

Analysis of the sporulating microfungal community in decomposing fallen leaves of *Rinorea guatemalensis* (Wats.) Bartlett (Malphigiales, Violaceae) in a Mexican rainforest

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Abstract – In Mexico, the study of the composition and changes of the sporulating microfungi on decaying fallen leaves in rainforest ecosystems has not been elucidated. In this study, we evaluated the species richness, diversity, abundance and similarity of the sporulating microfungi inhabiting the leaf litter of *Rinorea guatemalensis*, a dominant evergreen tree in family Violaceae (Malphigiales) from the rainforest of the “Agua Blanca” park in the state of Tabasco. The study was done over a period of 210 days. In parallel we analyzed the loss of leaf biomass. The litter bag method was used. Fungal fruiting structures were detected and quantified using moist chambers. A total of 38 taxa were detected. Two main phases were detected in the biomass loss and in the changes in the microfungi community; the primary phase was characterized by the highest biomass loss and corresponded with the highest diversity, specific richness, as well the highest values of frequency of occurrence for the sporulating microfungi. In the secondary phase the biomass loss and diversity remain with little apparent changes and with a tendency of decrease gradually with increasing foliar decomposition. In the later stages of decomposition, the similarity in the microfungal community increased. The frequency and periodicity of occurrence values indicated that the sporulating microfungi community was structured mainly by rare and sporadic species. *Cylindrocladium scoparium*, *Microthyrium* sp., *Volutella ciliata* and *Volutella minima* were considered as residents throughout the study.

fungal diversity / leaf litter / leaf decomposition / Mexico / tropical microfungi

Résumé – Au Mexique, la composition et les changements de la microflore fongique sporulante qui est présente sur les feuilles mortes en décomposition dans les écosystèmes de forêt humide n’ont pas encore été élucidés. Au cours de cette étude, nous avons évalué la richesse en espèces, les indices de diversité, d’abondance et de similarité de la microflore fongique sporulante détectée dans la litière de *Rinorea guatemalensis*, un arbre sempervirent de la famille Violaceae présent dans la forêt humide du parc « Agua Blanca » dans l’état de Tabasco. L’étude a été réalisée sur une période de 210 jours. En parallèle, nous avons analysé la perte de biomasse des feuilles en utilisant la méthode des sacs de litière. Les structures de sporulation ont été détectées et quantifiées en utilisant une chambre humide. Au total, 38 taxons ont été détectés. Deux phases principales ont été identifiées au cours des phénomènes de perte de biomasse et de changement de microflore fongique. La première phase était caractérisée par la plus forte perte de biomasse et correspondait à la diversité, la richesse en espèces et la fréquence d’occurrence les plus élevées de la microflore fongique sporulante. Au cours de la deuxième phase, la perte de biomasse et la diversité ont montré de faibles changements avec une tendance à diminuer

graduellement au fur et à mesure que la décomposition foliaire augmentait. Au cours des derniers stades de décomposition, l'indice de similarité au sein de la microflore fongique a augmenté. Les valeurs de fréquence et de périodicité d'occurrence ont indiqué que la microflore fongique sporulante était principalement structurée par des espèces sporadiques et rares. *Cylindrocladium scoparium*, *Microthyrium* sp., *Volutella ciliata* et *Volutella minima* ont été considérés comme espèces dominantes au cours de l'étude.

décomposition des feuilles / diversité fongique / litière / microflore fongique tropicale / Mexique

INTRODUCTION

From the early stages of development until they die and are broken down in soil, leaves provide a physical environment suitable for a diverse community of microfungi. Until a decade ago, the composition of fungal species on leaf litter and successional changes along the decomposition process had been investigated mainly in species from temperate regions (Visser & Parkinson, 1975; Tokumasu *et al.*, 1994; Valenzuela *et al.*, 2001). In recent years a considerable number of papers dealing with tropical species as *Ficus pleurocarpa* (Paulus *et al.*, 2006), *Manglietia garrettii* (Promputtha *et al.*, 2002) and *Anacardium occidentale* (Shanthi & Vittal, 2010) have been published. Most of these studies have been conducted with material collected in tropical regions of Asia (Promputtha *et al.*, 2002; Wang *et al.*, 2008; Shanthi & Vittal, 2010) and Africa (Lee *et al.*, 2004; Rambelli *et al.*, 2004). For the Neotropic region, as far as we were able to investigate in the available scientific literature the few contributions include the pioneer work of Kiffer *et al.* (1981), with leaf litter of *Eupera falcata* in a rain forest on French Guyana, and those of Bills & Polishook with leaf litter from Costa Rica (1994) and Polishook *et al.* (1996) with decaying leaves of *Guarea guidonia* and *Manilkara bidentata* from Puerto Rico. In Mexico, the contributions dealing with tropical litter microfungi have mostly concentrated on description and inventory of new or interesting taxa (Mercado-Sierra *et al.*, 1997; Heredia *et al.*, 1997; Castañeda-Ruiz *et al.*, 2004). Until now, changes in the community of microfungi along the leaves decomposition in the soil have not been elucidated from any of the Mexican rainforests. The only information with an ecological approach to the leaf litter microfungi community is that of Heredia (1993) with leaf litter of the dominant tree species in a cloud forest (*Quercus sartorii*, *Q. germana* & *Liquidambar styraciflua*) from the northeast region of the Mexican Republic.

The objective of this study is to evaluate the composition, diversity and changes of the sporulating microfungi associated with the fallen leaves of a tropical dominant tree species – *Rinorea guatemalensis*, during the course of decomposition in their natural habitat. In parallel, we analyzed the loss of biomass of the leaves to find out its eventual relation with the diversity and specific richness of the microfungi community.

MATERIALS AND METHODS

Study site. — The study was conducted at the “Agua Blanca” State Park, located in Tabasco (Fig. 1) in the southeastern region of Mexico (17°35' and 17°40' N and 92°24' a 92°20' W; alt. 100-200m asl), covering an area of 2, 025 ha

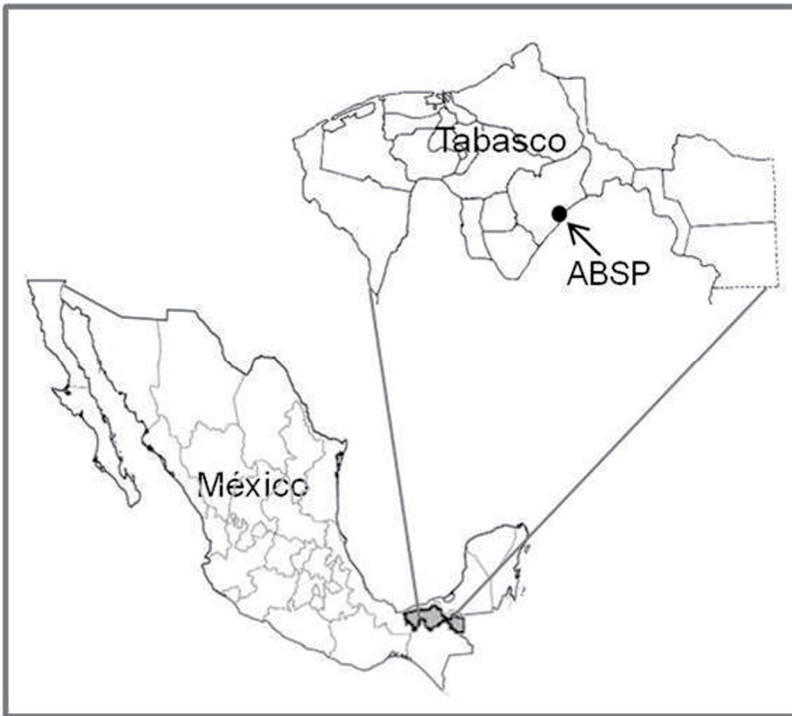


Fig. 1. Geographical location of the “Agua Blanca” State Park (ABSP).

(INEGI, 2001). The climate is hot and humid with rain all the year. The average annual rainfall is from 2100 to 3200 mm, with maximum in September and minimum in February. During the period when the study was conducted (November 2010 to June 2012), the mean annual temperature was 27.1°C and the mean monthly temperature ranges from 22.5°C in January and 31.5°C in May (Data from the Tabasco Statistical Yearbook, 2011). Monthly mean temperatures and precipitations are shown in Figure 2. The predominant ecosystem in the park is the rainforest, where the major canopy species include *Astrocarium mexicanum*, *Brosimum alicastrum*, *Lonchocarpus guatemalensis*, *Quararibea alicastrum*, *Psycrotia chiapensis*, *Dendropanax arboreus*, *Heliocarpus appendiculatus* and *Rinorea guatemalensis* (Castillo & Zavala, 1996). The latter species, *R. guatemalensis* (family Violaceae), known as “cafetillo” or “botoncillo” in the area, is one of the dominant canopy species in the rainforest of the park (Zarco-Espinoza *et al.*, 2010). This perennial plant grows as shrubs or small trees up to 10 m high; its leaves are opposite, elliptical, acuminate, measuring 7-15 cm long and 2.5-6 cm wide and have pedicels of 2-4 mm long (Standley & Williams, 1961).

Leaf collection, processing and installation of litter bags. — Changes in the sporulating microfungal community and in the remaining plant biomass (dry weight of the leaves of *Rinorea guatemalensis*) along their decomposition were monitored using the litter bag technique (Gilbert & Bock, 1960). To prepare the litter bags (15 × 20 cm/mesh size 2 mm), freshly fallen leaves of *R. guatemalensis* were collected from the rainforest floor on November 2011. Only

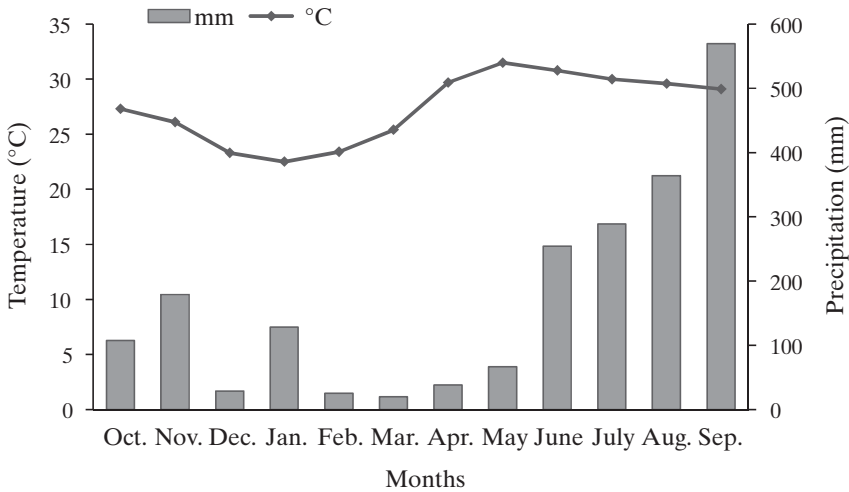


Fig. 2. Monthly average temperatures and precipitation in the study area during the study period (November 2010 to June 2011). Data from the Tabasco Statistical Yearbook (2011).

leaves without obvious fungal or faunal attack were used. The leaves were taken to the laboratory and air-dried at room temperature for two days, after that, ten leaves were enclosed in each mesh bag. A total of thirty litter bags were used for the mycological analyses. A second batch of thirty litter bags, each one containing 4 g of freshly fallen leaves (= 1.6 g dry weight) were prepared to monitor mass loss of the leaves during the course of decomposition. Thus a total of 60 litter bags were prepared. Before moving the litter bags to the study area ten litter bags (five with known weight and five for mycological analyses) were randomly selected to represent day 0. In the rainforest a plot of 10 × 20 m was established, the litter bags were placed in ten rows of five bags each. The litter bags were attached to the forest floor with metal pins to prevent movement. Ten litter bags (five weighted and five no weighted) were randomly removed from the rain forest floor each sampling time (60, 105, 129, 169 y 210 days) after the placement. Each bag was placed in a separate paper bag and transported to the laboratory.

Sampling for biomass loss and mycological analyses. — Foreign plant remains attached to the outside of the bags were carefully removed with forceps. The losses of mass were determined after drying the samples to a constant weight at 70°C (Lousier & Parkinson, 1976). Samples for mycological analyses were processed the same day that they were collected in the field. Each leaf was incubated individually in a moist chamber consisted of a Petri dish (100 × 15 mm) with a filter paper in the bottom which was periodically moistened with sterile water. The leaves were examined periodically over 30 days under a stereomicroscope for the presence of sporulating microfungi. Semi permanent (with lactophenol and lactic acid) and permanent (with alcohol polyvinyl) slides of fungal fruiting bodies were prepared. All sporulating fungi were recorded and identified to lowest possible taxonomic level. Taxonomic determination was based on morphological characters, using specialized literature (Ellis, 1971; 1976; Matsushima, 1971; 1975; 1980; 1983; 1987; 1993; Castañeda-Ruiz & Kendrick, 1990a; 1990b; 1991). Slides of all taxa are maintained at the Mycological collection of the Herbarium of the University of Tabasco (UJAT).

Parameters evaluated and data analysis. — For all fungi found we calculated the “frequency of occurrence” (% FOC) and the “periodicity of occurrence” (% POC) as follows:

$$\% \text{ FOC} = \frac{\text{Number of leaves in which a taxon was recorded in a litter bag sample}}{\text{Total number of leaves in a litter bag sample (10)}} \times 100$$

$$\% \text{ POC} = \frac{\text{Number of samplings in which a taxon was recorded}}{\text{Total sampling (6)}} \times 100$$

In each one of the five samples, from the 10 leaves of each litter bag the frequency of occurrence of each of the species detected was calculated and then the average of the five litter bags was calculated. In addition, the diversity by the Shannon-Wiener (H') index was calculated. To compare fungal composition between the different sampling dates, we used the Morisita's similarity index. To identify statistical differences in the diversity between samples, an ANOVA of one way was applied (program Statgraphics 5.1). Both analyzes were performed using the software BIO-DAP version 2.5. The Tukey's test was used for multiple comparisons.

Values of frequency of occurrence (% FOC) were considered to evaluate the abundance of the fungal species in the leaves. Based on this, the fungi were categorized into four categories: very abundant species (100-75%), abundant species (74-51%), moderately abundant species (50-25%) and rare species (24-1%). With respect to periodicity of occurrence (% POC) the following categories were established: resident (100%), frequent (83.3-66.7%), occasional (50-33.3%) and sporadic (16.7%).

RESULTS

The loss of biomass was higher for the early samplings and after 105 days of decomposition the samples had lost 50% of their biomass. After that time, the loss of biomass slowed down (Fig. 3) and at the end of the study, samples retained around 33% of their original biomass.

From the 300 analyzed leaves over the whole experimental period, a total of 38 taxa were detected: five ascomycetes and thirty-three anamorphic taxa (Table 1). At all samplings, there was a high proportion of species characterized as rare (Fig. 4). Species classified as very abundant and abundant were present mainly in the freshly fallen leaves and in the earliest stages of decomposition. *Colletotrichum* sp., *Cylindrocladium scoparium*, *Volutella ciliata*, *V. minima* and *Microthyrium* sp. were very abundant species in the freshly fallen leaves. With the exception of *Colletotrichum* sp., the other four microfungi were abundant in subsequent samplings. *Cylindrocladium scoparium* and *Microthyrium* sp. continued to be very abundant species up to 129 days after the start of decomposition. In contrast, *Volutella ciliata* and *V. minima* decreased significantly with increasing time of decomposition; for the last three samples both were characterized as rare species.

Despite the high abundance in the freshly fallen leaves, *Phomopsis* sp., *Fusarium* sp. and *Virgatospora echinofibrosa* were not detected on decayed leaves, unlike some species, such as *Menisporopsis multisetulata* and *M. theobromae*, which appeared only on the decayed leaves. Both the latter species and *Microthyrium* sp. were detected as very abundant to abundant even up to the very last samplings.

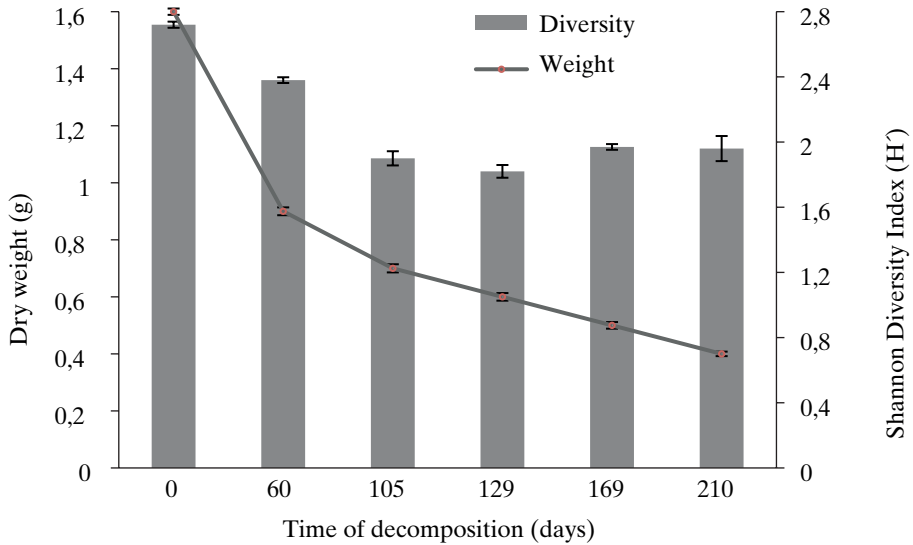


Fig. 3. Changes in the remaining mass (dry weight) and diversity (Shannon H' Index) in leaves of *Rinorea guatemalensis* along their decomposition in the rainforest soil. Values are means ($n = 5$). 0 = freshly fallen leaves. Bars indicate the standard error.

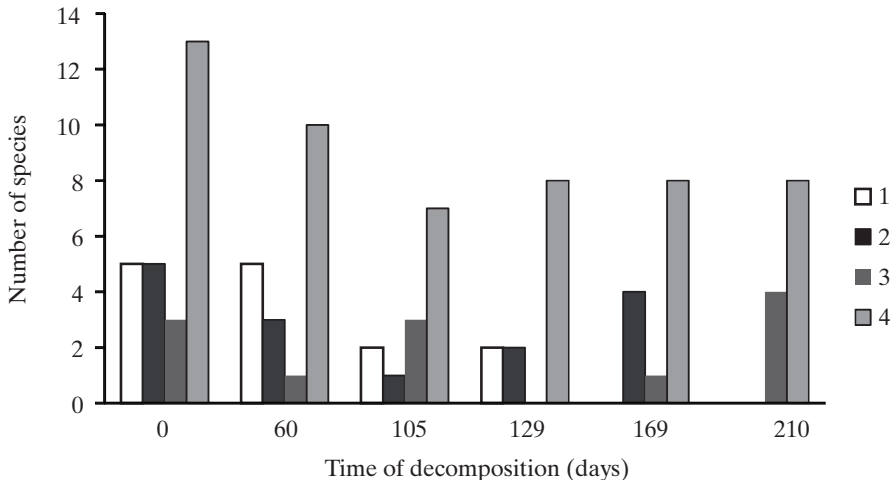


Fig. 4. Number of species in each one of the four categories established according to their frequency of occurrence values in the different sampling dates. 1. Very abundant species. 2. Abundant species. 3. Moderately abundant species and 4. Rare species. 0 = freshly fallen leaves.

Considering the periodicity of occurrence (Table 1), a high proportion of the species were characterized as sporadic (47.3%) or occasional (26.3%). Only *Cylindrocladium scoparium*, *Volutella ciliata*, *V. minima*, *Microthyrium* sp. and *Ophiocera fusiformes* were considered as residents. The frequent species were *Boerlagiomyces grandisporus*, *Menisporopsis multisetulata*, *M. theobromae*, *Wiesneriomyces laurinus* and *Verticillium fungicola*.

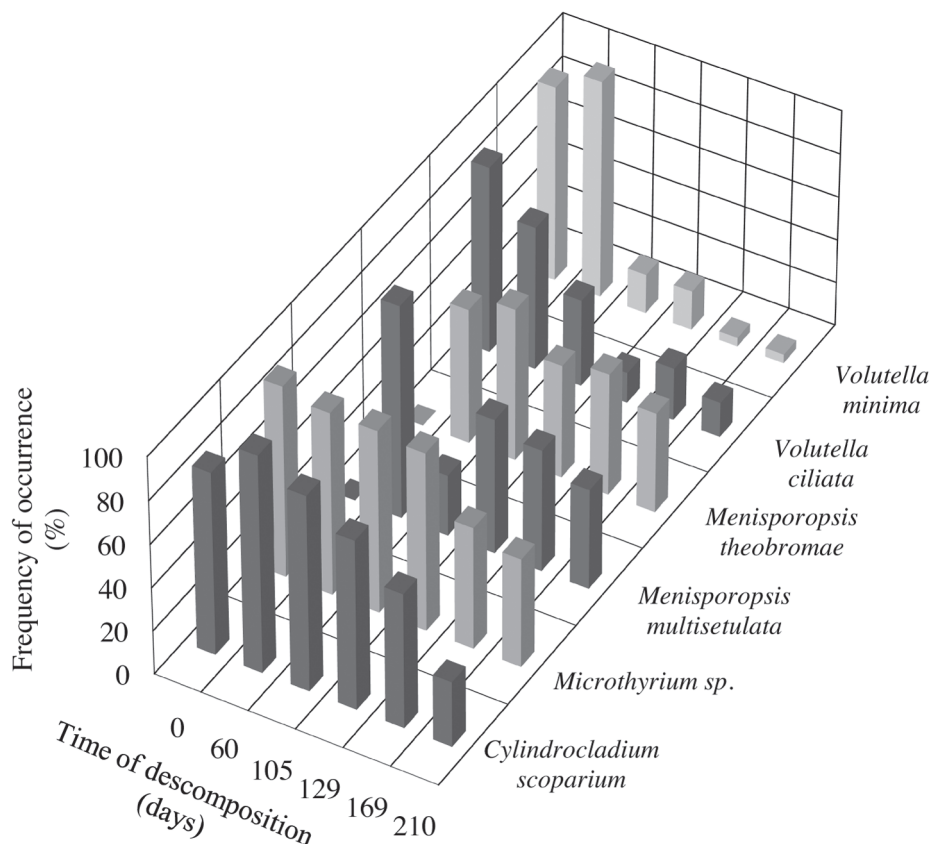


Fig. 5. Frequency of occurrence of the dominant sporulating microfungi on *Rinorea guatemalensis* leaves along their decomposition. 0 = freshly fallen leaves.

Cyndrocladium scoparium, *Menisporopsis multisetulata*, *M. theobromae*, *Microthyrium sp.*, *Volutella ciliata* and *Volutella minima* were very abundant in at least two samplings and scored as residents or frequent. We considered these fungi to represent the dominant species of the *Rinorea guatemalensis* leaves during their decomposition on the rainforest soil (Fig. 5).

During the entire decomposition process, species richness ranged from 12 to 26 species with the highest values found in the freshly fallen leaves. After 105 days of decomposition the number of species decreased, and in subsequent samplings there were no notorious decreases noted (Table 1). Diversity was in a range from 1.8 to 2.7 (Fig. 3). We found significant differences ($P < 0.05$) among the samplings over time. According to the Tukey's test the diversity was highest in the freshly fallen leaves and at 60 days of decomposition. The percentage of similarity in the composition of the microfungi among the six sampling times indicated that as the decomposition increases, the similarity with the original fungal community in the freshly fallen leaves decreases (Table 2) but remained very similar (over 90%) in subsequent samplings (at 129, 169 and 210 days).

Table 1. Frequency of occurrence (% FCO*) and periodicity of occurrence (PCO) of all recorded sporulating microfungi on *Rinorea guatemalensis* leaves along a time gradient.

Fungi	Time of decomposition (days)						PCO
	0*	60*	105*	129*	169*	210*	%
Anamorphic fungi							
<i>Acrogenospora sphaerocephala</i>	–	2	–	2	–	–	33.3
<i>Anungitea uniseptata</i>	–	–	–	6	32	16	50
<i>Arthrotrrys oligospora</i>	–	–	–	–	6	–	16.7
<i>Beltraniella porosa</i>	2	–	–	–	–	–	16.7
<i>Beltraniopsis asperisetifer</i>	4	16	–	2	–	–	50
<i>Chaetopsina fulva</i>	–	12	–	–	–	–	16.7
<i>Cladosporium oxysporum</i>	4	–	–	–	–	–	16.7
<i>Clonostachys compactiuscula</i>	58	78	–	–	–	20	50
<i>Colletotrichum</i> sp.	90	–	2	–	–	–	33.3
<i>Cylindrocladium scoparium</i>	84	100	90	78	62	30	100
<i>Dyctiochaeta assamica</i>	–	–	2	–	–	–	16.7
<i>Dyctiosporium subramanianii</i>	4	–	–	–	–	–	16.7
<i>Exosporium ampullaceum</i>	–	2	2	–	–	–	33.3
<i>Fusarium</i> sp.	44	–	–	–	–	–	16.7
<i>Gliomastix</i> sp.	52	10	–	–	–	–	33.3
<i>Graphium putredinis</i>	24	–	–	–	–	–	16.7
<i>Helicosporium phragmitis</i>	–	–	4	–	–	–	16.7
<i>Libertella</i> sp.	10	–	–	–	–	–	16.7
<i>Menisporopsis multisetulata</i>	–	98	28	62	56	46	83.3
<i>Menisporopsis theobromae</i>	–	62	70	52	56	46	83.3
<i>Microsporium</i> sp.	16	–	–	–	–	–	16.7
<i>Myrothecium roridum</i>	20	20	–	–	–	–	33.3
<i>Periconia jabalpurensis</i>	2	–	–	–	–	–	16.7
<i>Phomopsis</i> sp.	70	–	–	–	–	–	16.7
<i>Selenodriella perramosa</i>	–	–	–	–	6	–	16.7
<i>Stachylidium bicolor</i>	2	–	–	–	–	–	16.7
<i>Tetraploa aristata</i>	6	2	–	–	–	–	33.3
<i>Veronaea coprofila</i>	2	–	–	–	–	–	16.7
<i>Verticillium fungicola</i>	54	54	–	–	4	10	66.7
<i>Virgatospora echinofibrosa</i>	48	–	–	–	–	–	16.7
<i>Volutella ciliata</i>	86	66	40	14	24	16	100
<i>Volutella minima</i>	90	100	18	18	4	4	100
<i>Wiesneriomyces laurinus</i>	–	4	8	2	2	4	83.3
Ascomycetes							
<i>Astrophaeriella</i> sp.	6	2	–	–	–	–	33.3
<i>Boerlagiomyces grandisporus</i>	–	4	2	6	8	14	83.3
<i>Glomerella cingulata</i>	32	–	–	–	–	–	16.7
<i>Microthyrium</i> sp.	88	84	84	82	56	50	100
<i>Ophiocera fusiformes</i>	68	40	38	10	12	4	100
Species richness	26	19	13	12	13	12	

* Values are means (n = 5). 0 = freshly fallen leaves.

Table 2. Morisita's similarity index (top right corner) and number of shared species (lower left corner) between the different days of decomposition of the *Rinorea guatemalensis* leaves.

Days	0*	60	105	129	169	210
0		68	52	43	41	42
60	12		76	77	76	81
105	6	8		93	89	83
129	6	11	9		94	90
169	6	10	9	10		94
210	7	11	9	10	11	

* = freshly fallen leaves

DISCUSSION

This is the first synecological study dealing with the microfungal community associated with the decomposition of leaves in a Mexican lowland rainforest. Taking into account the methodology used, the information of this study covers only those microfungi that are able to sporulate in humid chambers. This technique has frequently been used in similar studies (Shenoy & Ahmad, 1994; Pasqualetti *et al.*, 1999; Promputtha *et al.*, 2002; Shanti & Vittal, 2012) and allows for identification to species or genus level of most fungi detected.

The number of species detected is in the range reported in other studies on tropical leaves, in which the direct observation in moist chambers was used; e. g. in Thailand Promputtha *et al.* (2002) detected 22 taxa in *Manglietia garrettii* leaves and Wang *et al.* (2008) reported a range from 24 to 33 taxa in leaves of five species of *Ficus*, in Puerto Rico Polishook *et al.* (1996) detected 24 species from leaves of *Manilkara bidentata* and *Guarea guidonia*, and in a tropical Australian forest Paulus *et al.* (2006) reported a range from 31-81 species from six tree species. Studies using direct and indirect methods (Polishook *et al.*, 1996; Paulus *et al.*, 2006) have shown that a considerably greater number of taxa could be detected using indirect methods (e.g. washing-plating of particle suspensions), however this method will also detect dormant spores and therefore may not serve as a good reflection of the fungi involved in decaying leaves (Promputtha *et al.*, 2002). In addition, culture plating would exclude fungi that cannot grow on agar plates.

As in other studies with tropical plants (Álvarez-Sánchez & Becerra, 1996; Loranger *et al.*, 2002), the dynamics of the *Rinorea guatemalensis* leaf decomposition can be characterized by two main phases: a primary phase in which a high amount of biomass is lost (in this case 53.1% total mass loss during 105 days), and a secondary in which the decomposition decreases, and leaf debris are degraded more slowly (Fig. 3). The leaves of tropical plants can be break down completely in periods of few months to two or three years (Olson, 1963). Unfortunately we could not find out how long it takes to break down the leaves of *R. guatemalensis* completely. Contrary to what we expected, by the end of the study the samples still retained around a third of their biomass. This could be due to the reduced decomposition rates occasioned by the period in which the study

was conducted (November to June) (Fig. 2) which included the months with less rainfall. For this situation the number of the litter bags used was not sufficient to assess the loss of biomass until complete disappearance of the leaves on the soil of the rainforest.

With the data obtained, we could expect that if the same decomposition rate is maintained, the leaves that fall on the ground during November will be decomposed completely in approximately 300 days. Future studies must be performed to corroborate this and to compare the dynamics of decomposition with leaves that fall on the ground during the months with highest precipitation (June-September). Despite this, the information obtained gave us an overview of the composition and changes in the microfungal community during the two main phases of decomposition in the leaves studied. Figure 3 shows that the highest biomass loss (primary phase) is likely to correspond with the highest diversity, specific richness, and the highest values of abundance (calculated as frequency of occurrence) for the species (Fig. 4). It is well documented that the initial loss in mass is related to leaching of initially present soluble C, and to the high microbial activity derived from the most easily degradable compounds, such as sugars and amino-acids (Berg & Staaf, 1980; McClaugherty & Berg, 1987). Under these conditions, the greater biodiversity that we found could correspond to increased microbial enzyme diversity and, consequently, to the increased availability of nutrients that favour the abundance of the fungal species present (Yanna & Hyde, 2002).

On the other hand, it is quite probable that the simultaneous presence of endophytic and saprobic fungi in the freshly fallen leaves of *R. Guatemalensis* causes increased diversity during the early decomposition stages, since some of the very abundant species found in the freshly fallen leaves, such as *Clonostachys compactiuscula*, *Colletotrichum* sp. and *Phomopsis* sp. (Table 1), have been isolated from the green leaves of several tropical plant species (Subramanian & Vittal, 1979; Lee & Hyde, 2002; Urdaneta & Delgado, 2007). Their capacity for survival as saprobes has been shown by Promptuttha *et al.* (2007), using molecular analyses. Further studies of endophytes from *R. guatemalensis* are necessary to fully test this hypothesis.

The secondary phase of the leaf decomposition lasted from day 105 until the end of the study. Our results showed that during this phase, the loss of biomass and diversity remains with little apparent changes and with a tendency of decreasing diversity with increasing foliar decomposition. Similar results have been reported with leaves of *Ficus pleurocarpa* (Paulus *et al.*, 2006), bamboo (Zhou & Hyde, 2002) and fronds of *Phoenix hanceana* (Yanna & Hyde, 2002). It has been shown that this phase is influenced negatively by slowly degradable compounds as lignin, phenols, tannins (Palm & Rowland, 1997), which can be a selective factor in the microfungal community.

The frequency and the periodicity of occurrence values indicated that during the decomposition process of the leaves, the sporulating microfungal community was structured mainly by rare and sporadic species. Similar results have been obtained with the microfungi from leaves from other tropical and subtropical regions (Shanthi & Vittal, 2010; Bills & Polishook, 1994; Heredia, 1993). Few species were present during the entire decomposition process. Of the 38 species detected, the following six were classified as dominant: *Cylindrocladium scoparium*, *Menisporopsis multisetulata*, *M. theobromae*, *Microthyrium* sp., *Volutella ciliata* and *Volutella minima*. In particular *Cylindrocladium scoparium* and *Microthyrium* sp. stand out for their high abundance at all sampling times, although they tend to decrease with increasing

leaf decomposition. *Cylindrocladium scoparium* (anamorphic stage of *Calonectria morganii*) is widely distributed in the tropics and associated with plant diseases (Crous, 2002). *Cylindrocladium scoparium* was also found frequently during the decomposition of *Liquidambar styraciflua* leaves in a Mexican cloud forest (Heredia, 1993).

The two *Menisporopsis* species can be considered as dominant in leaves with moderate and advanced decomposition stages. *Menisporopsis multisetulata* had not been collected since it was described by Tsui *et al.* (1999) from samples collected in Hong Kong. On the other hand, *M. theobromae* is a common species reported frequently in tropical and subtropical leaf litter (Matsushima, 1993; Delgado-Rodríguez & Mena-Portales, 2004; Heredia, 1994). *Volutella ciliata* and *V. minima* were dominant in the freshly fallen leaves and at 60 days of leaf decomposition. Both have a wide distribution and *V. ciliata* also was reported as dominant in leaf litter of *Celtis tala* in a xeric forest from Argentina (Allegrucci *et al.*, 2007) and in *Ficus virens*, *F. altissima* and *F. semicordata* in Thailand (Wang *et al.*, 2008). *V. minima* was isolated by Polishook *et al.* (1996) as the second most abundant species from leaf litter of *Guarea guidonia* in Puerto Rico.

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