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Arbuscular mycorrhiza and their effect on the soil structure in farms with agroecological and intensive management

Hongos formadores de micorrizas arbusculares y su efecto sobre la estructura de los suelos en fincas con manejos agroecológicos e intensivos

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Abstract

Arbuscular mycorrhizal fungi help to reduce the damage caused by erosion and maintain soil structure through the production of mycelium and adhering substances. This study evaluated the structural stability; estimated the diversity and density of mycorrhizal spores present in three soil systems (eroded, forest and coffee plantations) in the rural area of Dagua, Valle del Cauca, Colombia. The systems evaluated were classified as farms with intensive or agroecological management. There were 25 morphospecies of mycorrhiza grouped in 13 genera, being *Glomus* and *Entrophospora* the most representative. The values of the indexes for geometric mean diameter (GMD) and weighted mean diameter (WMD) and diversity of mycorrhizal spores were statistically higher in farms with agroecological management than in farms with intensive management. The aggregate stability analysis revealed that eroded soils have significantly lower stability than forest and crop soils. A statistically significant correlation was found between diversity (r = 0.579) and spore density (r = 0.66) regarding GMD, and WMD with Shannon diversity (r = 0.54). Differences in practices, use and soil management affect mycorrhizal diversity found on farms and its effect such as particle aggregation agent generates remarkable changes in the stability and soil structure of evaluated areas. It is concluded, that agroecological management tends to benefit both mycorrhizae and the structure of soils.

Key words: Aggregate stability, agroecology, arbuscular mycorrhizal, conventional agriculture, erosion.

Resumen

En el suelo, los hongos formadores de micorrizas arbusculares (HMA) ayudan a reducir los daños causados por erosión y a mantener la estructura mediante la producción de micelio y sustancias adherentes. En el estudio se evaluó la estabilidad estructural del suelo y se estimaron la diversidad y la densidad de esporas de HMA presentes en tres sistemas de suelo (erosionado, bosque, cultivo de café) en una zona rural del municipio de Dagua, Valle del Cauca, Colombia. Las fincas se clasificaron en sistemas con manejos intensivos o agroecológicos. Se encontraron 25 morfoespecies de micorrizas agrupadas en 13 géneros, siendo *Glomus* y *Entrophospora* los más representativos. Los valores de los índices de los diámetros geométrico medio (DGM) y ponderado medio (DPM) y la diversidad de esporas de micorrizas fueron significativamente más altos en las fincas clasificadas con manejos agroecológicos que en aquellas con manejo intensivo. Los análisis de estabilidad de agregados revelaron que los suelos erosionados tienen significativamente menor estabilidad que los de bosque y cultivo. Se encontró una relación estadísticamente significativa en la diversidad (r = 0.579) y densidad de esporas (r = 0.66) con respecto al DGM, y del DPM con el índice de diversidad H' (r = 0.54). Las diferencias en las prácticas, uso y manejo del suelo se reflejan en la diversidad de micorrizas encontradas en las fincas y su efecto, como agentes de agregación de partículas, genera cambios notorios en la estabilidad y estructura del suelo en suelos de las zonas de evaluación. En síntesis, el manejo agroecológico tiende a favorecer las micorrizas y la estructura de los suelos.

Palabras clave: Agricultura convencional, agroecología, erosión, estabilidad de agregados, micorrizas arbusculares.

Introduction

Intensification of conventional agriculture in the tropics together with many human activities that accelerate erosion due to deforestation, significantly decrease the quality of the soil. This is particularly critical in areas subject to adverse changes in the physical, chemical and biological properties of soils agricultural vocation. This leads to negative effects at the social, environmental, economic and human health levels, as reflected in the rapid action of erosion due to deforestation and degradation of large areas (Arshad and Martin, 2002) In order to avoid the negative effects caused by intensive farming, research is done in agro-ecological handling solutions that reduce the use of chemical inputs and promote the rational use of natural resources, mixed cropping, use of local varieties tolerant to droughts or implementing traditional techniques (Altieri and Koohafkan, 2008). Some of these researches, focused on the study of microorganisms and their role in the soil matrix, seeking to reduce production costs and generate positive effects on the environment (Jaizme and Rodriguez, 2008).Among the communities of microorganisms living in the soil, forming arbuscular mycorrhizal fungi (AMF) are key to ensuring the sustainability of the soil-plant system (Oehl *et al.,* 2004). The symbiosis between mycorrhizal fungi and plant, can be used as bio-inoculant to reduce nutrient deficiency in plants and to participate in processes of aggregation and soil retention through physical (production of mycelium) and chemical mechanisms (production of adherent substances), in order to alleviate the effects caused by erosion (Jaizme and Rodriguez, 2008).

Research on the role of mycorrhizae in soil quality and sustainable agriculture have focused mainly on soils of the temperate zones. However, since the role of the mycorrhizal association depends on the interaction between fungus - plant and abiotic environment, is, therefore necessary to assess the role played by mycorrhizae on the fertility of tropical soils, as there are differences between temperate and tropical zones. Agriculture in temperate regions is characterized by excess conditions, whereas in the tropics the characteristic is the access conditions, especially the macronutrient phosphorus which some mycorrhizal fungi solubilize and provide to the plants (Cardoso and Kuyper, 2006).In the municipality of Dagua, Valle del Cauca, Colombia, agricultural intensification on coffee and banana crops in the search for higher yields has triggered a sharp deterioration in soil quality. Intensive processes of plowing and fertilizing with chemical inputs, cause reduction in soil fertility by decreasing the communities of AMF species and by the effect on the ecological functions performed by these organisms in the soil matrix, especially the retention of aggregates and prevention of erosion (Beare *et al.*, 1997). The above situation can be contrasted with farms of agroecological management in the same region, where farmers use less aggressive methods with the soil in terms of disturbance, use of pesticides and synthetic chemicals. It is therefore important to determine whether there are differences between different soil management practices present in the area, including small areas of forest that act as natural reference. In that vein, in the present study on farms in a rural area of Dagua, Valle del Cauca, indices of structural stability in eroded soils, forest areas and coffee and banana plantations with different agricultural managements were assessed and the relationship between soil structure maintenance and observed AMF communities was analyzed.

Materials and methods

Area of study and selection of farms

The study was done in farms located at the watershed of the Dagua river, Valle del Cauca (Colombia). This area has vegetation belonging to the humid subtropical forest with average temperatures between 23 and 25°C, at 1460 MASL in average (Espinal, 1968). Five farms were selected and classified according to their agroecologic status based on the qualitative method by Altieri and Nicholls (2002) (Table 1), in farms with agroecological management and farms with intensive management, in order to evaluate the soil quality and health of the crops by a series of sustainability indicators. These have values that oscillate between 1 and 10: one means the least desirable, 5 is middle and 10 is the most desired, according to the soil and crop characteristics. Farms with values equal to 5 are in the sustainability threshold, whereas those with lower averages are below the threshold and are hereinafter referred as farms with intensive management; whereas those with higher average than 5 are referred as farm with agroecological management.

Table 1. Assigned values to the quality indicators for soil and crop health in coffee farms of the rural zone of Dagua, Valle del Cauca. Based on Altieri and Nicholls, 2002.

Indicator	Farm				
	La Meseta	El Brillante	La Esperanza	Villama-ría	El Cedro
Soil quality					
Structure	5	7	9	9	8
Color, odor and organic matter	3	6	7	9	10
Soil porosity	3	6	9	8	9
Soil coverage	3	5	9	8	10
Erosion	3	6	8	9	10
Biological activity	2	6	9	10	9
Average	3.2	6	8.3	8.8	9.3
Crop health					
Aparence	5	7	9	9	9
Weed control	3	6	8	8	10
Crop fertilization	1	5	8	8	9
Plant diversity	2	7	8	9	9
Natural surrounding diversity	2	7	10	10	9
Management system	1	7	8	8	9
Average	2.3	6.5	8.5	8.7	9.2
Combined average	2.75	6.25	8.4	8.75	9.25

Experimental design

In the research a random block design with three systems was used: (1) crop area with coffee and banana plantations; (2) eroded zone, characterized by high degree of erosion with presence of reddish gullies and without apparent organic layer; and (3) forest area, belongs to secondary forest without agricultural intervention. In each system five plots were identified. Samplings were done in April, 2012. As the research was done in five farms, the number of plots per system was also five with two samples per plot, for a total of 10 samples.

Evaluation of spores from mycorrhizal fungi

From each field sample two subsamples of 100 g of soil were taken at 10 cm depth and were homogenized to get one of 200 g. Spores were extracted from 20 g of soil following the protocol proposed by Sieverding (1983) with modifications described by Sanchez de Praguer *et al.* (2010). Moisture was determined in other 20 g of soil. Samples were passed through successive sieves of 2 mm, 450 µm, 120 µm and 40 µm pore sizes; then the contents were centrifuged at 3600 rpm for 4 min, using a sucrose solution (70%) and water. Finally, the extracted spores in the solution were counted and mounted on PVGL (polyvinyl glycerol). Counting was expressed as spores/100 g of dry soil, using the equation proposed by *et al.* Sieverding (1983):

$$\text{No. spores} = \left(\frac{\text{counted spores}}{\text{sample weight}}\right) * \frac{wi}{wf} * 100$$

where:

wi: initial weight of the simple used to determine moisture.

wf: final weight of the sample to determine moisture content.

Spores were determined till morphospecies based on the taxonomic keys of Sánchez de Práguer *et al.* (2010); Oehl *et al.* (2011) and Redecker *et al.* (2013).

Analysis of agregate stability

The aggregate stability is evaluated by Yoder method described by Kemper *et al.* (1965) and modified by Jaramillo (2001), from two 100 g samples. With one gravimetric moisture was calculated and with the other, the Yoder method was used for 15 min using sieves of pore size of 2.00, 0.85, 0.50 and 0.25 mm. Samples were dried out for 24 h in oven at 105 °C. The geometric mean diameter (GMD) and weighted mean diameter (WMD) were calculated using the equations:

$$\text{GMD} = \sum_{i=1}^{n} \bar{x}_1 \frac{w_i}{100}$$

$$\text{WMD} = \exp\left(\frac{\sum_{i=1}^{n} w_i \ln \bar{x}_i}{\sum_{i=1}^{n} w_i}\right)$$

where:

$\bar{x}_i$: Average diameter of the fraction from the corresponding size.

w_i: Percentage of the weight of the respective fraction of the aggregates of determined size range divided by 100.

Statistical analysis

For these analysis the inviable spores were not taken into account. Data were cleared to calculate the spore diversity using the Shannon (H´) and Simpson (α) indexes. Differences in the structure of communities of the present mycorrhizal fungi species in all three soil types were evaluated by PERMANOVA analysis in PC-ORD® v5.0 software; in addition, the visual comparison of the evaluated variables was performed. By one-way analysis of variance the differences in the values of the aggregate stability index, abundance, richness and density of spores of mycorrhizal fungi in forest areas, crop and erosion and between farms with different agricultural managements were assessed. The Statistica® v7.0 was used. Normality of data was evaluated with the Shapiro-Wilk test and the homogeneity of variance with the Levene test. By means of the Spearman correlation the spores density and morphospecies richness of the arbuscular mycorrhizae were determined according to soil type and structural stability.

Results and discussion

Identification of mycorrhiza spores

25 morphospecies of AM spores were identified belonging to 13 genera (Table 2), among them *Glomus*, *Diversispora*, *Acaulospora*, *Entrophospora* and *Scutellospora* that have been reported in research done in coffee and plantain crops (Sánchez de Práguer, 1999; Bolaños *et al.*, 2000). *Glomus* and *Entrophospora* were the most abundant with 62 and 19 individuals, respectively. 23 morphospecies of AM were observed in the cropping zones, 12 in the forest and 6 in the eroded soil (Table 2). *Glomus* is the genus with more species in the Glomeromycetes and therefore, the most representative one with six morphospecies. This is an expected result since includes species that tend to survive in both healthy and perturbed environments (Oehl *et al.*, 2004). Important to notice is that till now and according to the consensus of arbuscular mycorrhizae fungi, the fungi from genus *Entrophospora*, Entrophosporaceae family, are under an uncertain taxonomic position (Redecker *et al.*, 2013).

Indexes of spore diversity of mycorrhizal fungi

The diversity index of Shannon in the farms with agroecological management (El Brillante,

Table 2. List of mycorrhizal morphospecies observed in cropping (C), forest (B) and eroded (E) areas of farms in the rural area of Dagua, Valle del Cauca, Colombia.

Morphospecies	La Meseta			El Brillante			La Esperanza			Villamaría			El Cedro		
	C	B	E	C	B	E	C	B	E	C	B	E	C	B	E
Acaulospora sp.1	0	0	0	1	0	0	1	1	0	0	0	0	7	0	0
Acaulospora sp.2	0	0	0	0	0	0	3	1	0	0	0	0	0	0	0
Acaulospora sp.3	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0
Ambispora sp.1	0	1	0	0	0	0	0	0	0	0	0	0	5	2	0
Ambispora sp.2	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0
Diversispora sp.1	0	0	0	0	0	0	0	1	1	1	0	0	5	3	0
Diversispora sp.2	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Entrophospora sp.1	0	4	0	0	0	0	2	0	0	1	0	0	16	8	1
Entrophospora sp.2	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Funneliformis sp.1	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0
Gigaspora sp.1	0	0	0	1	0	0	1	0	0	5	0	0	1	1	1
Gigaspora sp.2	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
Glomus sp.1	0	1	0	2	0	0	11	2	0	31	1	0	18	6	1
Glomus sp.2	0	0	0	0	0	0	1	0	0	0	0	0	2	0	0
Glomus sp.3	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0
Glomus sp.4	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0
Glomus sp.5	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Glomus sp.6	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0
Pacispora sp.1	0	1	0	0	0	0	2	0	0	7	0	0	8	1	0
Pacispora sp.2	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Paraglomus sp.1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Racocetra sp.1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Redeckera sp.1	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0
Sacullospora sp.1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Scutellospora sp.1	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0
Total	0	10	0	3	0	1	20	4	2	50	1	0	69	22	3

La Esperanza, Vilamaría and El Cedro) were higher than the ones observed in the farm La Meseta (Table 3). This difference may be related to factors such as disturbance in the hyphae network, the breaking of the thin cell walls of the spores, changes in nutrient content, alteration in soil microbial activity and stress caused in fungi by progressive loss of organic matter, which consequently is reflected in the decline of communities of species of AMF (Oehl *et al.*, 2003).

Similar as other studies (Helgason *et al.*, 1998), in this study the richness and abundance of spores AM on farms with agroecological management were higher in cultivation areas than in the forest areas and eroded soil (Table 3). Diversity indices of Shannon and Simpson are a reflection not only the richness of species of spores but how equal populations of these species are distributed in the community. It is considered that a system is healthier to the extent that the equitability is greater. Unlike Shannon index, which increases as the diversity grows, the Simpson index decreases in value as diversity decreases (Maurer and McGill, 2011). Based on the above, these results are interpreted in the sense that agroecological practices favor mycorrhizae. In other words, practice reduced tillage or plow and the limited application of chemical inputs, including fungicides, on farms with agroecological managements allow easy establishment of high densities of AMF spores in soil (Andrade *et al.*, 2009). However, soils in forests may be subject to aggression, for example to remove organic waste (compost) for sale in nurseries, this would explain the disparity of results obtained in the forest. Moreover, environmental differences are also important factors determining the density of AMF spores in these systems; it is known that with higher temperature and luminosity on the soil, the production of spores of HMA increases (Guadamarra and Alvarez, 1999). The coffee growing areas produce little shade than forests, therefore, there is an increase in temperature and light conditions on these soils, allowing more reproduction of these spores in these arable areas (Cardoso *et al.*, 2003). Additionally, the rapid degradation of forest areas generated by the expansion of the agricultural frontier, plus indirect harmful effects resulting from agricultural activity (Bethlenfalvay, 1993), cause a reduction in diversity and abundance mycorrhizal spores in the forest. The low density and diversity of spores found in eroded soil is a reasonable result and agree with the results obtained by

Table 3. Indexes of Simpson, Shannon and spores density of arbuscular mycorrhizae in 100 g of dry soil in farms of the rural area of Dagua, Valle del Cauca, Colombia.

Farm	Area	Simpson D	Shannon H'	Spores density	GMD	WMD
		Agroecological management (farm)				
El Brillante	Crop	0.375	1.4	6.71	3.936	3.197
	Forest	—	—	—	4.24	3.634
	Erosion	1		1.43	2.58	1.637
El Cedro	Crop	0.1333	2.373	13.4	3.498	3.28
	Forest	0.2397	1.633	27.8	3.461	2.553
	Erosion	0.3333	1.99	3.57	2.664	1.92
La Esperanza	Crop	0.3197	1.5	31.92	3.961	3.225
	Forest	0.28	1.332	6.9	3.57	2.858
	Erosion	0.5	0.6931	2.7	2.815	1.116
Villamaría	Crop	0.4176	1.322	56.23	4.656	4.335
	Forest	1	—	1.8	4.599	4.249
	Erosion	—	—	—	2.433	1.558
		Intensive agricultural management (farm)				
La Meseta	Crop	—	—	—	2.815	1.936
	Forest	0.22	1.748	11.55	1.713	1.7
	Erosion	—	—	—	2.43	1.554

Values of indexes for aggregate stability by farm divided according to the agricultural management.

Munyanziza *et al.* (1997). Simpson indices were similar to each other in the cropping areas, forest and eroded soil, showing low levels of dominance between morphospecies.

Analysis of aggregate stability

Differences in the index for aggregate stability were observed GMD (P = 0.023), WMD (P = 0.0007) and spores bulk density (P = 0.0231) among the cropping areas, forest and erosion. With the post anova analysis was found that the highest indices of aggregate stability were in the cropping areas (P = 0.0044 GMD and 0.0013 WMD) and forest (P = 0.0048 GMD and 0.0002 WMD) followed by eroded soils. No differences in the values of abundance and richness of spores between the areas studied were found (P = 0.0656 and 0.0529). However it appears that the abundance and richness of spores tended to be higher in cropping areas and lower in forest areas and eroded soil (Figure 1). These differences are influenced by the management previously performed on crops, which may cause different changes in soil properties (Osorio, 2009). Therefore, in the farm La Meseta intensive agricultural managements may be causing changes by reduction of the average size of aggregates and, thus in the stability of these. In that farm, the size was 2.8 mm for the GMD and 1.9 mm for WMD (Table 2), when the optimal value for aggregates in cropping areas should be around 3 mm (Jaramillo, 2001). mycorrhiza mycorrhizae mycorrhizal fungi In contrast, on farms El Brillante, La Esperanza, Villamaría and El Cedro, values were recorded indices of aggregate stability above 3 mm in cropping areas, suggesting greater stability and agricultural management practices that cause less negative impact on the soil. As a result of agroecological managements on these farms, the diversity of mycorrhizae in cropping areas was higher than in the farm intensive agricultural management.

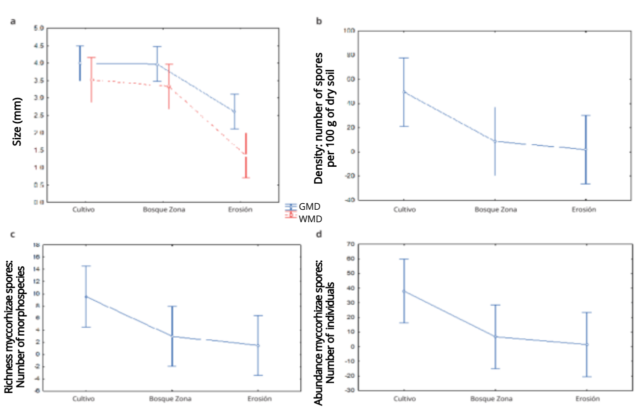

Figure 1. Values for the stability indexes of GMD and WMD (aggregate size in millimeters, mm) (a), spore (b), richness (c) and abundance (d) among cropping, forest and eroded areas in evaluated farms in the rural zone of Dagua, Valle del Cauca, Colombia.

GMD, WMD = Indexes of the geometric mean diameter (GMD) and weighted mean diameter (WMD).

The characteristics of agroecological practices are key to determining the differences in the values of the indexes for GMD, WMD, diversity and density of spores in soils of eroded and cropping areas. These features allow establishing in cultivation areas increased plant cover and facilitate the presence of temporary binders as roots and mycorrhizal mycelia networks on the soil. In turn, these agents provide organic matter to the system allowing the microbiota in the soil to develop a structure of porous aggregates of rounded shape typical of horizons A called crumb structure, which provides greater stability to the soil (Jaramillo, 2001).

Generally soil depends largely on the organic material to maintain structural stability. Therefore, the loss of the organic layer can produce a reduction in aggregate from 0.25 to 2 mm diameter, which are responsible for increased stability (Wilson *et al.*, 2009). Similarly, it was observed that the soils in the forest area had an aggregates state similar to the ones in the cropping area (Figure 1). This similarity is due, like in the forest, in the coffee plantations there are low or no plowing practices, which favors the permanence of a high percentage of stable macroaggregates, indicating the absence of disturbance in these soils, especially in networks of mycelia and roots (Verbruggen and Kiers, 2010).

Relationship among the AMF diversity index and the aggregate stability

The structural stability index WMD had a significant relationship with diversity (r = 0.579; P = 0.023) and spores density of the arbuscular mycorrhizae (r = 0.66; P =0.007). On the other hand, GMD also showed a significant relationship with the spore density of the mycorrhizal fungi (r = 0.54; P = 0.038). This analysis shows that the variables of aggregate stability increases proportionally as does the density and diversity of arbuscular mycorrhizal spores evaluated in soils of the farms (Table 4).

The relationships found between variables of aggregate stability, density and diversity of

Table 4. Significant correlations (P < 0.05) among spore density per 100 g of soil and GMD and WMD and the diversity index of Shannon and WMD.

Index	GMD	WMD	Density	H diversity
GMD	1.00			
WMD	0.95	1.00		
Density	*0.54*[b]	*0.67*	1.00	
H diversity	0.48	0.*58*	0.80	1.00

a. Indexes of the geometric mean diameter (GMD) and weighted mean diameter (WMD).
b. Significant values and of importance are shown in italic.

mycorrhizal spores suggest that communities of AMF species can influence soil aggregation at different scales. First, the biochemicals secreted by mycorrhizae are considered an important mechanism in soil aggregation, including the glomalin, a fungal protein that acts as a binder forming sticking string bags that they are secreted by hyphae and are able to stabilize the aggregates (Picone, 2003). Like glomalin, other compounds found in mycorrhiza as polysaccharides, hydrophobins and mucilage, which may have a functional role in aggregate stability (and Mummey Rilling, 2006). On the other hand, the growth of broad networks of mycelium of mycorrhizal fungi due to a high density of spores, generates a direct mechanical action on the soil organic matter by agglomerating small particles that bind hyphae and plant waste which, in turn, form aggregates. As these aggregates increase in size, the contribution of mycorrhizae increases in importance (Cardoso *et al.*, 2003).

Furthermore, the low amount of organic matter, as in the case of eroded areas constitutes an unfavorable factor that impedes maintenance of mycorrhizal fungi communities and, therefore, the stability of aggregates that maintain optimum soil structure. It is not excluded that some forests have suffered soil degradation processes due to the removal of the organic layer for use as a substrate in nurseries and gardens.

In the Permanova analysis no statistical differences were detected in the structure of the AMF communities' structure among the systems (P > 0.31). The high abundance of *Glomus* and *Entrophospora* morphospecies, which are independent from the degree of disturbance or type of soil, can be a determinant factor for the absence of those differences in the structure of the mycorrhizal fungi communities (Muleta *et al.*, 2008).

Conclusions

The agroecological management farms provide adequate characteristics to support a higher density of arbuscular mycorrhizal spores. One of those features is the structural stability that in soils of the study reached higher values in farms with these types of managements, compared with soils on farms with intensive agricultural managements.

The positive relationship between soil structure and density of mycorrhizal spores suggests that they have a key role to generate more aggregation and stability by preventing erosion.

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