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# **Functional strategies of arbuscular mycorrhizal fungal diversity: significance of analyzing glomeromycotan spores numbers or biovolumes**

Estrategias funcionales de la diversidad fúngica micorrízica arbuscular: importancia del análisis del número de esporas de hongos glomeromicetos o de los biovolúmenes

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#### **ABSTRACT**

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*Eduardo Furrazola Gómez http://orcid.org/0000-0002-6367-5238* In this study, we investigated the relationship between plants and arbuscular mycorrhizal fungal communities by evaluating the species composition of this latter associated with two adjacent, yet distinct vegetative types in Las Peladas Ecological Station, Sierra del Rosario, Cuba: a montane shrubby savanna and a microphyllous evergreen forest. These vegetation types differ markedly in plant species composition as well as in other vegetative characteristics, including plant structure, turnover rates and striking environmental changes at the soil level. Microphyllous evergreen forest and shrubby savanna at Las Peladas are also markedly influenced by topography. Soil samples were collected along transepts at four plots, two of them growing microphyllous forest (FW and FE) and two more growing shrubby savannah (SW and SE). Plant cover and glomeromycotan species distribution were studied and correlated. Entropy values were assessed and statistically compared. The obtained results demonstrate that glomeromycotan communities' diversity are determined firstly by environmental prevailing conditions, and secondly by their relationships towards fitting better a particular host plant. These host plants seemed to be favored by the reduction of competitiveness at FW and SW because of the reduced number of plant species surpassing 10 % of plant cover, and at SE because of plant adaptation to water stress evenly favoring plant growth and fungal fitness.

**Key words:** arbuscular mycorrhizal fungal communities, environmental variables, evergreen forest, fungal fitness, shrubby savanna

#### **RESUMEN**

En el presente estudio se investigó la relación existente entre las plantas y las comunidades de hongos micorrizógenos arbusculares mediante la evaluación de la composición de especies de estas últimas asociadas a dos tipos de vegetación distintas adyacentes en Las Peladas, en Sierra del Rosario, Cuba: una sabana arbustiva montana y un bosque siempreverde micrófilo. Estos tipos de vegetación difieren marcadamente en la composición de las especies vegetales, así como en otros tipos de características vegetales como estructura de las plantas, tasas de renovación y cambios ambientales contrastantes a nivel del suelo. El bosque siempreverde micrófilo y la sabana arbustiva de Las Peladas también se encuentran altamente influidos por la topografía de la región. Las muestras de suelo fueron colectadas a lo largo de transeptos en cuatro parcelas, dos de ellas en bosque micrófilo con exposiciones este y oeste (BE y BO, respectivamente) y dos en sabanas arbustivas igualmente con exposición este y oeste (SE y SO). La cubierta vegetal y la distribución de las especies de hongos glomeromicetos fueron estudiadas y correlacionadas. Los valores de entropía fueron medidos y comparados estadísticamente. Los resultados obtenidos demuestran que la diversidad de las comunidades de hongos glomeromicetos está determinada, en primer lugar, por las condiciones ambientales prevalecientes y, en segundo lugar, por sus relaciones para adaptarse mejor a una planta huésped en particular. Estas plantas hospederas particulares parecieron verse favorecidas por la reducción de la competitividad en FW y SW debido al número reducido de especies de plantas que supera el 10% de la cubierta vegetal, y en SE debido a la adaptación de las plantas al estrés hídrico que favorece el crecimiento de las plantas y el acoplamiento fúngico con las especies vegetales.

**Palabras clave:** acoplamiento fúngico, bosque siempreverde, comunidades de hongos micorrizógenos arbusculares, sabana arbustiva, variables ambientales

## **INTRODUCTION**

Arbuscular mycorrhizal fungi (AMF; Glomeromycota) form the broadest range of symbiotic relationships between fungi and plants in nature (Tedersoo *et al*., 2018). This association has been shown to be critical in the physiology and nutrition of plants, with some plants being more dependent upon the presence of the fungi than others (Janos, 1980a; Brundrett, 2017; Hoeksema *et al.,* 2018). Because of the differing dependence of plants on mycorrhizal fungi, the presence of these fungi has been predicted to exert considerable influence on the process of succession (Janos, 1980b; Koziol and Bever, 2015). While the mechanism remains to be investigated, several studies have demonstrated substantial influence of mycorrhizal fungi on plant communty composition and ecosystem processes. For example, the plant species composition during succession (Medve, 1984; Koziol and Bever, 2019), the plant diversity and evenness of communities (Grime *et al.,* 1987; Vogelsang *et al.,* 2006), and the outcome of interspecific plant competition (Hartnett *et al.,* 1993; Zhou *et al.,* 2018) have been shown to depend upon the presence and composition of AM fungi.

Increasing evidence indicates that species of AM fungi differ in attributes, which will influence plant community processes. For example, fungal species have been shown to differ in their effectiveness in plant growth promotion, with the species of fungi which best promote growth differing between plant species (Ravnskov and Jakobsen, 1995; van der Heijden and Scheublin, 2007; Koziol and Bever, 2016; Cheeke *et al*., 2019) as well as environmental condition (Ji *et al.,* 2012; Xiang *et al*., 2016). Fungal species have also been shown to differ in their ability to bind soil particles into microaggregates (Miller and Jastrow, 1994; Duchicela *et al.,* 2013). Thus, it is possible that plant community composition and processes will be influenced not only by the presence of AM fungi, but also by the composition of the AM fungal community.

We presently know little about the specific associations of plant and fungal communities. Spatial associations have been found both across (Herrera *et al.,* 1997) and within (Schultz, 1996) vegetation types. These associations may result from the direct effects of the plant community composition on the fungal community (Bever *et al.,* 1996) or from the effect of the fungal community composition on the plant community (Koziol and Bever, 2019). If both of these processes are occurring, an active dynamic of plant and fungal composition may result which could lead to a variety of dynamical outcomes (Bever, 1999; Bever *et al.*, 2002). Regardless, of the precise dynamics, the identification of fungal communities specifically associated with plant communities highlights the potential importance of

AM fungal community composition in the dynamics of plant communities.

In this study, we investigate the relationship between plant and fungal communities by evaluating the species composition of the arbuscular mycorrhizal fungal communities associated with two adjacent, yet distinct vegetative types in Las Peladas Ecological Station, Sierra del Rosario, Cuba: a montane shrubby savanna and a microphyllous evergreen forest. These vegetation types differ markedly in plant species composition as well as in other vegetative characteristics, including plant structure, turnover rates and striking environmental changes at the soil level. Microphyllous evergreen forest and shrubby savanna at Las Peladas are also markedly influenced by topography. Therefore, the influence of abiotic environmental conditions on the glomeromycotan community cannot be discarded. If plant and fungal community composition are causatively related, then the composition of the fungal communities should be similarly distinct.

On the other hand, the analysis of entropy of glomeromycotan fungal community has been not carried out previously in Cuba. Glomeromycotan species differ too much on their sizes so that species producing large spores supposedly require more carbon to form than those ones producing smaller spores. Therefore, we assume that when characterizing entropy values for glomeromycotan fungi working with the spore's biovolumes would give a better approach to understand the species diversity than working with spore numbers, which are enormously variable from one to a second fungus. We therefore test the null hypothesis that the glomeromycotan fungal communities fit somehow plant species composition and abundance and prevailing environmental conditions.

#### **MATERIALS AND METHODS**

# **Study area**

The study was conducted within the UNESCO Biosphere Reserve (BR) Sierra del Rosario. The reserve is located at the Easternmost section of the Sierra del Rosario mountains within the provinces of Pinar del Río and La Habana and is south of the Cabañas Bay at the geographical co-ordinates of 24° 45' and 23° 00' North and 82° 50' and 83° 10' West. The mountain system at the reserve is sharply sectioned with slopes averaging  $25$  to  $45^{\circ}$  and reaching altitudes of  $100$  to 565 m a.s.l. The annual temperature and rainfall at the reserve (averaged over more than 20 years) are 24.4°C and 2,014 mm, respectively. Minimal values of temperatures and rainfall occur from November to April (dry season), and the maximal ones from May to October (rainy season). Additional information

on the geography, natural history, and human history of the reserve is published elsewhere (Herrera *et al.*, 1988).

The study focused on the northern section of the massif "Las Peladas" which main watershed is oriented West to East at the North-Eastern quarter of the BR Sierra del Rosario. The massif is dissected by a sectioned system of ridges and creeks, which reach heights from 250 to 370 m a.s.l. and extend approximately perpendicularly to the North and South (**Fig. 1**).

Geologically, the Cretaceous Rock Formation "Polier" is predominant (Furrazola-Bermúdez, 1987). For the lithology of "Polier" fine grain well stratified grayish-brown calcareous stones predominate intermixed with fine layers of quartz and argillite sandstone. Predominant soils to the North belong to the type Yellowish-Red Lixiviated Fersialithic. Their textures vary from sandy clayed loams to sandy loams. **Table 1** shows data about soil characteristics (first 15 cm layer) at "Las Peladas". On ridges, soils are frequently shallow and extremely stony. According as the distance around the ridge's watershed increases, these soils are deeper, the deepest soils being found at valleys between ridges.



**Ecological Station Las Peladas, Biosphere Reserve** Sierra del Rosario.

Figure 1. Aerial photograph of the study area. F, valleys covered by evergreen microphyllous forest; S, ridges surface covered by shrubby savannas. The photo was taken in the morning (the easternmost slopes of ridges hills growing savannas are illuminated by sun. A white line indicates the limits between forest and savannas. White spots show the sampling sites. FW, SW, SE and FE refer to the study plots: Forest West, Savanna West, Savanna East and Forest East, respectively.

**Figura 1.** Fotografía aérea del área de estudio. F, valles cubiertos por bosque siempreverde micrófilo; S, cañadas cubiertas de sabanas arbustivas. Foto tomada en la mañana (las pendientes más al este de las cimas de las cañadas se encuentran iluminadas por el sol). La línea blanca indica los límites entre los bosques y las sabanas. Los puntos blancos muestran los sitios de muestreo. BO, SO, SE y BE se refieren a las parcelas estudiadas: Bosque Oeste, Sabana Oeste, Sabana Este y Bosque Este, respectivamente.

The ridges of "Las Peladas" are dominated by a shrubby savanna, which is floristically related with those plants typical for xerophytic shrublands over serpintinites. Edaphic drought is the major stress determining the occurrence of this tropical mountain savanna. Shrubs represent 33% of species at the savanna. These shrubs reach commonly up to 2 m height, being all microphyllous. Herbaceous plant species, mainly belonging to Gramineae and Cyperaceae represent 27% of total flora at the savanna. Mosses, lichens, lycopods and grassy ferns are also frequently observed. Phytogeographically, the flora of the savanna is connected also with the Caribbean but mostly with the Northernmost Caribbean and Antilles. Plant endemism at these savannas reach 26% composed mainly by Pan-Cuban, Western Cuba and Pinar del Río province elements.

Microphyllous Evergreen Forest predominates at V-valleys between ridges with savanna communities. At these valleys, collecting sources from the surroundings (mainly rainwater and nutrients), water stress is reduced if compared with the neighbor savannas. These forests show a continental structure, *i.e.*, emerging trees up to 20 m are observed over the dominant stratum reaching 6 to 15 m height and averaging about 10 m height. Predominant tree species are mostly sclerophyllous and microphyllous, though exceptions as the notophyllous *Calophyllum spp.*and the mesophyllous *Amaioua corymbosa* are common. Tree density is very high if compared with the Mesophyllous Evergreen Forest, which generally grow at Sierra del Rosario over richer soils (Herrera *et al.*, 1988). The understory is also dense, showing a shrubby stratum 1 to 3 m height composed by tree species saplings in addition to shrubby species. Tree ferns, climbers, vines and epiphytes (Bromeliaceae, Orchidaceae, Piperaceae, and different ferns) are commonly observed. An herbaceous stratum reaching up to 1 m height shows many grasses belonging mostly to Gramineae, Cyperaceae and Melastomataceae. Mosses, liverworts and grassy ferns can be also observed. In addition, forest tree and shrubby species seedlings commonly occur at the understory.

Phytogeographycal relationships of "Las Peladas" forests are highly associated with the Caribbean (39.5%). Plant endemism is as high as 34%. Pan-Cuban elements represent half the endemics though those from Western Cuba and Pinar del Río province are well represented too. Several endemics are exclusive for Sierra del Rosario.

# **Extraction and identification of glomeromycotan spores**

Soil samples were collected along sets of transepts with a Western or Eastern exposure. For each exposure, two parallel transepts were sampled 100 to 150 m on either side of the distinct forest-

**Table 1.** Soil characteristics at "Las Peladas", Reserve of Biosphere Sierra del Rosario.



**Tabla 1.** Características del suelo en "Las Peladas", Reserva de la Bisofera Sierra del Rosario.

savanna border (FW-SW and FE-SE in **Fig. 1**). Along each of these transepts, five sub-samples (corners and center of a square surface) were taken and mixed within each of five samples along each transept being separated in between by 25 to 35 m. Soil subsamples were collected up to 15 cm depth. At the forests, each sample was completed inside 25 m<sup>2</sup> surface, while at the savannas the samples had  $6.25 \text{ m}^2$  surfaces each.

In the laboratory, the 25 compound samples were air-dried at room temperature. During air-drying soils were segregated by hand being the rootlets separated. All samples were then separately milled using a stone mill, sieved through a 2 mm mesh and mixed with their corresponding dry rootlets after being chopped into approximately 5 mm length segments. Samples were stored in sealed polyethylene bags at room temperature for 9 months.

References to spore populations or biovolumes on a soil weight basis are not a good choice at least for those substrates where raw humus necromass may strikingly differ among samples and this fact is not uncommon. A volumetric reference might be always desirable since mycorrhizal functioning (and the same occur for most of living beings associated to soils) is performed under a spatial basis. On the other hand, the assessment of soil bulk density using intact soil samples is also not good, since large stones, roots or other materials could be included in the measured volume. These undesirable materials might strikingly modify the expected spatial environment where living beings are functioning. Therefore, we prefer to use sieved soil to assess soil bulk densities, what could be mentioned as Sieved Soil Bulk Density (SSBD). For each sample SSBD values were estimated dropping approx. 150 ml of 2 mm sieved air-dried soil into a 250 ml glass cylinder and taping the cylinder over a surface in order to get the minimal possible volume. Subsequently each sample was weighed. Bulk densities were expressed in g.cm-3. The estimation of spore populations or biovolumes are referred to 1 dm<sup>3</sup>, separately for each sample in order to make them comparable, otherwise they are not. **Table 2** shows data about mean SSBD values and their variability at "Las Peladas".

For analyzing glomeromycotan communities 100 g by weight from each air-dried soil sample were processed as described in Herrera *et al.* (2004). Subsequently, 10%, by weight, of the 0.14 fraction and 5% of the 0.04 mm fraction from dried sieving's were prepared for the taxonomic identification and enumeration of spores. These fractions were soaked with approx. 20 ml water in 50 ml centrifuge tubes. Then, 20 ml of 2 M sucrose with 0.2% Tween 80 were subsequently layered below the water. These tubes were centrifuged at 2.500 r.p.m. for 10 minutes, the spores suspended in the supernatant were retrieved on a 0.04 mm mesh and washed into a dish.

Whole spores were examined under a dissecting microscope with magnification of up to 40 x and spores were also mounted on a slide in PVLG or PVLG with Melzer's reagent and examined under a compound microscope with magnification of up to 1000 x. Glomeromycotan spores were classified according to their shape, color, content, sporophores, auxilary cell characteristics, wall structure and ornamentation. Spore types were identified by comparison with the available descriptions (Schenck and Perez, 1990) and vouchers available from the Cuban National Herbarium (Collection HAC-G, including vouchers from Cuba, Venezuela, Costa Rica and Mexico) at the Institute of Ecology and Systematic (IES), and the International Culture Collection of Vesicular Arbuscular Mycorrhizal Fungi (INVAM). Individual spores were counted except for species of *Sclerocystis* which form tight sporocarps.

**Table 2.** Estimated sieved soil bulk densities (SSBD) at "Las Peladas". All values are mean of 5 samples..

**Tabla 2.** Densidad aparente aproximada del suelo tamizado (DAST) en "Las Peladas". Todos los valores son la media de 5 muestras.

	SSBD $(g.cm^{-3})$				
<b>STUDY PLOTS</b>	Mean values	Standard deviations	Variation coefficient $(\%)$		
FOREST WEST (FW)	1.016	0.111	10.88		
FOREST EAST (FE)	0.975	0.139	14.30		
SAVANNA WEST (SW)	1.010	0.112	11.09		
SAVANNA EAST (SE)	1.197	0.096	8.02		

For that latest genus, the species were analyzed taking into consideration the most ancient criteria about its taxonomical classification, due to these differences in biovolumes. For these fungi, sporocarps rather than spores were counted. All counting were carried out by two separate observers. Mean spore or sporocarp diameter were estimated for all the collected spore types in order to calculate the spore's biovolumes. Total spore numbers and biovolumes for each spore type were estimated as spores.dm<sup>-3</sup> or mm<sup>3</sup>.dm<sup>-3</sup>.

# **Analysis of plant species covering, mycotrophy and glomeromycotan spores**

Plant covering at each plot was also analyzed using squared samples along transepts as for soil collection. Thanks to the contingent spatial pattern of plant individuals among the studied communities, within each plot a representative sampling area replicate of  $25 \text{ m}^2$  for forest plots and 6.25 m<sup>2</sup> for savanna plots was adequate (Matteucci and Colma, 1982). Five of these replicates were sampled at each transept. The length of the transept at each plot was chosen following the recommendations of Richards *et al.* (1940) and Müeller-Dumbois and Ellemberg (1974), *i.e.* a transept length at least equivalent to five times the average height of individuals. The transept lengths measured approx. 100 m in all plots.

Plant species identification was recorded during field sampling and confirmed at the herbarium (HAC). For most of species the number of individuals were counted and their particular covering estimated as follows: 1) for trees at the forest plots the procedure was done directly; 2) for shrubs it was estimated that each individual show an average covering of 2% of 25 m2 in the case of forest plots, while for the savanna plots their covering was estimated directly; 3) grass individuals were estimated to show an average covering reaching 0.02% of 25 m<sup>2</sup> in the forest plots, while in the savanna plots they were considered to show an average covering of 0.2% of  $6.25$  m<sup>2</sup> for most of grass species, with the exception of those reaching 1 m height and estimated to cover 1% of 6.25 m2 . For *Andropogon spp*. and *Rhynchospora spp*. with too many individuals in the savanna plots, it was considered that they show altogether a covering reaching 91.5% and 8.5%, respectively (Leda Menéndez, personal communication). For their counting, forest grasses were classified under three classes, those showing more than 30 individuals (considered to be 40), the ones showing 10 to 30 individuals (considered to be 20) and those with less than 10 individuals (considered to be 5) at each sampling square.

Mycotrophy at Las Peladas was studied by sampling rootlets belonging to particular species at the study plots or using previous data for species collected from other places at the BR Sierra del Rosario. Sampled rootlets were washed after sampling, air-dried, cleared, and stained with 0.05% Trypan Blue in lactoglycerol (Phyllips and Hayman, 1970). Colonization levels were classified into three categories: high, medium and low. To analyze the mycotrophy of those species which rootlets were not sampled we considered the reports which are found in literature, or we accounted them as potential mycotroph if some other member of the family had been previously reported to be.

Other type of results included the analysis of spore populations and their biovolumes, and plant species covering presented as frequency distributions since this type of graph give a general idea about the species participation and their interrelationships at each community. Curves of abundance of species at each plot, considering spore population or their biovolumes were obtained too.

#### **Estimation of entropy among plots**

Entropy values were estimated separately for glomeromycotan spore population or biovolume data, considering forest and savanna plots for both cases. Entropy among species, H(S), among plots, H (P), species/plots, H(S/P), plots/species, H (P/S), and total entropy, H (SP), were calculated according to Pineda *et al.* (1981). In addition, H(S) MAX, H (P)MAX, H(S/P) MAX, H(P/S) MAX and H(SP)MAX, were calculated. These last values refer to the former ones as the ratio they are with respect to the maximal probable values to be obtained, therefore varying between 0 and 1 making data comparable. At the same time H-MAX, values give an idea about evenness at each plot.

#### **Statistical analysis**

In order to compare statistically entropy values corresponding to glomeromycotan spore population or biovolumes the procedure recommended by Magurran (1988) was followed. Variances for all entropy values (H') were estimated, then student- "t" values were calculated and finally the degrees of freedom.

# **Multivariate analysis of plant and glomeromycotan species relationships**

A matrix was generated containing the glomeromycotan fungi occurrences at each plot (presence, 1, absence, 0). The Jaccard index was estimated for plots to observe their similarity with respect to each glomeromycotan community. The resulting matrix was clustered by SAHN clustering according to Rohlf (1993) software NTSYS-pc version 1.8.

We also tested whether the composition of the glomeromycotan community varied between the forest and the savanna using the profile contrast within a multivariate analysis of variance (Bever *et al*., 1996) in which vegetation type, location and its interaction were used as predictors. Reflecting our paired sampling design, differences between vegetation types was tested over the vegetation\*location of pairing interaction. Analyses were performed using proc glm in SAS. The spore abundance of the 17 most frequently observed AM fungal species was analyzed. Because of the high number of zeros in the dataset, the spore counts were transformed to ranks prior to analysis as in Bever *et al*. (1996).

#### **RESULTS AND DISCUSSION**

#### **Glomeromycotan and plant species**

The list of all glomeromycotan species is given in **Table 3**, which were collected at the four plots. As observed in the table, variations in spore biovolume are very large when comparing, for example *Glomus microaggregatum* and *Gigaspora margarita*, the first being near 2000 times larger. *Sclerocystis spp*. produce also large biovolume sporocarps, however, their populations are commonly not too large.

A total of 96 plant species were found at the four plots (**Appendix 1**). Las Peladas plant formations are the most diverse plant communities at Sierra del Rosario. On the other hand, all the rootlet samples collected to analyze their mycotrophy proved to be arbuscular mycorrhizal (AM, **Table 4**).

This is an interesting result because commonly plant communities do not reach 100% of their plant species being AM. In order to have a general idea about the total mycotrophy at the whole region, Annex 2 give a list of botanical families which being represented at Las Peladas have been reported to contain unless one AM member. Summarizing the results in **Table 4** and **Appendix 2**, there are no doubts about the significance of glomeromycotan species in the studied plots.

Frequency distributions of plant covering in the studied plots are shown in **Figs. 2** and **3**. Though the species dominance and composition change between plots, those species contributing separately to plant covering more or less than 0.5% are approximately the same in their numbers. For FW 30 species contribute more and 22 species, contribute less than 0.5%, representing the last assembly a plant covering reaching 3.88%. For FE 32 species contribute more and 21 species, contribute less than 0.5%, representing the last assembly a plant covering reaching 2.06%. When **Fig. 2** is analyzed, it is possible to observe that there are large differences between



**Figure 2.** Frequency distribution of plant covering in forest plots (FW and FE) at Las Peladas.





**Figure 3.** Frequency distribution of plant covering in savannah plots (SW and SE) at Las Peladas.

**Figura 3.** Distribución de frecuencia de la cubierta vegetal en parcelas de sabana (SO y SE) en las Peladas.

both plots with respect to the plant species dominating each one. Dominance of plant covering is much larger in FW than in FE, giving the later a greater evenness. With respect to the species dominating each plot, they might be considered

**Table 3.** List of glomeromycotan species occurring at Las Peladas Ecological Station. Those species already described appear in bolded cursive. Other species are probably new too.

**Tabla 3.** Lista de especies de hongos glomeromicetos existentes en la Estación Ecológica Las Peladas, RB. Aquellas especies ya descritas aparecen en letra cursiva. Las otras especies resultan probables especies nuevas.



1 S1, White spores, 105-125 µm in diameter, thin walled (3-5 µm), surface covered by small papillae.

2 S3, Spores orange in color, 85-93 µm in diameter, thick walled (11-13 µm).

3 S5, Spores are light tan in color, µm in diameter, elliptical in form, pits uniformly distributed, thin spore wall, resembling *Ac. foveata*.

 4 S6, Spores light to dark yellow 70-90 µm in diameter, walls 3-7 µm, surface ornamented with rounded pits evenly distributed, each pit showing at the center small papillae.

<sup>5</sup> S8, Spores dark brown to black in color, forming loose aggregates with few spores embedded in a loose matrix of yellow to brown hyphae intermixed with "vesicles" or aborted spores. Spores are 70-109 µm in diameter, spore wall thickness variable (3-12 µm), sporophore thickness also variable 7-18 µm near the spore base. Known also from Venezuela.

 $6$  S9, Spores are light to dark tan in color, 130-150 µm in diameter, wall thickness 3-9 µm, all showing at the spore apex a rounded spore outgrowth - "pilum", cap - filled, like the spore content, with small oil droplets, opening between the spore and the "pilum" not occluded. Spore wall and sporophore similar to *Funneliformis mosseae*. Found also in Venezuela.

 7 S11, Spores brown to dark brown, commonly occurring single in soil, but also in loose aggregates, 71-153 µm in diameter. Wall thickness 3-16 µm, with an evanescent outer wall surrounding internal walls. Very common in tropics. Also observed in Costa Rica, Mexico and Venezuela.

8 S13, Spores occur single in soil or in loose aggregates, yellow to brown in color, 89-204 µm in diameter, wall thickness 6-19 µm, composed by two wall groups, an outer whitish laminate and evanescent and a second brownish, laminate and ornamented with small papillae when young. Papillae protruding the outer wall or growing freely and intermixed outwards giving a hiring appearance.

 9 S17, Spores are light brown to tan, opaque, generally found in loose aggregates with few to many (more than 30) spores, 66-93 µm in diameter, thin walled (2-4 µm) and showing relatively thick sporophores 6-13 µm near the spore base.

<sup>10</sup> S18, Spores are mostly dark brown in color, with a hyphal mantle around, generally found in loose aggregates with few spores, 52-130 µm in diameter, spore wall 8-12 µm.

<sup>11</sup> S27, Spores 140-170 µm in diameter, with a very thin wall (4-7 µm). Sporophore very thin walled and difficult to observe.

**Table 4.** Plant species proved to be AM in Las Peladas. In the list, species which rootlets were analyzed in the present study (**\***) or AM species previously reported for other sites of Sierra del Rosario (Ferrer and Herrera, 1988).

**Tabla 4.** Especies de plantas que resultaron ser MA en Las Peladas. En la lista, especies cuyas raicillas fueron analizadas en el presente estudio (\*) o especies MA reportadas previamente para otros sitios de la Sierra del Rosario (Ferrer y Herrera, 1988).



as being enough different. Well represented dominating plant species at FW are P53, P15, P40, P3, P41, P91, P30, P73, P69, P33, P83 and P37, while in FE they are P53, P30, P41, P33, P60, P91, P14, P73, P1, P15, P20, P58 and P3.

A similar result was observed when comparing the savannah plots (**Fig. 3**). An increase of plant covering, and species composition is observed at SE rather than a change in species dominance. However, those species contributing separately to plant covering more or less than 0.5% are approximately the same in their numbers. For SW 23 species contribute more and 16 species, contribute less than 0.5%, representing the last assembly a plant covering reaching 2.18%. For SE 21 species contribute more and 12 species, contribute less than 0.5 %, representing the last assembly a plant covering reaching 1.68%. Plant species with a relevant covering are enough different in both cases, however, the dominance of plant covering is larger in SE than in SW, so that an inversion occurred with respect to the forest plots where the dominance is large at FW. Undoubtedly the exposure is influencing the observed results, as the westernmost exposed plots (FW and SW) are more humid and protected against direct sunshine (frequency of clouds and rains are larger during the afternoons at Sierra del Rosario), and the easternmost ones are more dry and during more time exposed to direct sunshine.

## **Relationships between plots according to their Glomeromycotan communities**

**Figure 4** shows the phenogram resulting from the analysis of the matrix showed in **Table 5** according to the Jaccard Index. As shown in the figure, the glomeromycotan communities are more distinct in the plot FW followed by the plot SW, as the easternmost plots (FE and SE) are highly related. According to these results abiotic factors seem to be the most significant in determining the glomeromycotan community composition since, as mentioned before, the westernmost plots when compared with the easternmost ones are more humid and exhibit probably reduced heating along the day.

However, the different environmental factors distinctly influencing plots according to their exposition do not completely determine the composition of the AM fungal community. As observed in **Table 5**, there are 12 glomeromycotan species which occur in the four plots, therefore being more generalist



**Figure 4.** Phenogram of the studied plots at Las Peladas according to the distribution of glomeromycotan species. Analysis performed by Jaccard method.

**Figura 4.** Fenograma de las parcelas estudiadas en Las Peladas de acuerdo con la distribución de las especies de hongos glomeromicetos. Análisis desarrollado por el método de Jaccard

to the prevailing conditions at Las Peladas, even though some of them, e.g. *Scutellospora minuta* and *Glomus* "brown spiny", were relatively rare. Another group of species were found only in forest plots, in easternmost plots, or their occurrence is particularly influenced at only one or two plots. Obviously, the sampling was not perfect so that the observation of spores of AM fungal species in a particular plot could depend upon its life cycle (Pringle and Bever, 2002). But these concerns have reduced significance when groups of AM fungi shared distribution patterns.

Conversely, it is also true that the composition of the plant community exerted strong effects on the composition of the AM fungal community. Our profile analysis revealed that the composition of the AM fungal community was significantly different between the forests and the savanna (Profile\*veg  $F_{16,16}$  = 2.88, *P*=0.02). While most AM fungi can associate with a wide array of hosts, a growing body of work suggests that their performance relative to each other depends upon the host species involved. The sporulation rates of AM fungi have been found to be host-dependent in laboratory systems (Daft and Hogarth, 1983; Hetrick and Bloom, 1986; Bever *et al.,* 1996), and such differences in sporulation rates may explain changes in the composition of the AM fungal community in response to the changes in the plant community (Schenck and Kinloch, 1980; Johnson *et al.,* 1991; Sanders and Fitter, 1992; Johnson, *et al*., 1992; Hendrix *et al*., 1995; Bever *et al.,* 1996). In field studies, both plant and soil factors have been shown to be important determinants of fungal species distribution and abundance (Schultz, 1996; Stürmer *et al.,* 2018). Given such mutual interdependence of plant and fungal relative growth rates, coexistence of both plants and fungi would be determined by potentially complex dynamics (Bever, 1999; Bever *et al.* 2002).

**Table 5.** Groups of glomeromycotan species found at Las Peladas. Species organized according to their occurrence in the studied plots.

**Tabla 5.** Grupos de especies de hongos glomeromicetos encontrados en Las Peladas. Especies organizadas de acuerdo con su ocurrencia en las parcelas estudiadas.

	${\rm FW}$	FE	<b>SW</b>	SE
Ac. "orange thick wall"	1	1	1	1
Ac. longula	1	1	1	1
Ac. spinosa	1	1	1	1
Scut. minuta	$\mathbf{1}$	$\mathbf{1}$	$\mathbf{1}$	$\mathbf{1}$
Gl. "brown spiny"	$\mathbf{1}$	$\mathbf{1}$	$\mathbf{1}$	$\mathbf{1}$
Scl. pachycaulis	$\mathbf{1}$	$\mathbf{1}$	1	1
Gl. "dark brown S"	1	1	1	1
Par. occultum	1	$\mathbf{1}$	$\mathbf{1}$	$\mathbf{1}$
Gl. microaggregatum	1	$\mathbf{1}$	1	1
Funn. geosporum	1	1	1	1
Gl. aggregatum	1	$\mathbf{1}$	$\,1$	1
Gl. "light brown"	1	1	1	1
Gl. "Sclerocystis-like"	0	1	1	1
Gl. "with cap"	1	1	$\overline{0}$	1
Ac. "foveata-like"	$\mathbf{1}$	$\mathbf{1}$	$\overline{0}$	$\overline{0}$
Ac. "kentinensis-like"	1	$\mathbf{1}$	$\boldsymbol{0}$	0
Scl. microcarpus	1	$\mathbf{1}$	0	0
Ac. "white papilose"	$\overline{0}$	$\mathbf{1}$	$\boldsymbol{0}$	1
Scl. coremioides	$\mathbf{1}$	$\boldsymbol{0}$	$\mathbf{1}$	$\theta$
Gl. "dark brown M"	1	$\overline{0}$	$\boldsymbol{0}$	$\overline{0}$
Gl. sinuosum	1	$\overline{0}$	$\boldsymbol{0}$	0
Scl. rubiformis	1	$\overline{0}$	$\boldsymbol{0}$	$\overline{0}$
Gl. mortonii	1	$\overline{0}$	$\boldsymbol{0}$	$\overline{0}$
Scl. clavispora	$\boldsymbol{0}$	$\mathbf{1}$	0	0
Gig. margarita	$\overline{0}$	$\overline{0}$	$\mathbf{1}$	$\overline{0}$
Scut. "scutata-like"	$\boldsymbol{0}$	$\overline{0}$	1	$\overline{0}$
Scut. "yellow LP"	$\overline{0}$	$\overline{0}$	$\boldsymbol{0}$	1

## **Spore population vs. spore biovolume to interpret glomeromycotan diversity**

When analyzing the glomeromycotan species abundance distribution (**Figs. 5** to **8**) it is noted that all fungal communities follow the broken stick model. The broken stick model reflects a much more equitable than those suggested by other distribution models (Magurran, 1988) and communities that follow this model can be stable. Therefore, the glomeromycotan communities at the four analyzed plots are stabilized considering the AMF spores reproductively as



mycorrhizal propagules (populations) and energetically as a mycomass trapping a quantity of carbon dedicated by plants to their formation.



**Figure 5.** Abundance distribution of glomeromycotan spores according to their population or biovolume at the plot FW.

**Figura 5.** Distribución de abundancia de las esporas de glomeromicetos de acuerdo con su población o biovolumen en la parcela BO.



**Figure 7.** Abundance distribution of glomeromycotan spores according to their population or biovolume at the plot SW.

**Figure 7.** Distribución de abundancia de las esporas de glomeromicetos de acuerdo con su población o biovolumen en la parcela SO.



**Figure 6.** Abundance distribution of glomeromycotan spores according to their population or biovolume at the plot FE.

**Figura 6.** Distribución de abundancia de las esporas de glomeromicetos de acuerdo con su población o biovolumen en la parcela BE.



**Figure 8.** Abundance distribution of glomeromycotan spores according to their population or biovolume at the plot SE.

**Figura 8.** Distribución de abundancia de las esporas de glomeromicetos de acuerdo con su población o biovolumen en la parcela SE.

However, some changes referring to the number of dominant species were observed when comparing the spore population abundance or their biovolume abundance. **Fig. 5** show an increment of 3 dominant species (12 vs. 9) when analyzing the spore biovolume vs. their population. At the same time, the composition of dominant species differs for each case. A larger difference is observed in **Fig. 6** where the dominant species are 9 when analyzing the spore biovolume vs. 5 when analyzing their population.

Again, at FE the composition of dominant species differs for each case. These results suggest that: 1) the species abundance according to the spore biovolume fit better the broken stick model; 2) the spore biovolume reflects better the functional characteristics of the glomeromycotan community diversity since it approach more accurately the energy dedicated by the plant host to its constitution; and 3) the spore population fit better a binomial than a normal distribution and the contrary occur for the spore biovolume.

For the savannah plots (SW and SE) the results were inverted when compared with the ones obtained for the forest plots, i.e. the number of dominant species is reduced when analyzing the spore biovolume. This occurred somehow attenuated for SW but markedly for SE. In both cases and especially at SE the explanation could be due to the shortage of carbon dedicated to produce spores, what is not surprising due to the larger water stress occurring at the savannah plots which is surely larger for SE.

The functional relevance of analyzing spore population or biovolume is also observed in **Fig. 9**. In this figure it is possible to note that total biovolume change very little among plots, and even less among forest (FW and FE) or savannah plots (SW and SE), while a totally different result is observed when analyzing spore population.

# **Glomeromycotan community entropy values and their statistical variation among plots**

**Table 6** shows the results of estimating entropy (H') values for all the plots according to data expressed as spore population or biovolume. In general, it was observed that the diversity of AMF, H'(S) values, was larger at the westernmost exposed plots (FW and SW), although the results vary according to the origin of data (spore population or biovolume). On the other hand, the values obtained for H' (P) are similar what means that the variation of diversity did not depended from plots, i.e. plots were statistically representative.

Diversity of plots depending upon the species, H' (P/S), did not vary too much, what is confirmed when analyzing  $H'(P/S)$  **Table 6.** Entropy (H') and H' max values according to Pineda *et al*. (1981), and variances according to Magurran (1988). Values corresponding to forest (FW and FE) or savannah (SW and SE)

**Tabla 6.** Entropía (H') y valores de H' max de acuerdo con Pineda *et al*. (1981), y varianzas de acuerdo con Magurran (1988). Los valores corresponden a las parcelas de bosque (BO y BE) o sabana (SO y SE)



max values. Similar results are observed when analyzing H'(S/P) values. These results mean that the plot diversity was not greatly affected by the species diversity nor was the plot diversity greatly affected by the species diversity.

The smallest obtained total entropy values, H' (SP), were observed in the FE plots. When analyzing **Table 7** the first result observed is that spore population data lacked acceptable value to characterize the variations of diversity among different glomeromycotan community. With only one exception, all the differences obtained when comparing pairs of plots were statistically different. Data about spore biovolume fit much better the expected results as showed in **Table 6**. About H'(S) values all the plot pair values were statistically different except for comparing FW vs. SW and FE vs. SE. This result means that not only the glomeromycotan community composition (see **Fig. 4**) but also its diversity is affected primarily by the environmental prevailing conditions being drier and hotter at the easternmost exposed plots and more humid and fresher at the westernmost exposed ones.

None of the paired comparisons produced significant differences when analyzing H' (P) spore biovolume values. This result means that statistically all the samples were representative from each plot. A similar result was obtained

for  $H'$  ( $E/P$ ) values which produced significant differences only when comparing SW vs. SE plots. This means that according to the plots diversity the variation of species was more heterogeneous at SE (2.07) than at SW (1.67). Again, the more stressed environment found at SE reflects its influence on the glomeromycotan community.

The diversity of plots according to the species diversity, H' (P/S) values, produced significantly not differences when comparing FW vs. SW and SW vs. SE, what qualify the plot SW as an intermediate environment along a wetter (FW) to **Table 7.** Significant differences between plot pairs according to their entropy (H') values. All the analysis being carried out by the estimation of variances of H' values, student's-"t" test, and degrees of freedom according to Magurran (1988). Degrees of freedom ranged 158 663 - 285 953 for comparisons corresponding to spores number, and 162 - 257 for comparisons corresponding to spores biovolume.

**Table 7.** Diferencias significativas entre pares de parcelas de acuerdo con su entropía (H'). Todos los análisis fueron desarrollados por la estimación de las varianzas de los valores de H', la prueba "t" de student, y los grados de libertad de acuerdo con Magurran (1988). Grados de libertad variaron 158 663 - 285 953 para las comparaciones correspondientes al número de esporas, y 162 - 257 para las comparaciones correspondientes al biovolumen de las esporas.



ns, not significantly different; \* significantly different for *P* < 0,05 ("t" > 1.96); \*\* significantly different for  $P < 0.01$  ("t" > 2,58).

ns, no diferentes significativamente; \* diferentes significativamente para *P* < 05 ("t" > 1.96); \*\* diferentes significativamente para *P* < 0,01 ("t" > 2,58).

drier (SE) gradient. All the remaining pairs of compared plots were significantly different.

Finally, total entropy values, H'(EP), were not significantly different when comparing FW vs. SW, FW vs. SE and SW vs. SE, what can not be explained according to the environment prevailing abiotic factors, but to intrinsic characteristics occurring at the plot FE being significantly different from all the other plots.

Going back to **Table 5**, those glomeromycotan fungi being represented at all the examined plots can be considered as generalist species. When **Figs. 5** to **8** are analyzed looking for those species which abundance surpass 1 % (spore biovolume), generalist glomeromycotan are 58 % for FW, 78% for FE, 44 % for SW and 100 % for SE. These behaviors of glomeromycotans justify the largest biovolumes and spore populations for the easternmost exposed areas when forest and savannah plots are compared separately (**Fig. 9**).

Both at FE and SE the largest spore biovolumes and populations are produced by generalist AMF. At the same time, in FW, SW and SE the plant species dominating covering (plant cover larger than 10%) are 2 to 3, while in FE the species dominating plant cover are 7. These dominating species at FE are just the ones being reported as the dominant species for microphyllous evergreen forest at Las Peladas: *Matayba apetala*, *Coccoloba retusa*, *Guettarda valenzuelana*, *Cyathaea arborea*, *Nectandra corriacea*, *Symplocos salicifolia* and *Callophyllum antillanum*.



**Figure 9.** Total glomeromycotan participation for the studied plots, expressed as spore population or biovolume.

**Figura 9.** Participación total de los hongos glomeromicetos en las parcelas estudiadas, expresadas como poblaciones de esporas o biovolumen de las mismas.

Summarizing the obtained results, it would be possible to assume that the significantly smallest H' (SP) value obtained for the plot FE could be probably determined by the prevailing environmental conditions favoring the production of generalist glomeromycotans and leaving restricted possibilities to other specialist glomeromycotans to flourish because of the reduced occurrence of particular host to fit. These particular host plant species seems to be favored at FW and SW where not only generalist, but specialist AMF might produce significant spore biovolumes (surpassing 1% for each fungal species), therefore the large values of H' (SP). The SE showed a large value of H' (SP) and at the same time was dominated by generalist glomeromycotans. This may result from the following reasons: 1) the larger adaptation of both generalist and specialist glomeromycotans to water stress, and 2) the lack of competitiveness among host plants which could evenly favor both generalist and specialist AMF, therefore improving evenness values.

Overall, we have found that the glomeromycotans communities' diversity and composition are determined firstly by environmental prevailing conditions, and secondly by their relationships towards particular host plant species. These particular host plants seemed to be favored by the reduction of competitiveness at FW and SW because of the reduced number of plant species surpassing 10% of plant cover, and at SE because of plant adaptation to water stress evenly favoring plant growth and fungal fitness.

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**Appendix 1.** List of plant species at Las Peladas study plots. Endemics are marked with an **x**.

**Anexo 1.** Lista de plantas en las parcelas estudiadas en Las Peladas. Los endémicos están marcados con una **x**.





Note: Though P51 and P84 are listed in the table, they were not included in the analysis. In addition, P5 and P6 were considered together since it was not possible to separate them in the field because of lacking reproductive structures.

**Appendix 2.** Families represented at Las Peladas including at least one AM species member.

![](_page_16_Picture_357.jpeg)

![](_page_16_Picture_358.jpeg)

<sup>1</sup> Most of the families of the order Hypericales include species, which have been proved to be arbuscular mycorrhizal.