Soil Quality Indicators Associated With The Application Of Mycorrhizal Fungi In Coffee Plantations

Indicadores de calidad del suelo asociados a la aplicación de hongos micorrizales en plantaciones de café

Indicadores de qualidade do solo associados à aplicação de fungos micorrízicos em plantações de café

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Abstract

Introduction: This article is the result of the research work "Evaluation of the quality of the soil associated with the use of mycorrhizae in coffee plantations in the Municipality of Sasaima, Cundinamarca" carried out during the years 2018 and 2019 at the Universidad Libre.

Research problem: Several investigations report the use of mycorrhizal fungi in plant development, however, there are few studies regarding the relationship between mycorrhizae and soil quality indicators.

Methodology: Four plots were evaluated, applying: 1) Control; 2) Local native mycorrhizae isolated by the authors; 3) Commercial liquid mycorrhizae; and 4) Commercial solid mycorrhizae. The samples of the rhizospheric soils were taken and bimonthly monitoring was applied until 5 collection cycles were obtained. The physicochemical parameters, abundance of functional groups of microorganisms, and enzymatic activities of the soil were determined and analyzed by means of univariate and multivariate statistical analysis.

Conclusion: The research hypothesis was validated since the mycorrhizae inoculation significantly increased the values of the soil quality indicators, namely organic carbon (14%), moisture (19%), phosphorus (30%), abundance of nitrogen-fixing bacteria (5.5%), phosphate-solubilizing bacteria (7.8%), cellulolytic bacteria (9.3%), and phosphate-solubilizing fungi (2.6%). In addition, the local native mycorrhizae that were isolated by the authors showed greater benefits than those of the commercial mycorrhizae.

Originality: This research reveals the impact of mycorrhizae on the soil quality indicators over time during the first year of application, especially on the microbial and enzymatic parameters.

Limitations: Several authors have investigated the importance of the mycorrhizae on the physicochemical parameters of the plant, and not on the microbial and enzymatic parameters of the soil.

Keywords: Organic farming, soil enzymes, functional groups of microorganisms, soil nutrients.

Resumen

Introducción: El presente artículo es producto de la investigación "Evaluación de la calidad del suelo asociado al uso de micorrizas en cafetales del Municipio de Sasaima, Cundinamarca" realizado durante el año 2018 y 2019 en la Universidad Libre.

Problema de investigación: Diversas investigaciones reportan el uso de micorrizas en el desarrollo de plantas, sin embargo, se presentan pocos estudios en cuanto a las relaciones ente las micorrizas y los indicadores de calidad del suelo.

Metodología: Se evaluaron cuatro parcelas en las cuales se aplicó: 1). Control, 2). Micorrizas autóctonas de la zona aisladas por las autoras, 3). Micorrizas líquidas comerciales y 4). Micorrizas sólidas comerciales. Se realizó muestreo de suelos rizosféricos con seguimiento bimensual hasta obtener 5 ciclos de colecta. Se determinaron parámetros fisicoquímicos, abundancia de grupos funcionales de microorganismos y actividades enzimáticas del suelo, los cuales fueron analizados mediante análisis estadístico: univariado y multivariado.

Conclusión: La hipótesis de investigación fue validada ya que la inoculación de micorrizas aumentó significativamente los valores de los indicadores de calidad del suelo como el carbono orgánico (14%), humedad (19%), fósforo (30%), abundancia de bacterias fijadoras de nitrógeno (5.5%), bacterias solubilizadoras de fosfato (7.8%), bacterias celulolíticas (9.3%) y hongos solubilizadores de fosfato (2.6%). Adicionalmente, las micorrizas autóctonas de la zona de estudio, aisladas por las autoras presentaron mayores beneficios con respecto a las micorrizas comerciales.

Originalidad: Esta investigación revela el impacto de las micorrizas en los indicadores de calidad del suelo, especialmente en los parámetros microbianos y enzimáticos a lo largo del tiempo durante el primer año de aplicación.

Limitaciones: Los reportes de diversos autores investigan la importancia de las micorrizas en los parámetros fisicoquímicos de la planta y no sobre los parámetros microbianos y enzimáticos del suelo.

Palabras claves: Agricultura ecológica, enzimas del suelo, grupos funcionales de microorganismos, nutrientes del suelo.

Resumo

Introdução: Este artigo é o produto da investigação "Avaliação da qualidade do solo associada ao uso de micorrizas em plantações de café no município de Sasaima, Cundinamarca", realizada nos anos de 2018 e 2019 na Universidade Livre.

Problema de pesquisa: Várias investigações relatam o uso de micorrizas no desenvolvimento das plantas, no entanto, poucos estudos são apresentados sobre as relações entre micorrizas e indicadores de qualidade do solo.

Metodologia: Foram avaliadas quatro parcelas nas quais foram aplicadas as seguintes: 1). Controle 2). Micorrizas autóctones da área isolada pelos autores, 3). Micorrizas líquidas comerciais e 4). Micorrizas sólidas comerciais. Os solos rizosféricos foram amostrados com monitoramento bimensal até a obtenção de 5 ciclos de coleta. Foram determinados parâmetros físico-químicos, abundância de grupos funcionais de microrganismos e atividades enzimáticas do solo, os quais foram analisados por análise estatística: univariada e multivariada.

Conclusão: A hipótese da pesquisa foi validada, pois a inoculação de micorrizas aumentou significativamente os valores dos indicadores de qualidade do solo, como carbono orgânico (14%), umidade (19%), fósforo (30%), abundância de bactérias fixadoras nitrogênio (5,5%), bactérias solubilizantes de fosfato (7,8%), bactérias celulolíticas (9,3%) e fungos solubilizantes de fosfato (2,6%). Além disso, as micorrizas autóctones na área de estudo, isoladas pelos autores, apresentaram maiores benefícios quando comparadas às micorrizas comerciais.

Originalidade: Esta pesquisa revela o impacto das micorrizas nos indicadores de qualidade do solo, especialmente nos parâmetros microbianos e enzimáticos ao longo do tempo durante o primeiro ano de aplicação.

Limitações: Os relatos de vários autores investigam a importância das micorrizas nos parâmetros físico-químicos da planta e não nos parâmetros microbianos e enzimáticos do solo.

Palavras-chave: Agricultura ecológica, enzimas do solo, grupos funcionais de microrganismos, nutrientes do solo.

1. INTRODUCTION

The coffee plant originated in the tropical rainforests of modern Ethiopia. Initially, during the 10th century, it was cultivated by the Arabs; and centuries later, it arrived in the New World with European colonialism. It soon became an economically important crop, and its trade and exportation have been a fundamental element in the history of Latin American countries [1]. According to historical and statistical analyses from the International Coffee Organization (ICO), Colombia is the third largest coffee producing country in the world. The percentage participation in 2015 was Brazil

(30.2%), Vietnam (19.2%), and Colombia (9.4%), also taking the position of the third largest exporter during the same year, with a worldwide share of (11.9%) [2]. In the case of Cundinamarca, by 2013, coffee used 24% of the area that was allocated to agriculture, distributed among 63 municipalities in the department, benefiting nearly 36,000 households [3].

Likewise, producers are always looking for alternatives to improve crop yields through the excessive use of chemical fertilizers, in order to satisfy the basic needs of a continuously growing population. However, these different human activities have transformed the environment by altering the balance of the ecosystem. Currently, it has been indicated that the percentage of absorption of the fertilizer by the crops is between 30% and 50%, and the rest of the components are wasted into the soil, contaminating the groundwater [4]. Therefore, one of the most important exercises that the Coffee Sector has launched in order to improve the quality of coffee and increase produce is the implementation of good agricultural and manufacturing practices. Good agricultural practices are related to the preparation and maintenance of the crop and collection, also taking into account the requirements and principles of hygiene and safety, which include soil management, quality and health of the propagation material, selection of varieties and use of fertilization according to crop needs [5].

Several research projects have studied the effects of the arbuscular mycorrhizae on plants and roots, as mycorrhizae are considered to form symbiotic associations between fungi and the roots of the plant. Also, it has been reported that they are able to improve crop productivity thanks to an increase in nitrogen (N) and phosphorus (P), radical development, the storage of organic carbon (OC) and its protective effect against fungal root diseases [6], [7]; however, little has been reported about the relationships between mycorrhizae and soil quality indicators of the soils surrounding its application.

The biochemical properties of the soil that include C and N, microbial biomass, and enzyme activities, react rapidly to environmental fluctuations and anthropogenic disturbances [8], [9], [10]. In the same way, it is important to analyze the behavior of the parameters of soil quality after years of transformation, as microorganisms such as fungi and bacteria on the soil are also considered a potential indicator of soil health in response to the changes regarding soil use [7]. Furthermore, soil enzymes are critical for the biochemical catalytic reactions that allow for the decomposition of animals, plants, and microorganisms, providing information on the microbiological status and physicochemical properties of the soil [10].

In this order of ideas and according to the reports that have been released by several authors, mycorrhizae are of great relevance in the physicochemical parameters of the soil, however, little is known about their impact on other soil quality indicators, especially on microbial and enzymatic parameters over time during the first year of application.

Therefore, the objective of this research was to evaluate the possible changes in soil quality associated with the use of mycorrhizae in coffee plantations in the municipality of Sasaima; this, by monitoring the physicochemical parameters, the abundance of functional groups of mycorrhizae, and the enzymatic activities associated with biogeochemical cycles of nitrogen, phosphorus and carbon in soils that are used for coffee crops with and without the application of mycorrhizae. Our research hypothesis was that mycorrhizae generate a significant modification, representing a possible improvement in the behavior of the above-mentioned soil quality parameters.

1.1. Literature or research background review

The following background was consulted in the Scielo and Redalyc database, which reports the use of mycorrhizae in plant development; however, few studies have been carried out regarding the relationships between mycorrhizae and soil quality indicators:

During the year 2013 and in order to determine the effects of arbuscular mycorrhizae and *Meloidogyne spp.* on tomato crops (*Solanum lycopersicum L.*), Alarcón, Boicet, Godefoy, Bacilio, Ceiro, and Bazán from the University of Granma (Cuba) used seven treatments, consisting of simple and combined applications of a concentrate of native and non-native strains of mycorrhizae (*Glomus mosseae* [Gerdemann & Trappe] and *Glomus sp.* [Schenck & Smith]), including a control and two population levels of *Meloidogyne spp.* with 30 replicate samples. The gall index and plant growth parameters, such as average height (cm), average dry weight (g), and crop yield (kg. plant-1), were evaluated 60 days after nematode inoculation. The best results for the plant growth parameters were achieved when applying the native strain concentrate and its combination with *Glomus mosseae* and *Glomus sp.* The results obtained in this study indicate that there is no simple response in the arbuscular mycorrhizal plant nematode interaction, and that this response depends on factors such as the nematode, mycorrhizae, plant species, the environmental factors, the time of mycorrhization process, and the period of exposure to the nematode [11].

In the research developed by Villalobos in 2019, native arbuscular mycorrhizae from the Huamantanga Forest Municipal Conservation Area were used to favor the growth of cocoa seedlings (*Theobroma cacao L.*) during the nursery stage, obtaining a positive response in the growth of the main root and volume of secondary roots, attributed to a greater absorption of phosphorus. The seedlings of the *Theobroma cacao L*. were inoculated by the methods of root immersion and direct application to the soil. According to the author, the outputs for colonization by the method of submergence by spores of arbuscular mycorrhizae, native to the genus *Glomus*, were 80.25 % [12].

The objective of the research by Del Aguila, Vallejos, Arevalo, and Becerra during the year 2018, which was developed in important coffee provinces in the Region of San Martin: Lamas, Moyobamba, and El Dorado [13], was to determine the effect of nine arbuscular mycorrhizal consortia and the inoculation of *Coffea arabica* seedlings, of the Caturra variety, compared to a control (without inoculation), for a period of seven months in a plant nursery; this, due to the fact that coffee cultivation is one of the most important agricultural economic activities in the Region of San Martin. The application of mycorrhizae fungi could be one alternative to increase their production. They applied a Completely Randomized Design (CRD), nine treatments with Arbuscular Mycorrhizal Fungi (AMF) from Chirapa, Aviacion, Chontadillo, Calzada, Los Angeles, Potrerillo, Nueva Juventud, Buena Vista, and Monte de los olivos, and a control without inoculation consisting of three replicates (6 plants/repeat). The mycorrhizal consortia originate from coffee farms in the Region of San Martin, Peru. These sources of collected inoculum were sent to the plant nursery facilities for them to be multiplied in pots using *Zea mays* corn plant traps, for a period of 60 days. They evaluated the percentage of colonization, the length of extra-radical mycelium, the height of the plant, and the leaf area. The results showed that three of the nine consortia that were being studied in the provinces of Los Angeles, Buena Vista, and Monte de los Olivos (T5, T8, and T9) were more efficient in the growth and development of coffee plants.

Other investigations, such as the one by Cuenca, Cáceres, Oirdobro, Hasmy, and Urdaneta in 2007, presented the preliminary results of a project whose purpose was to produce commercial inoculants of arbuscular mycorrhizae to be used in acid soils, and the potential for its application in tropical areas, particularly in Venezuela. Crucial steps in the production of inoculants were shown, including their advantages in lettuce and cassava crops. Likewise, the document stated that mycorrhizae emerge as an ecological and productive tool in soils that are poor in available phosphorus; therefore, its implementation should be addressed soon and with a high ethical content by professionals engaged in agriculture [14]

Similarly, the research developed in 2016 by Garzón in the Colombian Amazon, indicated that *in vitro* crops can be grown in plant nurseries where native or commercial species of Arbuscular Mycorrhizal Fungus (AMF) are used for root inoculation techniques, with a previously known agronomic management. Through this method, the functional compatibility between mycorrhizae, soil, and plants can be established, which leads to an increase in production; although, a negative competition with other native fungi can be generated. The effectiveness of inoculation of mycorrhizal fungi proves to be high in edible species such as yucca brava (*Manihot esculenta*), porotillo (*V. luteola*), arazá (*Eugenia stipitata*), borojó (*Borojoa sorbilis*), and chontaduro (*Bactris gasipaes*) [15].

2. MATERIALS AND METHODS

STUDY SITE DESCRIPTION

This study was carried out in the municipality of Sasaima, department of Cundinamarca, Colombia, approximately 77 km from Bogotá, with coordinates (04° 56′ 44.9′′ N-74° 25′ 12′′ W). According to Holdridge life zones classification system [16], Sasaima is included in the premontane wet forest (PMwf) group. It is located near the Guane River between 900 and 1200 m.a.s.l., and has a warm, humid climate with average annual temperatures of 20.4 °C and average annual precipitation of 2567 mm [17].

According to the General Soil Study by the Instituto Geográfico Agustín Codazzi [18], the soils are of Class IV, which have limitations that restrict the selection of plants and require very careful tilling. These soils are suitable for the crops in the region and extensive stockbreeding, as long as appropriate conservation practices are observed. These practices should be aimed at making good use of the land through adequate crop location, rotation, and ditch systems in order to stop the advancement of erosion.

EXPERIMENTAL DESIGN AND SOIL SAMPLING

Four study plots (5x5 m) were selected as follows:

Plot 1: Control soil without application of mycorrhizae (CO by its abbreviation in Spanish).
Plot 2: Soil with application of mycorrhizae native to the area and isolated by the authors (MAZ by its abbreviation in Spanish)
Plot 3: Soil with application of commercial liquid mycorrhizae (ML by its abbreviation in Spanish)
Plot 4: Soil with application of commercial solid mycorrhizae (MS by its abbreviation in Spanish)

Bi-monthly samples of rhizospheric soil were collected until five sampling cycles were obtained, for a total of 10 sampling events. Ten sub-samples containing fine roots were obtained from each site, at a depth of 20 cm, and were mixed and homogenized until obtaining a sample. Three composite samples were taken for each plot, for a total of 60 rhizospheric soil samples. The samples were then stored for transport; in the case of microorganism analysis, they were kept at 4°C and in the case of enzymatic analysis at -20°C.

The solid mycorrhizae (Safer Mycorrhizae M.A.) that were applied contained spores, mycelium, and propagules (colonized roots, free mycelium, and spores) based on arbuscular mycorrhizae, native to the genera *Glomus, Acaulospora, Scutellospora* and *Entrophospora,* reporting a concentration of 300 spores/gram. The substrate used was disinfected soil, free from pathogenic microorganisms.

The liquid mycorrhizae (dry water-soluble powder) that were applied consisted of a biological inoculum based on mycorrhizae, native to the genera *Glomus*, *Acaulosphora* and *Entrophospora,* reporting a concentration of 75-80 spores/gram. The substrate used was disinfected soil, free from pathogenic microorganisms.

The autochthonous mycorrhizae that were isolated for the study were obtained by means of the extraction and isolation of spores from the soil of the area using the methodology by M. Sánchez de Prager, R. Posada, D. Velásquez, and M. Narváez [19] with the following adjustments: 100 g of sifted soil were taken, passed through 75, 45, and 25µm superimposed sieves. The contents of the smaller sieve were centrifuged with sucrose, making it possible to observe a separation of three phases: water-sucrose-soil; the sucrose where the spores accumulated was extracted, and the identification of the morphotypes was carried out by comparing the morphological characters, identifying the color, shape, and presence of ornamentation with the help of a stericroscope . These characteristics were compared with those that are detailed in the taxonomic keys, obtaining a *Glomus* and *Acaulosphora* genera classification. These were later multiplied in soils typical in the study area, having soil sown with fodder oats (*Avena sativa*) as substrate. Finally, the spores were counted directly, after their separation by wet sifting in a gradient of sucrose, and 25 spores/gram were reported.

PHYSICOCHEMICAL PARAMETERS OF THE SOIL AND PARAMETERS OF THE COFFEE PLANTATIONS

The physicochemical parameters of the soil were determined according to the methods proposed by IGAC [14] as follows: pH in water according to the potentiometric method in relation to soil: water (w/v) of 1: 1; total N (TN) with the micro-Kjeldahl method; assimilable P with the Bray II method; percentage of organic carbon (OC) according to the Walkley and Black method, and cation exchange capacity (CEC). The contents of OC, P, and TN were determined in triplicate for each of the samples in the plots.

Parallel to the soil sampling, the height and canopy of the coffee plants were recorded, the latter being obtained directly by measuring the area, angles, or positions of the organs by the method reported by [20].

FUNCTIONAL GROUPS OF SOIL MICROORGANISMS

For the determination of the abundance of functional groups of microorganisms, a count of Colony-Forming Units per gram of dry soil (CFU/g dry soil) was made and expressed in Log CFU/g dry soil, by means of the plate sowing method. For the case of the Nitrogen-Fixing Bacteria (NFB), sowing was performed in the reported NFB medium [21], maintaining incubation at 28°C for 47 hours.

The counting of the phosphate-solubilizing microorganisms, bacteria (PSB) and fungi (PSF), was carried out in an SRS medium, according to (Sundara Rao & Sinha, 1963) following the procedure reported by [21], incubating the bacteria at 28°C for 40 h and the fungi at 20°C for seven days.

To determine the abundance of cellulolytic microorganisms, bacteria (CB) and fungi (CF), the medium was counted with 1% carboxymethylcellulose as the only source of modified carbon from [22], incubating the bacteria at 28°C for 48 hours and the fungi at 20°C for seven days. For all cases of microorganism counts, three replicates were made for each of the samples.

SOIL ENZYME ACTIVITY

The methods reported by [23] were used to determine the following soil enzyme activities. Urease activity was determined using urea as a substrate; the method is based on the colorimetric determination of the ammonia that is released after incubating the soil samples with urea solution for 2 hours at 37°C. In order to determine this activity, 0.1 g of soil were collected, the urease activity is expressed as μg N g^{-1} dw 2 h^{-1}. The acid and alkaline phosphatase activity was determined by using p-nitrophenyl phosphate sodium as substrate, incubating the samples for 1 hour at 37°C. The β-glucosidase activity was determined by using p-nitrophenyl-β-D-glucoside as substrate; for these, 0.2 g of soil were collected, and the activities of acid phosphatase, alkaline phosphatase, and β-glucosidase were expressed as μg pNP g^{-1} dw h^{-1}. All the enzyme activities were determined in triplicate.

STATISTICAL ANALYSIS

The assumptions of normality and variance homogeneity were verified using the Shapiro-Wilk and Bartlett tests respectively. After that, the Kruskal-Wallis tests with Mann-Whitney and T-Student Post-Hoc analysis were used to determine significant differences. All the tests were carried out with the Stats package [24]; and finally, the principal component analysis (PCA) of the FactoMineR package was performed [25]. The analyses were performed with the statistical software version R-3.5.3 [24].

3. RESULTS

PHYSICOCHEMICAL PARAMETERS OF THE SOIL

The results of this research indicate that the humidity of the soils in the area under study is in a range between 48.5 and 61.6%, which allows them to be classified as usable soils according to their water content. Likewise, the pH varies between 4.8 and 5.3, placing the soil between the categories of very strongly acid and strongly acid. The organic carbon was presented in a range between 6.9 and 8.4%, which indicates a high content of carbon in the soil. The content of phosphorus varied from 1.1 to 1.6 ppm, which means that the phosphorus in the studied soil is low. Finally, total nitrogen presented a range between 0.4 and 0.5%, which translates into the availability of a high content of this element in the soil.

According to the results that are shown in Table 1, in which the physico-chemical parameters are correlated with the soils of the plots that were considered, the pH exhibits a significant decrease in the soils of the solid mycorrhizae plot with respect to the control plots and with the application of liquid mycorrhizae. Additionally, there was a statistically significant increase in the percentage of organic carbon in the soils of the plot where native and liquid mycorrhizae were applied with respect to the other plots. Likewise, the percentage of phosphorus exposed a statistically significant increase in the soils of the plots with mycorrhizae application with respect to the control plot. With regard to the cation exchange capacity, significant increases were observed in the plots that are native to the area and with the liquid mycorrhizae with respect to the solid mycorrhizae and control. Finally, the total nitrogen in the soil and the height of the coffee plants did not show significant differences between the plots.

Table 1. Physico-chemical parameters of the soils analyzed for each of the plots during all the sampling cycles.

PLOTS	MOISTURE (%)	pH	OC (%)	CEC (cmolckg-1)	P (ppm)	TN (%)	CANOPY (cm)	HEIGHT (cm)
CO	48.5 a	5.3 a	6.9 a	27.7 ab	1.1 a	0.5 a	316.8 a	77.9 a
MAZ	54.4 a	5.1 ab	8.4 b	29.4 a	1.6 b	0.4 a	243.5 b	63.6 a
ML	61.6 b	5.3 a	7.8 b	28.4 a	1.3 b	0.4 a	295.1 a	68.3 a
MS	56.6 a	4.8 b	7.4 a	26.0 b	1.4 b	0.4 a	241.7 b	71.5 a

Source: own work

Control soil without application of mycorrhizae (CO by its abbreviation in Spanish); Soil with application of mycorrhizae native to the area and isolated by the authors (MAZ by its abbreviation in Spanish); Liquid Mycorrhizae (ML by its abbreviation in Spanish); Solid Mycorrhizae (MS by its abbreviation in Spanish); Organic Carbon (OC); Cation Exchange Capacity (CEC); Phosphorus (P); Total Nitrogen (TN). Different letters in each parameter indicate significant differences; equal letters in each parameter indicate that there were no statistically significant differences ($P \leq 0.05$).

Table 2 shows the data of the physico-chemical parameters for each of the plots studied during the five sampling cycles. These data indicate that both the canopy and height showed a tendency to increase in all the sample soils, and the organic carbon displayed a similar behavior in soils with mycorrhizae application. However, the other chemical parameters that were evaluated did not show a defined trend over the time of application.

Table 2. Physicochemical parameters of the soils analyzed each month.

PLOTS	MONTH	MOISTURE (%)	pH	OC (%)	CEC (cmolckg-1)	P (ppm)	TN (%)	CANOPY (cm)	HEIGHT (cm)
CO	0	50.0 a	5.8 a	5.6 a	24.6 a	0.9 a	0.6 a	215.5 a	54.0 a
	2	60.7 ab	5.2 b	7.2 b	29.5 b	1.9 b	0.5 a	255.5 a	64.3 b
	4	29.5 b	5.0 b	7.9 bc	29.0 b	1.2 c	0.5 a	311.0 a	73.2 b
	6	63.9 a	5.4 b	8.5 c	31.8 b	0.9 a	0.3 b	364.5b	91.7 c
	8	38.4 ab	5.4 b	5.7 a	24.0 a	0.7 e	0.2 b	437.5 c	106.2 c
MAZ	0	52.9 a	5.7 a	6.3 a	30.8 a	1.7 a	0.5 a	210.5 a	37.0 a
	2	68.2 b	5.3 a	8.5 b	31.8 a	2.4 b	0.2 b	221.7 a	54.7 a
	4	26.3 c	4.5 b	8.2 b	32.6 a	2.1 c	0.6 a	243.7 a	61.8 a
	6	64.7 b	4.8 b	9.7 c	29.9 a	0.9 d	0.3 b	265.5 a	77.2 b
	8	60.4 ba	5.3 a	9.7 c	22.2 a	1.0 d	0.6 a	276.2 a	87.3 b

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PLOTS	MONTH	MOISTURE (%)	pH	OC (%)	CEC (cmolckg-1)	P (ppm)	TN (%)	CANOPY (cm)	HEIGHT (cm)
ML	**0**	61.8 a	5.5 a	6.1 a	27.0 a	1.4 a	0.5 a	224.7 a	52.7 a
	2	73.2 a	5.9 a	8.7 b	32.0 b	2.5 b	0.7 c	259.5 a	58.5 a
	4	45.4 b	4.9 b	7.1 c	25.6 a	1.0 c	0.3 d	303.2 b	65.7 b
	6	67.2 a	5.3 c	9.8 d	33.1 b	0.9 c	0.4 c	336.0 b	75.8 c
	8	60.8 a	5.1 c	7.6 c	24.5 a	0.8 c	0.3 d	352.4 b	89.0 d
MS	**0**	60.7 a	5.2 a	7.2 a	29.5 a	1.9 a	0.5 a	136.0 a	52.7 a
	2	65.3 b	4.9 b	7.1 a	23.9 b	1.5 b	0.6 b	205.7 b	58.6 a
	4	44.4 c	4.4 a	6.5 b	30.4 b	1.0 c	0.4 c	223.5 b	66.5 a
	6	60.6 a	4.6 c	8.1 a	21.4 b	1.5 d	0.3 d	306.7 c	83.5 b
	8	53.6 a	5.2 c	8.2 c	25.0 b	1.2 d	0.3 d	336.5 c	96.3 c

Source: own work

Control soil without application of mycorrhizae (CO by its abbreviation in Spanish); Soil with application of mycorrhizae native to the area and isolated by the authors (MAZ by its abbreviation in Spanish); Liquid Mycorrhizae (ML by its abbreviation in Spanish); Solid Mycorrhizae (MS by its abbreviation in Spanish); Organic Carbon (OC); Cation Exchange Capacity (CEC); Phosphorus (P); Total Nitrogen (TN). Different letters in each parameter indicate significant differences; equal letters in each parameter indicate that there were no statistically significant differences ($P \leq 0.05$).

FUNCTIONAL GROUPS OF MICROORGANISMS AND ENZYMATIC ACTIVITY IN THE SOIL

As can be seen in (Figure 1), the nitrogen-fixing microorganisms revealed significantly higher average abundances in the soil with application of mycorrhizae native to the area than in the control soil. In the same way, the plots with application of liquid and solid mycorrhizae presented an intermediate increase between these two and consequently, in (Figure 2) the Phosphate-Solubilizing Bacteria (PSB) showed significant increase in the plots with application of native and liquid mycorrhizae with respect to the control soil. Similarly, the solid mycorrhizae showed an intermediate increase in PSB abundance among all plots. On the contrary, the Phosphate-Solubilizing Fungi (PSF) showed significant increases in all plots with application of mycorrhizae with respect to the control soil.

Specifications for the figures: the name of the plot is presented on the X-axis of all the graphs. Control soil without application of mycorrhizae (CO by its abbreviation in Spanish); Soil with application of mycorrhizae native to the area and isolated by the authors (MAZ by its abbreviation in Spanish); Commercial Liquid Mycorrhizae application (ML by its abbreviation in Spanish); and Commercial Solid Mycorrhizae application (MS by its abbreviation in Spanish).

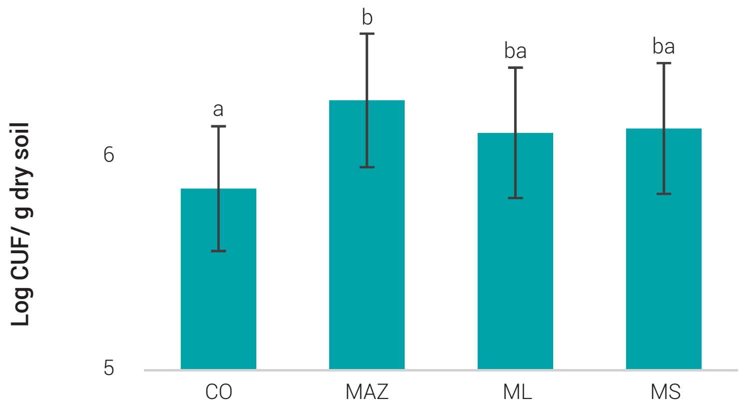

Figure 1. Abundance of the functional groups of microorganisms, Nitrogen-Fixing Bacteria analyzed for each of the plots studied during the five sampling cycles. Different letters in each parameter indicate significant differences; equal letters in each parameter indicate that there were no statistically significant differences ($P \leq 0.05$).

Source: own work

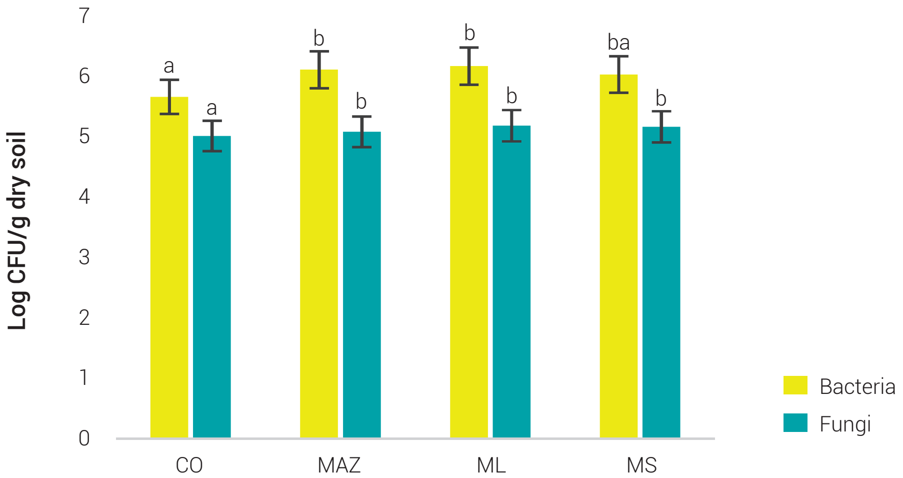

Figure 2. Abundance of functional groups of phosphate-solubilizing microorganisms analyzed for each of the plots studied during the five sampling cycles. Equal letters on the bars indicate that there are no statistically significant differences. Different letters on the bars indicate that there are statistically significant differences ($P \leq 0.05$).

Source: own work

Likewise, the abundance of Cellulolytic Bacteria (CB) (Figure 3) showed the same trend as the phosphate-solubilizing fungi (PSF), in relation to a significant increase in all the plots with mycorrhizae application with respect to the control plot. In the case of the Cellulolytic Fungi (CF), the same trend was not observed. Also, enzyme activities such as urease (Figure 4), acid phosphatase (Figure 5), alkaline phosphatase (Figure 6), and β-glucosidase (Figure 7) did not present statistically significant differences with respect to the control plot.

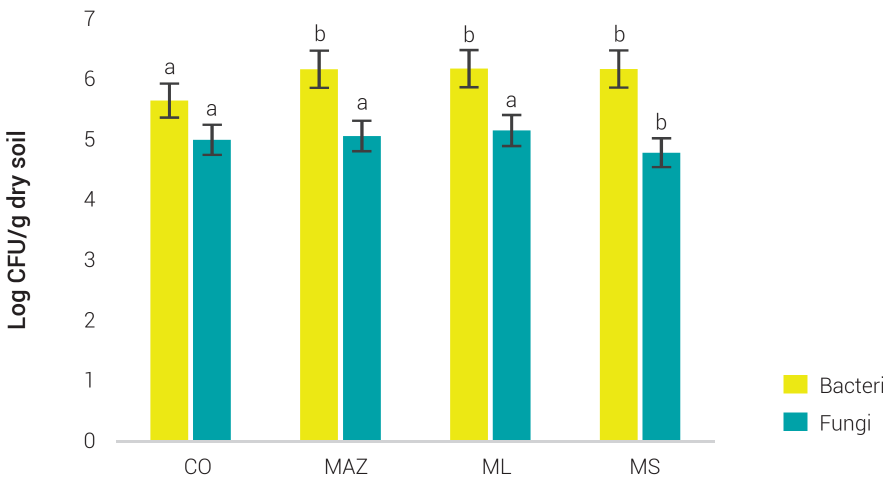

Figure 3. Abundance of functional groups of cellulolytic microorganisms (bacteria and fungi) analyzed for each of the plots studied during the five sampling cycles. Equal letters on the bars indicate that there are no statistically significant differences. Different letters on the bars indicate that there are statistically significant differences (P≤0.05).

Source: own work

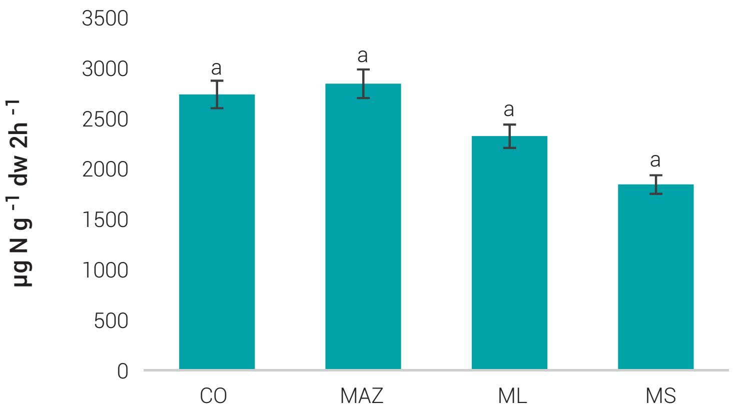

Figure 4. Urease enzyme activity analyzed for each of the plots studied during the five sampling cycles. Equal letters on the bars indicate that there are no statistically significant differences. Different letters on the bars indicate that there are statistically significant differences (P≤0.05).

Source: own work

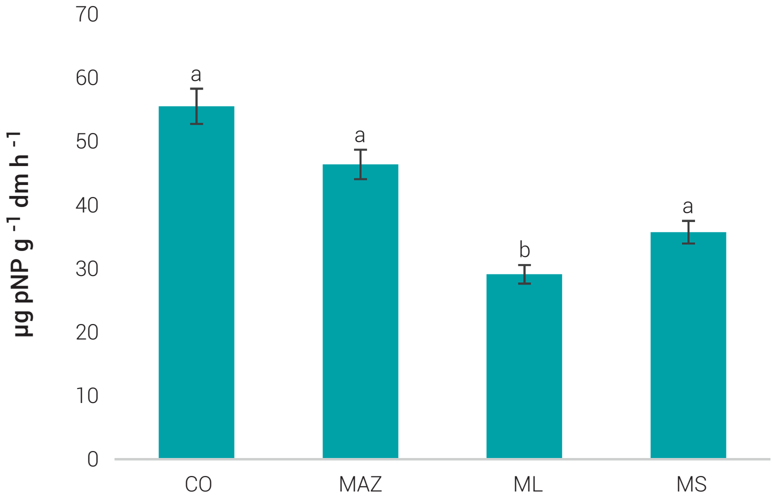

Figure 5. Acid Phosphatase enzyme activity analyzed for each of the plots studied during the five sampling cycles. Equal letters on the bars indicate that there are no statistically significant differences. Different letters on the bars indicate that there are statistically significant differences (P≤0.05).

Source: own work

Figure 6. Alkaline phosphatase enzyme activity analyzed for each of the plots studied during the five sampling cycles. Equal letters on the bars indicate that there are no statistically significant differences. Different letters on the bars indicate that there are statistically significant differences (P≤0.05).

Source: own work

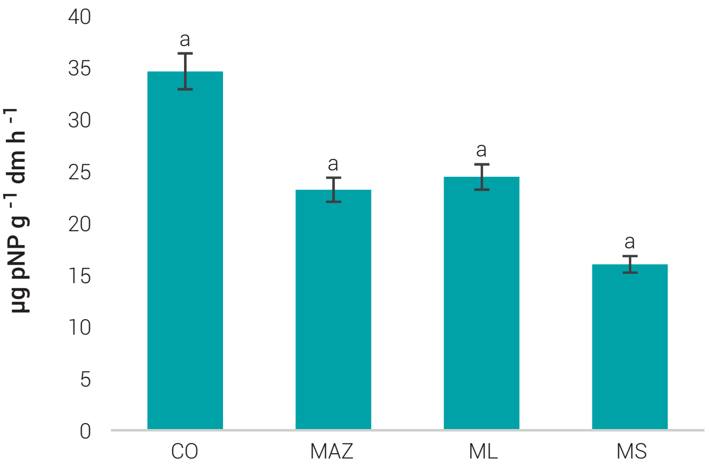

Figure 7. β-glucosidase enzyme activity analyzed for each of the plots studied during the five sampling cycles. Equal letters on the bars indicate that there are no statistically significant differences. Different letters on the bars indicate that there are statistically significant differences (P≤0.05).

Source: own work

The results that are stated in Table 3 show that, over the months, Nitrogen-Fixing Bacteria (NFB) showed an increasing trend in the different plots that were evaluated. The other microorganisms, such as the enzymatic activities of the soil, did not show a unique trend defined over time in the application of mycorrhizae.

Table 3. Functional groups of microorganisms in the soils that were analyzed, and monthly enzymatic activity

PLOTS	MONTH	NFB	PSB	PSF	CB	CF	Urease	Acid Phosphatase	Alkaline Phosphatase	β-Glucosidase
		Log CFU/g dry soil					µg tyr g – 1 dm 2 h – 1	µg pNP g-1 dm h-1		
CO	0	5.3 a	5.5 a	4.7 a	5.6 a	4.7 a	2027.7 a	43.5 ab	29.1 a	35.3 a
	2	5.4 a	5.6 a	4.7 a	5.6 a	5.3 b	2540.2 a	23.2 a	48.1b	9.9 b
	4	5.8 b	6.0 b	5.3 b	5.8 bc	4.7 a	6710.1b	62.9 b	105.7 c	79.2 c
	6	6.2 c	5.5 a	38.4 c	5.5 c	4.9 c	2269.6 a	37.1 ab	45.5 b	26.6 a
	8	6.4 d	5.7 c	5.2 b	5.5 c	5.1 c	126.8 a	110.8 c	43.7 b	22.0 a
MAZ	0	5.5 a	5.4 a	5.3 a	5.6 a	5.0 a	3688.2 a	7.1 a	28.1 a	24.4 a
	2	6.5 b	6.2 b	5.2 ab	6.5 b	5.4 b	3090.3 a	32.9 b	49.9 b	8.4 b
	4	6.5 b	5.9 c	5.1 b	6.0 c	5.1 a	5127.1a	162.7 c	67.2 b	40.8 c
	6	6.5 b	6.5 b	4.7 c	6.5 b	4.9 a	2087.4 a	19.9 ab	27.6 a	22.3 a
	8	6.2 c	6.5 b	4.9 d	6.0 c	4.7 c	209.9 c	9.1 a	27.8 a	19.9 a

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PLOTS	MONTH	NFB	PSB	PSF	CB	CF	Urease	Acid Phosphatase	Alkaline Phosphatase	β-Glucosidase
		Log CFU/g dry soil					µg tyr g – 1 dm 2 h – 1	µg pNP g-1 dm h-1		
ML	0	5.1 a	5.4 a	5.3 a	5.5 a	5.0 a	2146.5 a	22.2 a	15.0 a	30.4 a
	2	6.1 b	6.5 b	5.2 a	6.5 b	4.9 a	4716.1 b	56.4 b	42.8 b	15.0 b
	4	6.5 c	5.7 c	4.9 b	5.7 c	5.5 b	3941.7 b	21.6 a	63.0 b	27.0 a
	6	6.3 c	6.6 d	5.1c	6.5 b	5.3 b	685.7 a	38.6 b	27.9 c	29.2 a
	8	6.3 c	6.5 b	5.1 c	6.5 b	4.9 a	112.3 a	6.5 a	79.5 d	20.5 b
MS	0	5.2 a	5.5 a	5.8 a	5.5 a	5.1 a	2749.6 a	19.8 a	12.3 a	10.2 a
	2	6.4 b	6.1 b	4.6 b	6.5 b	4.7 b	2539.9 a	24.8 a	28.5 b	6.3 a
	4	6.2 b	5.6 a	5.1 b	5.8 c	4.8 b	3382.1 a	54.4 b	12.7 a	17.2 b
	6	6.4 b	6.6 c	4.7 b	6.6 b	4.6 b	534.8 b	33.2 c	11.4 a	22.7 c
	8	6.5 b	6.1 b	5.3 b	6.3 e	4.5 c	2.6 b	46.3 d	140.5 c	23.5 c

Source: own work

Control soil (CO by its abbreviation in Spanish); Mycorrhizae native to the area and isolated by the authors (MAZ by its abbreviation in Spanish); Liquid Mycorrhizae (ML by its abbreviation in Spanish); Solid Mycorrhizae (MS by its abbreviation in Spanish); Nitrogen-Fixing Bacteria (NFB); Phosphate-Solubilizing Bacteria (PSB); Phosphate-Solubilizing Fungi (PSF); Cellulolytic Bacteria (CB); Cellulolytic Fungi (CF). Different letters in each parameter indicate significant differences; equal letters in each parameter indicate that there were no statistically significant differences ($P \leq 0.05$).

PRINCIPAL COMPONENT ANALYSIS (PCA)

The cumulative variance of the principal component analysis (Figure 8) was 86.4%, which is a good indicator of the quality of the analysis that was performed. When analyzing the results globally, the plots with mycorrhizae application are substantially different from the control soil plot; however, the behavior of the soils in the plots with the mycorrhizae that were isolated from the study area and the liquid mycorrhizae have great similarity between them, with respect to the solid mycorrhizae.

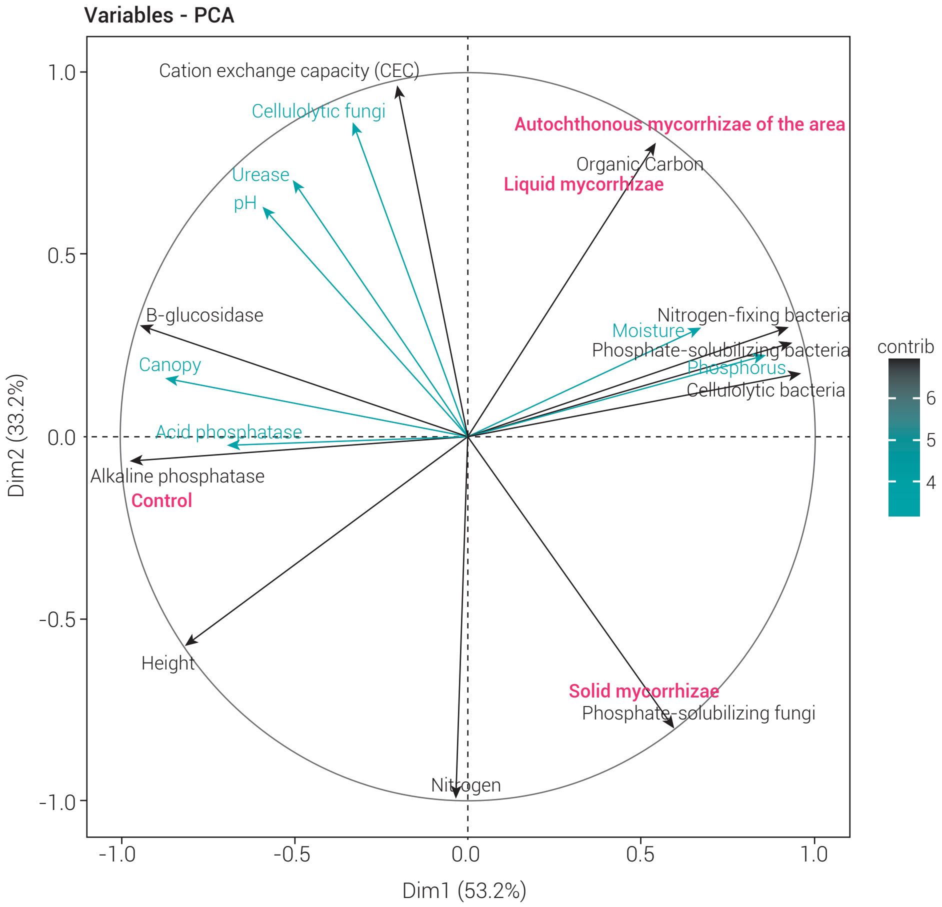

Figure 8. Principal Component Analysis (PCA)
Source: own work

Firstly, the soils in the plots with application of mycorrhizae native to the study area (Figure 2) and the liquid mycorrhizae, had a higher correlation with the following soil quality parameters: cation exchange capacity (CEC), organic carbon (OC), moisture, phosphorus (P), nitrogen-fixing bacteria (NFB), phosphate-solubilizing bacteria (PSB), and cellulolytic bacteria (CB); however, moisture and phosphorus had less contribution with respect to the other soil quality parameters. After that, the solid mycorrhizae showed a higher correlation with the phosphate-solubilizing fungi and by the contrary, the cellulolytic fungi did not show strong correlation with any of the plots. In the control plot (Figure 8), it was evident that the enzymes acid phosphatase, alkaline, β-glucosidase, and canopy showed a higher correlation; however, the acid phosphatase and canopy had less contribution in the analysis that was developed.

4. DISCUSSION AND CONCLUSIONS

The research hypothesis, that mycorrhizae application generates significant favorable changes in soil quality, was valid for the following parameters: organic carbon (14%), moisture (18.62%), phosphorus (30.3%), abundance of nitrogen-fixing bacteria (5.47%), phosphate-solubilizing bacteria (7.83%), cellulolytic bacteria (9.28%), and phosphate-solubilizing fungi (2.59%).

The soils with mycorrhizae application had significant changes in their content of phosphorus. These results coincide with what has been reported in the literature, in the sense that the presence of mycorrhizae has been found to favor phosphorus absorption in plants that are growing on acidic soils or soils with low to moderate fertility; as is the case of the study area [15]. Indeed, these results are similar in the study where the content of phosphorus in the coffee plants reported higher with the treatments that were inoculated with just the fungi or in conjunction compared with the control, as was observed in the coffee plantations of this study [26]. These results coincide with reports in which the availability of nutrients in the plants belonging to a crop may depend on some interactions between the plant and the mycorrhizae, which in turn influence the development of other microorganisms, contributing to the adaptation and production of plants [27].

In addition, the soils from the plots with mycorrhizal inoculation showed significant changes in the percentage of organic carbon, thus considering the importance of inoculation in nutrient absorption, and agreeing with those who claim that 5% to 10% of the photosynthetic carbon resides within the mycorrhizal component [28]. It should be noted that the content of organic carbon increased in soils with application of mycorrhizae native to the area during the five harvesting cycles, which is in line with those who believe that arbuscular mycorrhizal fungi are great conduits for the flow of carbon [29]. On the other hand, this research reports that the abundance of functional groups of microorganisms, such as bacteria and fungi, improve their behavior when mycorrhizae are inoculated into the soil. This also coincides with research works based on the interaction of the arbuscular mycorrhizal fungi with plants, in which, for example, it is stated that symbiosis not only occurs between microorganisms and plants but also among microorganisms. Therefore, the interaction between bacteria and mycorrhizae is symbiotic, insofar as bacteria help mycorrhizae to establish symbiosis, while the latter promote diversity and the potential for bacterial invasion [30].

In this regard, the high correlation between fungi and bacteria that was evidenced in the analysis of the main components in the soils of the plots that were inoculated with mycorrhizae shows that there is a symbiotic relationship between bacteria and fungi [30], where the latter depends on the compounds that are produced by the bacteria to survive. On the other hand, nitrogen-fixing bacteria showed significant changes in soils with mycorrhizal application. This coincides with studies that have been performed on mycorrhizae in legumes, in which mycorrhizal symbiosis improves nitrogen fixation and also coincides with those indicating that arbuscular mycorrhizal fungi accelerated the amount of bacteria and fungi over time in treatments where inoculation with mycorrhizae was used [31], [32].

Similarly, the results that have been reported indicate that phosphate-solubilizing microorganisms increased significantly in soils with mycorrhizal application; the same results were found by those who claim that phosphate-solubilizing bacteria and mycorrhizae can act synergistically, as phosphate-solubilizing bacteria can increase the amount of phosphorus that is available in the soil, meaning that mycorrhizal inoculation leads to changes in the bacterial composition of the root, as evidenced in this study [33].

Likewise, the populations of cellulolytic bacteria in the plots with mycorrhizal inoculation increased significantly in relation to the populations of cellulolytic bacteria in the control plot. This is consistent with what was reported in the study where the arbuscular mycorrhizal fungi increased the speed of decomposition of the organic material, indirectly influencing decomposition by means of the interactions with other soil microorganisms [34]. All in all, the data from the authors above demonstrate interactions between the arbuscular mycorrhizal fungi and the bacteria, reflecting the resource competition for decomposition products such as cellulolytic microorganisms that act as decomposers of plant and animal waste, releasing plant-assimilable nutrients [35]. Finally, a greater number of studies are required in order to document the storage of nutrients and their dynamics associated with the communities of microorganisms in the soil. This is a central issue considering that a high percentage of the carbon in the plant is aimed at maintaining the biomass of symbiont organisms, such as bacteria and mycorrhizal fungi [36].

Certainly, although the spores that were inoculated in the plot of the mycorrhizae native to the area were lower than those of the solid and liquid application, the behavior of quality parameters of the soil in the study area was significantly higher in the plot with native mycorrhizae, coinciding with the research that reports the efficiency in the inoculation of bacterial consortia with native mycorrhizae, in which the spores, being resistant structures typical of the place, can remain in the soil for a longer period of time compared to the remains of external mycorrhized vegetables, such as in the case of solid and liquid mycorrhizae [37]. Finally, according to the statistical study and the significance of the quality parameters of the soil reported in this research, we can state that the mycorrhizae native to the study area have a potential for isolation, so they can be used in future projects and they are of special consideration in sustainable agricultural strategies.

However, the hypothesis of this research work was not valid for the following soil quality parameters: pH, nitrogen, alkaline phosphatase activities, acid phosphatase, β-glucosidase, urease and cellulolytic fungi.

As for the pH, it did not vary over time and showed the same behavior as in the research where it was stated that greater mycorrhizal activity takes place in the presence of extremely acidic pH levels and that this does not depend on mycorrhization [15]. Nitrogen did not differ in the plots with mycorrhizal application. This concurs with the study that states that the concentration of nitrogen in the external hyphae of the arbuscular mycorrhizal fungi is 4-7 times higher than that of the plant shoots, and at least ten times higher than that of the roots. Consequently, the arbuscular mycorrhizal fungi must acquire large amounts of nitrogen from the soil for their own growth, and in nitrogen-limited systems, they are unlikely to transport biologically significant amounts of nitrogen to their host plants. Additionally, it is worth mentioning that the importance of the mycorrhizal fungi in plant nitrogen acquisition has been clearly demonstrated for the case of ectomycorrhizal fungi, but there is less evidence for other types of mycorrhizal fungi such as the arbuscular [38].

Likewise, the behavior of the acid and alkaline phosphatase was lower in the plots with mycorrhizal inoculation with respect to the control. The above is based on the fact that the mycorrhizae concentrate the phosphatase activity in the fungal mantle and therefore they diminish the integral phosphatase activity of the present investigation. It is also confirmed that the mycorrhizae have a limited capacity for enzymatic degradation [39], and this coincides with the study in which it was demonstrated that the phosphatase decreases over time in the soils that are inoculated with mycorrhizae [40].

In conclusion, the hypothesis of this investigation was validated in the sense that the inoculation of mycorrhizae significantly modified the behavior of the soil quality indicators such as the organic carbon (14%), moisture (19%), phosphorus (30%), abundance of nitrogen-fixing bacteria (5.5%), phosphate-solubilizing bacteria (7.8%), cellulolytic bacteria (9.3%), and phosphate-solubilizing fungi (2.6%). In addition, the mycorrhizae native to the study area and isolated by the authors showed a greater increase in quality parameters of the soil, with respect to the commercial mycorrhizae of solid and liquid application, which translates into a greater potential for use in future projects. However, the hypothesis was not validated with respect to the modification of the behavior of the pH, nitrogen, enzymatic activities, and cellulolytic fungi.

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