THE ECTOMYCORRHIZAL COMMUNITY IN A *PINUS OAXACANA* FOREST UNDER DIFFERENT SILVICULTURAL TREATMENTS

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VALDÉS M, PEREDA V, RAMÍREZ P, VALENZUELA R & PINEDA RM. 2009. The ectomycorrhizal community in a *Pinus oaxacana* forest under different silvicultural treatments. This study was conducted in a tropical mountain ecosystem to examine the ectomycorrhizal (EM) fungal communities in a secondary pine-oak forest under different silvicultural practices. Species richness was analysed by examining ectomycorrhizal root tips and sporocarps in a control, seed tree harvest and seed tree harvest plus prescribed burning stands. The fungal region ITS1-5.8S-ITS2 from single EM root tips was digested and compared with the ITS-RFLP of identified sporocarps of the same stands. Diversity of EM fungi was higher in terms of ITS-RFLPs for root tips compared with sporocarps. Fungal abundance was slightly affected by silvicultural treatments, but species composition was different. Fruiting bodies of *Laccaria laccata* and *Lactarius crysorrheus* were most abundant in all stands. Sporocarps of *Russula emetica, L. laccata, L. chrysorrheus* and sclerotia of *Cenococcum geophilum* were found in all three stands. However, *L. laccata* and *L. chrysorrheus* were found just in the roots of trees growing in the burned stand. *Cenococcum geophilum* was present in all stands. The seed tree treatment had the highest numbers of the root colonizing fungi as well as EM sporocarps, i.e. 24 RFLP-taxa and 30 sporocarp species, whereas the control stand had 19 RFLP-taxa as EM and 12 sporocarp species. Thirty five per cent of the identified sporocarp taxa were found in the roots. Thus, the seed tree treatment preserved the vital symbionts of the trees best.

Keywords: Tropical mountain forest, thinning, fire, ITS ectomycorrhizal fungi, sporocarp diversity

VALDÉS M, PEREDA V, RAMÍREZ P, VALENZUELA R & PINEDA RM. 2009. Komuniti ektomikoriza hutan *Pinus oaxacana* di bawah rawatan silvikultur berlainan. Kajian ini dijalankan dalam ekosistem tropika yang bergunung untuk menyelidiki komuniti kulat ektomikoriza (EM) dalam hutan pain-oak sekunder yang mengalami rawatan silvikultur berlainan. Kekayaan spesies dianalisis dengan memeriksa hujung akar ektomikoriza dan sporokarpa dalam dirian kawalan, dirian yang mengalami pembalakan pokok biji benih dan dirian yang mengalami pembalakan pokok biji benih serta pembakaran. Kawasan kulat ITS1-5.8S-ITS2 daripada hujung akar EM tunggal dicerna dan dibandingkan dengan ITS-RFLP sporokarpa yang telah dikenal pasti dari dirian yang sama. Kepelbagaian kulat EM dari segi ITS-RFLP adalah lebih tinggi pada hujung akar berbanding sporokarpa. Kelimpahan kulat tidak banyak dipengaruhi oleh rawatan silvikultur tetapi komposisi spesies adalah berbeza. Kandul spora *Laccaria laccata* dan *Lactarius crysorrheus* paling banyak di semua dirian. Sporokarpa *Russula emetica, L. laccata, L. crysorrheus* dan sklerotia *Cenococcum geophilum* dijumpai di ketiga-tiga dirian. Namun, *L. laccata* dan *L. crysorrheus* cuma dijumpai dalam akar pokok yang tumbuh di dirian yang telah dibakar. *Cenococcum geophilum* hadir di kesemua dirian. Dirian pokok biji benih mempunyai bilangan kulat pengkoloni akar serta sporokarpa EM yang terbanyak iaitu 24 takson RFLP dan 30 spesies sporokarpa. Dirian kawalan pula mempunyai 19 takson RFLP sebagai EM dan 12 spesies sporokarpa. Sebanyak 35% daripada takson sporokarpa yang dikenal pasti terdapat dalam akar. Rawatan pokok biji benih paling sesuai untuk mengekalkan simbion penting pokok.

INTRODUCTION

There is a lack of knowledge about the effects of silvicultural harvest types and fire on the ectomycorrhizal (EM) community in tropical mountain ecosystems. The secondary forests of the Sierra Norte at the State of Oaxaca, Mexico provide an ideal setting for studying the effects of different silvicultural treatments (including fire) applied for timber harvest and forest regeneration. Human settlements have existed in the region for centuries and long-term disturbance has played an important role in shaping the present landscape (Asbjornsen

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1999). These forests established because agriculture was given up more than 50 years ago. The combination of human pressure and the extreme climatic conditions common to dry tropical montane ecosystems creates a system that is highly stressed. Although Oaxaca is recognized as biologically rich and ethnically diverse in Mexico, the state is one of the country's most ecologically degraded and economically poor regions (Carvajal 1991).

The effects of forest management practices on the persistence of mycorrhizal associations on root systems are particularly important with respect to plant responses to and recovery from disturbances (Vogt *et al.* 1997). Ectomycorrhizal (EM) communities are sensitive to disturbance, and a reduction in fungal species richness and soil inocula have been reported following forest harvesting (Perry *et al.* 1982) and wildfire (Visser 1995). Fire may cause substantial nutrient loss through volatilization, changes in soil porosity and chemistry, and significantly reduce soil in biomass and reduce particularly root symbiosis such as EM fungi (Neary *et al.* 1999, Stendell *et al.* 1999). Intense fires have been reported to alter the EM community composition, reducing the number of species and triggering a succession in the species of EM found on roots (Torres & Honrubia 1997). Fire has been implicated in a significant change in the relative abundance of certain EM morphotypes (de Román & de Miguel 2005), decreasing significantly diversity and production of edible fungi (Martín-Pinto *et al.* 2006), and influencing the diversity and distribution of EM species (Visser 1995, Torres & Honrubia 1997) which could result in the colonization of roots by less effective symbiotic associations (Allen 1991). Therefore, the effect of forest management on the ectomycorrhizal symbiosis needs to be understood.

Currently, the most common way of estimating the species diversity of EM fungi in an ecosystem is to count the frequency and abundance of their sexual fruit bodies. This characterization of a fungal community requires long-term site monitoring because of the strong effect of environmental factors on the sexual reproduction of macromycetes and because of long intervals in reproduction of many species (Vogt *et al.* 1982). Sporocarp surveys poorly reflect the composition of belowground EM fungal communities as shown in several studies (Gardes & Bruns 1996a, Dahlberg *et al.* 1997).

Other approaches for identifying fungal symbionts include morphological and structural descriptions of ectomycorrhizae (Agerer 1987–1993, Ingleby *et al.* 1990) and direct genetic fingerprinting of ectomycorrhizae using molecular methods (Gardes *et al.* 1991, Gardes & Bruns 1993, 1996b, Amicucci *et al.* 1998, Gherbi *et al.* 1999, Jonsson *et al.* 1999a). Although molecular tools based on polymerase chain reaction present problems related to the sorting of fungal morphotypes and root tip sample bias (Horton & Bruns 2001), they are still among the most useful approaches for identification of fungi from mycorrhizae or mycelium because they require only small sample sizes and can be targeted at several taxonomic levels.

In the present study we investigated the above and belowground ectomycorrhizal (EM) communities in secondary natural *Pinus oaxacana* stands following silvicultural treatments using molecular and morphological identifications. The internal transcribed spacer (ITS) regions (rDNA) of sporocarps and roots tips were examined and subject to ITS-restriction length polymorphism (RFLP) analysis. Patterns of EM root tips were compared with those obtained from sporocarps.

MATERIALS AND METHODS

Location and description of the study sites

Three sites were selected within an area of 19 500 ha in the region known as Sierra Norte in the State of Oaxaca, Mexico (Figure 1). According to Koppen's classification system, modified by García (1981), both sub-humid temperate and humid temperate climatic zones are represented in the region and are characterized by summer rains.

The forest has been established on abandoned agricultural lands. All three forest stands show minor differences in altitude, slope, soil texture and compaction, composition and silvicultural treatments, as indicated in Table 1 (Valdés *et al.* 2003).

Sites had previously been subjected (five years before) to the following silvicultural treatments: (1) stand of intermediate disturbance intensity, consisting of a seed tree harvest treatment (ST), (2) stand of high disturbance intensity, consisting of seed tree harvest with burning (STB), and (3) non-harvested 'reference site' (C), consisting of

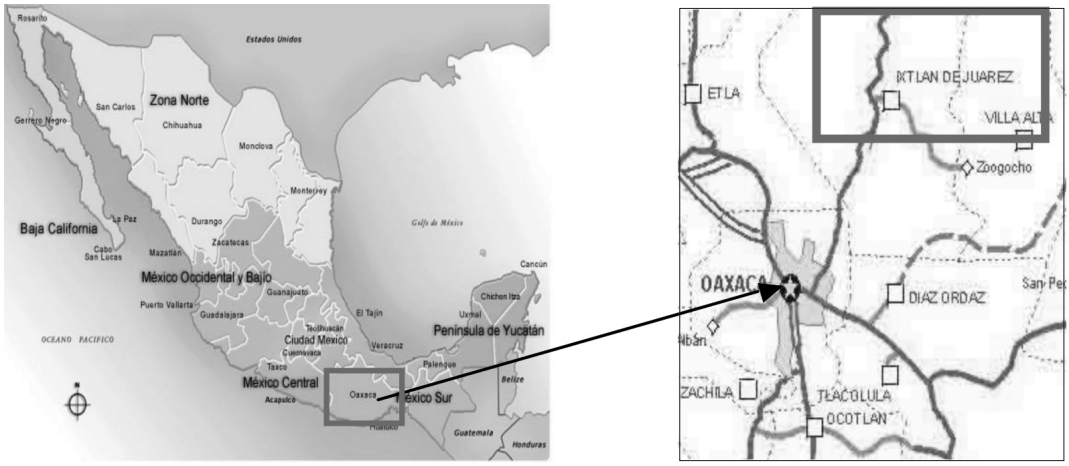

Figure 1 Location map of Ixtán de Juárez, Oaxaca, Mexico and study site

Table 1 Description of the forest stands (Valdés *et al.* 2003)

Characteristic	Seed tree (ST)	Seed tree + burning (STB)	Control (C)
Elevation (m)	2180	2280	2250
Slope (%)	36.5	36.5	40.8
Texture	Sandy loam	Sandy loam	Clay loam
Soil compaction	High	High	Intermediate
Silvicultural treatment	Seed tree	Seed tree	None
Additional disturbance	Light slash burning	Intense slash burning	None
pH	5.2	5.4	6.8
EC (mmhos cm^{-1})	0.03	0.05	0.16
OM (%)	4.3	4.6	12.8
Total N (%)	0.12	0.14	0.35
P (ppm)	9.7	3	10

a site having similar ecological characteristics to other sites prior to treatment.

Study design

Five 6 × 6 m plots were established in each of the three study stands. The sporocarps of the EM fungi and the fine roots were collected in these plots in 1997, 1998 and 1999. Conifer fine roots (< 2 mm) were sampled according to the method described by Vogt *et al.* (1982), with the exception that a PVC cylinder (5 cm inside diameter) was used instead of a metal cylinder.

Roots were sampled two times during the rainy season and two times during the dry season of 1997, 1998 and 1999. For each sampling period, five cores were collected from each five-plot plots for determination of fine roots. All the organic layer and 5 cm of the mineral soil were sampled. After removal, the two open sides of the PVC cylinder were covered with plastic to minimize disturbance and individually stored at 4 °C prior to processing.

Collection and study of sporocarps

To obtain a more complete picture of the EM communities present in each forest stand, sporocarps of epigeous EM fungi were surveyed in each of the five plots. Sporocarps outside of the subplots were also collected to ensure that as many fruiting epigeous EM species as possible were recorded to compare with morphotype species. During the fruiting (rainy) season of 2002 and 2003 an additional survey was done. These data were used to compare the number and abundance of EM species aboveground with the number belowground.

Morphological examination and macrochemical tests were conducted using protocols outlined by Cifuentes *et al.* (1986). Taxonomy keys for identifying sporocarps included Pegler (1983), Smith and Smith (1985), McKnight and McKnight (1987) and Guzmán (1990). Sporocarp collections were deposited at the Herbarium of the Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional (ENCB) and the Etnomycological Herbarium of the Instituto Tecnológico Agropecuario de Oaxaca at Oaxaca State. Herbarium ENCB is abbreviated according to Holmgren *et al.* (1990).

The sporocarp species similarity between each stand was determined by the Sørensen and Jaccard similarity index (Mueller-Dambois & Ellemberg 1974).

Preparation of EM roots from soil cores

The roots were rinsed with water to remove soil particles using 2.0, 0.85 and 0.5 mm mesh sieves. Pine fine roots were removed from the soil core under a stereoscope using fine forceps. Roots belonging to other species were also removed from the sample. Pine live roots were separated from dead roots and only live roots were included in subsequent DNA and PCR studies. Live pine roots were recognized by their turgidity, light coloured cortex and steel, and plumpness. A subsample of root tips was examined under a stereomicroscope to assess the presence of EM colonization. A minimum of 500 EM root tips were evaluated for each core sample. Most of the roots were found in the forest floor organic layer.

To supplement the macroscopic analysis, cross-sections of randomly selected root segments were prepared, stained with cotton blue and examined microscopically to assess the mantle and Hartig net of the ectomycorrhizae.

Entomycorrhizae short-roots were classified on morphotypes (Agerer 1987–1993). Mycorrhizae formed by *Cenococcum geophylum* had sufficient characteristics to be identified morphologically with certainty (Agerer 1987–1993). All samples were stored at -20 °C prior to DNA extraction.

Molecular identification of EM fungi

To identify the mycorrhizal sporocarps and the microsymbiont in the ectomycorrhizae, DNA was extracted individually from one to three mycorrhizal tips using the CTAB (cetyl trimethylammanium bromide) method and/or utilizing the DNeasy plant mini kit (Quiagen). The ITS region of the rDNA was amplified using ITS1/F and ITS4 as primers (Gardes & Bruns 1993) in PCR thermal cycler.

We characterized the ITS region by digestion with restriction enzymes *HinfI* and *AluI* (Gardes & Bruns 1993, 1996a). The resulting RFLPs were used to match fruiting bodies and mycorrhizae. The fragments were analysed using the Kodak ID 3.6.1 Image Analysis Software.

Our criterion for species-level identification was the exact RFLP matches between identified fungal material and unidentified EM for single digests of the two enzymes. The term RFLP-taxon refers here to the equivalent of a single species or a group of species showing a unique RFLP pattern.

RESULTS

Table 2 shows the difference between stands in terms of composition of the EM sporocarp species. Differences were supported by the Sorensen similarity index, 0.47, when comparing the species composition similarity between the control and ST stands, and 0.38 between the control and STB stands. *Laccaria laccata, Laccaria* sp., *Lactarius crysorrheus* and the ascomycete *C. geophilum* were found in all three stands. There was no consistent change in sporocarp species composition following fire as the most common and numerous species were found in nearly all stands but the numbers of those sporocarp species were different between stands. For example, mycorrhizal frequencies and sporocarps of *L. laccata* were more frequent in the undisturbed stand, less frequent in the medium disturbed stand and had low numbers in the most disturbed stand. *Laccaria amethystina* fruiting bodies were found in the control and ST stands, but not in the STB stand. The ST stand had the greatest abundance of EM sporocarps.

From the 41 sporocarp species collected, we distinguished 25 taxa using RFLP patterns of the ITS region. All 25 types could be distinguished by *HinfI* digests alone and 24 by *AluI* digests. The patterns produced by *HinfI* were often more easily compared than those produced by *AluI,* since *HinfI* patterns tended to have fewer and larger fragments.

Table 2 Total taxa of sporocarps collected from *Pinus oaxacana* under different silvicultural methods

Taxa	Control	Seed tree	Seed tree + burning
Amanita caesarea	x	x	
Amainita flavoconia		x	
Amanita gemmata			x
Amanita muscaria	x		
Amanita vaginata		x	
Amanita verna		x	
Boletellus sp.			x
Boletus sp.			x
Cantharellus cibarius		x	
Cantharellus tubaeformis		x	
Cenococcum geophilum	x	x	x
Chroogomphus rutilus		x	x
Clavariadelphus truncatus	x		x
Clytocibe gibba		x	
Cortinarius sp.		x	
Craterellus fallax	x	x	
Gomphus floccosus		x	
Helvella crispa		x	
Hydnum repandum		x	
Hygrocybe conica		x	
Laccaria amethystina	x	x	
Laccaria farinacea			
Laccaria laccata	x	x	x
Laccaria proxima		x	
Laccaria sp.	x	x	x
Lactarius affinis	x		
Lactarius chrysorrheus	x	x	x
Lactarius piperatus		x	
Lactarius scrobiculatus		x	
Lactarius sp.	x		x
Lactarius volemus		x	
Lycoperdon perlatum			x
Lycoperdon pyriforme			x
Ramaria sp.			x
Russula emetica	x	x	x
Russula sp.		x	
Scleroderma verrucosum		x	
Suillus granulatus		x	
Suillus luteus		x	
Tricholoma equestre		x	
Xerocomus sp.			x

Short roots sampled from all five samples of each plot of three studied stands were morphotyped. Dichotomous ramification, smooth shape of rhizomorphs and grainy mantle surface were the most frequent characteristics found in the morphotypes. No one type of ramification, shape of rhizomorph or mantle surface were prevalent in the stands as shown in Table 3.

Table 3 Description of the most important morphological and anatomical characteristics (Agerer 1987–2002) of the 42 EM morphotypes found in the studied stands of the pine-oak forest in Ixtlán, Oaxaca, Mexico

Morphotype	Type of ramification	Shape of unramified ends	Shape of rhizomorph	Mantle surface	Control	Seed tree	Seed tree + burning
EM 1	Dichotomous	Straight	Smooth	Grainy	+	+	+
EM 2	Coralloid	Globular	Smooth	Smooth	+	+	+
EM 3	Dichotomous	Straight	Smooth	Reticulate	+		+
EM 4	Dichotomous	Bent	Smooth	Grainy	+	+	+
EM 5	Monopodial-pinnate	Tortuous	Smooth	Cottony	+	+	+
EM 6	Dichotomous	Straight	Smooth	Smooth	+	+	+
EM 7	Monopodial-pinnate	Straight	Smooth	Smooth			+
EM 8	Dichotomous	Straight	Smooth	Reticulate			+
EM 9	Dichotomous	Straight	Interconnected filaments	Short-spiny			+
EM 10	Tubercle-like	Globular	Interconnected filaments	Reticulate-like		+	+
EM 11	Dichotomous irregular	Tortuous	Smooth	Grainy		+	+
EM 12	Tubercle-like	Bent	Smooth	Grainy			+
EM 13	Dichotomous	Straight	Smooth	Grainy			+
EM 14	Simple	Straight	Smooth	Smooth			+
EM 15	Dichotomous	Tortuous	Hairy	Short-spiny			+
EM 16	Simple	Folded end	Smooth	Smooth			+
EM 17	Dichotomous	Straight	Smooth	Smooth		+	+
EM 18	Coralloid	Bent	Smooth	Smooth		+	+
EM 19	Dichotomous	Bent	Interconnected filaments	Grainy		+	+
EM 21	Dichotomous	Bent	Smooth	Reticulate	+		
EM 20	Tubercle-like	Bent	Smooth	Grainy	+		
EM 22	Simple	Straight	Interconnected filaments	Smooth	+		
EM 23	Tubercle-like	Straight	Smooth	Smooth	+		
EM 24	Dichotomous	Straight	Smooth	Smooth	+		
EM 25	Dichotomous	Straight	Smooth	Reticulate	+	+	
EM 26	Dichotomous	Straight	Smooth	Short-spiny	+		
EM 27	Dichotomous	Bent	Smooth	Reticulate	+		
EM 28	Simple	Straight	Hairy	Woolly	+		
EM 29	Dichotomous	Bent	Smooth	Grainy	+	+	
EM 30	Dichotomous	Straight	Interconnected filaments	Woolly	+	+	+
EM 31	Simple	Straight	Smooth	Grainy	+		+
EM 32	Simple	Straight	Smooth	Smooth		+	
EM 33	Dichotomous	Straight	Smooth	Smooth		+	
EM 34	Tubercle-like	Tortuous	Smooth			+	
EM 35	Coralloid	Tortuous	Smooth	Smooth		+	
EM 36	Dichotomous	Straight	Smooth	Smooth		+	
EM 37	Dichotomous-pinnate irregular	Tortuous	Smooth	Reticulate		+	
EM 38	Tubercle-like	Tortuous	Smooth	Grainy		+	
EM 39	Dichotomous	Straight	Smooth	Smooth		+	
EM 40	Dichotomous	Straight	Smooth	Reticulate		+	
EM 41	Dichotomous	Straight	Smooth	Grainy		+	
EM 42*	Dichotomous	Straight	Not observed	Curly hyphae	+	+	+

* *Cenococcum geophilum*

Most of the EM processed by PCR were successfully amplified (two morphotypes were not amplified). RFLP were obtained for all the EM amplified. Of all the EM processed by PCR-RFLP, we distinguished 42 RFLP-taxa (Table 3). The ST stand had the highest number and diversity of the root colonizing EM fungi. From the total amount of morphotype RFLP-taxa, 24 were found in the seed tree stand, 22 in the STB stand and 19 in the control stand. The control stand showed 9 morphotypes (RFLP-taxa) in common with the ST and STB stands respectively.

Table 4 lists the species composition of EM fungi found within the pine roots. Sixteen of the 26 ITS-RFLP types matched patterns from DNA extracted from EM morphotypes, indicating the presence of basidiomycete species of EM inside the roots of the pines. Additionally, the typical ectomycorrhizae formed by the ascomycete *C. geophilum* was found within the roots of pine in all three stands.

Several of the common RFLP-taxa (*L. laccata, Laccaria* sp., *L. chrysorrheus* and *Russula emetica*) occurred in the roots of pine in the three forest stands according to the matched patterns from DNA samples extracted from identified basidiocarps.

Our sporocarp surveys showed to be different from the composition of belowground EM fungal communities. For instance *A. muscaria* ssp *flavivolvata, Clavariadelphus truncatus, Craterellus fallax, Gomphus floccosus, Hydnum repandum* and *Lactarius affinis* were found as sporocarps, and they were not found within the roots. Sporocarps of *L. chrysorrheus* and *L. laccata* which occurred in the three stands were identified within the roots of pines in the STB stand only.

DISCUSSION

Using the digested internal transcribed spacer (ITS region of rDNA) of fungi from single ectomycorrhizae and comparing these with the ITS-restriction length polymorphisms (RFLP) of EM sporocarps, we were able to document the belowground diversity of the fungal community of a pine forest under contrasting silvicultural treatments. Our data suggest that tropical mountain forests in Mexico differ in several respects from the effects reported in other studies on EM fungi conducted in other forest ecosystems. There was no evidence that disturbance caused by thinning or low-intensity fire affected the richness or composition of EM fungi. Other

Table 4 Species composition of EM fungi found within the pine roots from stands under silvicultural treatments in Oaxaca, Mexico

Identified fungus within the pine roots	Silvicultural treatment		
	Control	Seed tree	Seed tree + burning
Amanita caesarea		x	
A. muscaria		x	x
A. vaginata	x		
Cenococcum geophilum	x	x	x
Helvella crispa		x	x
Laccaria sp.	x	x	x
L. farinacea	x		x
L. laccata			x
Lactarius sp.	x	x	
L. chrysorrheus			x
Lycoperdon perlatum			x
L. pyriforme			x
Hygrocybe lonice	x		
Ramaria sp.		x	
Suillus luteus		x	
Tricholoma flavovirens		x	

studies pointed towards an alteration of the soil microbial community composition and activity through selective heat-induced mortality (De Bano *et al.* 1998). For instance, bacteria tend to be more resistant to heat induced by fire than fungi, and generally increase in abundance more than fungi following even moderate-intensity fire (Pietikäinen & Fritze 1995). The fact that in our study the treatments were applied five years earlier may explain the difference.

Pre-fire forest composition has an important influence on the post-fire community (Macdonald 2007) and can, in turn, influence other ecosystem processes (Hart *et al.* 2005). Besides, the proximity of seedlings to sources of EM inocula is also thought to be a significant factor in the rate of establishment and succession following disturbance (Jonsson *et al.* 1999b). Pine regeneration and oak sprouting recorded previously in our forest (Valdés *et al.* 2003) were higher in the ST stand, which also had higher EM fungal richness as reflected by sporocarps and mycorrhizae. Indeed, opening of the overstorey favours development of shrub forms which were not present in the control stand probably due to their intolerance to shade (Valdés *et al.* 2003) and may represent a microbial reservoir. According to Amaranthus and Perry (1987) as well as Allen (1991), the ability of microbial symbionts on plant roots to persist following fire depends, in part, on their relative ability to survive in the absence of their hosts, which tends to be lower for EM than arbuscular mycorrhizal (AM) fungi. Indeed, Hart *et al.* (2005) hypothesized that the relative abundance of EM and AM fungi will be influenced indirectly by the comparative dominance of their plant host following fire.

In contrast to the above-mentioned studies, we did not find any consistent change in sporocarp species composition following fire, as the most common and numerous species were found in nearly all stands. Nevertheless, the numbers of those species varied considerably between stands, e.g. mycorrhizal frequencies and sporocarps of *L. laccata* were more frequent in the undisturbed stand, less frequent in the medium disturbed stand and least in the most disturbed stand (Valdes *et al.* 2003). *Laccaria amethystina* fruiting bodies were found in the control and ST stands, but not in the STB stand. These findings can be attributed to the significance of fire intensity, since a low-intensity burn only slightly affects the litter layer where the majority of ectomycorrhizae are located, and rarely affects the fruiting bodies of basidiomycetes (Palmer *et al.* 1994).

As observed by other authors (Gardes & Bruns 1996b, Dahlberg *et al.* 1997, Kårén *et al.* 1997), our sporocarp surveys poorly reflected the composition of belowground EM fungal communities. For instance *A. muscaria* ssp. *flavivolvata, C. truncatus, C. fallax, G. floccosus, H. repandum* and *L. affinis* were found as sporocarps, and were not found within the roots.

Diversity was not as high as that reported for older forests. However, it was similar to diversity levels reported for young forests. The belowground diversity of EM fungal species was higher than the aboveground species diversity. The identified (RFLP-taxa) fruiting species found in this study formed 57% of the total number of RFLP-taxa as mycorrhizae. Some abundant fruiting species apparently produced few mycorrhizae, whereas other common species observed on roots were poorly represented above-ground. *Laccaria laccata, Laccaria* sp., *L. crysorrheus* and the ascomycete *C. geophilum,* the common species in all three stands, suggest that these species are tolerant to the disturbance caused by the silvicultural practices. However, *L. laccata* and *L. crysorrheus,* which had the most abundant fruiting bodies in all stands, were found in the root tips of the most disturbed stand only.

Climatic conditions in different areas play an important role. The time period for this study encompassed both a drought year (El Niño phenomenon) and a normal year; the ectomycorrhizal root biomass was reduced by almost 60% in the drought year compared with the non-drought year (Valdés *et al.* 2006), and only ecosystems sharing the same climatic, edaphic and tree species compositions should be compared to define the impact of stress produced by silvicultural treatments. The regeneration of pine seedlings and sprouting of oak trees could have an effect on the increase over time of EM individuals in the burned stand, but more detailed investigations are required. In the undisturbed stand, a little change was detected in the EM fruiting bodies that can be explained by the irregular reproduction of many species of basidiomycetes.

The implications of this study are constrained by the pseudoreplications and the contrasting reports in the literature. It is not possible to draw definitive conclusions on the silvicultural dynamics of the EM communities in pine-oak forests of tropical mountain ecosystems.

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