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Soil Microbiological Attributes Under Different Vegetation Covers

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Abstract

The aim of the study was to characterize the microbiological activity of the soil and its relationship with soil attributes in a toposequence under different vegetation covers at the UFRRJ Botanical Garden. Soil samples were collected from the surface layer of the shoulder, backslope and footslope. These show little variation in slope, but have different vegetation cover. The shoulder and footslope are covered by tree species from different botanical families, and the middle third by grasses. The tree species (shoulder) and the grasses (backslope) showed higher values of microbial biomass carbon, soil enzyme activity, sporulation and species richness of arbuscular mycorrhizal fungi. The correlations found suggest the effect of chemical and physical attributes, especially the lower levels of P and coarse sand, on the increase in these soil microbiological attributes. Greater deposition of glomalin-related soil protein was observed in areas with tree species, with correlations with pH and TOC.

Keywords: Arbuscular mycorrhizal fungi, enzymatic activity, glomalin, soil microbial biomass, tree and grass species.

1. INTRODUCTION

Soil microbiological attributes are considered to be important ecological indicators in view of their role in various relevant processes in the soil, promoting the maintenance of ecosystem services (Cusset et al., 2024). The use of information related to soil microbiological properties or processes represents an important tool for assessing and interpreting the effects of natural or anthropogenic interference in the soil. Especially since this compartment of the ecosystem is the product of climate and organisms acting on the source material over time. Soil microbial biomass corresponds to the living and most active fraction of soil organic matter, participating in biological and biochemical processes that occur in the soil (Pinto et al., 2019). Soil microbial biomass

carbon is considered an excellent indicator of soil quality, as it is intensely influenced by management and environmental conditions (Bargali, 2024).

Likewise, enzymatic activity has been used as a bioindicator of soil quality due to its relevance in the dynamics of soil organic matter (SOM) and in other processes that occur in the soil, such as nutrient cycling and functional diversity (Liu et al., 2021). This is also due to the fact that most studies show the responsiveness of this attribute - considered an indicator of biochemical functions that occur in the soil - to changes imposed on this environment (Liu et al., 2021; Matos et al., 2020; Petter et al., 2019). Some important enzymes, such as arylsulfatase, β-Glucosidase, dehydrogenase, and those that hydrolyze fluorescein diacetate such as lipase, protease and esterase, are involved in biogeochemical cycles (carbon, nitrogen and sulfur) and, consequently, can reflect changes in the metabolic processes occurring in the soil (Notaro et al., 2018).

In turn, microorganisms such as arbuscular mycorrhizal fungi (AMFs) play a fundamental role in the survival, growth and development of plants (Wattenburger et al., 2020), as well as acting in various soil processes, including the formation of aggregates and the cycling of nutrients and carbon (Kim et al., 2022; Liu et al., 2022). These ecological functions of AMFs are partly due to the production of glomalin, a recalcitrant, insoluble and heat-resistant glycoprotein attached to the wall of AMF hyphae, which is secreted into the soil or released after the decomposition of hyphae and spores (Driver et al., 2005). AMFs and glomalin are good indicators of soil quality, given the roles they play in the soil-plant system and their responsiveness to soil use and management systems (Matos et al., 2022).

The aim of this study was to characterize the microbiological activity of the soil and its relationship with the chemical and physical attributes of the soil in a toposequence under different vegetation covers in the Botanical Garden of the Federal Rural University of Rio de Janeiro.

2. MATERIAL AND METHODS

2.1. Study area

The study was carried out at the Botanical Garden on the campus of the Federal Rural University of Rio de Janeiro, located in Seropédica, RJ. The municipality is situated at the geographical coordinates of south latitude 22°45'32.3" and west longitude 43°41'32.3", under the domain of the Atlantic Forest biome, with a sub-humid tropical climate classified as Aw according to Koppen. The sampling points were distributed in three areas (Figure 1), located in a toposequence (shoulder, backslope and footslope) with little variation in slope, but with different vegetation cover. The shoulder is dominated by tree species from the botanical families Rubiaceae, Lecythidaceae, Sapindaceae, Chrysobalanaceae and Fabaceae; the backslope is characterized by undergrowth from the Poaceae family and no tree species; and the footslope is made up of trees from the Euphorbiaceae, Fabaceae, Meliaceae, Myrtaceae and Sapotaceae families. The shoulder and backslope have clay-textured soil in the surface layer, and the footslope has a sandy texture (Lima et al., 2019).



Figure 1. Illustrative diagram showing the layout of the toposequence points under different vegetation covers in the UFRRJ Botanical Garden.

2.2. Collection of soil samples

Deformed samples were collected from the areas in the 0-10 cm depth layer. Four simple soil samples were collected to make up one composite sample, totaling six composite samples per area. Sampling was carried out at the end of the rainy and dry seasons. In the thirds with the presence of tree species, the samples were collected under the tree canopy. After collection, to assess the chemical attributes, the soil samples were air-dried, crumbled and passed through a 2 mm sieve and stored in the form of fine air-dried soil (TFSA) (Teixeira et al., 2017). For the analysis of arbuscular mycorrhizal fungi, the samples were only dried and crushed. The samples used for the microbiological analysis were stored at 4 °C until they were processed (Mondal et al., 2024).

2.3. Soil analysis

The granulometric analysis was carried out using the pipette method, thus quantifying the contents of the sand (final, coarse and total), silt and clay fractions (Teixeira et al., 2017). The characterization of the chemical attributes associated with soil fertility was carried out by determining: a) pH in water at a ratio of 1:2.5 (soil:water); b) exchangeable Ca^{2+} . Mg²⁺, Al³⁺ extracted with KCl 1 mol L⁻¹, analyzed by titrimetry; c) P, K⁺ and Na⁺ extracted with Mehlich-1; and d) H+Al evaluated using a 0.025 mol L⁻¹ calcium acetate solution (Teixeira et al., 2017). Based on this data, the values of the sorption complex were calculated: a) sum of bases (S-value); b) cation exchange capacity at pH 7.0 (T-value); and c) base saturation (V-value).

Total nitrogen (N) was determined in 0.5 g of TFSA passed through a 0.15 mm sieve (100 mesh) after being ground in a mortar. The material was subjected to sulphuric digestion (Tedesco et al., 1995) and the N measured by Kjeldahl distillation (Bremner & Mulvaney, 1982). Total organic carbon (TOC) was determined via wet oxidation of soil organic matter according to Yeomans & Bremner (1988). For the physical granulometric fractionation, the method proposed by Cambardella & Elliott (1993) was used, determining the particulate organic carbon (POC) and the fraction associated with minerals (or organic carbon associated with minerals - MAOC), linked to the silt and clay fractions. The Coam fraction was obtained by the difference between TOC and POC. Light organic matter in water (LOM-C) was obtained using the aqueous flotation method (Anderson & Ingram, 1989; Loss et al., 2014). LOM carbon (LOM-C) was quantified according to Yeomans & Bremner (1988).

To assess enzymatic activity, the hydrolysis of fluorescein diacetate (FDA) was quantified according to Schnurer

& Rosswal (1982). 1.0 g of soil and FDA stock solution were used. The reading was taken on a spectrophotometer at a wavelength of 490 nm to determine the amount of hydrolyzed fluorescein. The fumigation-extraction method was used to quantify soil microbial biomass carbon (MBC), as described in De-Polli & Guerra (1999). The microbial quotient (qMic) was calculated and obtained according to Anderson & Domsch (1993).

The extraction of total soil protein related to glomalin (PSRG) from the samples collected followed the procedure described by Wright & Updahyaya (1998). The amount of PSRG - Total (PSRG-T) was obtained from the extraction in an autoclave using 1.0g of soil and 8ml of 50mM sodium citrate, pH 8.0 at 121 °C, for 60 min. After autoclaving, centrifugation was carried out at 4000 rpm for 10 min, where the supernatant was removed for subsequent protein quantification. GRSP was quantified using the Bradford method (1976) modified by Wright et al. (1996), using bovine serum albumin as a standard. The GRSP concentrations were corrected to mg g⁻¹ of soil, taking into account the total volume of supernatant and the dry weight of the soil.

The extraction of AMF glomerospores was carried out on 50 g of soil from each composite sample using the techniques of decantation and wet sieving (Gerdemann and Nicolson 1963), followed by centrifugation in water and sucrose (45%) (Jenkins, 1964). For identification, the spores were prepared on slides with Melzer's fixative solutions and polyvinyl alcohol in lactoglycerol (PVLG) and identified according to the morphological description available on the Internet at the International Culture Collection of Arbuscular Mycorrhizal Fungi - INVAM, Schenck & Pérez (1987), and updates of the scientific names of the species according to the AMF species list (2024). The average richness (ASR-AMF) and total richness (TSR-AMF) of species were quantified. The frequency of occurrence of each species (FRO) was calculated using the equation $Fi = Ji/K \times 100$, where Ji is the number of samples in which species i occurs and K is the total number of samples.

2.4. Data analysis

The results obtained were analyzed for normality of error distribution (Lillifors test / SAEG 5.0) and homogeneity of variances (Cochran and Bartlett tests). When the data was not normally distributed, it was log-transformed and then the mean values were compared using the Bonferroni T-test at 5%. Pearson's correlation analysis was also carried out using the Past program, and multivariate cluster analysis and principal component analysis (PCA).

3. RESULTS AND DISCUSSION

3.1. Characterization of the soil's physical and chemical attributes

The clay content was higher in the backslope, differing only from the footslope of the landscape (Table 1). On the other hand, there were higher levels of total sand in the footslope when compared to the upper and backslopes, with a greater share of coarse sand (Table 1). The content of fine sand and silt did not differ between the thirds (Table 1).

Table 1. Contents of the granulometric fractions of the soil in the 0-10 cm layer of a toposequence with different vegetation covers, in the Botanical Garden of the Federal Rural University of Rio de Janeiro.

Third	Clay	Total sand	Fine sand g kg-1	Coarse sand	Silt
Shoulder	199.04 ab	677.50 b	11.07 a	566.83 b	123.46a
Backslope	230.38 a	632.83 b	13.70 a	495.83 c	136.79a
Footslope	73.92 b	859.00 a	13.70 a	731.83 a	67.07a

Means followed by the same letter in the column do not differ by Bonferroni's T-test at 5%.

The chemical attributes varied between the different thirds of the landscape. In both periods (rainy and dry), the highest pH values were found in the backslope, under the influence of grasses. These differed from the values observed in the footslope during the rainy season; and from the values observed in the shoulder during the dry season, with both thirds (upper and lower) containing tree species (Table 2). This corroborates the higher clay content and lower coarse sand content at this intermediate point in the toposequence.

The Ca^{2+,} Mg²⁺ and K⁺ contents differed between the thirds only in the rainy season, with the highest values observed under the grasses in the backslope compared to the upper and footslopes under the tree species (Table 2). On the other hand, the Al³⁺ and H+Al contents were higher in the area where the tree species occur in the shoulder, with a difference being observed compared to the backslope (grasses), in both the dry and rainy periods (Table 2). Na⁺ levels did not vary between the thirds in the rainy season, but in the dry season the highest values were observed in the backslope, in the grass area, when compared to the shoulder, under the tree species (Table 2).

Ca²⁺ A13+ pН Mg^{2+} Third cmol_dm-3 RS DS RS DS RS DS RS DS 1.37 b Shoulder 5.63ab* 4.90 b 1.63 a 1.22 b 1.52a 0.00* 0.55 a 2.12 a 0.00 b Backslope 5.84 a 5.62 a 1.70 a 2.38 a 1.92 a 0.00^{*} Footslope 5.48 b 5.24 ab 0.87 c 1.30 b 0.00* 0.27 ab 1.48 a 1.32 a H+Al **K**⁺ Na⁺ S-value Third cmol_dm-3 RS DS RS DS RS DS RS DS 5.66 a 3.34 a Shoulder 4.48 a 0.13 b 0.14 a 0.03 a* 0.05 b 2.75 b Backslope 2.97 a 3.02 b 0.22 a 0.20 a 0.04 a* 0.07 a 4.76 a* 3.89 a Footslope 2.36 a 2.01 b 0.07 c 0.13 a* 0.03 a* 0.06 ab 2.26 b 2.99 a **T-value** V-value р Ν Third cmol_c dm⁻³ % mg kg⁻¹ g kg-1 RS DS RS DS RS DS RS DS Shoulder 7.23 ab 9.01 a 39.10 b 36.94b 3.66 b 3.60 b 1.01 a* 3.35 a Backslope 7.73 a 6.91 ab 52.72 ab 56.41a 4.69 b 3.59 b 0.86 a* 3.38 a Footslope 4.63 b 5.00 b 62.37 a 61.49a 7.87 a 7.22 a 0.20 a* 3.22 a

Table 2. Content of chemical attributes associated with soil fertility in the 0-10 cm layer of a toposequence with different vegetation covers in the Botanical Garden of the Federal Rural University of Rio de Janeiro.

Means, by attributes, followed by the same letter in the column do not differ by Bonferroni's T-test at 5%. * Indicates significant difference at 5% between seasons. RS: Rainy season; DS: Dry season; S-value: Base sum; T-value: Cation exchange capacity pH in H₂O; V-value: Base saturation.

With regard to P, in both periods the highest levels of the attribute were observed in the footslope (presence of tree species) of the landscape (Table 2). The sum of bases (S-value), on the other hand, only varied in the rainy season and the highest levels were observed in the backslope, under the grasses (Table 2). In general, cation exchange capacity values (T-value) were lower in the footslope (tree species) when compared to the shoulder (tree species) and backslope (grasses). Base saturation (V-value) showed the opposite pattern in both periods (Table 2).

The levels of total organic carbon (TOC) and the fractions of soil organic matter (SOM) varied between the thirds of the landscape (Table 3). The highest levels of TOC, particulate organic carbon (POC) and carbon associated with minerals (MAOC) were observed in the upper and backslopes, with the presence of tree species and grasses, respectively (Table 3). The carbon content of the organic matter in water (LOM-C) differed between the collection periods, with higher levels of the fraction in the backslope of the landscape in the dry period. Also in the dry season, the highest levels of LOM-C were quantified in the upper and backslopes compared to the footslope (Table 3).

Table 3. Total organic carbon content and fractions of soil organic matter in the 0-10 cm layer of a toposequence with different vegetation covers in the Botanical Garden of the Federal Rural University of Rio de Janeiro.

	тос		РОС		МАОС		LOM-C	
Third			g kg ⁻¹					
	RS	DS	RS	DS	RS	DS	RS	DS
Shoulder	29.73 a*	15.48a	5.40 ab	5.96a	24.33 a*	9.52a	0.53 a	1.69a
Backslope	27.40 a*	13.32ab	8.22 a	6.04a	19.17 ab*	7.29ab	0.55 a*	1.60a
Footslope	18.75 b*	9.04b	3.27 b	3.64a	15.48 b*	5.39b	0.24 a	0.73b

Means followed by the same letter in the column do not differ by Bonferroni's T-test at 5%.

*Indicates significant difference at 5% between seasons. FDA: hydrolysis of fluorescein diacetate; RS: Rainy season; DS: Dry season; TOC: total organic carbon; POC: particulate organic carbon; MAOC: organic carbon associated with minerals; LOM-C: light organic matter.

3.2. Microbiological attributes and their relationship with soil physical and chemical attributes

Higher values of soil enzymatic activity, assessed by the hydrolysis of fluorescein diacetate (FDA), were observed in the upper and backslopes of the landscape, in the area with tree species and grasses, respectively, considering the rainy and dry periods (Table 4). This pattern may be associated with the higher levels of soil organic matter (SOM) in these areas (Silva et al., 2018), as well as soil physical characteristics such as texture (Bittar et al., 2013), which is corroborated by the correlation analysis between these soil attributes (Table 5).

Table 4. Microbiological soil attribute contents in the 0-10 cm layer of a toposequence with different vegetation covers in the Botanical Garden of the Federal Rural University of Rio de Janeiro.

	FI	FDA SMB-C ⁽¹⁾		qMic (2)		GRSP		
Third	μgFluoresc.g ⁻¹ SS h ⁻¹		mg	mg kg⁻¹		%		g g ⁻¹
	RS	DS	RS	DS	RS	DS	RS	DS
Shoulder	140.20 a	115.29a	199.68ab	266.84ab	0.69 a*	1.74a	3.66 a	5.02 a
Backslope	128.43 a	120.61a	288.42a	308.79a	1.08 a*	2.32a	4.12 a	2.59 b
Footslope	39.89 b	59.32b	136.36b	146.94b	0.71 a*	1.70a	2.92 a	3.12 ab
	SA-A	AMF	ASR-	AMF		TSR-	AMF	
Third	SA-A n. esporos	AMF em 50 cm ⁻³	ASR-	AMF		TSR-	AMF	
Third	SA-2 n. esporos RS	AMF em 50 cm ⁻³ DS	ASR- RS	AMF DS	R	TSR-	AMF) \$
Third Shoulder	SA-A n. esporos RS 2760 a	AMF em 50 cm ⁻³ DS 2675 a	ASR- RS 7.5 a*	AMF DS 5.50 a	R 14	TSR- S	AMF	DS 9
Third Shoulder Backslope	SA-4 n. esporos RS 2760 a 2824 a*	AMF em 50 cm ⁻³ DS 2675 a 1858 a	ASR- RS 7.5 a* 5.5 ab	•AMF DS 5.50 a 4.67 ab	R 14 8	TSR- S 4	AMF	DS 9 8

Means followed by the same letter in the column do not differ by Bonferroni's T-test at 5%.

* Indicates significant difference at 5% between seasons.

RS: Rainy season; DS: Dry season; C-SBM: carbon-soil microbial biomass; FDA: fluorescein diacetate hydrolysis; AMF: arbuscular mycorrhizal fungi; AE: spores abundance; ASR: average species richness; TSR: Total species richness; GRSP: Soil Protein Related to Glomalin.

Vaniárcaio	FDA	SBM-C	SA-AMF	ASR-AMF	FDA	SBM-C	SA-AMF	ASR-AMF	GRSP
variaveis		Rainy	season				Dry season		
Clay	-	-	0.58*	-	0.74***	0.51*	0.50*	-	-
Total Sand	-0.75***	-	-0.85***	-0.59**	-0.89***	-0.75***	-0.65**	-0.60**	-
Coarse Sand	-0.72***	-0.53*	-0.85***	-0.59*	-0.87***	-0.75***	-0.62**	-0.62**	-
Silt	0.52*	0.49*	0.49*	0.57*	-	-	-	0.55*	-
TOC	0.74***	-	0.69**	-	0.75***	0.67**	-	0.49*	0.70**
POC	0.47*	0.60**	0.65**	-	0.64**	0.58*	-	-	-
MAOC	0.65**	-	0.49*	-	0.62**	0.55*	-	-	0.72***
LOM-C	0.50*	-	0.62**	-	-	-	-	-	-
pН	0.48*	-	0.49*	-	-	-	-	-	-0.47*
H+Al	0.59*	-	-	-	0.63**	-	0.60**	0.61**	0.769**
Al	-	-	-	-	-	-	-	-	0.60**
Na	0.48*	-	-	-	-	-	-	-	-
Ca	-	-	0.61**	-	-	-	-	-	-
K	0.66**	0.58*	0.63**	-	-	0.63**	-	-	-
Р	-0.54*	-	-0.62**	-0.67**	-0.85***	-0.65**	-0.78***	-0.61**	-
Mg	-	0.49*	-	-	-	-	-	-	-
Ν	-	-	-	0.58*	-	0.56*	-	-	-
T-value	0.74***	-	0.55*	-	0.71***	0.56*	0.64**	0.62**	0.65**
V-value	-	-	-	-	-0.55*	-	-0.66**	-0.58*	-0.60**
S-value	-	0.54*	0.56*	-	0.48*	0.54*	-	-	-

Table 5. Pearson's correlation between microbiological activity and the physical and chemical attributes of the soil in a toposequence with different vegetation covers in the Botanical Garden of the Federal Rural University of Rio de Janeiro.

*Significant at 0.05; **Significant at 0.01; *** Significant at 0.001

TOC: Total organic carbon; POC: Particulate organic carbon; MAOC: Organic carbon associated with minerals; LOM-C: Light organic matter; T-value: Cation exchange capacity pH in H_2O ; V-value: Base saturation; SMB-C: Soil microbial biomass carbon; qMic: Microbial quotient; FDA: Fluorescein diacetate hydrolysis; AMF: Arbuscular mycorrhizal fungi; SA: spores abundace; ASR: Average species richness; TSR: Total species richness; PSRG: Soil protein related to glomalin

In both periods, there were positive and significant correlations between FDA and TOC and the fractions POC, MAOC and LOM-C (Table 5). In addition to significant correlations with the other chemical and granulometric attributes of the soil, these were positive correlations with pH (rainy), H+Al (rainy and dry), Na⁺ (rainy) and K⁺ (rainy), T-value (rainy and dry), S-value (dry), clay (dry) and silt (rainy); and negative correlations with P (rainy and dry), V-value and coarse sand content (rainy and dry) (Table 5). Soil texture is related to soil porosity and aeration, which can have important implications for microbial and enzyme activity, and consequently for the decomposition and mineralization of SOM.

The influence of SOM on enzymatic activity has also been reported in several studies (Maurya et al., 2020; Silva et al., 2021). The quantity and quality of the substrate added or present in the soil is important for increasing microbial activity (Silva et al., 2018), and the hydrolysis of fluorescein diacetate (FDA) - carried out by a variety of enzymes, including esterases, proteases and lipases - can be used as an indicator of microbial activity (Nikaeen et al., 2015; Komilis et al., 2011).

The carbon content of the soil microbial biomass (MBC) followed a similar pattern to that observed for FDA activity, with the lowest values quantified in the footslope. However,

for this variable, there was no difference between the lower and shoulders. Positive and significant correlations were observed in the rainy and/or dry periods between MBC and silt content (rainy), TOC (dry), POC (rainy and dry), MAOC (dry), K⁺ (rainy and dry), N (dry), T-value (dry), V-value (dry), S-value (rainy and dry); and negative correlations with coarse sand content (rainy and dry) and P content (dry). Once again, this shows the influence of texture and some of the soil's chemical attributes on microbial activity.

Although there were no differences in qMic values between the points in the landscape, in the backslope there was a percentage increase of 57% (higher) and 52% (lower) in the rainy season and 33% (higher) and 36% (lower) in the dry season compared to the other points. With regard to soil protein related to glomalin (GRSP), there was no difference between the thirds in the rainy season. However, in the dry period, a greater addition of protein was observed in the areas of tree species in the shoulder of the landscape, differing only from the grasses in the backslope, where the lowest values of the attribute were found (Table 4).

In the area of tree species in the footslope, intermediate values of GRSP were observed (Table 4). This pattern shows that the presence of tree species is contributing to greater incorporation of this protein into the soil during the dry season. The incorporation of GRSP can contribute to the storage of carbon in the soil and to the stability of aggregates (Matos et al., 2022). The accumulation of GRSP in the soil depends on different factors such as the species richness of the AMF community, plant community composition, as well as soil attributes (Singh et al., 2016).

In this study, it was found that the upper and footslopes of the landscape where there was greater floristic richness saw greater additions of GRSP, in at least one of the periods. In addition, significant correlations between GRSP and soil chemical attributes were observed in the dry period. There were also positive and significant correlations with TOC, MAOC, Al³⁺, H+Al and T-value (Table 5); and negative correlations with pH and V-value. Thus, it was found that higher values of CTC (T-value), base saturation (V-value), active (Al³⁺) and potential (H+Al) acidity and TOC had a positive effect on GRSP levels (Table 5). Previous studies have reported negative correlations between GRSP and pH (Lovelock et al., 2004), as well as positive correlations with TOC (Šarapatka et al., 2019; Wang et al., 2020; Silva et al., 2021).

With regard to the AMF, a greater abundance of spores (AS) was observed in the upper and backslopes of the landscape, in areas with tree species and grasses, respectively, when compared to the footslope, with tree species cover, in both sampling periods (rainy and dry) (Table 5). Higher populations of AMF spores in the higher thirds of the landscape may be associated, among other factors, with the influence of vegetation cover on the soil's chemical attributes, such as soil P content.

In the rainy season there were positive and significant correlations between SA and pH, K, S, T-value and TOC; and negative correlations with P (Table 5); and in the dry season SA correlated positively with H+Al; T-value; and TOC; and negatively with V-value and P (Table 5). The correlation results indicate that lower pH values and higher P contents may be reducing the number of spores. While higher values of cation exchange capacity (T-value), sum of bases and TOC content may be stimulating AMF sporulation. Other authors (Khakpour & Khara, 2012; Isobe et al., 2007) have also reported a negative correlation between soil P content and the population of AMF spores.

The average and total richness of AMF species in both periods was highest in the shoulder, under tree species, followed by intermediate values in the backslope, with the presence of grasses, and lowest in the footslope, with tree species (Table 5). There were significant correlations between the richness of AMF species and the chemical attributes of the soil in the rainy and dry periods (Table 5). In the rainy season, a negative correlation was observed with P; and in the dry season, positive correlations with H+Al, T-value and TOC, and negative correlations with P and V-value (Table 5). It can thus be seen that the higher P levels in the lower part of the landscape may have been one of the factors contributing to the lower richness of AMF species, as was also observed for the spore population.

A total of 16 AMF morphospecies were observed (13 at species level and 3 at genus level) distributed in seven genera and six families (Table 6; Figure 2). The genera *Glomus* (6) and *Acaulospora* (5) had the highest number of species, followed by *Ambispora, Claroideoglomus, Diversispora, Gigaspora* and *Rhizoglomus* with one species each (Table 6). The Acaulosporaceae and Glomeraceae families have the highest number of species identified within the Glomeromycota phylum (Silva et al., 2014), and studies show that these families are better able to adapt to soils subjected to different uses and management (Ferreira et al., 2012; Silva Junior & Cardoso, 2006).

Table 6. Relative frequency of occurrence (%) of species of arbuscular mycorrhizal fungi in a toposequence with different vegetation covers in the Botanical Garden of the Federal Rural University of Rio de Janeiro.

E:1:/C	Shoulder		Backslope		Footslope	
Families/Species	RS	DS	RS	DS	RS	DS
Acaulosporaceae						
Acaulospora foveata	16.67	16.67	-	-	-	-
A. mellea	-	16.67	-	-	-	-
A. scrobiculata	16.67	33.33	33.33	-	-	16.67
Acaulospora sp.	16.67	-	-	-	-	-
A. spinosa	-	-	16.67	-	-	-
Ambisporaceae						
Ambispora leptoticha	100	100	100	100	100	100
Claroideo-						
Glomeraceae						
Claroideoglomus	16 67	_	83 33	_	_	
etunicatum	10.07		05.55	-		
Diversisporaceae						
Diversispora tortuosa	-	-	-	33.33	-	-
Gigasporaceae						
Gigaspora sp.	16.67	-	-	33.33	-	-
Glomeraceae						
Glomus clavisporum	100	100	100	100	100	83.33
G. formosanum	16.67	-	-	16.67	-	-
G. glomerulatum	16.67	16.67	16.67	16.67	-	-
G. macrocarpum	100	100	100	100	100	83.33
Glomus sp. 1	83.33	66.67	33.33	-	-	-
Glomus sp. 2	83.33	-	-	-	16.67	-
Rhizoglomus microaggregatum	100	100	100	83.33	16.67	33.33

For the species *Glomus macrocarpum*, *Rhizoglomus microaggregatum* and *G. clavisporum*, a high relative frequency of occurrence (RFO) (>80%) was observed in most of the

areas and periods evaluated (Table 6). While *Acaulospora mellea*, *Acaulospora* sp., *A. foveata* and *Glomus* sp1 were identified only in the shoulder (tree species) and with a low RFO (Table 3); *Diversispora tortuosa* and *A. spinosa* were found in the backslope, under grasses, also with a low RFO (Table 6). There was no pattern of occurrence for the other species (Table 6).

The similarity between the thirds, considering the relative frequency of the AMF species, generally did

not vary with the sampling period, as can be seen from the hierarchical cluster analysis (Figure 2). Two groups were formed in both periods, the first consisting of the shoulder (tree species) and the backslope (grasses), which showed approximately 50% similarity to the second group, which consisted of the footslope (tree species) (Figure 2). There was greater similarity (~55%) in the composition of AMF species between the upper (tree species) and middle (grass) thirds of the landscape (Figure 2).



(A)

Figure 2. Dendrogram of similarity considering the relative frequency of occurrence of species of arbuscular mycorrhizal fungi in a toposequence with different vegetation covers in the Botanical Garden of the Federal Rural University of Rio de Janeiro. (A) Rainy season; and (B) Dry season.

The footslope was isolated from the others, possibly due to the more marked difference in the soil's chemical and physical attributes, especially P content and granulometry, which correlated significantly with both AMF, EMR and SA. Some studies have shown that the main factors influencing the composition of the AMF community are host plants, geographical and climatic characteristics and soil characteristics, such as P content (Davison et al., 2015; Qin et al., 2021).

3.3. Multivariate analysis integrating physical, chemical and microbiological soil atributes

The cluster analysis, integrating the soil's chemical and microbiological attributes, showed the formation of two

distinct groups (Figure 3). The first group, made up only of the footslope, was approximately 75% different from the second group, made up of the upper and backslopes. Greater similarity (~60%) was observed between the upper and backslopes, with the presence of tree and grass species, respectively (Figure 3).



Figure 3. Cluster and principal component analysis integrating the physical, chemical and microbiological variables of the soil of a toposequence under different vegetation covers, in the Botanical Garden of the Federal Rural University of Rio de Janeiro. (A and B) Rainy season; (C and D) Dry season. POC: particulate organic carbon; ASR-AMF: average species richness; SA-AMF: spores abundance; LOM-C: Light organic matter; FDA: hidrolysis of fluorescein diacetate; MAOC: organic carbon associated with minerals; GRSP: Soil protein related to glomalin; MBC: Soil microbial biomass carbon; qMic: Microbial quotient; TOC: Total organic carbon; pH: Active acidity; Ca²⁺: Exchangeable calcium; Mg²⁺: Exchangeable magnesium; H+Al: Potential acidity; K⁺: Exchangeable potassium; P: Available phosphorus; SB: Base sum; CEC: Cation exchange capacity at pH 7.0; and BS: Base saturation.

The principal component analysis (PCA) identified the main variables that contributed most to the dissimilarity/similarity between the thirds of the landscape (Figure 3). PCA showed an accumulated variance for principal components (PC) 1 and 2 of 69.6% and 68.3% in the rainy and dry periods, respectively. In both periods, most of the variables were more associated with PC 1 and more related to the upper and backslopes of the landscape.

According to the factor loadings, in the rainy season, the first PC (main axis), which explains 48.3% of the total variance, showed higher positive correlations (>0.7) with Ca^{2+} , K⁺, TOC, S-value, CEC, POC, FDA and SA; while in the dry season, the first PC, which explains 44.1% of the total variance, showed higher positive correlations (>0.7) with H+Al, TOC, CEC, POC, MAOC, and FDA, and negative

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correlations with P and coarse sand. These variables were the ones that contributed most to the dissimilarity of the upper and backslopes in relation to the footslope.

4. CONCLUSIONS

The tree species and grasses present in the higher thirds of the landscape are contributing to greater enzymatic activity in the soil. Their observed correlations suggest that the lower P contents and higher contents of the SOM fractions, pH, H+Al, Na⁺, K⁺ and cation exchange capacity are stimulating the enzymatic activity of the soil.

MBC levels were higher in the backslope of the landscape under the influence of species from the Poaceae family. Their correlations indicate the influence of physical attributes and chemical attributes to a greater or lesser degree on this microbiological attribute.

There was greater sporulation and species richness of AMF in the areas with the presence of tree species and grasses, in the higher thirds of the landscape. The results of their correlation show the effect of the chemical attributes of the soil, especially the lower levels of available P.

During the dry season, there is a greater deposition of GRSP in the soil in areas with tree species. The correlations were significant with pH and TOC. This indicates that the higher active acidity of the soil is contributing to greater incorporation of this protein. While the positive correlation with TOC suggests a greater contribution of this protein to the accumulation of carbon in the soil.

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