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Fungal diversity in coffee plantation systems and in a tropical montane cloud forest in Veracruz, Mexico

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Abstract We compared the abundance, species richness and diversity of saprobic filamentous microfungi in the forest and in coffee plantation systems (with different biophysical structures of the vegetation and agricultural management) and evaluated the degree of similarity in species composition among these sites. Soil washing was used to isolate the saprobic filamentous microfungi. Emerging colonies were quantified and transferred to tubes with culture medium and then mounted on semi-permanent slides for identification under the microscope. From 90 samples and 4,500 inoculated soils particles, 415 species were distinguished. The genera *Trichoderma*, *Penicillium*, *Fusarium, Chaetomium* and *Humicola* were the most frequent in all study sites. The transformation of the tropical montane cloud forest in coffee plantation had no significant effect on the abundance, species richness and diversity of the saprobic filamentous microfungi. Effects of the biophysical structures of the vegetation and the agricultural management of the sites were only detected at the level of dominant genera (*Fusarium, Trichoderma and Penicillium*) and on the evenness. The low degree of similarity among the six sites suggests the existence of a high exchange of species.

Keywords Diversity index · Species richness · Abundance · *Coffea arabica* L. · Filamentous fungi

Introduction

Coffee is the main exported agricultural product in Mexico; more than 70 % of the coffee plantations practice cultivation under shade (Moguel and Toledo 1999). Depending on the species of trees used for shade, intensity of pesticide and fertilizer use and the production and diversity of products for consumption, it is possible to distinguish the following systems in an order of lower to greater vegetal complexity: unshaded, shaded monoculture, commercial polyculture, traditional polyculture and rustic coffee systems (Moguel and Toledo 1996).

Due to the fact that the climatological requirements of altitude-grown coffee coincide with the distribution of tropical montane cloud forest in the central region of Veracruz, a high proportion of this forest area has been replaced by coffee plantations. This is in spite of the fact that tropical montane cloud forest covers just 0.8 % of the Mexican national territory, yet hosts between 10 and 12 % of all the plant species estimated for the country and is thus considered one of the most diverse ecosystems in Mexico (Rzedowski 1996).

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Tropical montane cloud forest also provides important environmental services such as carbon sequestration, water capture and purification and the control of soil erosion and river sedimentation, as well as the landslides and floods that affect large parts of lowland Mexico (Bruijnzeel and Hamilton 2001).

Due to their geographical location and biophysical structure, shaded coffee plantations are considered to be agroecosystems that host an important diversity of native plants and animals (Manson et al. 2008). Various studies have shown that, in contrast to other agricultural uses, the cultivation of shaded coffee, especially under the rustic management system, acts to conserve elements of the vegetal structure of the cloud forest that function as corridors or reserves for various organisms, such as birds (Tejeda-Cruz and Gordon 2008), ants (Valenzuela-González et al. 2008), bats (Sosa et al. 2008), amphibians and reptiles (González-Romero and Murrieta-Galindo 2008), ferns (Mehltreter 2008), vascular epiphytes (García-Franco and Toledo 2008) and saprobic and mycorrhizal fungi (Heredia and Arias 2008; Arias et al. 2012).

Little is known regarding the composition and structure of important groups within the soil microscopic communities, such as the microfungi: In the coffee producing central region of Veracruz state, there have been various studies of endomycorrhizal communities (Riess and Sanvito 1985; Trejo et al. 1998; Arias et al. 2012). However, there are fewer studies of the saprobic microfungi even though these play an important role in soil food chains, decomposition of plant and animal material (Dighton 2003) and solubilization of phosphorous (Pandey et al. 2008). Among the existing studies of the saprobic microfungi in coffee plantations are those of Siddiqi (1964) and Velmourougane et al. (2000), in Malawian and Indian, respectively. In Mexico, Persiani and Maggi (1988) analyzed the composition and structure of the saprobic microfungi in the rhizospheres of a greenhouse and two coffee plantations (unshaded and shaded).

The objectives of this study were to: (a) determine whether the biophysical structure of the vegetation and agricultural management of the different regional coffee plantation systems influence the composition, diversity and abundance of the communities of saprobic filamentous microfungi in the soil, and (b) determine the degree of similarity among these communities in the coffee plantation systems and those of the tropical montane cloud forest.

Materials and methods

This study formed part of a project entitled "An interdisciplinary study of the biodiversity conservation and environmental services of the tropical montane cloud forest in a management intensity gradient of coffee cultivation in central Veracruz" (SEMARNAT/2002-CO1-0194).

Study area

The study took place in the Coatepec-Huatusco area of central Veracruz state, Mexico (elevation 1,300–2,200 m asl). Annual mean temperature in the area ranges from 12 to 19 °C and typically there are three defined seasons: one relatively dry and temperate from October to March (season of cold northerly fronts "*nortes*"), another dry and warm from April to May and one humid and warm from June to October (Williams-Linera 1997).

The soils in all the coffee plantations included in the study correspond to andic Acrisols while those of the forest are umbric Andisols. In both cases, the surface horizons of the soil present similar edaphogenetic properties, enabling the comparison of edaphogenetic characteristics between the coffee plantations and the studied area of tropical montane cloud forest (Geissert and Ibáñez 2008).

The coffee plantations and the area of cloud forest form part of the study sites chosen for the project described previously. Together, they represent the main coffee plantation systems practiced in the region (Table 1). The area of tropical montane cloud forest is located within the private reserve known as "Las Cañadas" in the municipality of Huatusco, where the structure and composition of vegetal elements typical of tropical montane cloud forest are conserved.

Sampling design

For each of the six sites, three surveys were carried out (May 2004, and May and September 2005). In each site, five sampling points were chosen, each 50 m apart from the others in order to ensure their independence. To avoid edge effects, the distance between sampling points and the nearest road was 100 m. In the coffee plantations and forest, the center of each sampling point was a coffee plant and a tree, respectively. From these central points, two 1 m perpendicular axes were

Table 1 Characteristics of the study sites in central Veracruz: mean annual precipitation (MAP), tree composition, agricultural practices (for coffee plantations), index of the biophysical structure of the vegetation (IBS) and agricultural management index (AMI)

Study sites key	Latitude and longitude	MAP (mm) Dry season Wet season	Tree Composition[a]	Agricultural practices[b]	IBS[c]	AMI[a]
Unshaded monoculture system (UMO)	19°22′53″ 96°59′17″	309.2 1,554.0	No shade trees	Sparse use of agrochemical products	−0.06	0.34
Shaded simple system (SSL)	19°12′22″ 96°53′4″	275.9 1176.2	Canopy dominated by legumes (*Inga vera, Erythrina poeppigiana*)	Sparse use of agrochemical products	0.27	0.58
Shaded commercial polyculture system (SCP)	19°27′59″ 96°56′3″	304.02 1,499.3	Timber, legumes and fruit trees (*Inga jinicuil, Inga vera, Citrus* spp.)	Frequent use of agrochemicals products	0.58	0.32
Shaded traditional polyculture system (STP)	19°25′56″ 96°57′50″	298.2 1477.8	Some forest native trees, legumes, and timber and fruit trees (*Citrus* spp., *Inga vera, Trema micrantha* and *Musa* sp.)	Frequent use of agrochemicals products	0.51	1
Shaded traditional rustic system (STR)	19°12′57″ 96°53′7″	365.9 1,794	Native forest canopy, some legumes (*Quercus sartorii, Inga* sp., *Citrus* spp. and *Tapirira mexicana*)	No use of agrochemical products. Pest control by biological alternatives	0.61	0
Tropical montane cloud forest (FOREST)	19°11′23″ 96°59′11′	283.5 1,396.5	Native forest canopy (*Beilschmiedia mexicana, Brunellia mexicana, Quercus insignis, Q. leiophylla,Q. sartorii, Hampea intergerrima* and *Turpinia insignis*)	None	1	0

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

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

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

marked. At the ends of each axis, a 100 g sample of soil was taken from a depth of 0–10 cm. In the laboratory, the four soil samples per sampling point were mixed to obtain a compound sample. The soil was dried at ambient temperature and stored at 5 °C until processing for isolation of fungi. In total, 90 compound samples were analyzed (6 sampling sites × 5 sampling points × 3 samples).

Isolation, quantifications and identification of saprobic filamentous microfungi

For the detection and isolation of the microfungi, a soil particle filtration technique was used (Chapter 13, Bills et al. 2004). In each survey, for each site and individual sampling point, 50 soil particles were sown in dichloran rose bengal chloramphenicol agar (DBRC) in ten Petri dishes (50 particles × 5 points = 250 particles × 6 sites = 1,500 particles per survey × 3 surveys = total of 4,500 inoculated particles utilized in the study).

The Petri dishes containing the particles were incubated for 15 days and all emerging colonies were quantified and transferred to tubes with the culture medium potato dextrose agar (PDA) for subsequent quantification and identification.

For each of the species, semi-permanent preparations were produced in lactic acid, and identification was performed using taxonomic keys and specialized literature (Domsch et al. 1980; Ellis 1971, 1976). To identify species of the genera *Penicillium, Fusarium, Aspergillus* and *Trichoderma*, we used specialized methodologies recommended for each genus (Pitt 1991; Booth 1971; Klich and Pitt 1988; Gams and Bisset 1998). Non-fruiting fungal colonies were quantified and classified as sterile mycelia.

Analysis of variables

In each survey, for each of the six sites, we evaluated: (i) total abundance of colonies; considered as the total number of colonies isolated from 750 particles of soil (50 particles × 5 sampling points = 250 × 3 surveys = 750 particles per site), (ii) relative abundance of each species (RA %); calculated by dividing the number of colonies of a given species by the total number of isolated communities for a given site, and multiplying by 100, (iii) frequency of occurrence (Fro %); the number of sampling dates on which a given species was isolated divided by the total number of sampling dates, multiplied by 100, (iv) species richness (S); obtained by quantification of the total number of species present in each site, (v) species diversity; using Shannon Wiener (H′) index, which considers both the species richness and the uniformity of distribution of the number of individuals of each species, calculated using the formula: $H' = -\Sigma ((ni/n) \ln (ni))$ where ni = number of individuals of species i and n = number of all the individuals of all species, and (vi) evenness; obtained by dividing the Shannon diversity index by the logarithm of the number of taxa. These analyses were conducted using the program Past (version 1.84). With the objective of determining dominance patterns, Whittaker (abundance–dominance) curves were produced for each site, plotting the total abundance of each species against the range of species (ordered from highest to lowest abundance).

In order to detect differences in the abundance, richness and diversity of species among the sites, one-way analysis of variance was conducted (In each survey, 15 replicates were utilized per site for abundance and five replicates per site for richness and diversity). When the effects of the factors were shown to be significant by the ANOVA, paired Tukey HSD post hoc tests were performed. These analyses were conducted using the program Statistica (version 5.5).

Similarity in species composition between sites was evaluated using Sørensen (presence–absence data) and Bray Curtis (abundance data) indices; in the latter, a cluster analysis was conducted and a dendrogram generated showing the affinity of the sites based on species composition. These analyses were conducted using the PAST software (version 1.84).

A simple linear regression was used to determine the relationship of the abundance, species richness and diversity (dependent variables) with the physicochemical properties of the soil (data from Geissert e Ibañez 2008), the index of the biophysical structure of the vegetation and the index of agricultural management (independent variables).

Results

Taxonomic composition

A total of 415 species were distinguished from the 90 analyzed samples and 4,500 inoculated soil particles in

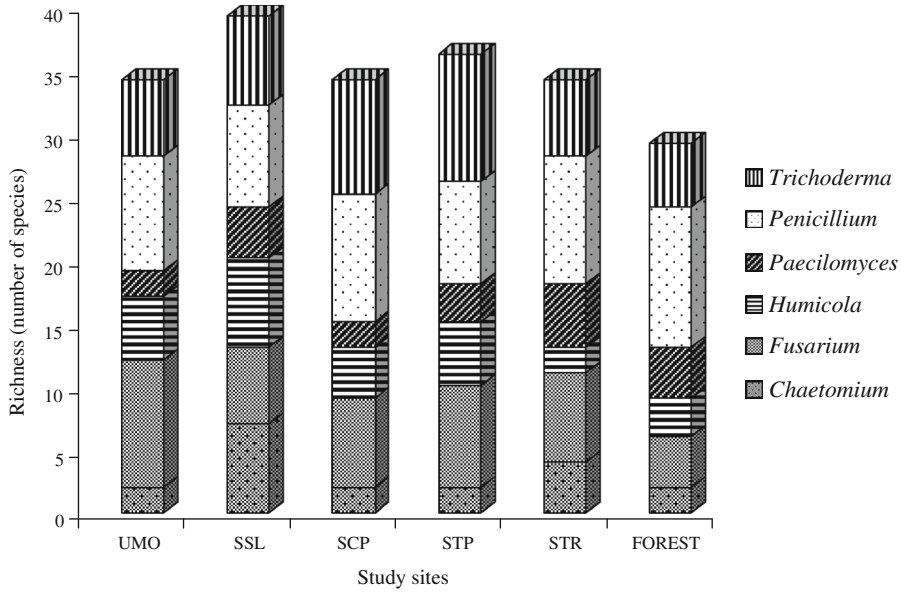

Fig. 1 Species richness of genera most common isolated of study sites. *UMO* unshaded monoculture system, *SSL* shaded simple system, *SCP* shaded commercial polyculture system, *STP* shaded traditional polyculture system, *STR* shaded traditional rustic system, *FOREST* tropical montane cloud forest

Table 2 Abundance total[†a] (number of colonies isolated from 750 soil particles), species richness[‡] (S), diversity[‡] (Shannon index H′) and evenness of saprobic filamentous microfungi in the study sites

Study sites	Abundance		Species richness (S)		Diversity (H′)		Evenness
	Total	Mean ± se	Total	Mean ± se	Total	Mean ± se	Total
UMO	359	23.93 ± 2.76[a]	121	39.59 ± 3.84[a]	4.16	3.37 ± 0.09[a]	0.86
SSL	344	22.93 ± 3.12[a]	130	41.20 ± 1.90[a]	4.29	3.07 ± 0.04[a]	0.88
SCP	270	17.93 ± 8.52[a]	118	34.0 ± 2.91[a]	4.36	3.33 ± 0.11[a]	0.91
STP	331	22.06 ± 12.24[a]	124	39.20 ± 4.88[a]	4.3	3.39 ± 0.11[a]	0.89
STR	334	22.26 ± 12.84[a]	126	36.20 ± 2.74[a]	4.09	3.25 ± 0.07[a]	0.84
FOREST	277	18.46 ± 10.19[a]	131	36.20 ± 4.07[a]	4.55	3.40 ± 0.11[a]	0.93

Abundance total (Number of colonies isolated from 750 soils particles)

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

[†a] Average of fifteen replicates by pooling data from three samples. *se* standard error

[‡] Average of five replicates formed by pooling data from three samples

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

culture media. Of these, 186 were identified to species level, 61 to genus level and 31 to group level, while the remaining 137 (33 %) were classed as sterile mycelia (Online Resource 1). The genera most abundant genera in each site were: *Penicillium* (24 species), *Humicola* (17), *Fusarium* (14), *Trichoderma* (13), *Chaetomium* (12) and *Paecilomyces* (7) (Fig. 1). The cultures were deposited in a culture collection in Micromycetes Laboratories, Instituto de Ecologia A.C.

Total abundance, relative abundance, dominance and frequency of occurrence of the saprobic filamentous microfungi

The total abundance of colonies varied from 277 to 359; the highest value corresponded to the unshaded monoculture system and the lowest to the tropical montane cloud forest. Statistical analysis showed that there were no significant differences in total abundance among sites (Table 2). The relative abundance

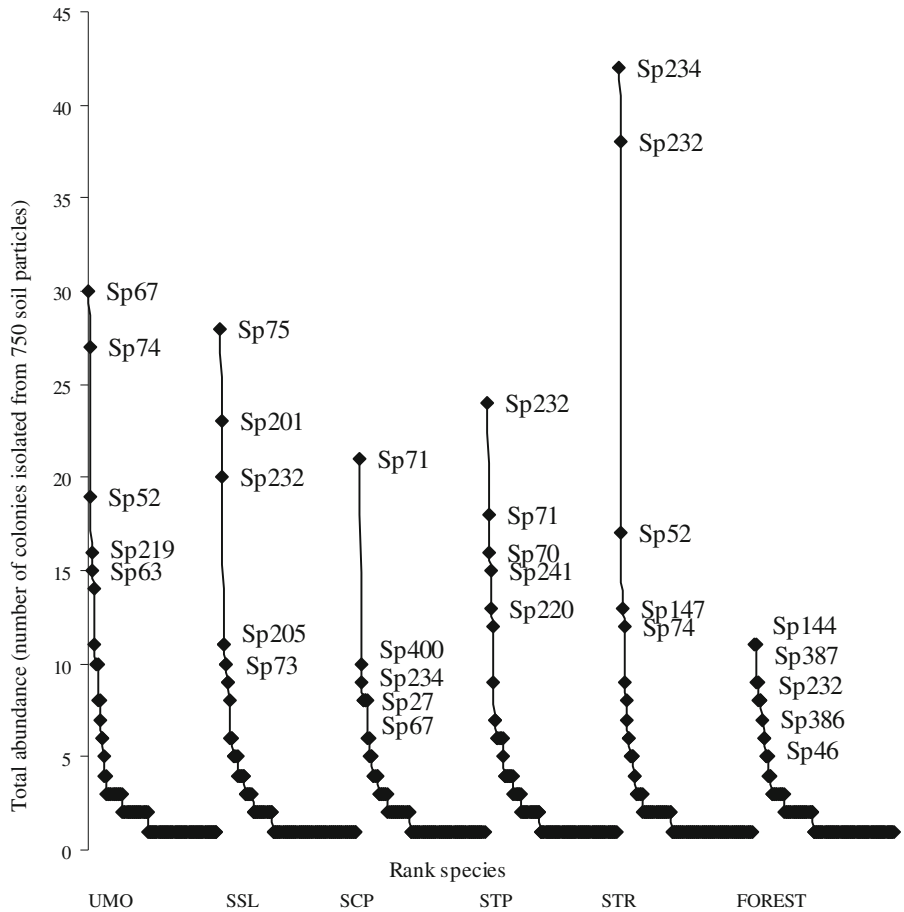

Fig. 2 Whittaker plots (Rank/abundance plots) illustrating the abundance of colonies of the saprobic filamentous microfungi detected in the study sites. Numerals refer to the list of species in Table 3. Species are plotted in sequence from highest to lowest abundance along the X axis. The Y axis represents the total colony abundance of each specie. 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

of the species ranged from 0.4 to 16.7 % (Online Resource 1). A high percentage of the species (approximately 97 %) presented values <5 % whenever they were detected. Species for which relative abundance values of between 10 and 16.7 % were found in at least one survey were: *Fusarium poae*, *F. tricinctum, Trichoderma cremeum* and *T. koningii.*

With the results of the percentage of frequency of occurrence (Online Resource 1), the following categories were established for species: very frequent (Fro = 100–76 %), frequent (Fro = 75–50 %), occasional (Fro = 49–25 %) and sporadic (Fro = 24–5 %). Fungi were mainly classed as sporadic species (383 species = 92.1 %), with a low number of species as occasional (29 = 6.98 %) and very few as frequent (3 = 0.72 %). No species were classed as very frequent. The most common species were: *F. chlamydosporum, Paecilomyces lilacinus* and *T. cremeum,* with the former two species only appearing in the coffee plantation soils.

In terms of species distribution at landscape level, only *Geotrichum candidum, Penicillium implicatum, Myrmecridium schulzeri* var. *schulzeri* and *Trichoderma koningii* were detected across all the studied sites in at least one of the three surveys (Online Resourse 1).

The distribution patterns of abundance–dominance, or Whittaker plots, of the species were notably similar among the coffee plantation systems and different in the forest (Fig. 2; Table 3); while 1–3 dominant species were clearly distinguishable in the coffee

Table 3 Abundance total (number of colonies isolated from 750 soil particles) of the saprobic filamentous microfungi with the highest abundance in the study sites

Species	Key	UMO	SSL	SCP	STP	STR	FOREST
Chaetomium cochlioides	Sp27	0	0	8	0	0	0
Cladosporium cladosporioides	Sp46	3	4	0	4	1	8
Cylindrocarpon obtusisporum	Sp52	19	3	0	0	17	0
Eupenicillium sp. 2.	Sp63	15	6	0	0	0	0
Fusarium tricinctum	Sp75	6	28	0	0	4	0
Fusarium chlamydosporum	Sp67	30	10	8	9	5	0
Fusarium moniliforme	Sp70	0	0	1	16	0	0
Fusarium moniliforme f. *subglutinans*	Sp71	0	0	21	18	1	0
Fusarium oxysporum	Sp73	2	11	6	0	0	0
Fusarium poae	Sp74	27	0	1	1	12	0
Myrmecridium schulzeir var. *schulzeri*	Sp205	3	11	8	4	3	3
Paecilomyces carneus	Sp144	0	1	0	4	2	11
Paecilomyces lilacinus	Sp147	8	9	8	12	13	0
Pseudeurotium sp.	Sp201	3	23	0	0	0	0
Talaromyces flavus	Sp219	16	4	0	0	0	0
Talaromyces flavus var. *flavus*	Sp220	16	4	4	13	5	0
Trichoderma cremeum	Sp232	6	20	0	24	38	8
Trichoderma koningii	Sp234	3	10	9	1	42	1
Trichoderma viride	Sp241	1	1	2	15	1	0
Cepa estéril 131	Sp386	0	0	0	0	0	9
Cepa estéril 132	Sp387	0	0	0	0	0	11
Xylarial type 3	Sp400	1	0	10	2	1	0

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

plantations, no species was found to dominate in the forest. Moreover, the majority of species in the forest presented low abundance. A high percentage of the species detected in the forest were not found in any of the coffee plantation sites (53 species = 40 %).

The dominant species of the five coffee plantations were different; in the unshaded monoculture systems, shaded simple system and shaded commercial polyculture system, species of the genus *Fusarium* (*F. chlamydosporum, F. tricinctum* and *F. moniliforme*, respectively) dominated, while in the coffee production with greater vegetation structure (shaded traditional polyculture and shaded traditional rustic systems), the dominant species were of the genus *Trichoderma* (*T. cremeum* and *T. koningii*).

Richness, diversity and evenness

No significant differences in richness and diversity were found among the sites. The highest number of species was found in the tropical montane cloud forest (131 species) and the lowest in the shaded commercial polyculture system (118 species) (Table 2). The Shannon diversity index ranged from 4.09 to 4.55 and again the highest value was in the tropical montane cloud forest while the lowest corresponded to the shaded traditional rustic system (Table 2). Evenness among all the sites was within the range 0.84–0.93; the highest value was found in the tropical montane cloud forest (Table 2), with a marginal significant difference (ANOVA $F = 2.36$, $P = 0.05$) compared to the shaded traditional rustic system ($t = P = 0.05$).

Similarity in saprobic filamentous microfungi species composition

According to the Sørensen index, similarity among sites was low (Table 4). The two polyculture systems (shaded commercial polyculture system and shaded traditional polyculture system) presented the highest similarity, while the lowest value of similarity was found between the shaded commercial polyculture system and the tropical montane cloud forest.

Table 4 Similarity matrix in the composition of saprobic filamentous microfungi calculated by the Sørensen index (above diagonal) and number of species shared (under diagonal) in the study sites

Sites	UMO	SSL	SCP	STP	STR	FOREST
UMO	*121*	0.22	0.18	0.2	0.18	0.14
SSL	46	*130*	0.16	0.19	0.2	0.15
SCP	37	35	*118*	0.24	0.2	0.12
STP	42	42	48	*124*	0.2	0.18
STR	39	43	41	47	*126*	0.18
FOREST	31	34	28	39	40	*131*

Numbers in italics 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

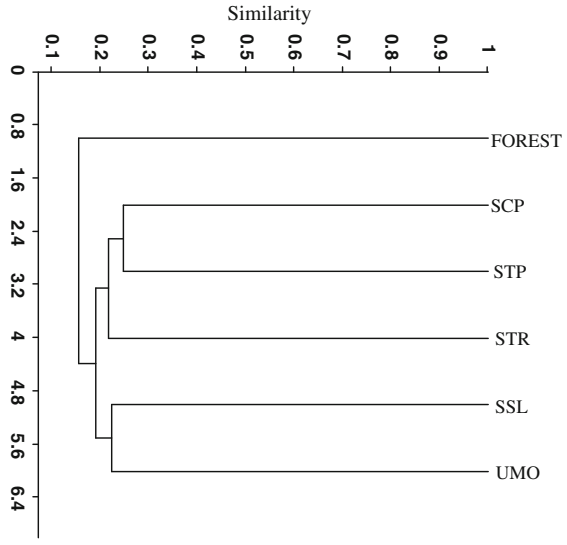

Fig. 3 Dendrogram showing similarity of coffee plantation systems and a tropical montane cloud forest in the saprobic filamentous microfungi composition obtained by a cluster analysis based on the Bray Curtis index. *UMO* unshaded monoculture system, *SSL* shaded simple system, *SCP* shaded commercial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

Similarity values among the coffee plantation sites were between 0.16 and 0.24, but ranged from 0.12 to 0.18 between the coffee plantations and the forest (Table 4). The shaded traditional rustic system and the shaded traditional polyculture system shared the

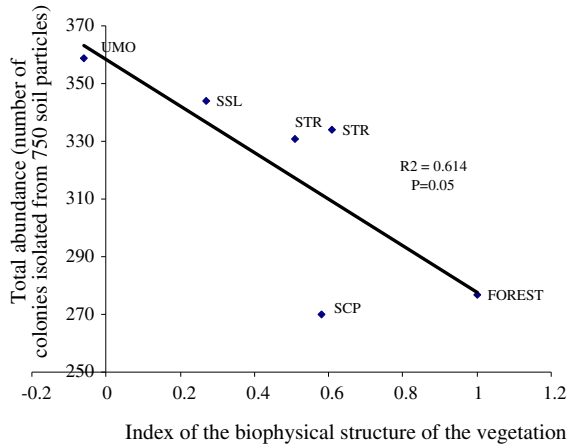

Fig. 4 Relationship between colonies total abundance of saprobic filamentous microfungi and the index of the biophysical structure of the vegetation from study sites. *UMO* unshaded monoculture system, *SSL* shaded simple system, *SCP* shaded commercial polyculture system, *STP* shaded traditional polyculture system, *STR* shaded traditional rustic system, *FOREST* tropical montane cloud forest

greatest number of species with the tropical montane cloud forest. In the dendrogram (Fig. 3), the mycobiota of the tropical montane cloud forest was grouped in an independent branch while that of the coffee plantation sites occupied another clade, which placed the shaded traditional rustic system together with the shaded traditional polyculture system and shaded commercial polyculture system in one group, and with the two monocultures (shaded simple system and unshaded monoculture system) in another.

Relationship between the abundance, species richness and species diversity of saprobic filamentous microfungi and the physicochemical properties of the soil, agricultural management and biophysical structure of the vegetation

No significant relationships were found between the variables analyzed (indices of agricultural management and biophysical structure of the vegetation and soil physico-chemical properties of soil and the richness and diversity of the saprobic microfungi in the study sites. Only a marginal ($r = 0.61$, $P = 0.05$) relationship was detected between the abundance of colonies and the index of the biophysical structure of the vegetation (Fig. 4); a higher number of colonies were present in sites with lower biophysical structure.

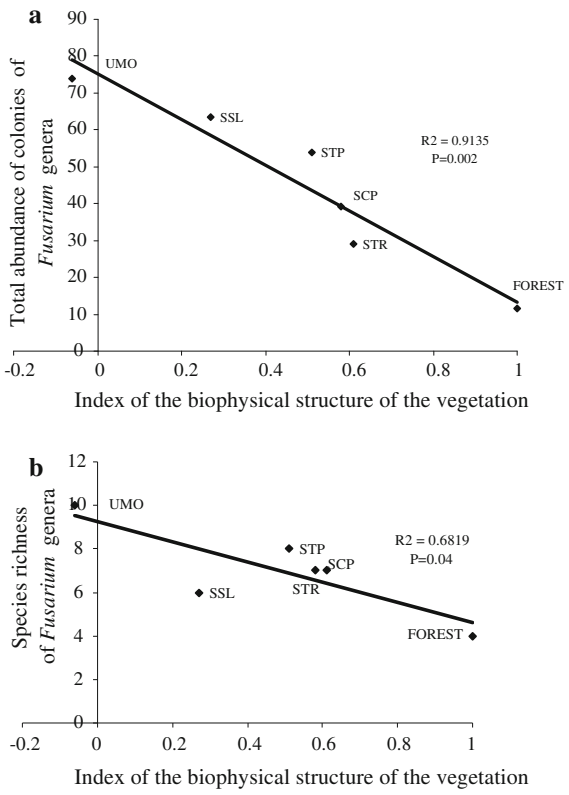

Fig. 5 Relationship between total abundance colonies (**a**) and species richness (**b**) of *Fusarium* genera and the index of the biophysical structures of the vegetation from study sites. *UMO* unshaded monoculture system, *SSL* shaded simple system, *SCP* shaded commercial polyculture system, *STP* shaded traditional polyculture system, *STR* shaded traditional rustic system, *FOREST* tropical montane cloud forest

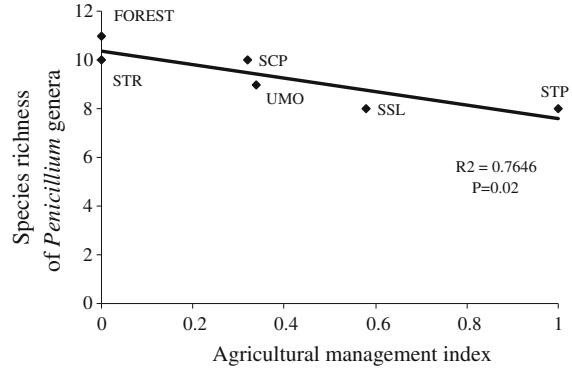

Fig. 6 Relationship between species richness of *Penicillium* and agricultural management index of study sites. *UMO* unshaded monoculture system, *SSL* shaded simple system, *SCP* shaded commercial polyculture system, *STP* shaded traditional polyculture system, *STR* shaded traditional rustic system, *FOREST* tropical montane cloud forest

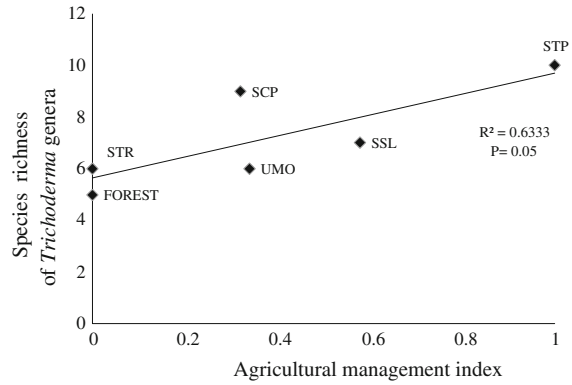

Fig. 7 Relationship between colonies species richness of *Trichoderma* genera and agricultural management index of study sites. *UMO* unshaded monoculture system, *SSL* shaded simple system, *SCP* shaded commercial polyculture system, *STP* shaded traditional polyculture system, *STR* shaded traditional rustic system, *FOREST* tropical montane cloud forest

In the regression analysis at the level of the dominant genera (*Fusarium, Penicillium* and *Trichoderma*), however, significant relationships were found between the abundance and richness of species and the indices of biophysical structure of the vegetation and agricultural management of the sites.

For the genus *Fusarium,* abundance and richness of species were negatively related to the index of the biophysical structure of the vegetation ($r = 0.91$, $P = 0.002$ and $r = 0.68$, $P = 0.04$, respectively); in the coffee plantations with vegetation of greater biophysical structure, lower numbers of colonies and species of this genus were presented (Fig. 5a and b). For the genus *Penicillium*, species richness was related negatively with the index of agricultural management ($r = 0.76$, $P = 0.02$); the sites with a higher agricultural management index presented lower species richness (Fig. 6). In contrast, the genus *Trichoderma* presented a higher number of species in the coffee plantations with higher indices of agricultural management (Fig. 7).

Discussion

In quantitative studies of soil saprobic microfungi, the number and type of species detected depend to large

extent on the technique employed (Gams 1992). Particle filtration, also known as soil washing, has replaced the practice of suspension planting, a technique widely applied in recent studies of soil microorganisms. Particle filtration favors the growth of the fungi found as hyphae associated with the soil particles, and for this reason they are considered to be in active form in the soil (Bills et al. 2004). With this technique, the washes eliminate the spores of the fast growing species and allow the development of those fungi that require more time for germination.

In common with the majority of the studies of soil fungi, an important proportion of the isolates did not present fructification, making their taxonomic identification impossible by traditional methods. These isolates must have specific requirements for inducing sporulation or at least require the presence of natural substrates. Considering these limiting factors, the application of molecular techniques would be of great assistance in identifying the strains. Thus a detailed morphological description was made of each of the 137 sterile strains, and their abundance was quantified in each survey. In this way, all of the isolates were accounted for enabling calculation of the evaluated parameters.

Composition of the saprobic filamentous microfungal species

Of the 82 genera distinguished, *Penicillium, Humicola, Fusarium, Trichoderma, Chaetomium, Paecilomyces* and *Talaromyces* presented the highest species richness. All of them have been isolated frequently in agroecosystems, including coffee plantations (Siddiqi 1964; Persiani and Maggi 1988; Velmourougane et al. 2000; Okoth et al. 2007; Belayneh et al. 2010).

In all of the studied coffee plantations, the genera *Fusarium* and *Trichoderma* (Fig. 1) presented the highest number of species. Both of these genera are characterized by a considerable competitive saprophytic ability, related to their potential for producing a large quantity of spores that germinate rapidly and colonize practically any type of substrate. They also produce mycotoxins, which gives them a competitive advantage over other soil microorganisms. Within the genus *Fusarium* are phytopathogenic species that cause root rotting, leaf spotting and death of plants ("damping off") in seedbeds (Agrios 1978). It is important to note that, despite the presence of these fungi, there were not major problems with diseases on the coffee plants in any of the plantations, which leads us to believe that a balance exists in all the analyzed soils between the saprobic fungal communities and parasites that impede their pathological expression.

The genus *Trichoderma* is also widely distributed in nature; various studies have confirmed their abundance in Mexican (Persiani and Maggi 1988), Indian (Velmourougane et al. 2000), Kenyan (Okoth et al. 2007, 2009) and Ethiopian (Belayneh et al. 2010) coffee plantations. Some *Trichoderma* species are used in agriculture, for example *T. harzianum*, which has a proven potential as a growth promoter in various crops including coffee. The species produces substances that act as catalyzers or accelerators of the primary meristematic tissues in the young parts of the plants (Castro and Rivillas 2005). The presence of *T. viride* in the coffee plantations is of great significance, since it has been shown that various strains of this species have the capacity to accelerate the germination of coffee (Cupull et al. 2000, 2003), as well as acting as a biological control of the phytopathogen *Rhizoctonia solani*, which causes "damping off" in coffee seedbeds (Rincón 1992; Andreu and Cupull 2000; Cupull et al. 2003).

Similarly, the presence in the coffee plantations of *Paecilomyces lilacinus, Metarhizium anisopliae* and *Pochonia suchalsporia* is important, since these entomopathogenic species are potential agents for the biological control of the coffee berry borer (*Hypothenemus hampei)*, a pest that causes significant losses in coffee production in the region (Vega et al. 2009). Some strains of *P. lilacinus* and *P. suchalsporia* also present activity against gall-forming phytopathogenic nematodes (Kerry and Jaffee 1997).

Colony abundance, species richness, diversity and evenness

Our results indicate that neither the biophysical structure of the vegetation nor the agricultural management of the coffee plantations in the region cause significant changes to the culturable populations of saprobic microfungi, in terms of abundance, species richness and diversity. Similar results were found by Persiani and Maggi (1988) in two coffee plantations (unshaded and shaded) in the region. This may be related to the high capacity of the microfungi to recover following disturbances (Persiani and Maggi

1988). The resilience of the soil microfungi could be related to their high functional plasticity given the fact that they possess a great biochemical capacity for degradation of substrates (Taylor et al. 2010). Moreover, these fungi are highly competitive, effective colonizers, present rapid life cycles and produce resistant structures (chlamydospores, sclerotia) and abundant propagules from fragments of mycelium and from sexual and asexual spores. These characteristics enable the microfungi to present a dynamic and efficient response to disturbance events, in terms of their dispersal and colonization of new environments (Zak 1992).

Unlike other transitory crops, in which the agricultural practices of leaving land fallow and constantly removing herbaceous plants can cause a negative impact on the mycobiota (Wu et al. 2008), the perennial nature of the coffee crop, together with its management within agroforestry systems, are factors that actually favor the maintenance of fungal communities. Even in the unshaded monoculture, the proliferation of weeds and grasses in the herbaceous stratum are favorable for saprobe fungi growth.

Differences were only detected between the coffee plantations and the forest in terms of the evenness and dominance of species (Fig. 2); while species of the genera *Fusarium* and *Trichoderma* were the most abundant in the coffee plantations, no species was found to dominate in the forest, where the genus *Paecilomyces* was the most abundant. This result could be due to the greater diversity of biological resources in the forest soil because of the presence of deciduous and perennial species. These species constantly provide the humus with an input of leaves, branches and fruits thus creating a wide range of niches for colonization by different types of saprobic fungi. Similarly, the interactions between the edaphic meso- and macrofauna are factors that regulate the fungal communities. In particular, species of the genus *Paecilomyces* could be linked with the populations of arthropods and nematodes that proliferate in the forest soils because of the high content of organic material that accumulates in the upper soil horizons.

In spite of the fact that the differences in total abundance of colonies between the sites were non-significant, the biophysical structure of the vegetation and the agricultural management was observed to have an effect on the abundance of the dominant genera. Persiani and Maggi (1988) also detected the influence of management practices on the dominance of particular groups in two coffee plantations. Linear regression analysis revealed that there was a higher abundance of fungal colonies in sites with lower biophysical structure of the vegetation, (Fig. 4). This result is closely related to the high proliferation of certain species of the genus *Fusarium* in these kinds of coffee plantations, such as the unshaded monocultures and the shaded simple system (Fig. 5a). The incidence of *Fusarium* species in sites with a higher density of coffee plants and lower tree coverage could be related to the rhizosphere environment of the weeds, grasses and coffee, the root exudates of which favor the germination of *Fusarium* chlamydospores (Thornton 1960).

The contrast between the high richness of species of the genus *Trichoderma* and the lower number of *Penicillium* species in sites with a high index of agricultural management demonstrates the complexity of species responses and the need for detailed study of the autoecology of soil microfungi.

Similarity in species composition of the saprobic filamentous microfungi

Of the 415 species isolated for this study, 53 were only present in the forest. These mainly corresponded to sterile strains and xylarial type mycelia.

The low percentage of similarity among the different coffee plantation sites suggests that the management practices and the structure of the regional coffee plantations cause substantial differences in the species composition of saprobic filamentous microfungi, since this group of fungi does not exhibit a random distribution, but typically presents clustering patterns at very different scales. This type of structure is governed by a series of factors, including substrate availability. The dendrogram obtained using the Bray Curtis index corroborates these results by separating the forest from the coffee plantations. The results suggest that, by maintaining a greater similarity to the biophysical structure of the vegetation in the tropical montane cloud forest and presenting a high concentration of organic material, the shaded traditional rustic system has a higher similarity in terms of species composition compared to the other coffee plantation systems. The degree of similarity in composition of the study sites is clearly very low (range 0.12–0.24), suggesting a high exchange of species at the regional

level; these results differ from those reported by Arias et al. (2012) for endomycorrhizal fungi, where there was no notable loss of the native morphospecies of the forest (range 0.59–0.85).

Our results lead us to conclude that transformation of the tropical montane cloud forest in the coffee plantations of central Veracruz does not cause significant changes to the abundance, species richness and community diversity of the soil saprobic filamentous microfungi. The effects of agricultural management and the biophysical structure of the vegetation in the coffee plantations are only detected at the level of the dominant genera *Fusarium, Trichoderma* and *Penicillium*. The low degree of similarity among the six sites indicates the existence of a high exchange of species without affecting the diversity and richness of the microfungal community.

It is important to highlight the fact that this is the first study to present a detailed ecological analysis of a community of saprobic fungi in a tropical montane cloud forest soil. Our results reveal the existence of high species richness in this ecosystem and, unlike the communities of saprobic fungi in the coffee plantations, the assembly of species in the forest contains a more equitative range of species of anamorphs, ascomycetes and basidiomycetes. With increased understanding of edaphic fungal community ecology, it is possible to develop strategies to conserve and use the native microfungal species in order to contribute to the organic management of the coffee plantations and allow producers to get better prices on the international market.

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References

Agrios GN (1978) Plant pathology, 2nd edn. Academic Press, New York

Andreu RC, Cupull SR (2000) Un manejo integrado de *Alternaria solani* causante de la Alternariosis del tomate. Internet http://www.Iicasaninet.Net/boletines/2000/a4n43.htmt

Arias RM, Heredia G, Sosa V, Fuentes-Ramirez L (2012) Diversity and abundance of arbuscular mycorrhizal fungi spores under different coffee plantation systems and in a tropical montane cloud forest patch in Veracruz, Mexico. Agrofor Syst 85:179–193

Belayneh TM, Kubicek CP, Druzhinina IS (2010) The rhizosphere of *Coffea arabica* in its native highland forests of Ethiopia provides a niche for a distinguished diversity of *Trichoderma*. Diversity 2:527–549

Bills GF, Christensen M, Thorm G (2004) Saprobic soil fungi. In: Muller, Bills G, Foster MS (eds) Biodiversity of fungi: inventory and monitoring methods. Elsevier, Academic Press, p 271–301

Booth C (1971) The genus Fusarium. Commonwealth Mycological Institute, England

Bruijnzeel L, Hamilton L (2001) Tiempo decisivo para las selvas de neblina. IHP Humid Tropics Programme Series No 13, DFID, UNESCO International Hydrological Programme, IUCN

Castro A, Rivillas C (2005) Bioregulación de *Rhizoctonia solani* en germinadores de café. Boletín Cenicafé. Avance Técnico No 336, Chinchiná, Colombia

Contreras A, Hernández-Martínez G (2008) ¡Que bien huele, mejor sabrá! La organización de productores de proyecto Biocafé. Instituto de Ecología A.C (INECOL), Xalapa, Veracruz

Cupull SR, Sánchez CC, Andreu C, Cupull MC, Pérez NC (2000) "Efecto de *Trichoderma* y *Azotobacter* en el control de *Rhizoctonia solani* y la estimulación del crecimiento de posturas de cafetos". Cuadernos de fitopatología. Revista técnica de fitopatología y entomología 17(66): 208–221

Cupull SR, Andreu C, Pérez NC, Delgado YP (2003) Efecto de *Trichoderma viride* como estimulante de la germinación, en el desarrollo de posturas de cafetos y el control de *Rhizoctonia solani* Kuhn. Centro Agrícola 30(1):21–25

Dighton J (2003) Fungi in ecosystem processes. Marcel Dekker, New York

Domsch KH, Gams W, Anderson TH (1980) Compendium of soil fungi, vol 1. IHW-Verlag, Berlin

Ellis MB (1971) Dematiaceous hyphomycetes, 1st edn. Comm. Mycol. Inst., Kew

Ellis MB (1976) More dematiaceous hyphomycetes, 1st edn. Comm. Mycol. Inst., Kew

Gams (1992) The analysis of communities of saprophytic microfungi with special refrence to soil fungi. In: Winterhoff W (ed) Fungi in vegetation science. Kluwer Academic, Dordrecht, Netherlands, pp 183–223

Gams W, Bisset J (1998) Morphology and identification of *Trichoderma. Trichoderma* y *Gliocladium* basic biology, taxonomy and genetics. In: Kubicek CP, Harman GE (eds) *Trichoderma* y *Gliocladium*. Taylor and Francis, Londres, pp 3–34

García-Franco J, Toledo T (2008) Epífitas vasculares: bromelias y orquídeas. In: Manson RH, Hernández-Ortiz V, Gallina S, Mehltreter K (eds) Agroecosistemas cafetaleros de Veracruz: Biodiversidad, Manejo y Conservación. Instituto de Ecología, A.C. (INECOL) e Instituto de Nacional de Ecología (INE-SEMARNAT), Ciudad de México, México, p 69–77

Geissert D, Ibáñez A (2008) Calidad y ambiente físico-químico de los suelos. In: Manson RH, Hernández-Ortiz V, Gallina

S, Mehltreter K (eds) Agroecosistemas cafetaleros de Veracruz: Biodiversidad, Manejo y Conservación. Instituto de Ecología, A.C. (INECOL) e Instituto de Nacional de Ecología (INE-SEMARNAT), Ciudad de México, México, p 213–221

González-Romero A, Murrieta-Galindo R (2008) Anfibios y Reptiles. In: Manson RH, Hernández-Ortiz V, Gallina S, Mehltreter K (eds) Agroecosistemas cafetaleros de Veracruz: Biodiversidad, Manejo y Conservación. Instituto de Ecología, A.C. (INECOL) e Instituto de Nacional de Ecología (INE-SEMARNAT), Ciudad de México, México, p 135–143

Heredia G, Arias R (2008) Hongos saprobios y endomicorrizógenos en suelos. In: Manson RH, Hernández-Ortiz V, Gallina S, Mehltreter K (eds) Agroecosistemas cafetaleros de Veracruz: Biodiversidad, Manejo y Conservación. Instituto de Ecología, A.C. (INECOL) e Instituto de Nacional de Ecología (INE-SEMARNAT), Ciudad de México, México, p 193–203

Kerry BR, Jaffee BA (1997) Fungi as biological control agents for plant parasitic nematodes. The Mycota 4:203–218

Klich MA, Pitt JI (1988) A laboratory guide to common *Aspergillus* species and their teleomorphs. Commonwealth Scientific and Industrial Research Organization, Australia

Manson RH, Hernández-Ortiz V, Gallina S, Mehltreter K (eds) (2008) Agroecosistemas cafetaleros de Veracruz: Biodiversidad, Manejo y Conservación. Instituto de Ecología, A.C. (INECOL) e Instituto de Nacional de Ecología (INE-SEMARNAT), Ciudad de México, México, pp 330

Mehltreter K (2008) Helechos. In: Manson RH, Hernández-Ortiz V, Gallina S, Mehltreter K (eds) Agroecosistemas cafetaleros de Veracruz: Biodiversidad, Manejo y Conservación. Instituto de Ecología, A.C. (INECOL) e Instituto de Nacional de Ecología (INE-SEMARNAT), Ciudad de México, México, p 83–93

Moguel P, Toledo VM (1996) El café en México. Ecología, cultura indígena y sustentabilidad. Ciencias 43:40–51

Moguel P, Toledo VM (1999) Biodiversity conservation in traditional coffee systems of Mexico. Conserv Biol 13:11–21

Okoth SA, Roimen H, Mutsotso B, Muya E, Kahindi J, Owino JO, Okoth P (2007) Land use systems and distribution of *Trichoderma* species in Embu Region, Kenia. Trop Subtrop Agroecosyst 7:105–122

Okoth SA, Okoth P, Muya E (2009) Influence of soil chemical and physical properties on occurrence of *Trichoderma* spp. In Embu, Kenia. Trop Subtrop Agroecosyst 11:303–312

Pandey A, Namrata D, Bhavesh K, Rinu K, Pankaj T (2008) Phosphate solubilization by *Penicillium* spp. isolated from soil samples of Indian Himalayan region. World J Microbiol Biotechnol 24:97–102

Persiani A, Maggi O (1988) Fungal communities in the rhizosphere of *Coffea arabica* L. in Mexico. Mycol Ital 2:21–37

Pitt JI (1991) A laboratory guide to common Penicillium species. CSIRO Divition of Food Science, Australia

Riess S, Sanvito A (1985) Investigations on vesicular arbuscular mycorrhizae in different conditions of coffee cultivations in Mexico. Micol Ital 14:57–62

Rincon G (1992) Control Biológical de *Rhizoctonia solani* con *Trichoderma* spp. en semilleros de café. Cenicafé 43(3):73–83

Rzedowski J (1996) Análisis preliminar de la Flora vascular de los bosques mesófilos de montaña de México. Acta Botánica Mexicana 35:25–44

Siddiqi MA (1964) Fungus flora of *Coffea arabica* in Nyasaland. Trans Br Mycol Soc 47(2):281–284

Sosa V, Hernández-Salazar, Hernández-Conrique, Castro-Luna A (2008) Murciélagos. In: Manson RH, Hernández-Ortiz V, Gallina S, Mehltreter K (eds) Agroecosistemas cafetaleros de Veracruz: Biodiversidad, Manejo y Conservación. Instituto de Ecología, A.C. (INECOL) e Instituto de Nacional de Ecología (INE-SEMARNAT), Ciudad de México, México, p 181–191

Taylor DL, Herriott IC, Kelsie E, Stone, McFarland JW, Booth MG, Leigh MB (2010) Structure and resilience of fungal communities in Alaskan boreal forest soils. Can J For Res 40:1288–1301

Tejeda-Cruz, Gordon C (2008) Aves. In: Manson RH, Hernández-Ortiz V, Gallina S, Mehltreter K (eds) Agroecosistemas cafetaleros de Veracruz: Biodiversidad, Manejo y Conservación. Instituto de Ecología, A.C. (INECOL) e Instituto de Nacional de Ecología (INE-SEMARNAT), Ciudad de México, México, p 149–160

Thornton RH (1960) Fungi of some forest and pasture soils. N Z J Agric Res 3:699–711

Trejo AD, Ferrera-Cerrato DR, Escalona MA, Rivera A (1998) Ecología de la endomicorriza arbuscular en el cultivo del café (*Coffea arabica* L.) La Ciencia y el Hombre 23:7–20

Valenzuela-González, Quiroz-Robledo L, Martínez-Tapia D (2008) Hormigas (Insecta: Hymenoptera: Formicidae). In: Manson RH, Hernández-Ortiz V, Gallina S, Mehltreter K (eds) Agroecosistemas cafetaleros de Veracruz: Biodiversidad, Manejo y Conservación. Instituto de Ecología, A.C. (INECOL) e Instituto de Nacional de Ecología (INE-SEMARNAT), Ciudad de México, México, p 107–121

Vega FE, Infante F, Castillo A, Jaramillo J (2009) The coffee berry borer, *Hypothenemus hampei* (Ferrari) (Coleoptera: Curculionidae): a short review, with recent findings and future research directions. Terr arthropod rev 2:129–147

Velmourougane K, Panneerselvam P, Shanmukhappa DR, Gopinandan TN, Srinivasan CS, Naidu R (2000) Microflora associated with high and low grown *Coffee arabica* and *C. robusta*. J Coffee Res (India) 28:9–19

Williams-Linera G (1997) Phenology of deciduos and broadleaved-evergreen tree species in a Mexican tropical lower montane forest. Glob Ecol Biogeogr 6:115–127

Williams-Linera G, López-Gómez AM (2008) Estructura y diversidad de la vegetación leñosa. In: Manson RH, Hernández-Ortiz V, Gallina S, Mehltreter K (eds) Agroecosistemas cafetaleros de Veracruz: Biodiversidad, Manejo y Conservación. Instituto de Ecología, A.C. (INECOL) e Instituto de Nacional de Ecología (INE-SEMARNAT), Ciudad de México, México, p 55–68

Wu T, Chellemi DO, Grahamb JH, Rosskopf EN (2008) Assessment of fungal communities in soil and tomato roots subjected to diverse land and crop management systems. Soil Biol Biochem 40:1967–1970

Zak JC (1992) Response of soil fungal communities to disturbance. In: Carrol GC, Wicklow DT (eds) The fungal community: its organization and role in the ecosystem. Marcel Dekker, Inc., New York City, pp 403–425