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Mycotrophic capacity and diversity of native arbuscular mycorrhizal fungi isolated from degraded soils

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Abstract

Arbuscular mycorrhizal fungi (AMF) are organisms that form mutualistic associations with most plants, favoring their development, especially those located in degraded areas. In order to identify the different predominant native AMF morphotypes, and determine the percentage of colonization, and spore density in soils of the Cumbaza sub-basin in San Martin, Peru, soil samples were taken from degraded areas of Chirikyacu, Vista Alegre, El Chontal, San Antonio de Cumbaza, Aucaloma and Shapumba, and they were associated with 4 legumes cover crops among them, *Cajanus cajan, Canavalia ensiformis, Crotalaria juncea* and *Vigna unguiculata*. A completely random design was used, considering 6 zones and 4 legumes with 3 replications. The results showed that the treatments with legumes had greater influence in the mycorrhizal colonization in comparison with the zones of study, being *Vigna unguiculata* the one that had greater colonization (75%). However, the number of spores was influenced mainly by the zones, where the Aucaloma treatment had the highest number (252 spores / 10 g of soil). Eleven native AMF morphotypes were identified, being those of the genus *Acaulospora* the most predominant.

Keywords: cover crops; degraded soil; legume; spores; symbiosis.

1. Introduction

Arbuscular mycorrhizal fungi (AMF) form mutualistic symbiotic associations with roots of approximately 80-90% of higher plants on the planet and in all habitats of the earth (van der Heijden *et al.*, 2015). The plant provides the fungus with products of photosynthesis and the fungus supplies nutrients to the plant, in particular immobile nutrients such as phosphorus (Cardoso *et al.*, 2017; Ma *et al.*, 2019). They can also increase plant resistance against abiotic or biotic stress (Islas *et al.*, 2016), exercise control over pathogens (Mora-Romero *et al.*, 2015; Alvarado-Herrejón *et al.*, 2019), increase the proportion of carbohydrates in the root (Chen *et al.*, 2018) and participate in the formation of soil aggregates (Peng *et al.*, 2013). Its extensive extra radical mycelium favors the link between the plant and the soil (Rojas-Mego *et al.*, 2014) allowing to explore a greater volume of soil than the roots, which increases the absorption of nutrients for the plant (Rajtor and Piotrowska-Seget, 2016).

The study of the diversity of AMF in natural conditions is carried out through the selection and quantification of the number of spores, as well as the identification of morphotypes associated with a specific plant. Native AMF selected from areas with particular soil and climate conditions can be used as inoculants, with a better chance of adapting to degraded areas (López-Gómez *et al.*, 2015). The AMF obtained in this way when they multiply and reintroduce in certain areas can increase their richness and together with the installation of legumes favor the recovery of degraded soils. The legumes (Peña-Venegas and Arias, 2009) associated with AMF improve the physical characteristics (decompaction of soils), chemical (accumulation of organic matter and greater viability of nutrients) and biological (greater activity and microbial diversity) of the soil, in addition to reduce the mortality rate in plants (Duval *et al.*, 2015). While recent

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studies have investigated the effects of soil disturbances on the composition and richness of AMF in both agricultural soils (Mickan *et al.*, 2018) and pasture soils (Stover *et al.*, 2018), there is little information on whether soil disturbance from migratory agriculture influences spore numbers and AMF colonization in acid pH soil areas.

Currently, the biological properties of the soil have become important criteria to evaluate their use and management (Vallejo-Quintero, 2013). These are considered a new and emerging tool for the maintenance and restoration of soils, since they act as an index of soil fertility and their symbiotic interactions with plants favor the assimilation of nutrients (Singh, 2015). The presence, in turn, of certain plant species, product of plant succession processes, reveals soil fragmentation, which leads to changes in communities of microorganisms such as AMF (Majewska *et al.*, 2018). The San Martín region, Peru, product of unsustainable anthropogenic actions, such as migratory agriculture, presents areas with soils that have lost their fertility (Carranza *et al.*, 2012; Ravikumar *et al.*, 2017), demonstrated by the presence of expansive herbaceous species such as *Pteridium aquilinum* and *Imperata brasiliensis*. The recovery of these areas with cover crops may require the restoration of beneficial soil microorganisms, such as AMFs, and therefore more knowledge is needed about the diversity of AMFs and their interaction with plant communities.

Therefore, the objective of the present work was to select different morphotypes of native arbuscular mycorrhizal fungi predominant in degraded soils of the Cumbaza sub-basin and determine their mycorrhizal potential associated with legume plants. The hypothesis was that there is a great diversity of AMF in degraded areas of the Cumbaza sub-basin, whose number and mycrotrophic capacity is increased by multiplying in trap plants. This would allow an adequate number of AMF propagules to be re-introduced into areas of degraded soils. The information obtained will contribute to a better understanding of the interactions between AMF, legumes and soil, in order to improve the restoration strategies of native plants in disturbed areas.

2. Materials and methods

2.1 Places of study and collection of soil samples

Soil samples were collected in degraded areas of 6 zones of the Cumbaza sub-basin located in the provinces of Lamas and San Martin, Peru. These areas were Chirikyacu (6°29.065'S; 76°22,038'W), Vista Alegre (6°22.830'S; 76°31.132'W), El Chontal (6°20.435'S; 76°30.769'W), San Antonio de Cumbaza (6°24.286'S; 76°25.181'W), Aucaloma (6°26.296'S; 76°25.440'W) and Shapumba (6°25.747'S; 76°28.977'W). Cumbaza sub-basin is characterized by presenting areas of altitude between 410 and 1199 meters above sea level, with shallow, acid and low fertility soils. These are classified as inceptisols, entisols and alfisols. The average annual rainfall is 1200 mm, the average temperature is 26 °C and the relative humidity is 70%, considering these characteristics as an ecosystem of tropical dry forest and tropical premontane rainforest (Figure 1).

For the sampling of the soil, parcels of 2500 m² were selected in each area where the plants of *Shapumba (Pteridium aquilinum)* and *Cashaucsha (Imperata brasiliensis*) predominated and are indicators of degraded soils. Sampling was randomized in a zig-zag fashion, taking 1 kg of soil at a depth of 0-20 cm in 10 equidistant points (approximately 10 m), making a total of 10 sub samples per zone.

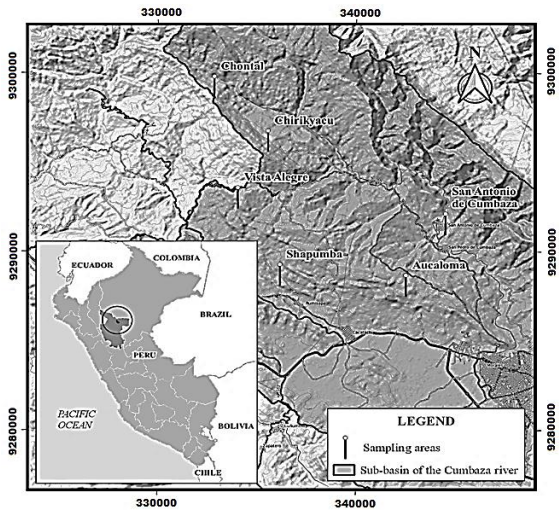


Figure 1. Map of the Cumbaza sub-basin that indicates the location of the 6 soil sampling zones.

The soil samples were homogenized and deposited in polyethylene bags and labeled with the name of the area and the collection date. Two fractions of each sample were separated, one for the selection of AMF spores and the other for physicochemical analysis. The remaining part was used as a substrate for the development of trap plants (legume cover crops). The soil samples were collected on April and May 2016, a season considered rainy, with rainfall ranging from 55 to 255 mm in the collection areas (SENAMHI, 2016).

Table 1
Physicochemical characteristics of soils in areas of the Cumbaza sub-basin

Areas	pH	EC µs/cm	N %	OM %	P ppm	K ppm	Mechanical analysis			Textural class
							Sandy %	Silt %	Clay %	
Chirikyacu	3.78	171.3	0.217	4.47	3.48	110.76	42	36	22	Loam
Vista Alegre	4.89	68.3	0.136	2.71	5.81	41.33	53	30	17	Sandy loam
Chontal	3.99	156.7	0.228	4.56	4.68	52.63	38	33	28	Sandy loam
Aucaloma	4.62	88.8	0.184	3.67	4.62	50.74	35	33	33	Clayish loam
Shapumba	4.47	57.8	0.129	2.57	4.18	28.61	54	28	18	Sandy loam
San Antonio de Cumbaza	6.40	275.2	0.264	5.22	7.94	233.8	32	24	45	Clayish

Analysis methodologies: pH (potentiometry, soil:water ratio 1:2.5, SI Analytics Lab 850 potentiometer, Germany), EC = Electrical conductivity (Conductimetry, soil:water ratio 1:2.5, SI Analytics Lab 960, Germany), N = Nitrogen (Micro Kjeldahl), OM = Organic matter (Walkley and Black), P = extractable phosphorus (modified Olsen), K = (By extraction of ammonium acetate and quantification in atomic absorption spectrophotometer GBC, SavantAA, Australia) and Texture (Bouyoucos Hydrometer).

2.2 Physicochemical characteristics of soils and spore density

The physicochemical characteristics of the soils collected are shown in Table 1, where the high acidity and low fertility of these soils can be observed. Table 2, on the other hand, shows the number of AMF spores isolated from the soils of the study areas.

Table 2
Number of AMF spores isolated from soils in areas of the Cumbaza sub-basin

Areas	N° of spores / 10 g of soil*
Chirikyacu	25
Vista Alegre	20
Aucaloma	13
El Chontal	13
Shapumba	28
San Antonio de Cumbaza	17

* Determined by the methods described by Gerdemann and Nicolson (1963) and by Schenck and Pérez (1990).

2.3 Trap plants and multiplication of AMF

The soils collected from each of the zones under study were crushed and screened separately. These, with the addition of vermiculite in a ratio of 1:1, became the substrate for the development of trap plants. The vermiculite was washed and sterilized previously in an autoclave (Fravill, AVDA50, Peru) at 121 °C for 1 hour. For the sowing 3.5 kg pots were used, each of which contained substrate with soil from each zone and were placed under nursery conditions. Disinfected seeds were used (with alcohol (70%) for 1 minute, rinsing with distilled H_2O, NaOCl (2%) for 5 minutes and rinsing with distilled H_2O) of 4 legumes adapted to tropical climates: Puspino (*Cajanus cajan*), Canavalia (*Canavalia ensiformis*), Crotalaria (*Crotalaria juncea*) and Caupi (*Vigna unguiculata*). The plants were kept in greenhouse conditions for 2.5 to 3 months, being watered until maintaining the humidity in field capacity. Nutritious solution of Hoagland without phosphorus was applied every 2 weeks. Irrigation was suspended between 70 and 80 days to stimulate the multiplication of AMF spores. At the end of this period, soil samples from the pots were dried and then sieved, keeping 500 g for further analysis. On the other hand, the roots of each of the legumes were washed with abundant water and cut into segments of approximately 2 cm in length. Then, these were placed in 50 ml falcon tubes adding alcohol (70%) for preservation.

2.4 Density of spores

The isolation of spores from the soil samples was carried out according to the wet sieving and decantation method, proposed by Gerdemann and Nicolson (1963) with certain modifications. To do this, a sample of 10 g of soil was sieved through sieves of 710 µm and 53 µm (ELE International, USA) in sequence, to isolate spores of different sizes. They were then placed in 50 ml Falcon tubes and resuspended with distilled H_2O for centrifugation (in Hettich centrifuge, Rotofix 32 A, Germany) at 3500 rpm for 5 minutes. A second centrifugation was performed in aqueous sucrose solution (70%) at 3500 rpm for 5 minutes. This stage was repeated twice. The spores were quantified according to the method proposed by Schenck and Pérez (1990). Thus, 10 ml of each sample processed by the sieving and decanting technique was taken and deposited in a concentric plate to facilitate spore counting. The spores were counted with the help of a manual counter and a binocular stereomicroscope (Carl Zeiss, Stemi 305, Germany) with a 40-fold increase.

2.5 Mycorrhizal colonization

Root staining of the legume cover crops was carried out according to the methodology of Vierheilig *et al.* (1998). Root samples that were preserved in alcohol (70%) were washed with running water and heated in a water bath (Selecta, Precisdig 12 l, Spain) at 90 °C with a solution of KOH (10%) for 40 minutes. Then, H_2O_2 (10%) was added until clarified. To follow, blue ink (Parker, Quink blue, France) was added for 60 seconds at 90 °C in a mary bath (Selecta, Precisdig 12 l, Spain). Finally, the stained roots were preserved in lactoglycerol for further evaluation. The determination of the percentage of mycorrhizal colonization was carried out according to the methodology

of Giovannetti and Mosse (1980). For this, the rootlets conserved in lactoglycerol were randomly distributed in a gridded Petri dish (1.27 x 1.27 cm), observing the binocular stereomicroscope (Carl Zeiss, Stemi 305, Germany) fungal structures of the AMF (hyphae, vesicles, arbuscules, spores). In each visual field the intersection of the roots with the horizontal line was observed, noting the presence or absence of mycorrhizal colonization of the root segment located on the grid line.

2.6 Isolation and identification of native AMF

For the development of this activity, the spores were placed on a clock plate and observed in the binocular stereomicroscope (Carl Zeiss, Stemi 305, Germany). With the help of a dissection needle, the spores were grouped according to the morphotypes, taking into account the morphological characteristics of shape, color and diameter, and then extracted with the help of a micropipette. The spores of the predominant morphotypes of each zone and of each legume was separated and placed on slides using polyvinyl alcohol and glycerol lactic acid (PVLG) and PVLG mixed with Melzer's reagent (1:1 v/v). Each preparation was kept at room temperature for 72 hours, and then observed in a binocular microscope (Carl Zeiss, Primo Star, Germany) at 100X and 400X. With the help of the Axio Vision system from Carl Zeiss, all the morphological characteristics were evaluated according to the parameters used in the illustrated arbuscular mycorrhizae catalog of the Colombian Amazon (Peña-Venegas *et al.*, 2006) and description of the species in the international collection of vesicular arbuscular mycorrhizal fungi from the University of West Virginia, USA (INVAM, 2017).

2.7 Experimental design and evaluation of variables

The present work was developed using a completely randomized design (DCA), with a factorial arrangement of 6A x 4B, considering factor A, areas of the Cumbaza subbasin, which include: Chirikyacu, Vista Alegre, Aucaloma, El Chontal, Shapumba and San Antonio de Cumbaza and factor B, legume cover crops, among them: *Canavalia ensiformis*, *Crotalaria juncea*, *Vigna unguiculata* and *Cajanus cajan*. The interaction of each of the factors gave rise to 24 treatments, with 3 repetitions, which makes a total of 72 experimental units. All the data obtained were subjected to analysis of variance. Once the homogeneity of the variances and the normal distribution of the data were verified, the means were compared using Tukey's multiple range test. For all cases, a significance level of $p < 0.05$ was used with $n = 3$. The data was analyzed with the SAS 9.2 program (SAS Institute Inc., 2008). To normalize the distribution of the data and stabilize the variances, the data expressed in percentage (mycorrhizal colonization) and quantifiable data (number of spores) were transformed to arcsine $\sqrt{x}$ and log (x), respectively.

In relation to the evaluation of the variables, the percentage of colonization of roots was calculated according to the methodology proposed by Giovannetti and Mosse (1980), using the following formula: Mycorrhizal colonization (%) = number of colonized segments / total number of evaluated segments x 100. The evaluation of the number of spores and the selection of predominant morphotypes was carried out in the third month of the experiment, with viable spores quantified in 10 g of soil and morphotypes found in a greater number of individuals (spores) in a determined sample of soil.

3. Results and discussion

3.1 Mycorrhizal colonization

According to the analysis of variance, it was observed that only the legume factor significantly influenced the mycorrhizal colonization ($p < 0.05$ and $R^2 = 61\%$), but not the zones factor. However, according to the mean comparison analysis ($p < 0.05$) (Figure 2), the legume roots of Chirikyacu showed the highest colonization (74.32%) and the lowest colonization (64.27%) which was observed in the roots of the Aucaloma area.

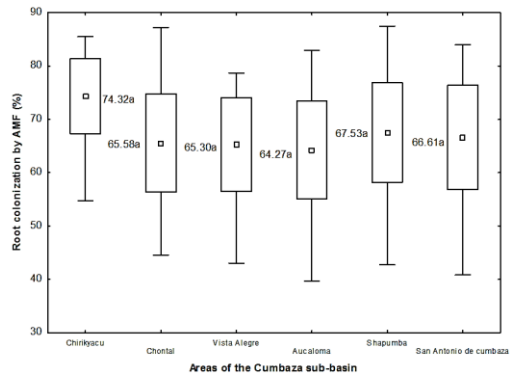


Figure 2. Box and whisker plot shows the mean, range and significant variation of root colonization by AMF in the different study areas. Different letters indicate a significant difference in the level of 0.05.

These results show high levels of colonization that can be closely linked to the pH of the soil and to the lower amount of available phosphorus present in the soils of

the different zones under study (Table 1). Corpoica (1998) found mycorrhization values of 50% to 84% in areas with low pH soils (3.5 to 4.2), results that agree with those obtained in the present work. In relation to phosphorus, some studies report an increase in mycorrhizal colonization in soils with low levels of available phosphorus (Chen *et al.*, 2008). In the present work, in the Chirikyaku area, less available phosphorus was found (3.48 ppm) (Table 1), greater mycorrhizal colonization was also observed (74.32%) (Figure 2).

Recently Ma *et al.* (2019), reported that under low phosphorus soil conditions the inoculation of AMF improved yield and zinc concentrations in wheat grain. On the other hand, in an AMF propagation trial using trap plants, but in this case corn, Alvarado-Herrejón *et al.* (2019) determined that corn plants grown in pots with sterile substrate, without phosphorus supplementation but supplemented with soil containing native AMF communities, reached the flowering stage unlike uninoculated corn plants that did not flower. This demonstrates the importance of knowing the type of vegetation and the behavior of AMF native to a given area, in order to establish future strategies for restoring degraded soils (Stover *et al.*, 2018), as is the case of the soils of the Cumbaza sub-basin, the study area of this paper.

Regarding the effect of legumes on mycorrhizal colonization, significant differences were observed between *Vigna unguiculata* (75.01%), *Crotalaria juncea* (73.73%) and *Canavalia ensiformis* (67.66%), in relation to *Cajanus cajan* (52.68%) that had the least colonization (Figure 3).

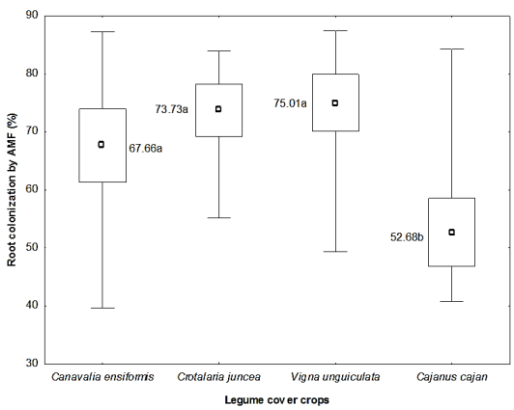


Figure 3. Box and whisker plot shows the mean root colonization (%) by AMF and its significant variation according to the legume cover crop used as a trap plant. Different letters indicate a significant difference in the level of 0.05.

These data are consistent with that reported by other authors such as Martín *et al.* (2007) that found 62.69% mycorrhizal colonization in roots of *Canavalia ensiformis* and Pérez-Luna *et al.* (2012) that reported 80.3% of colonization in legumes. Similarly, Higo *et al.* (2015) compared in an experiment under greenhouse conditions the yield of four cover crops between grasses and legumes (pea, hairy vetch, wheat and barley), finding differences in the percentage of root colonization by AMF between crops. The authors attributed these differences to the host selectivity. Thougnon-Islas *et al.* (2014), on the other hand, evaluated the mycotrophyc capacity of native AMF in soils of 7 zones in Buenos Aires (Argentina) using trap plants and observed that mycorrhizal colonization varied from 4.8% to 56.7%, without finding a uniform pattern among areas due to the edaphic differences existing among them.

Molecular analyzes performed by Turrini *et al.* (2018) revealed that the growth and nutrition of the plants (*Allium cepa*, *Capsicum annuum* and *Lactuca sativa*) were significantly affected by the diversity of native AMF that colonized the roots of the plants, varying their yield. Both native and non-native plant species influence the physicalchemical properties of the soil and thus the colonization of AMF (Majewska *et al.*, 2018). According to Basu *et al.* (2018), the biochemical mechanisms that occur between the symbionts directly affect the process of colonization and the formation of mycorrhizal structures, as well as the carbohydrate ratio and tensile strength of the plant root (Chen *et al.*, 2018). García-González *et al.* (2016) pointed out that the mycorrhizal structures formed (spores, hyphae, vesicles and arbuscules) and observed in the present work (Figure 4), fulfill different functions during the symbiosis, such as: reproduction, propagation, infection, absorption, reserve and exchange of nutrients between the plant and the fungus.

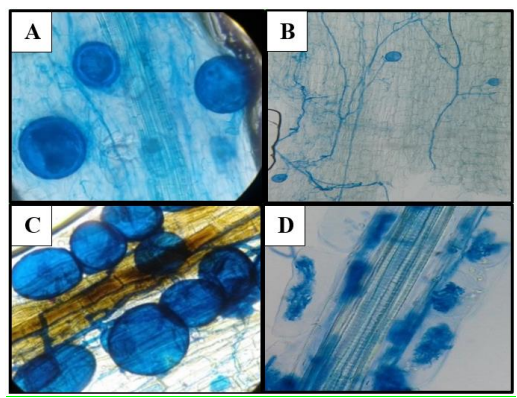


Figure 4. AMF structures in legume roots observed under a binocular microscope. A) Spores, B) Intraradical hyphae, C) Vesicles, D) Arbuscules.

3.2 Number of native AMF spores

When performing the analysis of variance, both the zones factor and the legume factor showed independent and significant effects on the number of spores ($p < 0.05$ and $R^2 = 54\%$). In relation to the effect of the zones on the number of spores, it was verified that the area of Aucaloma had a higher number of spores (252 spores / 10 g of soil) and, the area of Chirikyacu however had the lowest number (190 spores / 10 g of soil) (Figure 5).

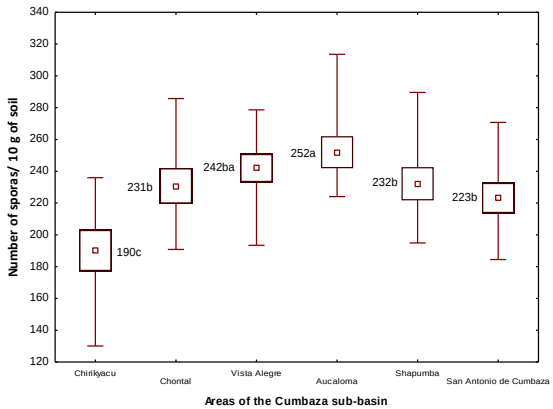


Figure 5. Box and whisker plot shows the mean, range and significant variation of the number of AMF spores in the different study areas. Different letters indicate a significant difference in the level of 0.05.

According to García-González *et al.* (2016) the physicochemical characteristics of soils can generate variations in the number of spores. The soil analysis of the study areas shows differences in pH, fertility level in phosphorus and nitrogen and in physical parameters such as texture and structure (Table 1), factors that could be influencing the variation in the number of spores described in the present study. Influences of the climatic characteristics of the sites on the number of spores were observed by Haro *et al.* (2018), who after selecting and identifying AMF from 6 sites in Burkina Faso, West Africa, found a significant negative correlation with respect to the level of phosphorus in the soil, reached between 339-2181 spores per 100 g of soil. Becerra *et al.* (2014), meanwhile, affirmed that, in addition to climatic factors, the production of AMF spores also depends on the type of host plant. The authors evaluated soils of 4 plants of the Chenopodiaceae family finding different numbers of spores in each of them (between 3 and 1162 per 100 g of soil). Differences in spore numbers were also observed by Majewska *et al.* (2018), studying the interaction of AMF with invasive exotic plants and expansive native plants and their relationship to soil properties. In the Cumbaza sub-basin, where soils were obtained for the development of this work, invasive species of *Pteridium aquilinum* and *Imperata brasiliensis* predominate, which have expanded in this area due to low soil fertility (Suazo-Ortuño *et al.*, 2015), so understanding the mechanisms of interaction between symbionts is essential to establish soil restoration programs in degraded areas.

In relation to the variation in the number of spores produced in the trap plants, compared to those found in the field (28-32 spores per 10 g of soil, Table 2), a considerable increase was observed, varying between 190-252 spores per 10 g of soil (Figure 5). Similar results were observed by Thougnon-Islas *et al.* (2014) who found between 80-1175 spores / 100 g in the substrate of trap plants, a greater number, according to the authors, than those found in the field. It is likely, then, that the mycorrhizal propagules in the soil samples were mainly segments of hyphae and colonized roots, instead of spores.

In relation to the effect of the legume cover crops on the number of spores, significant differences were found between *Canavalia ensiformis, Crotalaria juncea* and *Cajanus cajan*, with respect to *Vigna unguiculata* that had the smallest number of spores (Figure 6).

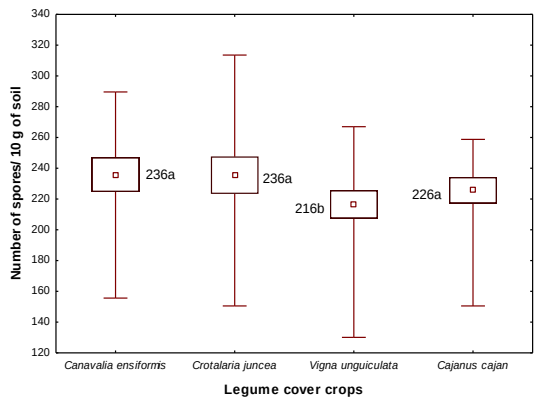


Figure 6. Box and whisker plot shows the mean, range and significant variation of the number of AMF spores in different legume cover crop. Different letters indicate a significant difference in the level of 0.05.

The climatic factors of the study area could be influencing the presence of AMF propagation structures. The soils used in the installation of trap plants were extracted in a rainy period. Oliveira and Oliveira (2005) when studying the seasonal dynamics of AMF of 2 fruit species, in an upland ecosystem in the central Amazon, observed a significant positive correlation between the increase in the number of AMF

spores with the rainy period, concluding that both the colonization and the sporulation of AMF are seasonal and depend on the species of host plant, the climatic factors and the chemical composition of the soil of a given area.

3.3 Predominant native AMF diversity

According to the morphological characteristics observed in the AMF spores of the soils of the Cumbaza sub-basin associated with 4 legume cover crops, the identification and selection of 11 predominant native morphotypes belonging to 4 genera (*Ambispora, Acaulospora, Glomus* and *Rhizoglumus*) was achieved. The most predominant was the genus *Acaulospora* with 8 morphotypes (Table 3). Bearing in mind that not all AMF species have the same capacity to form spores in a given period (Schenck and Pérez, 1990), it is very likely that other AMF genera exist in the area studied, not described in the present work.

Comparing the number of spores found in the soils of the different collection areas (Table 1) with respect to the number of spores of the different morphotypes found in the trap plants (Table 3), an increase of spores of up to 900% was observed and a wide diversity of efficient AMF morphotypes in degraded soils, which confirms the proposed hypothesis, hence the importance of these studies that support the application of techniques that use inoculants in soil recovery programs.

This report is consistent with that found by Lopes *et al.* (2016) in soils with acid pH and multiplied in trap plants using *Brachiaria decumbens* where they observed a total of 15 AMF morphotypes being the majority belonging to the genus of *Glomus* and *Acaulospora*. Pérez-Luna *et al.* (2012), in turn, reported morphotypes in acid soils in Mexico, in corn plots associated with cover crops, with the genera *Glomus* and *Acaulospora* being the most abundant with 11 and 8 morphotypes, respectively. Banni and Faituri (2013) pointed out that the genera *Glomus* and *Acaulospora* adapt better to the stressful conditions of acid soils. Likewise, it was observed that in trap plants there is a succession pattern in the sporulation of AMF, with *Glomus* species being the first to sporulate, while *Acaulospora* species do so later than a seasonal period (Oehl *et al.*, 2003). Stürmer and Siqueira (2011) when studying the richness and abundance of AMF spores in soils of 6 areas with different land uses (mature virgin forests and converted pasture sites, crops, agroforestry systems, secondary forests, young and old), in the Amazon region of Brazil. They observed that the fungal communities were dominated by the *Glomus* species, although the *Acaulospora* species produced more abundant sporulation, which shows that the cultural practices adopted in this region maintains a great diversity of AMF in the different areas. Alvarado-Herrejón *et al.* (2019), on the other hand, identified twenty-seven morphotypes of AMF at the species level, which belonged mainly to the families of Gigasporaceae, Glomeraceae and Acaulosporaceae. The wide distribution of the genera *Acaulospora* and *Glomus* observed in the present study coincides with the reports of the majority of the researches carried out on degraded soils (Sousa *et al.*, 2014, Torres-Arias *et al.*, 2017; Stover *et al.*, 2018).

Table 3 also shows that morphotypes 2 (*Acauslospora ignota*), had the highest abundance of spores with 923 individuals, being the most representative in this study. Morphotypes 1 reached a total of 430 individuals and morphotypes 3, 122 individuals. The morphotypes 4, 5, 6, 7, 8 and 9 belonging to the genus *Acaulospora* had between 14 and 91 spores / 10 g of soil. In relation to the morphotypes 10 and 11 belonging to the genera *Glomus* and *Rhizoglomus* they had 110 and 28 individuals respectively. Coelho *et al.* (2014) found that the type of substrate used in plant development also causes variations in the production of AMF spores and infectious propagules. Likewise, spore production and the distribution of AMF communities are related to soil pH and micronutrient levels (Mn and Zn) (Alguacil *et al.*, 2016). Table 4 shows the structures of the spores of the different AMF morphotypes found in the present work.

Table 3
Total number of spores per predominant AMF morphotypes, obtained from each of the substrates of the different study areas where each of the legumes used as trap plants grew

Morphotypes	M1	M2	M3	M4	M5	M6	M7	M8	M9	M10	M11
N° of spores / 10 g of soil	430	923	122	91	85	76	20	18	14	110	28

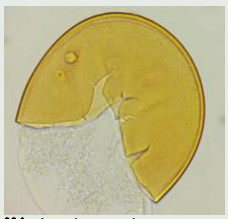
AMF morphotypes: M1 = *Ambispora appendiculata,* M2 = *Acaulospora ignota*, M3 = *Acaulospora rugosa*, M4 = *Acaulospora herrerae*, M5 = *Acaulospora* sp. 1, M6 = *Acaulospora* sp. 2, M7 = *Acaulospora foveata*, M8 = *Acaulospora tuberculata*, M9 = *Acaulospora* sp. 3, M10 = *Glomus* sp., M11 = *Rhizoglomus* sp.

Table 4
Identification of predominant native morphotypes of the 6 zones of the Cumbaza sub-basin and the 4 legume cover crops used as trap plants

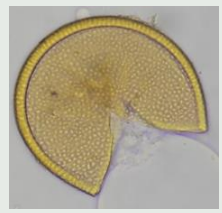
M1. *Ambispora appendiculata*
Color: yellow
Shape: globose
Diameter: 170-390 µm
Number of walls: 03
Ornamentation: yes
Scar: yes
Suspension hypha: no
Germination plate: no
Reaction in Melzer: yes

M2. *Acaulospora ignota*
Color: light yellow
Shape: Globose
Diameter: 60-95 µm
Number of walls: 03
Ornamentation: no
Scar: yes
Suspension hypha: no
Germination plate: no
Reaction in Melzer: yes

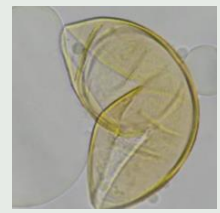
M3. *Acaulospora rugosa*
Color: light yellow
Shape: Globose
Diameter: 70-128 µm
Number of walls: 03
Ornamentation: no
Scar: yes
Suspension hypha: no
Germination plate: no
Reaction in Melzer: yes



M4. *Acaulospora herrerae*
Color: yellowish brown
Shape: spheric
Diameter: 50-112 µm
Number of walls: 03
Ornamentation: yes
Scar: yes
Suspension hypha: no
Germination plate: no
Reaction in Melzer: yes



M5. *Acaulospora* sp. 1
Color: light yellow
Shape: spheric
Diameter: 70-112 µm
Number of walls: 03
Ornamentation: yes
Scar: yes
Suspension hypha: no
Germination plate: no
Reaction in Melzer: yes

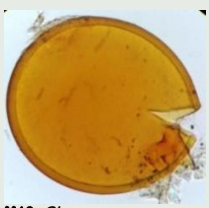


M6. *Acaulospora* sp. 2
Color: light yellow
Shape: spheric
Diameter: 75-100 µm
Number of walls: 03
Ornamentation: no
Scar: no
Suspension hypha: no
Germination plate: no
Reaction in Melzer: yes

M7. *Acaulospora foveata*
Color: reddish-brown
Shape: spheric
Diameter: 300 a 400 µm
Number of walls: 03
Ornamentation: yes
Scar: yes
Suspension hypha: no
Germination plate: no
Reaction in Melzer: yes

M8. *Acaulospora tuberculata*
Color: dark yellow
Shape: spheric
Diameter: 255-340 µm
Number of walls: 03
Ornamentation: yes
Scar: yes
Suspension hypha: no
Germination plate: no
Reaction in Melzer: yes

M9. *Acaulospora* sp. 3
Color: light yellow
Shape: spheric
Diameter: 220-320 µm
Number of walls: 03
Ornamentation: no
Scar: no
Suspension hypha: no
Germination plate: no
Reaction in Melzer: yes



M10. *Glomus* sp.
Color: light brown
Shape: spheric
Diameter: 80-200 µm
Number of walls: 01
Ornamentation: no
Scar: no
Suspension hypha: no
Germination plate: no
Reaction in Melzer: no

M11. *Rhizoglomus* sp.
Color: hyaline
Shape: globose
Diameter: 75-100 um
Number of walls: 02
Ornamentation: no
Scar: no
Suspension hypha: si
Germination plate: no
Reaction in Melzer: no

4. Conclusions

As demonstrated in this study, AMF constitute an important component of soil in degraded areas. The knowledge of the diversity and richness of AMF is a priority to later understand the processes that allow its adaptation in certain environments and the setting of symbiosis with plants. The selection of predominant native AMF and its multiplication in trap plants are actions that favor the increase of spores that could then be reintroduced in the degraded areas and thus improve the development of the plants through a greater absorption of nutrients from the soil. The AMF morphotypes selected in this way could be used as inoculants of legume cover crops in the recovery processes of degraded areas, an activity that is also favorable for the environment.

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