

Original Article

Occurrence and diversity of arbuscular mycorrhizal fungi in yerba mate (*Ilex paraguariensis* – Aquifoliaceae) cultivation environments

Ocorrência e diversidade de fungos micorrízicos arbusculares em ambientes com cultivo de erva mate (*Ilex paraguariensis* – Aquifoliaceae)

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Abstract

Yerba mate (*Ilex paraguariensis*) represents a culture of economic, social, and ecological importance for the cultivation regions. Due to the chemical, physical, and biological variations that occur in the different soils where yerba mate is economically exploited, the symbiotic associations with arbuscular mycorrhizal fungi (AMF) guarantee the plant's ability to absorb nutrients. The purpose of this study was to identify and quantify the occurrence of arbuscular mycorrhizal fungi in different environments of yerba mate cultivation. The research was performed in four areas located in the rural area of the municipality of Seberi/RS: Environment with production of yerba mate in the conventional system, silvopastoral system, organic system, and native forest. The normality of residuals and homogeneity of variances assumptions were verified using the Lilliefors and Chi-square tests and the averages compared by the Tukey's test at 5% probability of error. In addition to calculations of diversity, equivalent species, and evenness indices. The presence of AMF spores showed a direct relationship with the phosphorus (P) availability in each treatment, with a count reduction in the organic system, with P content lower than 3 mg kg⁻¹ of soil. The species with the highest predominance were the Acaulosporaceae (*Acaulospora colombiana*, *A. delicata*, and *A. tuberculata*), followed by the Glomaceae (*Glomus ambisporum* and *Glomus pansihalos*) in the conventional and silvopastoral systems. The silvopastoral and conventional systems showed the highest levels of Shannon-Weaver diversity (H') and Pielou's evenness, demonstrating greater diversity and consequently greater richness and uniformity.

Keywords: soil microbial diversity, ecology, symbiotic associations, phosphorus.

Resumo

A erva-mate (*Ilex paraguariensis*) representa uma cultura de importância econômica, social e ecológica para as regiões de cultivo. Devido às variações químicas, físicas e biológicas que ocorrem nos diferentes solos onde a erva-mate é explorada economicamente, as associações simbióticas com fungos micorrízicos arbusculares (FMA) garantem a capacidade da planta de absorver nutrientes. O objetivo deste estudo foi identificar e quantificar a ocorrência de fungos micorrízicos arbusculares em diferentes ambientes de cultivo de erva-mate. A pesquisa foi realizada em quatro áreas localizadas na zona rural do município de Seberi/RS: Ambiente com produção de erva-mate no sistema convencional, sistema silvipastoril, sistema orgânico e mata nativa. As suposições de normalidade dos resíduos e homogeneidade das variâncias foram verificadas pelos testes de Lilliefors e Qui-quadrado e as médias comparadas pelo teste de Tukey a 5% de probabilidade de erro. Além de cálculos de diversidade, espécies equivalentes e índices de equivalência. A presença de esporos de FMAs apresentou relação direta com a disponibilidade de fósforo (P) em cada tratamento, com redução na contagem no sistema orgânico, com teor de P inferior a 3 mg kg⁻¹ de solo. As espécies com maior predominância foram as Acaulosporaceae (*Acaulospora colombiana*, *A. delicata* e *A. tuberculata*), seguidas pelas Glomaceae (*Glomus ambisporum* e *Glomus pansihalos*) nos sistemas convencional e silvipastoril. Os sistemas silvipastoril e convencional apresentaram os maiores níveis de diversidade de Shannon-Weaver (H') e equitabilidade de Pielou, demonstrando maior diversidade e consequentemente maior riqueza e uniformidade.

Palavras-chave: diversidade microbiana do solo, ecologia, associações simbióticas, fósforo.

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1. Introduction

From South America, Yerba mate (*Ilex paraguariensis* A. St.-Hil. AQUIFOLIACEAE) is a plant highly consumed in Argentina, Paraguay, Uruguay, and southern Brazil, due to its high economic, social, and ecological value. Typically, its consumption in the countries of origin is in the form of *chimarrão* (infusion with hot water) and *tereré* (infusion with cold water). In addition, industrialized beverages, food supplements, drugs, and cosmetics are made from its leaves (Cardozo Junior and Morand, 2016).

Yerba mate is one of the most specific Brazilian systems and is explored in the native, conventional, agroforestry, and silviagricultural way, aiming at greater productivity and product quality (Sturion and Resende, 2010). This species occurs naturally in soils of low fertility, with high aluminum content and low pH; however, its cultivation is recommended in soils with medium to high natural fertility (Ricardi et al., 2020). In addition, it is necessary that the soils present certain physical characteristics for its planting, such as clayey texture, good depth, being well-ventilated, good drainage, and not being compacted (Silva et al., 2012).

The most limiting nutrients to the growth of forest species are nitrogen (N) and phosphorus (P), consequently, the most demanding (Acha et al., 2016). Slow-growing species, accustomed to low fertility soils, present lower efficiency in the use and response to nutrient viability (Lambers and Poorter, 1992).

The imbalance of nutrients in the soil is one of the main limiting factors for plant growth (Taiz et al., 2017); given this nutritional variation in the soil, symbiotic associations stand out through mycorrhizal fungi, which have the ability to increase the absorption of nutrients by plants, mainly phosphorus, which is the element found at lower levels in the soil (Berude et al., 2015; Trovato et al., 2024). Thus, the importance of research identifying and characterizing the occurrence of arbuscular mycorrhizal fungi (AMF) species in different yerba mate crops becomes evident, since the symbiotic association promoted by AMF is present in several species, generating benefits for the plants.

The purpose of this study was to identify and quantify the occurrence of arbuscular mycorrhizal fungi in different *Ilex paraguariensis* cultivation systems.

2. Material and Methods

The research was performed in four areas of yerba mate production, located in the rural area of the municipality of Seberi municipality, in Rio Grande do Sul State, southern Brazil: Conventional system, silvopastoral system, organic system, and native forest. According to the Köppen classification, these areas are located in a cfa2 climate, humid subtropical, with 1,800 to 2,100 mm average annual rainfall and 18°C average annual temperature (Alvares et al., 2013).

The conventional yerba mate production system has 1.4 ha, 2 x 3 m spacing (row, inter-row), fertilization at the time of planting when 1 kg of poultry litter weathered in the pit is added, harvested every two years, with 100%

of the leaves and branches being removed, and every 6 months a desiccation of the inter-row is performed to control, mainly, grasses. The silvopastoral system has 7 ha; it has the same spacing, fertilization, and harvest as the conventional system, containing a vegetation composed of spontaneous grasses and herbaceous plants from the region for cattle grazing, with an animal load of one unit per ha. The area with organic production of yerba mate has 8 ha, 2 x 3 m spacing (row, inter-row), with the inter-row being used for organic cultivation (yerba mate + native species + swine, and this organic system is certified by the Rede Ecológica de Agroecologia). The native forest area represents a fragment of Mixed Ombrophilous Forest, with a 9-ha area without anthropogenic interventions, serving as a witness for the accomplishment of the present study.

In each of the areas, eight samples of 2.0 kg containing soil and yerba mate roots were randomly collected, at a 0-20 cm depth. After collection, the samples were kept in plastic packages and stored for biological and chemical analysis. The analyzes were performed in the microbiology and soil chemistry laboratories of the Santa Maria Federal University – Campus Frederico Westphalen/RS, from May to October 2019.

The methodology by Brundrett et al. (1996) was adopted for the root clearing and staining procedure, in which 0.40 grams of roots cut at 1.0 cm length were used. The fragments were immersed in 10% KOH, in a water bath at 80°C for 40h, then transferred to a new 10% KOH solution for 24h at room temperature, and then to an alkaline solution of ammoniacal hydrogen peroxide (0.5% NH₄OH and 0.5% H₂O₂ in water) for 2h at room temperature. After clearing, the samples were neutralized in 1% HCl for 24h, and subsequently stained with 0.05% trypan blue (1:1:1- lactic acid, glycerol, water) in a water bath at 80°C for 30 min.

The selected root fragments were mounted in polyvinyl lacto-glycerol (PVLG), perpendicular to the longitudinal axis of the microscope slide. The presence of mycorrhizae was observed at 400x magnification with an optical microscope. For the categorization regarding colonization, the species were classified as very high, high, medium, low, and absent, when they presented a colonization degree >80%, 79-50%, 49-20%, 19-1%, and 0%, respectively, according to Carneiro et al (1998).

To verify the spatial heterogeneity of AMF, spores were extracted from 50 g of soil using the wet sieving method (Gerdemann and Nicolson, 1963) and centrifugation in sucrose (Jenkins, 1964).

After extraction, the spores were grouped by morphotypes (color, shape, and size), under a stereoscopic microscope, and placed on slides with polyvinyl alcohol-lactic acid-glycerol (PVLG) and Melzer. Species identification was performed using an optical microscope, based on the morphological characteristics of the spores, according to descriptions obtained from the International Culture Collection of (Vesicular) Arbuscular Mycorrhizal Fungi database (INVAM, 2020). The taxonomic classification used was the classification proposed by Redecker et al. (2013).

Microsoft Excel was used to calculate the diversity, equivalent species, and evenness indices from the experiment data.

The Shannon-Weaver diversity index (H') assigns equal weight to rare and abundant species (Magurran, 1988). The greater the H' value, the greater the studied population diversity. This index can express richness and uniformity (Santos et al., 2018), and is represented for Equation 1:

$$H' = \frac{[N \ln(N) - \sum_{i=1}^S n_i \ln(n_i)]}{N} \quad (1)$$

Where: H' = Shannon-Weaver index; n_i = number of sampled individuals of the i -th species; N = total number of individuals sampled; S = total number of species sampled; \ln = Napierian logarithm.

For the Shannon-Weaver equivalent species index (SH'), the following equation was used: $SH' = e^{H'}$ (measured in species), which represents the number of species that would be expected to be found in the community if all species had the same abundance (maximum evenness). As for the Pielou's evenness index (J), which belongs to the interval $[0, 1]$, where 1 represents the maximum diversity, that is, all species are equally abundant (Pielou, 1966), this index is calculated according to Equation 2:

$$J = \frac{H'}{H_{\max}} \quad (2)$$

Where: $H_{\max} = \ln(S)$; J = Pielou's evenness; S = total number of species sampled; H' = Shannon-Weaver diversity index.

For the statistical analysis, the normality of residuals and the homogeneity of variances assumptions were verified using the Lilliefors and Chi-square tests, respectively, with 5% probability. When these assumptions were not met, the variables were transformed using the BoxCox method. Subsequently, the data was submitted to analysis of variance and, when there was a statistically significant difference, the means were compared using Tukey's test at 5% probability of error. For the analyses, the statistical software GENES (Cruz, 2006) was used.

3. Results

3.1. Determining the area attributes

The result of the soil analysis in the areas with mate production system showed differences in chemical

properties, with greater variation in the phosphorus (P) content, being three times greater in the conventional system compared to the organic system (Table 1).

In the native forest (MN), silvopastoral (SP), and conventional (C) production systems, the potassium (K) content was high (Table 1). The pH values were low in the organic and silvopastoral systems, associated with high aluminum saturation and low base saturation in these areas.

3.2. Classification and density of spores

Analyzing the data in Table 2, we observed that initially there was the presence of several species of arbuscular mycorrhizal fungi in the yerba mate rhizosphere, as well as a very high count of spores per 50 g of soil, despite the low percentage of root colonization. Also, there was a predominance of the Acaulosporaceae family, followed by Glomeraceae and Gigasporaceae families, respectively. The most abundant spores were of the genus *Acaulospora*, characterized mainly by the easy identification of the germinal saccule, while other characteristics, such as the spore shape and size, the presence or absence of a germinal shield, germ tubes, suppressor hyphae, and number of layers in the spore wall, were evaluated for species-level identification (INVAM, 2020).

The AMF *Gigaspora albida*, *Scutellospora dipurpurascens*, and *S. biornata* showed variations in the number of spores in the yerba mate cultivation systems, with a predominance of *G. albida* in the silvopastoral system and of *S. dipurpurascens* and *S. biornata* in the conventional system (Table 2). This same trend was also verified for the *Acaulospora* and *Glomus* genera, mainly in the conventional system, not differing from the silvopastoral system, but with lower counts verified in the organic system.

Mycorrhizal colonization was higher in areas of native forest and organic system, and lower in silvopastoral and conventional systems (Figure 1), where the predominant structures were intraradical vesicles and hyphae, with low visibility of arbuscules. The conventional system presented the highest total number of spores, not differing from the silvopastoral system, being greater than the native forest and organic cultivation systems (Figure 1).

3.3. Diversity indices

There were higher diversity indexes and higher richness in the silvopastoral and conventional systems and lower richness in the organic system (Table 3). However, there is

Table 1. Soil analysis in native forest (MN), silvopastoral (SP), conventional (C), and organic (O) yerba mate production systems.

Area	pH _w	SMP	MO %	P mg/L	K	Ca	Mg	Al	Al+H cmolc/L CTC		Saturation	
										Effective	pH _r	Bases	Al
												 % ...
MN	5.5	5.9	3.9	3.8	480	3.9	2.9	0.3	4.7	8.4	12.8	63.0	3.6
SP	4.9	5.3	3.9	7.3	61.8	1.3	0.5	3.0	10.0	5.0	11.9	16.4	60.5
C	6.2	6.5	2.3	10.2	191.3	5.3	2.2	0.0	2.5	8.0	10.5	76.0	0.0
O	4.4	4.7	3.5	2.9	57	0.5	0.3	4.2	19.9	5.2	20.8	4.8	80.9

Table 2. Identified species and spore density of arbuscular mycorrhizal fungi in areas of yerba mate production: Native forest (MN), silvopastoral (SP), conventional (C), and organic (O).

Identified species of AMFsYerba mate production systems.....			
	MN	SP	C	O
	----- Spores density* -----			
<i>Gigaspora albida</i>	50.0 b**	150.0 a	60.0 b	50.0 b
<i>Scutellospora dipurpurascens</i>	23.7 b	22.8 b	40.0 a	27.3 b
<i>Scutellospora biornata</i>	21.5 ab	12.0 b	35.0 a	13.5 b
<i>Acaulospora colombiana</i>	180.0 b	170.0 b	370.0 a	170.0 b
<i>Acaulospora delicata</i>	285.0 a	215 ab	260.0 a	130.0 b
<i>Acaulospora tuberculata</i>	235.0 ab	245.0 ab	440.0 a	115.0 b
<i>Glomus ambisporum</i>	87.5 ab	146.0 a	94.5 ab	52.8 b
<i>Glomus pansihalos</i>	122.5 b	189.0 a	150.5 ab	62.3 c
Others	301.0 b	450.0 a	455.3 a	320.3 b

*Density = number per 50 g of soil; **Different lowercase letters on the lines indicates a significant difference by the Tukey's test at 5% probability of error.

Table 3. Shannon-Weaver diversity index (H'), Shannon-Weaver equivalent species (SH'), and Pielou's evenness (J) in the soil of yerba mate production areas: Native Forest, silvopastoral, conventional, and organic.

IndexesYerba mate production systems.....			
	Native forest	Silvopastoral	Conventional	Organic
H'	0.963	1.047	1.029	0.819
SH'	2.63	2.84	2.79	2.27
J	0.463	0.503	0.495	0.389

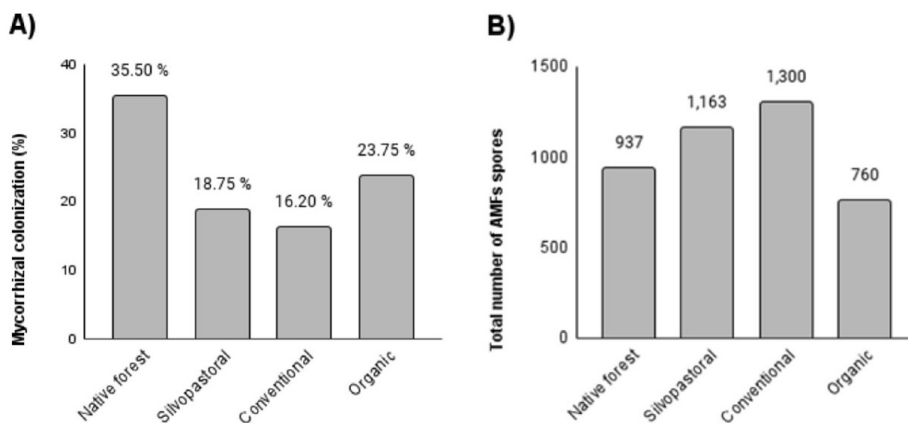


Figure 1. A) Mycorrhizal colonization; B) Total number of arbuscular mycorrhizal fungi spores in native forest area and in yerba mate production systems: Silvopastoral, conventional, and organic. Comparison of means by Tukey's test at 5% probability of error.

uniformity of species with basically the same ones found in all areas, showing greater affinity for these in the yerba mate mycorrhization.

The results obtained for the Shannon-Weaver equivalent species (SH') were 2.63 species, on average; it was higher

in silvopastoral systems (2.84) and lower in the organic system (2.27) (Table 3). The Pielou's evenness index showed low uniformity, ranging from 0.39 to 0.50, and being higher in the silvopastoral and conventional systems and lower in the organic system (Table 3).

4. Discussion

4.1. Determining the area attributes

Phosphorus content for clayey soils, such as those in this study, can be classified from 0 (Zero) to 4 as low, from 4.1 to 8 as medium, and greater than 8 mg.dm⁻³ as high (Sobral et al., 2015). Therefore, the soils of the native forest and organic systems were classified with low levels of phosphorus, the silvopastoral cultivation system with medium levels of phosphorus, and the conventional system with high levels of phosphorus (Table 1).

The phosphorus (P) content in the soil is considered the most important in relation to the symbiosis between the yerba mate crop and the arbuscular mycorrhizal fungi. The phosphorus concentration available in the soil has an effect on the symbiosis, with a reduction in mycorrhization at high concentrations of P and its stimulation in environments lacking this nutrient (Moreira and Siqueira, 2006). Thus, the greater the availability of this nutrient, the less the plant is encouraged to establish symbiosis.

However, the P content in the soil must be in accordance with the crop needs, because if the concentration in the soil is too low, there will be no stimulus to the growth of the yerba mate roots and, therefore, the mycorrhization will be lower. However, if it is high, the photosynthetic rate will increase and the translocation of sucrose to the roots will be greater, consequently inhibiting the germination of mycorrhizal fungi spores (Siqueira et al., 2010).

Potassium is a macronutrient responsible for water absorption, regulation of nutrient translocation, it participates in carbohydrate transport and storage, and also contributes to increased nitrogen absorption and protein synthesis in plants (Taiz et al., 2017). Furthermore, when analyzing mycorrhizal colonization in plant communities in Cerrado soils, Correia et al. (2004) showed a positive correlation with potassium levels in the soil. Arbuscular mycorrhizal fungi (AMF) contribute to increase the efficiency in the transport and absorption of nutrients in medium to low fertility soils, especially those with low mobility in the soil, such as P, Zn, and Cu (Oliveira et al., 1999; Taiz et al., 2017).

4.2. Classification and density of spores

The result found in the evaluation of the spores, where the abundance of the *Acaulospora* genus was verified corroborate those found by Gaiad and Lopes (1986), in which they demonstrated an abundant association of AMF fungi in the rhizosphere of yerba mate seedlings, with a predominance of the *Acaulospora* and *Glomus* genera such association with vascular plant seedlings provides a greater increase in their biomass and quality (Souza et al., 2023). In studies with forage peanut (*Arachis pintoi* cv. Belmonte) developed by Miranda et al. (2010), colonization by AMF was observed, with the genera *Glomus*, *Acaulospora*, and *Scutelospora* being the most representative.

The association of yerba mate with arbuscular mycorrhizal fungi is fundamental for the plants' maintenance in the productive environment, due to the increase in plant development provided by the symbiosis,

as well as the photosynthetic process and product quality are directly related to the diversity of mycorrhizal fungi in the soil (Tomazelli et al., 2022).

The variations found in the number of spores in the yerba mate cultivation systems can be explained by the phosphorus availability in each system. Thus, in the organic system a very low value (2.9 mg/L) is observed, which may have hindered the symbiosis and the AMF establishment (Siqueira et al., 2010). However, in the conventional and silvopastoral systems, there was greater availability of P and total number of AMF spores, which is in agreement with what was verified by Mattei (2013), but with an inverse relationship in the root colonization. Regarding mycorrhizal colonization in cropping systems, the use of AMF host grasses in integration with commercial crops can be a strategy, as it also ends up increasing colonization in commercial crop (Lammel et al., 2015).

Despite the phosphorus availability being low in the native forest yerba mate cultivation system (3.8 mg/L), this presence was enough for the plant to emit a signal for the occurrence of mycorrhization by *Acaulospora delicata*, *A. tuberculata* and *Glomus ambisporum*, Studies have already demonstrated that the association between plants and mycorrhizal fungi occurs differently and in different ecological conditions, and is normally negatively influenced by the presence of phosphorus. Thus, in soils poor in P, the use of AMFs becomes an excellent alternative, as it allows the plant an adequate development, even in adverse conditions (Mitra et al., 2022). Plants are able to develop symbiosis with mycorrhizae and be colonized by different rates, and there may be a preference for a particular species (Smith et al., 2011).

Mattei (2013) also found a predominance of intraradical vesicles and hyphae in native Myrtaceae. The native forest and organic systems showed medium root colonization, while the silvopastoral and conventional systems can be classified as low (Carneiro et al., 1998). However, the percentages of mycorrhizal colonization should not be used as the only parameter or efficiency guarantee, as certain AMF species can be highly infective, but inefficient in providing nutrients to the plant, not favoring its growth (Carvalho et al., 2022). On the other hand, efficient mycorrhizae increase the root absorption area of the plant, contributing to the absorption of nutrients, especially those with low mobility in the soil (Smith and Read, 2008; Berude et al., 2015), as well as plant tolerance to environmental stresses (Tang et al., 2009).

The silvopastoral and organic systems are characterized by the presence of grasses between the yerba mate intrarows, and this may have influenced the greater number of spores found. This is because these plants have a fasciculate and abundant root system, which allows an increase in sporulation in the soil due to the greater contact between the roots and the AMF propagules, with a great ability to provide photosynthates to the fungus (Daniels-Hetrick and Bloom, 1986).

The results showed greater root colonization (Figure 1) the lower the phosphorus content in the soil (Table 1). These results corroborate those found in the literature, in which low levels of phosphorus in the soil stimulate the

plant to form mycorrhiza, resulting in a higher colonization percentage (Moreira and Siqueira, 2006).

4.3. Diversity indices

The Shannon-Weaver diversity index (H'), which, despite considering the same weight for rare and abundant species, takes into account the sampling performed and has inference on the population under study (Magurran, 1988), it was higher in silvopastoral, conventional, and native forest systems. Therefore, the yerba mate production systems analyzed in this study maintained the fauna diversity compared to the native forest, corroborating the study performed by Marques et al. (2019), who concluded that the yerba mate activity is of great importance for the socio-environmental conservation.

The Shannon-Weaver equivalent species (SH') was affected by the predominance of species *Acaulospora colombiana*, *A. delicata*, and *A. tuberculata* (Table 2), in practice, indicating that if all species had the same abundance, it would be expected to find 2.84 species (three species) in the silvopastoral system, while in the organic system, something around two species (2,27).

The low uniformity showed for Pielou's evenness index can be explained by the large amount of other unidentified spores (Table 2). These low values found demonstrate the ecological dominance of a few species that predominate in the community (Scolforo et al., 2008). With Pielou's evenness indices similar to those from the present study, Santos et al. (2018), with the purpose of evaluating the abundance and diversity of AMF in Dry Forest ecosystems in the north of Minas Gerais state, in pasture areas and different succession stages (early; intermediate, and late), obtained Pielou's evenness indexes reducing from 0.79 in pasture areas to 0.52 in late succession.

5. Conclusions

The presence of AMF spores showed a direct relationship with the phosphorus (P) availability in each treatment, with a count reduction in the organic system, with P content lower than 3 mg kg⁻¹ of soil.

The species with the highest predominance were those from the family Acaulosporaceae (*Acaulospora colombiana*, *A. delicata*, and *A. tuberculata*), followed by the Glomaceae (*Glomus ambisporum* and *Glomus pansihalos*) in the conventional and silvopastoral systems

The silvopastoral and conventional systems showed a higher total number of spores and higher indices of Shannon-Weaver diversity (H') and Pielou's evenness, demonstrating greater diversity and consequently greater species richness and uniformity.

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