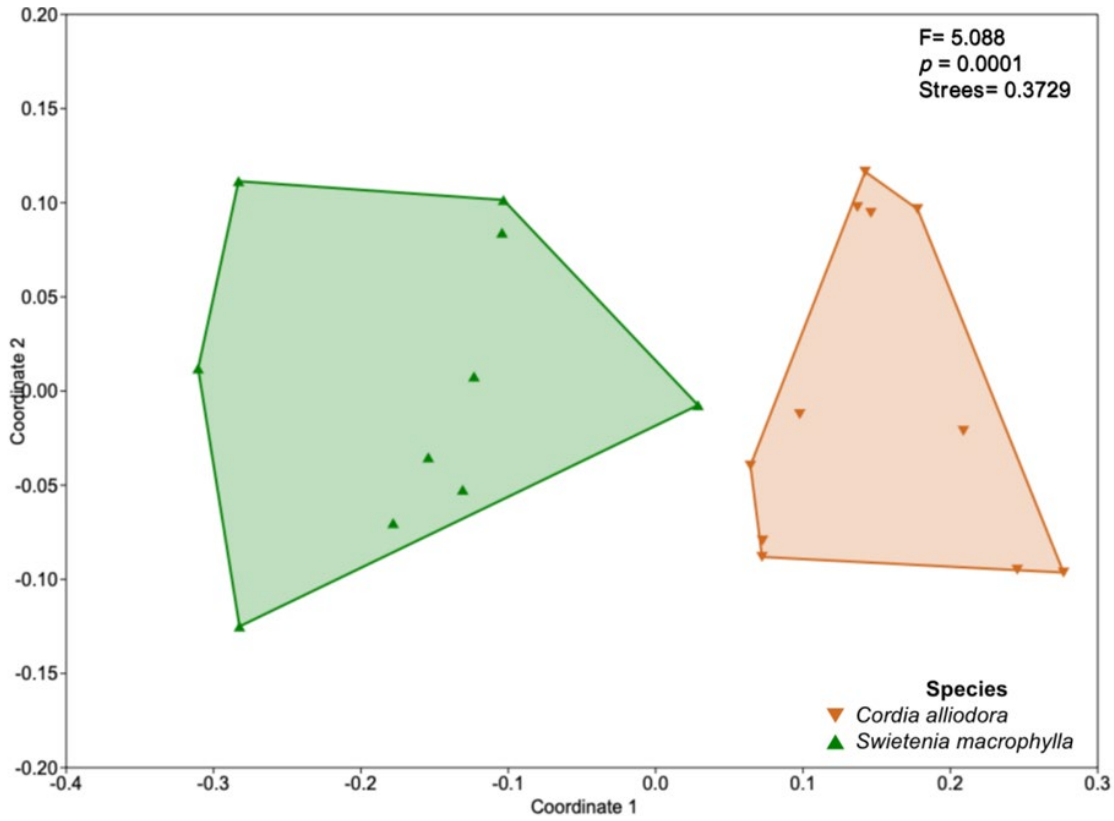

Figure 5: Non-metric multidimensional scaling analysis (NMDS) of AMF communities associated with two forest species in Costa Rica.

The richness of this study is the highest one recorded so far in Costa Rica with 57 species, representing 93.44 % of the total known in the country and only in two life zones. Additionally, we report 15 new geographical records of AMF increasing the richness to 76 species with respect to the 61 spp. currently reported (Mardones et al. 2024; Alarcón et al. 2025). This species richness represents approximately 20% of all AMF species currently described within the phylum Glomeromycota (Goto et al., 2024; Wijayawardene et al., 2024).

One of the properties that most influences the presence and diversity of AMF is pH (Zhang et al. 2021). A pH range between 5.7 and 6.6 is considered favorable for mycorrhizal symbiosis (Hernández-Acosta et al. 2021), values that are reflected in the site sampled for the rhizosphere of *S. macrophylla* and not so for the site with rhizosphere of *C. alliodora* (Table 1). However, according to da Silva et al., (2021) AMF can thrive in more acidic soils, as in the case of the Platanillo site (Table 1).

On the other hand, the influence of pH on the diversity of species found in this work was reflected, according to Laurindo et al. (2021) in a study of agroforestry systems in Brazil in which the authors reported that, Acaulosporaceae species are more common in soils with a pH below 4.0, while Glomeraceae are usually found more frequently in soils with a pH above 5.0. Similar results were obtained in both sampling sites, where in Platanillo Acaulosporaceae predominated (Figure 5) with a pH of 4.5 and in Santa Rosa National Park the second most predominant species was Glomeraceae (Figure 5) with a pH of 5.9.

Another important soil factor is the availability of nutrients, especially phosphorus, carbon, and nitrogen (Maitra et al. 2021). In this study, a low concentration of available P (Table 1) was obtained for both sites that is usually for soils in Costa Rica. Nonetheless, it is considered that there was a high richness of AMF, like that reported by da Silva et al. (2021) in no-till monoculture systems and agroforestry systems with low phosphorus availability. According to Hernández-Acosta et al. (2021), low phosphorus availability in the soil can stimulate the production of strigolactones by the plant, which are necessary to activate the metabolism and branching of AMF hyphae, favoring the establishment of symbiosis.

Regarding the richness and abundance (Figure. 2 and Figure 4) found at the Platanillo site, an agroforestry system with *C. alliodora*, these represent values like those reported by Laurindo et al. (2021) in agroforestry systems in different regions of Brazil and Polo-Marcial et al. (2023) in agroforestry systems with *C. odorata* in Costa Rica. For the Santa Rosa National Park site associated with *S. macrophylla* rhizosphere, slightly higher richness values were obtained than those reported for Amazonian forests (Zhang et al.

2021) and secondary forests associated with *C. odorata* in Costa Rica (Polo-Marcial et al. 2023).

The genera *Glomus* and *Acaulospora* are frequently reported as dominant or with high species richness in diverse ecosystems, including tropical forests, agroforestry systems, and grasslands (Olanipon et al. 2024), similar results were obtained in this study (Table 3). The results of the study showed differences in the composition and structure of AMF communities between *C. alliodora* and *S. macrophylla* (Figure 5), which seem to be strongly influenced by local environmental factors such as edaphic conditions and the type of ecosystem management. Similar results are reported by Laurindo et al. (2021) in their study of different ecosystems in Brazil, where the species identified are distributed in the orders Diversisporales and Glomerales.

According to the alpha diversity analysis, rare species are key to determining the differentiation between AMF communities for each sampled site, being a common pattern in AMF studies in tropical ecosystems (Polo-Marcial et al. 2023). Also, the high sample coverage (>0.9) at both sites validates the robustness of comparison and suggests adequate sampling (Figure 3). The ordination analysis (NMDS) and SIMPER supported this differentiation obtained with species such as *A. foveata* and *Halonatospora* sp. 1, this suggests that certain AMF species could fulfill specific functions according to the ecological context where they are found, as mentioned by Li et al. (2024) there are AMF communities sensitive to vegetation type, ecosystem management and successional state of the forest, reflecting the results obtained for the AMF communities present in the rhizosphere of two contrasting sites associated with *C. alliodora* y *S. macrophylla* in Costa Rica.

CONCLUSION

The AMF communities associated with *Cordia alliodora* in Platanillo, an agroforestry system, *and Swietenia macrophylla* in Santa Rosa National Park, a mature forest without intervention, present differences in their structure and composition, due to the physicochemical conditions of the soil and the type of management of each ecosystem. Despite not having detected differences in the total abundance of spores between sites, specific patterns were observed in the modes of formation and indicator species, highlighting *Acaulospora* in more acidic soils and *Glomus* in soils with higher fertility. The richness of morphospecies found (57) reflects a high potential diversity in the ecosystems sampled. This type of study highlights the importance of considering the identity and diversity of AMF associated with forest species of commercial interest and ecological importance, and in different types of ecosystems, to contribute to decision-making regarding the management of forest ecosystems.

AUTHORSHIP CONTRIBUTION

Project Idea: DMA; MRS; MHPM; LALP

Funding: DMA; MRS; MHPM; LALP

Database: DMA; MRS; SJP; MHPM; WWG; LALP

Processing: DMA; MRS; SJP; MHPM; WWG; LALP

Analysis: DMA; MRS; SJP; MHPM; WWG; LALP

Writing: DMA; MHPM; MRS; LALP; SJP; WWG; DAA; WRM

Review: DMA; MHPM; MRS; LALP; SJP; WWG; DAA; WRM

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DATA AVAILABILITY

The datasets supporting the conclusions are included in the article.

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