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Diversity and abundance of arbuscular mycorrhizal fungi spores under different coffee production systems and in a tropical montane cloud forest patch in Veracruz, Mexico

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Abstract We evaluate the arbuscular mycorrhizal fungi (AMF) community as measured by spores in different coffee production systems (at the depth of 0–15 cm). In addition, we analyze the similarities between the AMF communities in coffee production systems and those that occur in a tropical montane cloud forest patch in order to evaluate the capacity of coffee production systems to preserve the native AMF community. We carried out four samplings in five coffee production systems representative of a vegetation structure gradient, and in a forest. From 120 soil samples, 33 morphospecies were detected. In all the sites, the dominant morphospecies were *Glomus clarum* and *Glomus* sp. 3. We found no significant difference in AMF spore richness between sites. Diversity was similar in most of the coffee production systems. Significant differences were only detected in spore abundance; during the dry season the forest, shaded traditional rustic system and shaded simple system presented the highest spore abundance. With the exception of one species exclusive to the forest, the coffee production systems all share the same AMF species as the forest. The coffee production systems with the greatest similarity to cloud forest were the shaded traditional rustic system and the shaded simple system. It is suggested that control of weeds and fertilization could be important factors influencing the composition and abundance of AMF spores in coffee production systems.

Keywords Agroforestry · *Coffea arabica* L. · Arbuscular mycorrhizal fungi · Vegetation structure

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Introduction

For the last 25 years, coffee (*Coffea arabica* L.) has been the major export crop of Mexico (SAGARPA 2007), covering an area of 806,000 ha throughout the mountainous ranges of western, eastern, and south-eastern Mexico, at altitudes ranging from 300 to almost 2,000 m above sea level. In Mexico, the following five main coffee production systems can be distinguished according to floristic composition, vegetation structure and management level (Nolasco 1985): (1) The traditional rustic or mountain coffee: the lowest strata of the forest and the original tree cover are maintained, under which the coffee bushes are planted; (2) The traditional polyculture: as in the rustic system, coffee is introduced under the cover of

the original forest, but numerous other useful plants are planted; hence there is a great variety of domestic and wild plants in this system; (3) The commercial polyculture: involves complete removal of the original forest canopy trees and the introduction of selected shade trees such as legumes and commercial species (*Citrus* sp., bananas and other cash crops); (4) The simple shaded: in this system, leguminous trees (species of *Inga* sp.) are used to provide shade for coffee bushes; and (5) The unshaded monoculture: in which the coffee bushes are exposed to direct sunlight.

In the central region of Veracruz, the 5 coffee production systems described above have replaced the primary vegetation composed of tropical montane cloud forest, dramatically reducing its range. In recent years, around 40% of the area of cloud forest has disappeared (Williams-Linera 2002). This situation is of concern because the cloud forest hosts around 10–12% of the animal and plant species known in Mexico (Ramamoorthy et al. 1993). According to Rzedowski (1996), the complexity of its vegetation causes this forest to be the ecosystem with the highest plant diversity per unit area. Furthermore, because of its distribution along mountain ranges, cloud forest is the source of important ecosystem services, such as water capture and purification and soil erosion prevention (Hamilton 1995).

Notwithstanding the substitution of the cloud forest by coffee production in the region, the landscape retains an evergreen and exuberant appearance due to the diverse canopy of shade trees maintained as part of the production management. Previous studies have underlined the importance of shade coffee as a refuge for native biota (Perfecto et al. 2003). From 1990 to 2007, more than 50 articles presented data on the biodiversity found within shaded coffee systems, these studies have analyzed the composition and structure of vegetation and different groups of animals that live in the canopy and understory of the coffee production, including mammals, birds and several groups of arthropods (Manson et al. 2008).

In contrast, below-ground microscopic communities have been poorly studied, despite their importance to ecosystem structure and functionality. It is well documented that changes in the canopy can dramatically influence soil physico-chemical characteristics and therefore also the soil microscopic communities. Light infiltration and the quantity and quality of the litter are important parameters that regulate nutrient availability as well as microclimatic conditions in the first edaphic horizons (Wardle et al. 2004). Among soil microscopic organisms, the arbuscular mycorrhizal fungi (AMF) play a very important role in natural ecosystems as well as in agroecosystems due to their capacity to form arbuscular mycorrhizae, which are considered to be the most widely distributed and important symbiotic association in nature (Brachmann and Parniske 2006). It is well documented that AMF increase plant nutrient uptake ability, particularly for phosphorus and zinc in nutrient-poor soils (Scheneiger and Jakobsen 2000).

Since the pioneering work of Janse (1897), many studies have confirmed the presence of arbuscular mycorrhizal associations in coffee plants (Lopes et al. 1983; Cardoso et al. 2003; Muleta et al. 2008). Most of the literature concerning AMF in coffee describes greenhouse and field experiments to assess treatments with selected AMF isolates in order to improve coffee bean yield and quality (Colozzi-Filho and Siqueira 1986). Few contributions have addressed the ecological aspects of the AMF. Recently, Cardoso et al. (2003) and Muleta et al. (2007, 2008) analyzed the distribution and abundance of indigenous arbuscular fungi spores from Brazil and Ethiopia, in agroforestry and monoculture coffee systems respectively. In Mexico, the sole ecological study concerning AMF in coffee production systems is that of Trejo et al. (1998) who analyzed spore abundance and root colonization under three different coffee management systems. However, no detailed information exists to date regarding the species composition and diversity of AMF spores in different coffee production systems, and the capacity of these systems to preserve the native AMF germplasm of the primary vegetation.

Given the importance of coffee production systems in the central region of Veracruz, a multidisciplinary project was conducted by the Instituto de Ecología A. C. over the period 2004–2006, with the aim of evaluating the ecological importance of different coffee production systems in terms of biodiversity conservation and other environmental services. In addition to social and economical aspects, 13 taxonomic groups were studied, including AMF. A more detailed description of the project and preliminary results is provided in Manson et al. (2008).

Characterization of the AM fungal community using the diversity and relative abundance of AMF spores has been a common practice in studies of AMF ecology (Oehl et al. 2005; Lovera and Cuenca 2007); this makes it possible to compare results from different ecosystems. It is recognized that not all the fungi present may be sporulating at the time of sampling, and that the abundance and specific richness of spores found may not reflect the entire AMF community. Furthermore, it is acknowledged that spore counts may overestimate the abundance of those species that sporulate heavily in the field (Sanders 2004). However, spores represent an important feature of the life history of AMF, and their isolation and quantification is a relatively straightforward process and accessible for most researchers.

The objectives of the present study were: (a) to investigate whether the different coffee production systems—which represent a gradient of the vegetation structure and the intensity of crop practices—of central Veracruz influence the composition and diversity of AMF spores; and (b) to evaluate their capacity to preserve an AMF spore community similar to that of the tropical montane cloud forest.

Materials and methods

Study sites description

Five coffee production systems with vegetation structures of different complexity, and a patch of tropical montane cloud forest were selected in the central region of Veracruz State (Table 1). The extremes of the vegetation structure gradient were an unshaded monoculture system (least complex) and the forest (most complex). They are all located in the coffee growing region of Coatepec-Huatusco, at a maximum distance of 40 km apart. These coffee production systems are approximately 150–200 years old, since they were established by directly replacing the cloud forest. The climate of the area is semi-tropical with an annual mean temperature range of 12–19°C and three clearly defined seasons: the cold-front season (from October to March), the dry season (from April to May) and the wet season (from June to September; Williams-Linera 1997). A detailed study of the physico-chemical characteristics of the soils of each of our sampling points was carried out by Geissert and Ibáñez (2008), who classified the soil of the coffee production systems as an Andic acrisol and the soil of the cloud forest as an Umbric andosol (Table 2). These authors concluded that, in this region, the superficial horizons of these two types of soils share soil morphogenesis properties that allow comparisons between them.

Soil sampling at the study sites

We collected soil samples from 5 sampling points within each field site. The shortest distance between two contiguous points was 50 m. The area of the polygon delimited by the sampling points in each site ranged from 1 to 1.5 ha. Within each field site the distance from the sampling points to the nearest road or coffee production system edge was at least 100 m, in order to avoid edge effects. At each sampling point, four subsamples (each of 250 g, from a depth of 0–15 cm) were collected, 1 m from the center point in each of the cardinal points, and pooled to produce composite soil samples. Soil samples were collected in the dry season (May) and wet season (September) over two consecutive years (2004–2005). A total of 120 samples were analyzed. The composite samples (20 for each production systems or forest patch) were air-dried and stored at 4°C until analysis.

Soil physico-chemical analysis

Details of the methodology and results of the soil analysis can be consulted in Geissert and Ibáñez (2008) who applied the following techniques: organic carbon (OC) by Walkley–Black, pH by potentiometry, cation exchange capacity (CEC) with ammonium acetate 1 N (pH 7.0), total nitrogen (N) by micro-Kjeldahl, available phosphorus (P) by Bray 1 and phosphorus fixation (P) by Blakemore.

AMF spore isolation and identification

AMF spores were extracted from 50 g of air-dried soil by the wet sieving method, in a nest of three soil sieves (425, 106 and 45 μm), and by sucrose density gradient centrifugation (Brundrett et al. 1994). Spores were collected and quantified in Petri dishes using a dissecting microscope. Representative morphospecies were mounted on slides with polyvinyl lactophenol

Table 1 Characteristics of the study sites in central Veracruz: mean annual precipitation (MAP), location, tree composition, agricultural practices (for coffee plantations), and plant density

Production system key	MAP (mm) Dry season Wet season	N latitude W longitude	Tree composition[a]	Agricultural practices[b]	Coffee plants/ha[b]	Trees/ha[b]
Unshaded monoculture system (UMO)	309.2 1554.0	19°22′53″ 96°59′17″	No shade trees	Sparse use of agrochemical products	2500	0
Shaded simple system (SSL)	275.9 1176.2	19°12′22″ 96°53′4″	Canopy dominated by legumes (*Inga vera*, *Erythrina poeppigiana*)	Sparse use of agrochemical products	4346.6	266.1
Shaded commercial polyculture system (SCP)	304.02 1499.3	19°27′59″ 96°56′3″	Timber, legumes and fruit trees (*Inga jinicuil*, *Inga vera*, *Citrus* spp.)	Frequent use of agrochemicals products	1554.1	165.7
Shaded traditional polyculture system (STP)	298.2 1477.8	19°25′56″ 96°57′50″	Some forest native trees, legumes, and timber and fruit trees (*Citrus* spp., *Inga vera*, *Trema micrantha* and *Musa* sp.)	Frequent use of agrochemicals products	2287.5	160.0
Shaded traditional rustic system (STR)	365.9 1794	19°12′57″ 96°53′7″	Native forest canopy, some legumes (*Quercus sartorii*, *Inga* sp., *Citrus* spp. and *Tapirira mexicana*)	No use of agrochemical products. Pest control by biological alternatives	1328.9	140.8
Tropical montane cloud forest (FOREST)	283.5 1396.5	19°11′23″ 96°59′11″	Native forest canopy (*Beilschmiedia mexicana*, *Brunellia mexicana*, *Quercus insignis*, *Q. leiophylla*, *Q. sartorii*, *Hampea intergerrima* and *Turpinia insignis*)	None	0	751.0

[a] Data from Williams-Linera and López-Gómez (2008)

[b] Data from Contreras and Hernández-Martínez (2008)

Table 2 Soil physicochemical properties[a] of the production systems and patch forest studied (organic carbon (OC), organic matter (OM), cation exchange capacity (CEC), carbon–nitrogen ratio (C/N), phosphorus (P) available and fixation and soil coverage[b]

Soil physicochemical properties[a]									Soil coverage[b]			
Production system	OC (g/kg)	OM (g/kg)	pH	CEC (cmol/kg)	C/N	Available P (mg/kg)	Fixation P (%)	Soil type	Texture	Weed height (cm)	Weed cover (%)	Leaf litter cover (%)	Naked soil (%)
UMO	24.01	54.4	4.6	16.22	8.2	0.7	79.7	Andic acrisol	Loam—silt loam	29.6	45.7	28.4	25.9
SSL	25.98	59.8	5.1	20.86	9.0	0.2	62.5	Andic acrisol	Loam—silt loam	11.8	16.4	70.7	12.9
SCP	23.06	51.0	4.5	13.64	9.7	6.0	57.8	Andic acrisol	Loam—silt loam	13.1	13.3	72.5	14.2
STP	28.23	61.0	4.8	16.97	10.7	9.1	53.8	Andic acrisol	Loam—silt loam	34.4	55.1	35.3	9.7
STR	30.08	69.2	5.2	23.11	12.0	1.8	62.1	Andic acrisol	Loam—silt loam	24.6	30	54.6	15.9
FOREST	41.33	100.8	4.5	18.39	9.9	0.1	90.6	Umbric andosol	Sandy loam—sand	25.2	23.6	63.6	13.1

UMO unshaded monoculture system, *SSL* shaded simple system, *SCP* shaded commercial polyculture system, *STP* shaded traditional polyculture system, *STR* shaded traditional rustic system, *FOREST* tropical montane cloud forest

[a] Data from Geissert and Ibáñez (2008)

[b] Data from Manson et al. (2008)

glycerol (PVGL), with and without Melzer's reagent, for identification. Taxonomic determinations were carried out based on morphological characteristics using descriptions provided by the International Culture Collection of Vesicular/Arbuscular Mycorrhizal Fungi (http://invam.caf.wvu.edu; 2005), and following descriptions by Schenck and Pérez (1990). Voucher specimens were deposited at the Instituto de Ecología A. C. in Xalapa, Veracruz.

Calculations and statistical analysis

Spore abundance was expressed as the total number of spores found in 100 g of dry soil. Frequency of occurrence (Fr%) was calculated as the number of sampling dates in which a given morphospecies was isolated divided by the total number of sampling dates (T = 24) and multiplied by 100. Relative abundance for each morphospecies (RA%) was calculated as the ratio of the number of spores of a given morphospecies to the total number of spores, multiplied by 100.

Species richness (S) was measured as the total number of different spore morphospecies; diversity as the Shannon index (H′) calculated with the formula: $H' = -\sum((n_i/n)\ \ln\ (n_i/n))$ where: n_i = number of individuals of species i and n = number of all individuals of all species; evenness was measured by Shannon diversity divided by the logarithm of number of taxa. These analyses were conducted using the software PAST (ver. 1.84). Species-abundance distributions were plotted (Whittaker plots) to elucidate AMF dominance patterns in each one of the study sites. Distributions were obtained by plotting the logarithm (in base 10) of the abundance of each dispersed plant species ($Log_{10}(n_i/n)$), against the abundance species rank (from most abundant to least abundant species).

To identify differences in species richness, diversity and spore abundance in all the coffee production systems and the cloud forest, we conducted one- and two-way analyses of variance, after checking requirements for normal distribution and homogeneity of variance of data using Kolmogorov–Smirnov and Bartlett's tests, respectively. Only the spore abundance data did not match the requirements, and therefore these were log-transformed prior to analysis. Species richness (S) and AMF diversity (H′) were compared between sites using one-way ANOVAs (with 5 replicates per site calculated with the total number of morphospecies from both seasons and both

years to approach completeness of morphospecies composition). Spore abundance was compared between sites and seasons using repeated-measures ANOVA (10 replicates per site per season; sites functioning as statistical blocks for seasons within sites). When the effects of factors were significant in the ANOVAs, a Tukey's HSD *post hoc* test was run to test for pair-wise mean differences at $P = 0.05$. To investigate differences between seasons within each site in detail, we ran *post hoc t* tests. These analyses were conducted using Statistica (ver. 5.5) software. In order to test the relationship between soil physico-chemical properties (independent variables) and abundance, species richness and diversity (dependent variables), simple linear regressions were performed.

The similarity of AMF morphospecies composition between the coffee systems and the forest patch was evaluated through the Sørensen (presence data) and Bray Curtis (abundance data) indices. Based on the latter, we ran a cluster analysis to construct a dendrogram showing affinity of sites based on morphospecies composition. The grouping linkage was done with the unweighted pair-group method of arithmetic averages (UPGMA) by the software PAST (ver. 1.84).

Results

AMF morphospecies taxonomic composition

From the 120 samples analyzed, a total of 33 morphospecies were distinguished on the basis of morphological criteria (Table 3). The most common genera were *Glomus* (16 spp.) and *Acaulospora* (12 spp.). Very few morphospecies from *Ambispora* (1 sp.), *Entrophospora* (1 sp.), *Gigaspora* (1 sp.) and *Scutellospora* (2 spp.) were detected, and in all cases these appeared infrequently. Ten morphospecies remained unidentified because their morphological characteristics did not fit with any description of known species.

Frequency of occurrence, relative abundance and dominance of AMF morphospecies

A high percentage (72%) of the morphospecies had a frequency of occurrence of below 30%. The morphospecies *Acaulospora delicata*, *A. scrobiculata*, *Glomus clarum* and *Glomus* sp. 3 had the highest values for frequency of occurrence; the first two appeared in all the study sites and on almost all the sampling dates, while the other two appeared in all the sites and sampling dates (Table 3).

The dominance of the 33 morphospecies is illustrated by Whittaker plots (Fig. 1). At the top of the plots, *G. clarum* and *Glomus* sp. 3 are among the most abundant morphospecies in all the sites. Beside these two morphospecies, we found *A. delicata* with high abundance values in the shaded polyculture system and in the shaded traditional rustic system, while in the unshaded monoculture system, *Glomus intraradices* was the third more abundant morphospecies. In the forest *Acaulospora dilatata* and *Glomus fasciculatum* appeared abundantly (Table 3).

AMF spore abundance, richness and diversity

Spore abundance differed between study sites (ANOVA $F = 3.56$, $P = 0.005$) and seasons ($F = 15.37$, $P = 0.01$). Shaded traditional polyculture system had lower abundance than the shaded simple system ($t = -3.16$, $P = 0.006$) and the forest ($t = 3.04$, $P = 0.03$; Fig. 2). Spore abundance was higher in the dry season (387.3/100 $g^{-1} \pm 37.26$) than in the wet season (137.27 ± 20.32; Table 4). The site-season interaction effect was significant ($F = 2.92$, $P = 0.01$). With the exception of the unshaded monoculture system, production of spores was significantly higher in the rest of the sites during the dry season (shaded simple system $t = 3.49$, $P = 0.002$; shaded commercial polyculture system $t = 2.40$, $P = 0.02$; shaded traditional polyculture system $t = 5.72$, $P = 0.02$; shaded traditional rustic system $t = 4.21$, $P = 0.0005$; forest $t = 5.23$, $P = 0.00005$). No differences in abundance were found between the study sites in the wet season. In the dry season, however, the unshaded monoculture system had significantly lower abundance than the forest ($t = 3.59$, $P = 0.02$) (Table 4).

The number of morphospecies detected in the study sites ranged from 16 to 24 (Table 4). The shaded simple system and the shaded traditional rustic system had the highest number of morphospecies (both 24). The forest had the lowest number of morphospecies (16), however, no significant differences were found in the statistical analysis. The Shannon diversity index ranged from 1.2 to 1.9; the highest values were recorded for the shaded traditional polyculture system and the forest and the lowest for the shaded traditional rustic system (Table 4). In this case, we found statistically significant differences

Table 3 Composition, frequency of occurrence (Fr%) and relative abundance (RA) of the AMF spore morphospecies found at the study sites in dry (D) and wet (W) seasons over the sampling years 2004 (1) and 2005 (2)

	AMF spore morphospecies	Fr%	UMO[a]				SSL				SCP				STP				STR				FOREST			
			D		W		D		W		D		W		D		W		D		W		D		W	
			1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
1	*Acaulospora bireticulata*	20.8	0.3		0.5			0.3											0.4		3.4					
2	*Acaulospora delicata*	79.2	18.0	2.1	7.9		8.3	0.5	6.6	0.8	35.6	1.1	4.4		37.1		1.2	0.8	24.0	0.2	3.8	3.7	6.6			0.9
3	*Acaulospora dilatata*	66.7	1.5	1.0	0.9	0.6	4.7						0.5	0.6	0.8		0.6		1.1	0.2	1.8	3.0	30.2		4.7	0.9
4	*Acaulospora excavata*	29.2			0.7				2.2			1.1	4.4				0.6				5.7				2.1	
5	*Acaulospora laevis*	20.8			4.3						1.4	0.8			3.8				0.7							
6	*Acaulospora mellea*	62.5		2.2			5.6	0.7	17.3	0.4	3.6	1.5	29.4		2.0	1.2	45.5		1.0	0.5	6.4	3.7				
7	*Acaulospora scrobiculata*	79.2	5.3	6.4	0.7	1.2	2.5	1.3		1.7	0.3	1.4	5.8		1.4			0.8	1.7	1.2	1.9	3.7	12.8		0.4	6.4
8	*Acaulospora* sp. 1	50.0	0.6	2.5	1.0		1.4		6.2		0.9		19.6		4.1		9.7		0.9		9.1		16.0			
9	*Acaulospora* sp. 2	25.0	0.6				0.4				2.2				3.2				3.0				4.1			
10	*Acaulospora* sp. 3	4.2																	0.6							
11	*Acaulospora* sp. 4	4.2	1.0																							
12	*Acaulospora spinosa*	25.0		1.1		0.6			0.6		0.3	0.6													0.8	
13	*Ambispora gerdemannii*	20.8					0.2	0.4			0.2									0.2		1.5				
14	*Entrophospora infrequens*	8.3					0.4												1.1							
15	*Gigaspora* sp.	20.8								0.4				0.6							1.3				0.8	0.5
16	*Glomus claroideum*	25.0	7.2				4.6	0.6			2.6				4.0				1.9							
17	*Glomus clarum*	100.0	0.5	40.7	43.3	54.0	5.6	17.6	48.3	80.6	0.7	79.7	31.5	98.3	1.0	83.8	16.9	57.6	0.5	1.0	52.1	47.2	9.0	0.6	47.8	62.3
18	*Glomus constrictum*	12.5	1.2				0.2												1.4							
19	*Glomus coronatum*	4.2			0.9																					
20	*Glomus etunicatum*	54.2	1.4	0.4	0.2		4.8	0.9	1.0		0.8	4.6			4.8	3.6	2.4		1.1			3.0				
21	*Glomus fasciculatum*	25.0	1.2				1.2				0.5								10.7				21.3	18.9		
22	*Glomus glomerulatum*	12.5									0.3				0.2				0.2							
23	*Glomus intraradices*	33.3		11.8	2.6	36.4		0.7										1.6		1.4		2.2				0.9
24	*Glomus microaggregatum*	16.7					0.4				1.2				0.9				0.2							
25	*Glomus mosseae*	4.2						0.6																		
26	*Glomus rubiforme*	29.2			9.6			3.6	0.3											1.3		1.5		6.4	0.4	
27	*Glomus* sp. 1	16.7	0.3				0.2				0.2				0.2											
28	*Glomus* sp. 2	12.5						0.5				0.9			1.4											
29	*Glomus* sp. 3	95.8	62.2	31.9	27.7	7.2	59.5	72.2	17.5	16.1	49.2	6.4	4.4	0.6	35.0	11.0	23.1	39.0	49.7	94.2	14.7	29.3		74.1	40.2	28.2

Table 3 continued

	AMF spore morphospecies	Fr%	UMO[a]				SSL				SCP				STP				STR				FOREST			
			D		W		D		W		D		W		D		W		D		W		D		W	
			1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
30	*Glomus* sp. 4	4.2									0.8															
31	*Glomus* sp. 5	4.2																								0.9
32	*Scutellospora biornata*	12.5										1.9				0.5						2.0				
33	*Scutellospora dipapillosa*	8.3																		1.5				0.8		

[a] *UMO* unshaded monoculture system, *SSL* shaded simple system, *SCP* shaded commercial polyculture system, *STP* shaded traditional polyculture system, *STR* shaded traditional rustic system, *FOREST* tropical montane cloud forest

($F_{5,24} = 4.64$, $P = 0.0042$); the AMF diversity of the shaded traditional rustic system is significantly lower than that of both the shaded traditional polyculture system and the forest (Tukey test: $P = 0.013$, $P = 0.011$ respectively).

Relationship between AMF abundance, species richness and diversity and soil physico-chemical properties

Linear regression analyses showed no significant relationships between the soil physico-chemical properties of soil (Table 2) of the study sites and the AMF spores parameters analyzed. Only for available P was a tendency found consisting of a lower number of spores in those production systems (shaded commercial polyculture and shaded traditional polyculture) with the higher concentrations of P available ($r = 0.57$, $P = 0.08$).

Similarity in AMF morphospecies composition

According to Sørensen's similarity index, the similitude of the different coffee systems ranged from 0.70 to 0.85 (Table 5). The shaded traditional polyculture and the shaded commercial polyculture systems shared the highest number of morphospecies, while the unshaded monoculture system and the shaded simple system had the lowest. Similarity values between the coffee systems and the forest were lower, and ranged from 0.59 to 0.65. The shaded traditional rustic system, the shaded simple system and the unshaded monoculture system shared the highest number of morphospecies with the forest, while the shaded traditional polyculture system had the lowest.

In the cluster analysis (Fig. 3), the AMF spores community of the forest was grouped together with the shaded traditional rustic system and shaded simple system. The three remaining production systems were concentrated into a separate group, with the two polycultures forming one subgroup and the unshaded monoculture system forming another.

Discussion

Composition, species richness, diversity and dominance of the AMF morphospecies spores

Taking into account the fact that Lopes et al. (1983) recorded 42 morphospecies in 27 coffee production

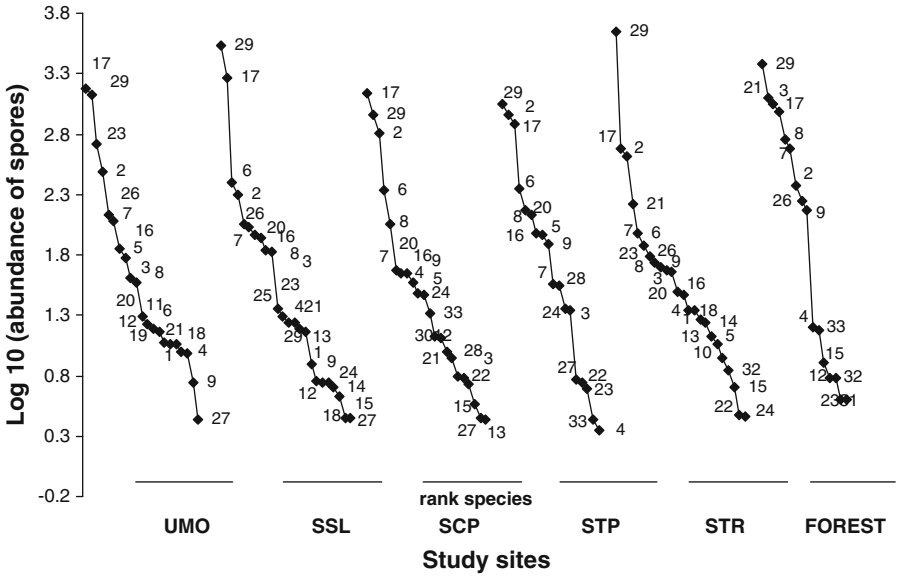

Fig. 1 Whittaker plots (rank/abundance plots) illustrating the richness and abundance of the AMF spore morphospecies detected in the study sites. *Numerals* refer to the list of morphospecies in Table 3. Morphospecies are plotted in sequence from highest to lowest abundance along the *X* axis. The *Y* axis represents the $\log_{10}$ (total spore abundance) of each morphospecies. Vegetation structure complexity increases from left to right. *UMO* unshaded monoculture system, *SSL* shaded simple system, *SCP* shaded commercial polyculture system, *STP* shaded traditional polyculture system, *STR* shaded traditional rustic system, *FOREST* tropical montane cloud forest

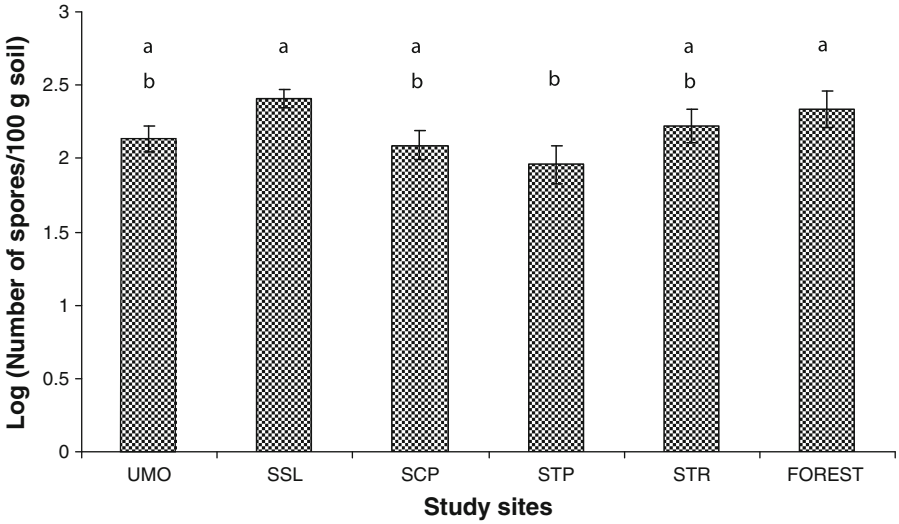

Fig. 2 Abundance of AMF spore morphospecies (average number of spores in 100 g^{-1} ± standard error; 20 replicates = 5 sampling points × 2 seasons × 2 years) at the study areas. Vegetation structure complexity increases from left to right. *UMO* unshaded monoculture system, *SSL* shaded simple system, *SCP* shaded commercial polyculture system, *STP* shaded traditional polyculture system, *STR* shaded traditional rustic system, *FOREST* tropical montane cloud forest. *Shared letters in a row* indicate no significant differences between sites

systems from Brazil, the number of morphospecies recorded in this work (32) in 5 coffee production systems indicates that the region hosts a considerable AMF spore richness. In common with studies carried out in coffee production soils from America (Lopes et al. 1983; Cruz 1989) and Africa (Muleta et al.

Table 4 Abundance[†] (number of spores·100 g^{-1}), species richness[‡] (S), diversity[‡] (Shannon index H′) and evenness of AMF spore morphospecies in five coffee agroforestry systems and a cloud forest patch of central Veracruz, Mexico

Study sites	Abundance		Species richness (S)		Diversity (H′)		Evenness
	Dry	Wet	Total	Mean ± se	Total	Mean ± se	Total
UMO	171.4 ± 38.52^{b}	253.9 ± 104.47^{a}	21	12.6 ± 1.12^{a}	1.8	1.51 ± 0.086^{ab}	0.28
SSL	455.8 ± 74.54^{ab}	180.6 ± 22.03^{a}	24	13.0 ± 0.84^{a}	1.43	1.30 ± 0.133^{ab}	0.17
SCP	302.0 ± 80.65^{ab}	68.6 ± 16.67^{a}	22	14.2 ± 0.86^{a}	1.73	1.59 ± 0.048^{ab}	0.26
STP	243.6 ± 42.02^{ab}	114.5 ± 32.20^{a}	18	11.0 ± 0.63^{a}	1.91	1.69 ± 0.064^{a}	0.38
STR	527.7 ± 114.63^{ab}	86.6 ± 15.92^{a}	24	12.8 ± 0.97^{a}	1.21	1.16 ± 0.155^{b}	0.14
FOREST	623.2 ± 94.92^{a}	119.3 ± 26.08^{a}	16	10.3 ± 1.25^{a}	1.92	1.70 ± 0.085^{a}	0.43
Total	2323.8	823.5					

Shared superscripts (a, b) by study sites indicate no significant differences between them

UMO unshaded monoculture system, *SSL* shaded simple system, *SCP* shaded commercial polyculture system, *STP* shaded traditional polyculture system, *STR* shaded traditional rustic system, *FOREST* tropical montane cloud forest, *se* standard error

[†] Average of ten replicates formed by pooling data from two consecutive years

[‡] Average of five replicates formed by pooling data from two climatic seasons in two consecutive years

Table 5 Similarity matrix in the composition of AMF spore morphospecies calculated by the Sørensen index (*above diagonal*) and number of species shared (*under diagonal*) in the study sites

Study sites	UMO	SSL	SCP	STP	STR	FOREST
UMO	**21**	0.80	0.70	0.72	0.76	0.65
SSL	18	**24**	0.78	0.71	0.83	0.65
SCP	15	18	**22**	0.85	0.74	0.63
STP	14	15	17	**18**	0.71	0.59
STR	17	20	17	15	**24**	0.65
FOREST	12	13	12	10	13	**16**

Numbers in bold indicate the total number of species for each site. Vegetation structure complexity increases from left to right

UMO unshaded monoculture system, *SSL* shaded simple system, *SCP* shaded commercial polyculture system, *STP* shaded traditional polyculture system, *STR* shaded traditional rustic system, *FOREST* tropical montane cloud forest

2008; Jefwa et al. 2009), we found the spores of the genera *Acaulospora* and *Glomus* to be dominant. This result is to be expected since both genera have a broad distribution in all kind of agroecosystems in the world (Oehl et al. 2003), as well as in forest ecosystems (Zhao et al. 2003). Different authors have associated the high incidence of *Glomus* and *Acaulospora* spores with their capacity to produce more spores in a shorter time than genera such as *Gigaspora* and *Scutellospora* (Bever et al. 1996). However, further investigation is necessary since very little is currently known regarding the sporogenous cycle and autoecology of AMF.

According to their relative abundance and frequency of occurrence (Table 3), *Glomus* sp. 3 and *G. clarum* were the dominant morphospecies at regional level (Fig. 1). *G. clarum* has been detected in primary ecosystems (Irrazabal et al. 2005; Li et al. 2007; Lopes et al. 2009) as well as in agricultural systems, including coffee production system (Bhattacharya and Bagyaraj 2002). In addition to the effectiveness of *G. clarum* in coffee seedling growth in glasshouses (Fernández-Martín et al. 2005), it has been demonstrated that this species can increase P concentration in *Gliricidia sepium* (Twum-Ampofo 2008). These features makes this fungus a promising morphospecies to be considered in future studies, since most of the soils in the study area are low in available P (Geissert and Ibáñez 2008).

Concerning to the species richness, statistical analysis showed that there were not significant differences (Table 4). However, the low number of morphospecies

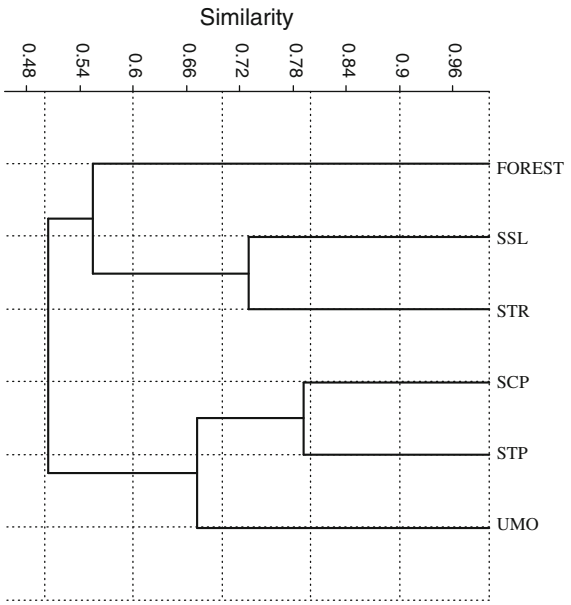

Fig. 3 Dendrogram showing similarity of coffee production systems and a cloud forest patch in the composition of AMF spore morphospecies obtained by a cluster analysis based on the Bray Curtis index. *UMO* unshaded monoculture system, *SSL* shaded simple system, *SCP* shaded commercial polyculture system, *STP* shaded traditional polyculture system, *STR* shaded traditional rustic system, *FOREST* tropical montane cloud forest

spores (16) found in the forest is surprising in view of the high plant diversity in this ecosystem. This result suggests that the species richness of AMF spores may not correlate with plant diversity; a feature that concurs with the findings of Allen et al. (1998) in a deciduous tropical forest and Zhao et al. (2003) in a tropical rain forest. This has been attributed to the fact that AMF species display no restriction of host range (Allen et al. 1995). For ecosystems with a high plant diversity, the possibility that one species of AMF can be associated with several plant species is an advantage that ensures symbiotic colonization even with a low richness of AMF species in a competitive nutrient-poor environment.

On the other hand, since we only analyzed the depths of 0–15 cm, there is the possibility that some AMF species were not detected in the samples analyzed. Oehl et al. (2005) found an important diversity of AMF species in subsoil samples from 20 to 70 cm. Thus, to get a complete comparison of AMF diversity between coffee production systems and the tropical forest they replace, future studies should analyze soil samples from greater depths.

With regard to spore diversity, with the exception of the shaded traditional rustic system, the AMF diversity in the other coffee systems is similar and does not differ from that of the forest. The lower diversity in the shaded traditional rustic system (Table 4), despite being one of the two sites with more morphospecies, is due to the dominance of *Glomus* sp. 3 (Fig. 1). Thus, in contrast to studies of macroscopic aboveground communities from monocultures and agroforestry coffee production (Philpott et al. 2008), the richness and diversity of the AMF spores from the production systems featuring a simpler vegetation structure had similar and, in some cases, higher values (although not significantly so) than those found in the systems with a more complex vegetation structure (in terms of tree height, density, basal area, diversity etc.), including the forest.

These results may be due to the main location of the AMF habitat in the plant rhizosphere, where the biophysical environment in the herbaceous stratum and in the first layers of soil could have a more direct influence than the structure of the tree layer. Because of the low coffee prices in the market over the 2 years of the study, limited weed control was carried out in all the production systems and consequently a dense herbaceous coverage was found during sampling in all the sites. The resulting accumulation of leaf litter and weed cover reduced the exposure of naked soil (Table 2) in all the production systems and created a similar microenvironment in all the sites, that also all featured virtually the same climatic conditions and soil type (Tables 1, 2). We suggest that this situation could explain the similarity of the species richness and diversity values as well as the dominance of the same AMF morphospecies at all the sites.

Among the weeds growing in the coffee production systems in the region are grasses of the genera *Andropogon*, *Brachiaria*, *Cynodon*, *Dachanthelium*, *Digitaria*, *Eragrostis* and *Paspalum* spp. as well as dicotyledons of the genera *Commelina*, *Solanum*, *Chenopodium* and *Melampodium* spp. In the unshaded monoculture, and in the shaded simple system, a considerable abundance of grasses was found over the four sampling dates. We do not have data regarding weed distribution and abundance in the study sites since this aspect was beyond the objectives of this study. Considering that some grasses have root systems that grow quickly and promote sporulation of the AMF (Daniels-Hetrick and Bloom 1986), and that weeds

could promote the decompression of soil and protect it against erosion (Coelho et al. 2004), it is important to carry on future studies in the region to evaluate the mycorrhizal status of the weeds.

Another aspect that could be influencing the high similarity among the AMF community of the evaluated coffee production systems is that in contrast with the annual crops, coffee is a perennial crop that does not undergo intensive tillage, meaning that the AMF community does not undergo drastic disturbance.

Finally, due to the impossibility of finding plantations of each type of production system with identical management practices, the possibility exists that our sampling points (at minimum distances recommended to guarantee spatial independence for microbial communities; Huising et al. 2008; Negrete-Yankelevich et al. 2006) may in fact represent pseudoreplicates, potentially obscuring differences in AMF richness and diversity.

Abundance and seasonal spore production

There were significant differences in the abundance and seasonal spore production between the coffee systems. Management practice and shade tree composition may explain these differences. The lowest abundance of spores in the shaded traditional polyculture system could be a consequence of the frequent chemical fertilization conducted in this coffee system. Several studies have reported suppression of mycorrhizae in the presence of high nutrient contents (Sieverding 1991). On the other hand, we attribute the high AM spore counts in the shaded simple system to the high coffee plant density and to the dominance of leguminous shade trees in this plantation. In agroforestry coffee systems in Ethiopia, Muleta et al. (2007), reported a positive correlation between AMF spores counts and the density of coffee plants and leguminous shade trees.

The higher spore abundance during the dry season in the different agroforestry systems and in the forest may be related to the phenology of the shade plants and to changes in their root physiology. The reduced water supply during the dry season could be an important factor in the induction of sporulation as a fungal survival strategy to deal with water stress and the death of fine roots (Redhead 1975). Similarly higher spore abundance in the dry season has been reported in other tropical ecosystems (Allen et al. 1998; Guadarrama and Álvarez-Sánches 1999; Picone 2000). On the other hand, the decline of the abundance of spores during the wet season has been related to spore germination and subsequent mycorrhizal colonization (Sigüenza et al. 1996). The absence of seasonal spore production in the unshaded monoculture system clearly indicates that the shade plants influence this process; similar results were found by Trejo et al. (1998).

Similarity between coffee production systems and the TMCF

Of the total number of morphospecies isolated from the five coffee production systems studied (32 morphospecies), except for the *Glomus* sp. 5, all were detected at least in one coffee production system. This allows us to assume that at the regional level there is not a notorious loss of the forest native morphospecies. Due to the elimination or introduction of different host plants in the different coffee systems, some forest AMF might be affected and produce fewer spores, for this reason it requires a greater sampling effort to detect them.

Interestingly, according to the Sørensen index, the three coffee production systems (unshaded monoculture system, shaded simple system and shaded traditional rustic system) with the greatest similarity to the forest are of quite different plant composition (Tables 1, 5). The only common factor between them and the forest is the low available P and high fixed P in the soil (Table 2). Miranda and Harris (1994) showed that some AMF morphospecies are more sensitive to P than others, and this may therefore be a selective factor in the morphospecies taxonomic composition. The Bray Curtis index confirmed the Sørensen index results. However, in the dendrogram, the unshaded monoculture system is located in another group together with the two polyculture systems that had the lower total spore abundance. The higher percentage of similitude between the coffee production systems with polycultures and their grouping in a different clade may be related to the high concentration of available P in the soils resulting from the frequent application of chemical fertilizers in both of these sites (Tables 1, 2). Siqueira et al. (1998) reported a reduction in spore number in coffee plants amended with high P rates. Given that the Bray Curtis index consider abundance values it provides a useful and better overview of the similarity between

the coffee production systems and the forest, since for conservation and restoration of ecosystems it is necessary to have a minimum abundance of native species.

Thus, taking into account the composition and relative abundance of AMF morphospecies, the shaded traditional rustic system and the shaded simple system are the coffee plantation systems with AMF communities most similar to those of cloud forest. However, because the different coffee plantations studied are under management, they maintain an adequate AMF spore community. This supports the idea that coffee production is an environmentally friendly practice.

Conservation of the AMF spore communities is very important for the coffee production systems in order to address the low concentration of available phosphorus in soils of the region, as well to reduce erosion in those coffee production systems typically located on sloping terrain. Regional-scale studies are necessary in order to select the suitable indigenous AM fungal strains to replace chemical fertilizers and thus to support producers in achieving certification of their coffee, allowing them to command a higher price for the product in the world market.

In conclusion, the vegetation structure and management of the coffee plantation systems studied does not influence the specific richness of AMF spores. Diversity was similar in most of the coffee plantation systems. However, the seasonal production of spores is affected by the production system. Some factors related to management practices such as weed control, soil microenvironment and soil chemical fertilization may be influencing the abundance and composition of the AMF spores at coffee plantations. This type of study allows us to say that the type of management practiced in the different coffee plantations in central Veracruz region does not cause a deleterious impact on the communities of AMF spores.

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