ARBUSCULAR MYCORRHIZAL FUNGAL COMMUNITIES IN PASTURE AND TROPICAL RIPARIAN FOREST ECOSYSTEMS IN GUAJARÁ-MIRIM, RONDÔNIA, BRAZIL

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Resumo

Comunidades de fungos micorrízicos arbusculares em ecossistemas de pastagem e floresta tropical ripária no município de Guajará-Mirim/RO. Compreender a dinâmica dos organismos do solo nos ecossistemas naturais pode ser uma ferramenta muito útil para determinar a qualidade dos ecossistemas florestais e agrícolas no estado de Rondônia. A hipótese deste trabalho é que a comunidade de fungos micorrízicos arbusculares (FMA) varia em função do sistema de uso do solo e da sazonalidade. O trabalho objetivou avaliar a composição e diversidade da comunidade de FMA em ecossistemas de pastagens e floresta tropical ripária em regeneração natural, no município de Guajará-Mirim/RO. Coletou-se, aleatoriamente, 10 amostras simples de solo, em cada um dos ecossistemas, em três épocas do ano (seca/2018; chuvosa/2018; e seca/2019). Foram avaliadas a riqueza de espécies de FMA e densidade dos esporos; a frequência de ocorrência de espécies; os índices de diversidade de Shannon-Wiener (H') e dominância de Simpson; e o número de amostra ideal. A riqueza de espécies de FMA variou entre as áreas e as estações, com tendência à maior riqueza no pasto, na estação seca/2018. As espécies dominantes de FMA foram *Glomus macrocarpum* e *Acaulospora mellea* nos dois ecossistemas; A pastagem, na estação seca/2018, apresentou maior diversidade de espécies (H') de FMA comparado a floresta tropical ripária na estação chuvosa/2018 e na estação seca/2019; a pastagem na estação seca/2019 diferiu em 100% da floresta tropical ripária, na composição da comunidade de espécies de FMA; são necessárias mais de 10 amostras simples para identificar um número maior de espécies de FMA em áreas de pastagem e floresta tropical ripária, no município de Guajará-Mirim/RO.
Palavras-chave: diversidade de espécies**;** ecossistema ripário; qualidade do solo.

Abstract

Understanding the dynamics of soil organisms in natural environments can be very useful for determining the quality of forest and agricultural ecosystems. The hypothesis of this work is that the FMA community varies depending on the land use system and seasonality. This study aimed to assess arbuscular mycorrhizal fungal (AMF) communities in pasture and regenerating riparian forest (Forest) sites in Guajará-Mirim, Rondônia, Brazil. Ten soil samples were randomly collected from each ecosystem in three seasons (2018 dry and rainy seasons and 2019 dry season). AMF species richness (SR), frequency, spore density, Shannon–Wiener (H') diversity index, Simpson's dominance index, and ideal sample size were determined. Species richness varied among sites and seasons, with a tendency toward higher richness in pasture soil in the 2018 dry season. The dominant AMF species in both ecosystems were *Glomus macrocarpum* and *Acaulospora mellea*. The pasture site in the 2018 dry season had higher AMF diversity (H') than the riparian forest in the 2018 rainy season and the 2019 dry season. The complete linkage distance between the pasture site in the 2019 dry season and all other sites/seasons was 100%. More than 10 soil samples are needed to accurately determine the richness of AMF species in pasture and riparian forest sites in Guajará-Mirim, Brazil.
Keywords: species diversity; riparian ecosystem; soil quality.

INTRODUCTION

Information on the dynamics of soil organisms in natural environments can be very useful for determining the quality of forest and agricultural ecosystems. Equilibrium between plant and soil microbial communities, including arbuscular mycorrhizal fungi (AMF), is necessary for maintaining soil fertility, organic matter decomposition, nutrient cycling and other biogeochemical processes. AMF contribute greatly to plant growth and development (CHEN *et al.*, 2018). These microorganisms help crops absorb nutrients, especially in low-P soils (CHEN *et al.*, 2018; KUMAR *et al.*, 2019), which are very common in Brazil. Further benefits include enhanced nutrient cycling and soil aggregate stability; AMF are important biotic factors for sustainable soil management

(CHEN *et al.*, 2018). These symbiotic fungi also contribute to forest succession, as they promote the establishment of mid- and late-successional species, paving the way for the return of climax communities of plants, animals and fungi (SOUSA *et al.*, 2014). These benefits help maintain plant biodiversity and ecosystem functions.

It is essential to understand the effects of riparian forest and agricultural species on AMF populations and vice versa (BRAGHIROLLI *et al.*, 2012). Various studies (SILVA *et al.*, 2016; WINAGRASKI *et al.*, 2019) have investigated the characteristics of AMF communities in different ecosystems, but controversial results were obtained. Cristo *et al.* (2018) found that the conversion of an Atlantic forest fragment to pasture reduced AMF species richness, suggesting that deforestation affected the diversity of AMF communities. Silva *et al.* (2016), on the other hand, observed that AMF species richness was not significantly altered by pasture establishment.

The dynamics of AMF communities may indicate which factors induce or inhibit processes associated with soil organic matter cycling and plant nutrition. For this analysis, it is necessary to first identify the plant and AMF species that occur in a given ecosystem. The hypothesis of this work is that the FMA community varies depending on the land use system and seasonality. This study aimed to investigate the relationship between land use, seasonality and AMF communities. AMF species richness, diversity, and spore density were determined in a riparian forest undergoing natural regeneration and a pasture site along the Madeira-Mamoré River, Guajará-Mirim, Rondônia, Brazil.

MATERIAL AND METHODS

The study was conducted in two sites located about 1000 m apart on the banks of the Madeira-Mamoré river in Guajará-Mirim, Rondônia, Brazil. The local climate is classified by the Köppen–Geiger system as tropical wet and dry (Aw), with most rainfall occurring between October and April and the lowest amounts (10 to 20 mm) between June and August. The average annual temperature is 26.6 °C and the average annual precipitation 1488 mm. The soil is dominated by sandy and clayey sediments. One site is a pasture on a private property on the city outskirts. The area was covered by tropical riparian vegetation until the 1990s, when deforestation began for cattle production. The other site is a tropical riparian forest fragment allowed to undergo natural regeneration since 1998. Table 1 summarizes the most abundant vegetation in each locality.

Table 1.   Floristic composition of pasture and regenerating forest sites located on the banks of the Madeira-Mamoré river in Guajará-Mirim, Rondônia, Brazil.
Tabela 1.   Composição florística das áreas de pastagem e floresta tropical ripária em regeneração natural ao longo do rio Madeira-Mamoré, no entorno da cidade de Guajará-Mirim/RO.

Site	Floristic information
Pasture	This area was occupied by "Juquira" 10 years ago. An area of 13 ha was plowed and planted with a mixture of grasses. Currently, it supports different types of livestock (horses, goats, cattle, and chickens). Vegetation is dominated by the genera *Genipa*, *Cariniana*, *Ficus*, *Chrysalidocarpus*, *Syagrus*, *Panicum*, *Brachiaria*, *Machaerium*, *Ziziphus*, *Handroanthus*, *Melissa*, *Struthanthus*, *Jacaranda*, *Pyrenoglyphis*, *Saccharum*, and *Swartzia*.
Regenerating tropical riparian forest	The area was isolated more than 20 years ago in an effort to promote natural regeneration. At the time, there was little understory vegetation. Currently, the most abundant genera are *Cordia*, *Syagrus*, *Clusia*, *Rollinia*, *Philodendron*, *Capsicum*, *Inga*, *Pinus*, *Schizolobium*, *Vanilla*, *Haworthia*, *Handroanthus*, *Astrocaryum*, *Cedrela*, *Monstera*, and *Theobroma*.

Sampling procedure

Soil samples (500 mL) were collected close to plant roots at depths of 0 to 20 cm in September 2018 (end of the dry season), December 2018 (rainy season), and May 2019 (beginning of the dry season). Ten samples were collected from randomly chosen points in each site. Then, samples were allowed to dry in the shade and divided into two parts, one of which was sent to the soil laboratory of the Federal Rural University of Rio de Janeiro for chemical and physical analysis (Table 2) (DONAGEMMA *et al.*, 2011) and the other was packed in plastic bags and stored at 10 °C until microbial analysis.

Table 2.   Chemical and physical attributes of soils under pasture and regenerating tropical riparian forest on the banks of the Madeira-Mamoré river in Guajará-Mirim, Rondônia, Brazil in the year 2018.

Tabela 2. Análises químicas e físicas das amostras de solos das áreas de pasto e de floresta tropical ripária em regeneração natural, nas margens do rio Madeira-Mamoré, município de Guajará-Mirim/RO, ano de 2018.

Site	pH	P	Ca	Mg	Na	K	H + Al
		mg kg^{-1}	----------------------------cmol$_c$ dm^{-3} ----------------------------				
Pasture	6.54	47.50	8.50	3.00	0.004	0.03	1.70
Forest	5.68	3.30	1.70	4.50	0.003	0.008	3.40
Site	SB	ECEC	BS	TOC	Clay	Sand	Silt
			%	g kg^{-1}		%	
Pasture	11.53	13.23	87.00	19.7	4.1	60.40	35.50
Forest	6.21	9.61	65.00	22.31	3.0	65.60	31.40

Analyses were conducted in 2018. SB, sum of bases; ECEC, effective cation exchange capacity; BS, base saturation; TOC, total organic carbon.

Characterization of AMF communities

Fungal spores were extracted from soil samples (100 mL) by the wet-sieving method (GERDEMANN; NICOLSON, 1963) followed by centrifugation in 50% (v/v) sucrose solution. An aliquot of the spore extract was transferred to a Petri dish for identification. Spores were grouped by size, color and shape. Selected spores from each group were mounted on microscope slides in polyvinyl alcohol/lactic acid/glycerol (PVLG) and crushed gently with a coverslip to expose the inner walls. Spores from the same group were mounted in a 1:1 (v/v) mixture of PVLG and Melzer reagent under another coverslip. Color results and morphological characteristics were used for fungal identification. AMF species were identified by comparison with descriptions from the International Culture Collection of (Vesicular) Arbuscular Mycorrhizal Fungi (http://invam.caf.wvu.edu) and Schenck and Perez (1988). Total spore counts and spore counts by species were determined by light microscopy with oil immersion.

Total spore density was calculated as the number of AMF spores per 100 mL of soil, and AMF species density (D_i) as the number of AMF spores of a given species per 100 mL of soil. The frequency of occurrence of each species (F_i) was calculated for each sampling period (dry and wet seasons) by the equation $F_i = J_i/K \times 100$, where J_i is the number of samples in which species i occurs and K is the total number of samples. Total species richness (S) was calculated as the total number of species identified in 100 mL of soil. Species dominance was classified into four categories based on frequency values (ZHANG et al., 2004): $F_i > 50\%$, dominant; $30\% < F_i \leq 50\%$, very common; $10\% < F_i \leq 30\%$, common; and $F_i \leq 10\%$, rare. The Shannon–Wiener diversity index (H') was calculated by the equation $H' = -\sum[(X_i/X_o) \times \log(X_i/X_o)]$, where X_i is the spore density of species i and X_o is the total spore density. Simpson's dominance index (C) was calculated as $C = S(X_i/X_o)^2$.

Species accumulation curves were constructed by plotting the cumulative number of species identified in each site on the y-axis and the cumulative number of samples on the x-axis (SCHILLING; BATISTA, 2008). These plots provide valuable information on the minimum sampling effort required to adequately represent fungal communities.

Spore density, diversity, and dominance data were subjected to the nonparametric Kruskal–Wallis test ($P < 0.05$) using Assistat version 7.7 (SILVA; AZEVEDO, 2016). Spore density data were subjected to complete linkage clustering with Pearson's distance ($1 - r$) as a metric to identify similarities and differences between sites and seasons. Cluster analysis was performed using Statistic version 8.0.

RESULTS

AMF community composition

In total, 40 AMF taxa were identified in pasture and forest sites, including 7 families (Acaulosporaceae, Ambisporaceae, Archaeosporaceae, Dentiscutataceae, Gigasporaceae, Glomeraceae, and Paraglomeraceae) and 12 genera (Table 3). Of the total taxa recorded, 33 (83%) were identified at the species level, 7 (17%) at the genus level, and 1 could not be identified. Considering all sites and seasons, it was found that the majority of AMF species belonged to the genus *Acaulospora* (11, 27.5%), followed by *Glomus* (7, 17.5%), *Ambispora* (5, 12.5%), *Scutellospora* (4, 10%), *Rhizophagus* (3, 7.5%), *Funneliformis* (2, 5%), and *Gigaspora* (2, 5%). *Appendicispora*, *Fuscutata*, *Racocetra*, and *Paraglomus* were represented by a single species each.

Table 3. Mean spore density (SD), frequency of occurrence (FO), and dominance level (D) of arbuscular mycorrhizal fungal species found in pasture (P) and regenerating tropical riparian forest (F) soils in September 2018 (late dry season), December 2018 (rainy season), and May 2019 (early dry season) in Guajará-Mirim, Rondônia, Brazil.

Tabela 3. Densidade média de esporos, frequência de ocorrência (FO%) e grau de dominância das espécies de FMAs em amostras de solos coletadas em setembro/2018 (final da época seca), dezembro/2018 (época chuvosa) e maio/2019 (início da época seca) nos ecossistemas de pastagem (P) e floresta tropical ripária (F), no município de Guajará-Mirim/RO.

Family/species	SD P	SD F	FO(D)	SD P	SD F	FO(D)	SD P	SD F	FO(D)
	September 2018			December 2018			May 2019		
Acaulosporaceae									
Acaulospora bireticulata F.M. Rothwell & Trappe	8	-	5(R)	-	-	-	-	-	-
Acaulospora denticulata Trappe & Gerd. (1974)	8	-	5(R)	-	-	-	-	-	-
Acaulospora excavata Ingleby & C. Walker (1994)	-	-	-	-	3	5(R)	-	-	-
Acaulospora foveata Trappe & Janos (1982)	7	-	10(R)	-	3	5(R)	-	-	-
Acaulospora koskei Błaszk. (1995)	-	-	-	-	-	-	-	29	10(R)
Acaulospora mellea Spain & N.C. Schenck (1984)	61	102	65(D)	124	123	45(D)	10	125	10(R)
Acaulospora morrowiae Spain & N. Schenck (1984) [as 'morrowae']	21	-	15(C)	6	3	10(R)	10	-	10(R)
Acaulospora rehmii Sieverd. & S. Toro (1987)	-	57	5(R)	-	-	-	-	-	-
Acaulospora scrobiculata Trappe (1977)	19	25	25(C)	86	-	15(C)	87	64	25(C)
Acaulospora sp1	-	51	15(C)	12	5	10(R)	-	-	-
Acaulospora tuberculata Janos & Trappe (1982)	35	-	20(C)	13	-	15(C)	85	-	15(C)
Kuklospora colombiana (Spain & N.C. Schenck) Kaonongbua, J.B. Morton & Bever (2010)	14	61	30(C)	48	21	25(C)	85	28	30(C)
Ambisporaceae									
Ambispora brasiliensis (B.T. Goto, L.C. Maia & Oehl) C. Walker, M. Krüger & A. Schüßler (2011)	10	-	10(R)	-	-	-	-	-	-
Ambispora callosa (Sieverd.) C. Walker, Vestberg & Schüßler	25	-	10(R)	38	-	15(C)	-	-	-
Ambispora granatensis J. Palenzuela, N. Ferrol & Oehl (2011)	103	25	15(C)	18	7	10(R)	-	-	-
Ambispora leptoticha C. Walker, Vestberg & Schüßler (2007)	-	-	-	37	-	15(C)	167	-	15(C)
Ambispora reticulata Oehl & Sieverd (2012)	-	29	5(R)	-	3	5(R)	-	-	-
Archaeosporaceae									
Appendicispora jimgerdemanni Spain, Oehl & Sieverd. 2006	8	-	5(R)	-	-	-	-	-	-
Dentiscutataceae									
Fuscutata heterogama Oehl, F.A. Souza, L.C. Maia & Sieverd. (2008)	14	23	20(C)	117	-	30(C)	383	5	45(VC)
Gigasporaceae									
Gigaspora decipiens I.R. Hall & L.K. Abbott (1984)	-	72	20(C)	-	-	-	17	8	15(C)
Gigaspora rosea T.H. Nicolson & N.C. Schenck (1979)	-	20	5(R)	-	-	-	-	-	-
Racocetra verrucosa Oehl, F.A. Souza & Sieverd. (2008)	-	85	15(C)	7	-	10(R)	28	58	15(C)
Scutellospora calospora C. Walker & F.E. Sanders (1986)	8	5	15(C)	30	19	25(C)	42	-	5(R)
Scutellospora cerradensis Spain & J. Miranda (1996)	5	-	5(R)	85	-	10(R)	9	-	10(R)
Scutellospora sp1	-	-	-	-	-	-	21	-	5(R)

Scutellospora weresubiae Oehl, F.A. Souza & Sieverd. (2008)	9	-	10(R)	9	-	5(R)	-	-	-
Glomeraceae									
Funneliformis caledonium (T.H. Nicolson & Gerd.) C. Walker & A. Schüßler (2010)	2	-	5(R)	-	-	-	-	-	-
Funneliformis geosporum T.H. Nicolson & Gerd.) C. Walker & A. Schüßler	83	-	25(C)	11	3	15(C)	10	12	15(C)
Glomus formosanum C.G. Wu & Z.C. Chen (1986)	5	-	5(R)	2	-	5(R)	-	-	-
Glomus heterosporum G.S. Sm. & N.C. Schenck (1985)	19	-	10(R)	-	-	-	-	-	-
Glomus macrocarpum Tul. & C. Tul. (1845)	159	437	85(D)	614	717	95(D)	1081	391	70(D)
Glomus sp1	-	-	-	-	-	-	104	40	35(VC)
Glomus sp2	29	32	35(VC)	18	16	20(VC)	17	62	20(C)
Glomus sp3	38	-	5(R)	-	-	-	-	-	-
Glomus sp4	-	-	-	-	3	5(R)	-	-	-
Rhizoglomus microaggregatum Sieverd., G.A. Silva & Oehl (2014)	118	14	30(C)	264	13	35(VC)	475	328	40(VC)
Rhizophagus clarus C. Walker & A. Schüßler (2010)	44	-	25(C)	205	-	25(C)	91	-	15(C)
Rhizophagus diaphanus C. Walker & A. Schüßler (2010)	11	7	15(C)	-	9	5(R)	70	19	25C)
Paraglomeraceae									
Paraglomus brasilianum J.B. Morton & D. Redecker (2001)	47	-	30(C)	4	-	5(R)	-	-	-
Unidentified species	35	104	35(VC)	39	44	50(D)	395	97	40(VC)
Species richness	28	17	34	22	16	27	20	14	21

R, rare; C, common; VC, very common; D, dominant.

Species richness varied among seasons and sites (Table 3). Thirty-four species occurred in the 2018 dry season, 27 in the 2018 rainy season, and 21 in the 2019 dry season. The number of AMF species recorded in the 2018 dry season was greater than that recorded in the 2018 rainy season, mainly in pasture soil. In total, 34 species were found in pasture soil, 28 in the 2018 dry season, 22 in the 2018 rainy season, and 20 in the 2019 dry season. Only 23 species were collected from forest soil, 17 in the 2018 dry season, 16 in the 2018 rainy season, and 14 in the 2019 dry season. Species richness also varied between early and late dry seasons, with 24 species recorded in September 2018 and 21 in May 2019.

The most frequently occurring species in all sites and seasons was *Glomus macrocarpum*, followed by *Acaulospora mellea* (Table 3). These AMF fungi were dominant in at least two of the three seasons analyzed, whereas most other species were rare or common.

Species diversity

Species diversity was significantly higher in the pasture site in the 2018 dry season than in other sites/seasons (Figure 1). Simpson's dominance index was higher in the riparian forest site in the 2018 rainy season, when the Shannon–Wiener diversity index was low, indicating low species evenness.
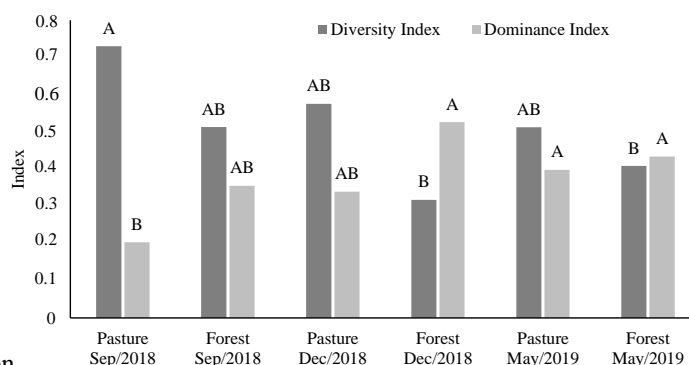

Figure 1. Shannon–Wiener diversity index and Simpson's dominance index of arbuscular mycorrhizal fungal species occurring in pasture and regenerating tropical riparian forest soils in September 2018 (late dry season), December 2018 (rainy

season), and May 2019 (early dry season) in Guajará-Mirim, Rondônia, Brazil. Different letters above bars indicate significant differences (Kruskal–Wallis test, $P < 0.05$).

Figura 1. Índices de diversidade de Shannon-Wiener e de dominância de Simpson de FMAs, em amostras de solos coletadas em setembro/2018 (final da época seca), dezembro/2018 (época chuvosa) e maio/2019 (início da época seca) nos ecossistemas de pastagem (pasto) e floresta tropical ripária (floresta), no município de Guajará-Mirim/RO. (Barras seguidas de mesma letra não diferem entre si nos ecossistemas nas três épocas de amostragens pelo teste de Kruskal-Wallis a 5% de probabilidade).

Complete linkage clustering of sites and seasons showed that the AMF community in the pasture site in the 2019 dry season was separated from other sites/seasons by a linkage distance of 1 (Figure 2). The other clusters were separated by distances lower than 0.5.
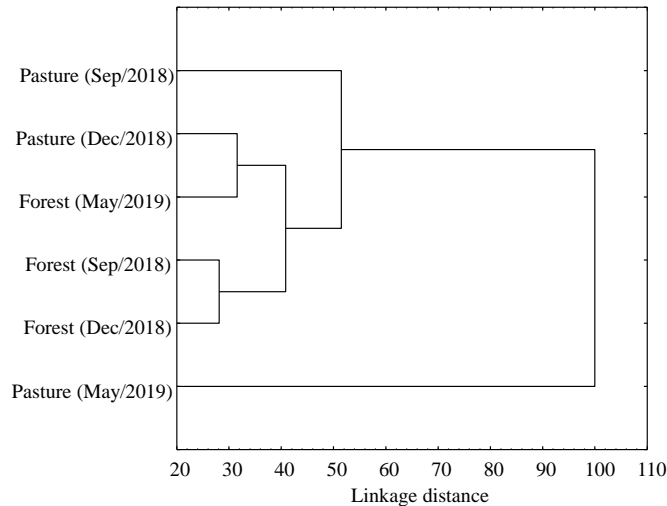

Figure 2. Complete linkage clustering of arbuscular mycorrhizal fungal communities in pasture and regenerating tropical riparian forest soils in September 2018 (late dry season), December 2018 (rainy season), and May 2019 (early dry season) in Guajará-Mirim, Rondônia, Brazil.

Figura 2. Análise de agrupamento das comunidades de FMAs de amostras de solo coletadas em setembro/2018 (final da época seca), dezembro/2018 (época chuvosa) e maio/2019 (início da época seca) nas áreas de pastagem (pasto) e floresta tropical ripária (floresta), no município de Guajará-Mirim/RO.

AMF spore density

The mean spore density of AMF did not differ significantly among sites and seasons ($P > 0.05$) (Figure 3). However, we observed a trend toward an increase in sporulation from the 2018 rainy season to the 2019 dry season. In these seasons, spore density tended to be higher in pasture than in forest soil.
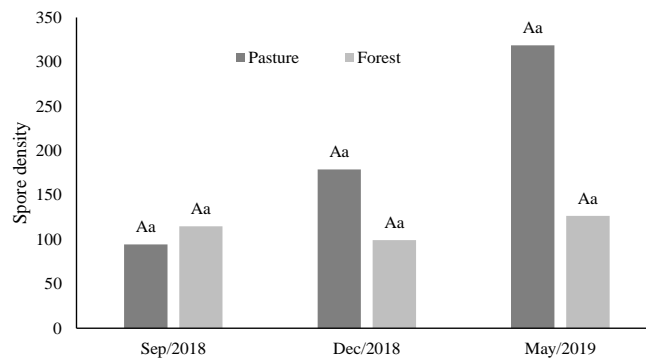

Figure 3. Mean density of arbuscular mycorrhizal fungal spores in pasture and regenerating tropical riparian forest soils in September 2018 (late dry season), December 2018 (rainy season), and May 2019 (early dry season) in Guajará-Mirim, Rondônia, Brazil. Different lowercase letters above bars indicate significant

differences between sites, and different uppercase letters indicate significant differences between seasons (Kruskal–Wallis test, $P < 0.05$).

Figura 3. Densidade média dos esporos de FMAs em amostras de solos coletadas em setembro/2018 (final da época seca), dezembro/2018 (época chuvosa) e maio/2019 (início da época seca) nas áreas de pastagem (pasto) e floresta tropical ripária (floresta), no município de Guajará-Mirim/RO. (Barras seguidas de mesma letra não diferem entre si nas áreas e três épocas de amostragens pelo teste de Kruskal-Wallis a 5% de probabilidade. Letras minúsculas comparam as áreas dentro de cada época, e letras maiúsculas comparam as épocas dentro de cada área)

Species accumulation curve

The species accumulation curve for the pasture site in the 2019 dry season reached a plateau with nine samples; on the other hand, 10 samples were not sufficient to reach a plateau in the 2018 dry and rainy seasons (Figure 1A). The species accumulation curves for the forest site did not reach a plateau, indicating that more than 10 samples are needed to accurately determine AMF species richness in tropical riparian forest soils, both in rainy and dry seasons (Figure 4B).
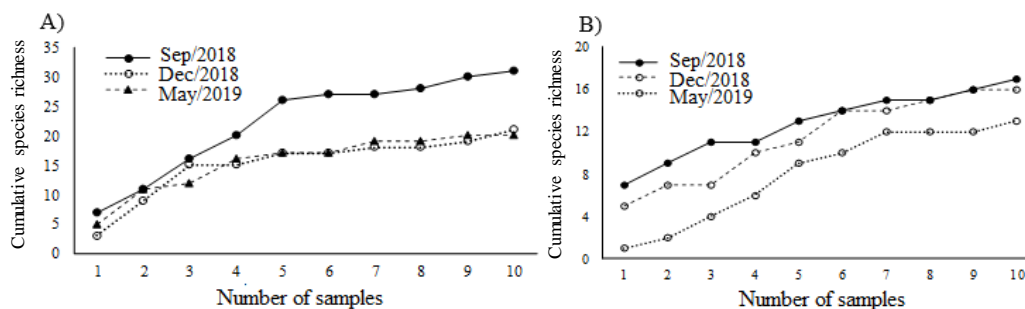

Figure 4. Species accumulation curve showing the number of arbuscular mycorrhizal fungal species plotted against the number of samples collected from pasture (A) and regenerating tropical riparian forest (B) soils in September 2018 (late dry season), December 2018 (rainy season), and May 2019 (early dry season) in Guajará-Mirim, Rondônia, Brazil.

Figura 4. Curva espécie-número de amostras de solos e número cumulativo de espécies de FMAs no ecossistema de pastagem (A) e floresta tropical ripária em regeneração natural (B) ao longo do Rio Madeira-Mamoré, localizado no município de Guajará-Mirim/RO, em três épocas (setembro/2018-final da época seca; dezembro/2018 - época chuvosa; e maio/2019 - início da época seca).

DISCUSSION

The number (forty) of AMF taxa found in this study was next to observed by Stürmer and Siqueira (2011) in a pasture and secondary forest (42 taxa) and lower than that observed by Caproni *et al.* (2003) in reforestation sites recovering from bauxite mining (57 taxa), both in the Amazon region.

AMF communities were dominated by *Glomus* and *Acaulospora*, evidence of the high adaptability and tolerance of these genera. Similar results were observed in several Brazilian (CRISTO *et al.*, 2018) and international (BI *et al.*, 2020) ecosystems. According to Pereira *et al.* (2014), the high number of species in *Glomus* and *Acaulospora*, added to their dissemination and adaptation capabilities, explains the dominance of these genera in most ecosystems. Miranda *et al.* (2005) identified *Acaulospora* and *Glomus* in different crop rotation and pasture systems in the Cerrado. Camara *et al.* (2016) reported that these two genera had the highest species richness in restinga fragments in Rio de Janeiro, Brazil.

G. macrocarpum and *A. mellea* were observed in all seasons and sites with high frequency and spore density. The former is commonly found with high frequency in Brazilian ecosystems. For instance, Santos *et al.* (2019) identified *G. macrocarpum* in native forest, Cerrado, and pasture soils. Camara *et al.* (2016) and Nogueira *et al.* (2016) identified the species in all samples collected from forest fragments (frequency of 100%). Similarly, *A. mellea* can be found in Cerrado (ASSIS *et al.*, 2014), Atlantic Forest (SILVA *et al.*, 2016), and Amazon (CAPRONI *et al.* 2003) biomes. *G. macrocarpum* and *A. mellea* were found in equal proportions with high frequency in pasture and agricultural sites and secondary forests at different stages of succession in the Médio-Paraíba region, Rio de Janeiro, in a study by Silva *et al.* (2016). The authors found other species (with lower frequencies) that were also observed in the present study, such as *Acaulospora foveata*, *Acaulospora scrobiculata*,

Kuklospora colombiana, and *Archaeospora leptoticha*, which indicates that these species occur in various Brazilian ecosystems.

Some AMF species occurred exclusively in the riparian forest site: *Acaulospora excavata*, *A. koskei*, *A. rehmii*, *A. reticulata*, and *Gigaspora rosea*. Of these, only *A. rehmii* was identified by Winagraski *et al.* (2019) as occurring in the Amazon region. The following species occurred only in the pasture site: *A. bireticulata*, *A. denticulata*, *A. tuberculata*, *A. brasiliensis*, *A. callosa*, *A. leptoticha* and *A. jimgerdemanni*, *F. caledonium*, *G. formosanum*, *G. heterosporum*, *Glomus* sp3, *P. brasilianum*, *R. clarus*, *S. cerradensis*, *Scutellospora* sp1, and *S. weresubiae* (Table 3). The higher number of exclusive species in the pasture site (*n* = 16) may be associated with the high soil pH (6.54) compared with that of riparian forest soil (pH 5.68). Higher soil pH and the presence of nonnative species in the pasture site are factors that contribute to nutrient availability, plant diversity, and soil moisture variations. Pasture soil had higher P, Ca, Na, K, sum of bases, base saturation, clay, and silt contents than forest soil (Table 2). However, Mg, H + Al, total organic carbon, and sand contents were higher in riparian soil (Table 2). Boerave *et al.* (2019) observed that AMF species richness increased with increasing soil pH, as found in the present study.

Some species occurred in both sites in all seasons, such as *A. foveata*, *A. mellea*, *A. morrowiae*, *A. scrobiculata*, *A.* sp1, *Ambispora granatensis*, *Funneliformis geosporum*, *Fuscutata heterogama*, *Gigaspora decipiens*, *Glomus macrocarpum*, *Glomus* sp1, *Glomus* sp2, *Kuklospora colombiana*, *Racocetra verrucosa*, *Rhizoglomus microaggregatum*, *Rhizophagus diaphanus*, and *Scutellospora calospora* (Table 3). Winagraski *et al.* (2019) argued that AMF species are selected by the environmental conditions under which the host develops. Some species are rare in forest ecosystems. Those that are ubiquitous could be used as inoculants for forest species.

Acaulospora sp1, *Glomus* sp1, *Glomus* sp2, *Glomus* sp3, *Glomus* sp4, and *Scutellospora* sp1 were identified only at the genus level, and probably, constitute species not yet described; more studies in the evaluated areas they can even provide the registration of species not yet known to science.

AMF species richness and Shannon–Wiener diversity varied among sites and seasons. There was a trend toward higher richness and diversity in the pasture site, especially in the 2018 dry season. The high Simpson's dominance and low Shannon–Wiener diversity indices observed in the forest site indicate low species evenness as a result of the dominance of *G. macrocarpum* and *A. mellea*. Boerave *et al.* (2019) stated that abiotic factors, such as climate, seasonality, geographical region, and land use and management may have greater influence over AMF communities than vegetation. In contrast, Pereira *et al.* (2014) argued that plant cover is one of the most important factors determining the occurrence of AMF species. In the current study, plant cover, soil moisture, and pH seemed to be determinant factors of AMF species richness and diversity.

The linkage distance of 1 between pasture in the 2019 dry season and other sites/seasons may be related to high sporulation of *Glomus macrocarpum*, *A. scrobiculata*, *A. tuberculata*, *A. leptoticha*, *F. heterogama*, *Glomus* sp1, *K. colombiana*, *R. microaggregatum*, *R. diaphanus*, *Scutellospora* sp1, and the unidentified species. In the study of Santos *et al.* (2019), pasture and native forest ecosystems had similar characteristics and were grouped together, attributed to the similar Shannon–Wiener indices. In contrast, in the current study, the Shannon–Wiener index did not support the differences observed in AMF populations between ecosystems.

The lack of significant differences in spore density between sites and seasons may be due to the high data variability. In the pasture site, spore density was 90% higher in the 2018 rainy season and 240% higher in the 2019 dry season in relation to the 2018 dry season. Furthermore, spore density was 80% higher in the 2019 dry season than in the 2018 rainy season. In the 2018 rainy season and 2019 dry season, the parameter was 80 and 150% higher in the pasture site than in the riparian forest, respectively. The higher values in the pasture site in the rainy season and early dry season (2019) can be attributed to the high seed germination of invasive plants. Seeds remain dormant in the dry season and then germinate, forming new roots and increasing symbiotic associations with AMF. The pasture site was dominated by *Brachiaria decumbens* Sptapf. throughout the year and had low livestock density, which can also contribute to plant diversity. These results are in line with other studies showing that plant cover may influence AMF sporulation (PEREIRA *et al.*, 2014; BI *et al.*, 2020). Silva *et al.* (2016) found more AMF spores in a pasture site than in an Atlantic Forest site.

AMF spore density was probably more affected by soil fertility and vegetation than by soil texture (Table 2). Soils with sandy texture, as those evaluated in this study, are characterized by excessive drainage. Pasture soils generally have higher drainage, evaporation, evapotranspiration, and density of fine roots than forest soils, thus promoting spore production, despite the high soil fertility.

In the riparian forest site, there was less variation in spore production between sampling times. This site has been regenerating for more than 20 years and has high occurrence of *Scheelea* sp. This palm species is commonly found in wet soils or dry land and may be influencing spore production. According to Carrenho *et al.* (2000), the sporulation pattern of AMF is related to root volume, which is lower in pioneer than in climax species. As natural regeneration progresses, the higher volume of roots in contact with AMF propagules stimulates sporulation. However, climax species seem to depend less on AMF in their initial stages of growth, as discussed

by Carrenho *et al.* (2000); therefore, AMF development in root systems is limited and the percentage of colonization is low, reducing spore production.

In general, species accumulation curves did not reach a plateau, except for the 2019 dry season. This analysis was also applied to natural and managed systems in the Amazon (STÜRMER; SIQUEIRA, 2011) and Atlantic Forest (PEREIRA *et al.*, 2014), and curve stabilization was not observed. It is known that total AMF richness is often underestimated when populations are evaluated by taking into consideration the presence of spores, as many species may colonize root fragments or occur in the soil only in the form of hyphae. Therefore, for greater accuracy, future studies should collect more than 10 soil samples and/or use trap cultures (PEREIRA *et al.*, 2014) to stimulate sporulation of AMF species generally not found in field samples.

In AMF community studies, it is important to determine the ideal sample size for each season so as to collect the highest number of AMF species, as populations may vary with climate change. Santos *et al.* (2019), in assessing AMF species diversity in native forest, pasture, and Cerrado ecosystems in Guajará-Mirim, Brazil, concluded that the ideal sample size in the dry season is greater than 15. In the rainy season, the ideal sample size is greater than 14, 17, and 6 in native forest, pasture, and Cerrado, respectively. However, according to the authors, to collect a high number of species, it is more important to sample many times in a year than to collect various samples in a given season, because sporulation varies greatly throughout the year.

CONCLUSIONS

- A total of 40 AMF taxa were identified in pasture and riparian forest ecosystems.
- *G. macrocarpum* and *A. mellea* were observed in all sites and seasons.
- AMF species richness varied between sites and seasons, with a tendency toward higher richness in the pasture site in the late dry season.
- The Shannon–Wiener diversity index was higher in pasture soil in the late dry season.
- There was a tendency for higher sporulation in the pasture site in the rainy season and early dry season.
- More than 10 soil samples are necessary to adequately determine AMF species richness in pasture and riparian forest ecosystems located on the banks of the Madeira-Mamoré River in Guajará-Mirim, Rondônia, Brazil.

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