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Hypocrealean (Hypocreales, Ascomycota) Fungal Diversity in Different Stages of Tropical Forest Succession in Costa Rica[1]

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ABSTRACT

The relationship between forest succession and microfungal diversity has been poorly studied. Fungi provide important ecosystem services that may deteriorate in deforested or highly disturbed forests. To determine the possible effects of deforestation and forest succession on microfungi, species diversity of hypocrealean fungi (Ascomycota) was compared in forest stands in Eastern Costa Rica representing three stages of succession: 1–2, 25–27 yr old, and an old growth forest. Species diversity in a second-growth forest fragment surrounded by timber plantations and second-growth forest was also compared to that of a stand surrounded by old growth forest. The results show that the overall diversity of hypocrealean fungi was inversely proportional to the age of the forest stand, and each family showed different successional trends. Clavicipitaceae was more diverse in the old-growth forest and was positively related to the age of the forest stand. Nectriaceae was highly diverse in the 1- to 2-yr-old stand and less diverse in the old-growth stand. Saprobic and plant pathogenic fungal species were more diverse in the 1- to 2-yr-old stand and their diversity was inversely proportional to the age of the forest stand. The diversity of insect pathogens was positively related to the age of the forest stand. The 20- to 22-yr-old forest fragment had the lowest number of species overall. Based on the data gathered in this study, hypocrealean fungal species diversity is related to the successional stage and fragmentation of tropical forest.

RESUMEN

La relación entre la sucesión de bosques secundarios y la diversidad de microhongos ha sido poco estudiada. Los hongos proveen servicios importantes al ecosistema, los cuales podrían deteriorarse en áreas deforestadas o altamente alteradas. Para determinar los posibles efectos de deforestación y sucesión del bosque en los microhongos, la diversidad de especies de hongos hipocreáceos (Ascomycota) se evaluó en varias localidades de Costa Rica que representaban tres estados de sucesión: 1–2, 25–27 años, y un bosque primario. La diversidad de especies en un fragmento de bosque rodeado de plantaciones forestales y bosque secundario también se comparó a un bosque de la misma edad rodeado de bosque viejo. Los resultados demuestran que la diversidad total de hongos hipocreáceos es inversamente proporcional a la edad del bosque. Cuando se analizó cada familia separadamente, se encontraron tendencias diferentes. Clavicipitaceae tuvo una alta diversidad en el bosque primario y una relación positiva a la edad del bosque. Nectriaceae tuvo una alta diversidad en rodales de 1–2 años y una baja diversidad en el bosque primario. Las especies de hongos saprófitas y fitopatógenas tuvieron una relación negativa con respecto a la edad del bosque. La diversidad de especies entomopatógenas tuvieron una relación positiva a la edad del rodal. El fragmento de bosque de 20–22 años tuvo el menor número de especies. Basado en los datos obtenidos en este estudio, el estado de sucesión del bosque y su fragmentación están relacionados a la diversidad de especies de hongos hipocreáceos.

Key words: Ascomycetes; biodiversity; Costa Rica; deforestation; forest disturbance; forest fragmentation; forest succession; fungi; tropical wet forest.

THE RAPID AND EXTENSIVE ALTERATION, INCLUDING DEFORESTATION, of tropical forests is a serious threat to their native biota. Some consider land-use changes, such as the conversion of primary forest to croplands, pastures, or other human dominated landscapes, as a primary driver of biodiversity loss in the tropics (Myers 1992; Sala *et al.* 2000). The influence of land-use history and the severity of disturbance events are increasingly being studied to determine the recovery of biodiversity in tropical forests after deforestation (Chazdon 2003). The recovery of tropical forests after such disturbance events is related to the type of disturbance, its duration, and its intensity, among others. In some regenerating tropical forests, diversity may increase, decrease, or remain unaffected over time. Site conditions also affect recovery, as does residual vegetation in adjacent stands (see examples in Chazdon 2003). In tropical forests, where regeneration is proceeding, understanding the potential to recover diversity is an important conservation endeavor.

The effects of deforestation, land-use change, and forest fragmentation on the biodiversity of plants, animals, especially birds, and insects have been studied in temperate and tropical forests (Okland 1994, Turner *et al.* 1997, Peña-Claros 2003, Fiedler & Schulze 2004, Naidoo 2004, Waltert *et al.* 2004, among others); however, the effects on microorganisms such as fungi have not been assessed or well documented (Staley 1997, Tsui *et al.* 1998, Moore *et al.* 2001). The few studies known for fungi have measured the effect of deforestation on macrofungi, such as mushrooms, bracket fungi, and mycorrhizal fungi in temperate forests (Albrecht 1991, Hagerman *et al.* 1999, Byrd *et al.* 2000).

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Ascomycetes (included in the artificial category known as "microfungi") play many important roles in natural and agricultural ecosystems and of the few studies relating biodiversity to forest disturbance, even fewer have considered this taxonomic group (Rambelli *et al.* 1984, 1991). For example, some species are saprobic on plant material, others are beneficial endophytes (organisms that live within healthy plants), and many are parasites of plants, insects (entomopathogens), and other fungi (mycoparasites) (Alexopoulos *et al.* 1996; Rossman *et al.* 1999; Kumar & Hyde 2004; Lee *et al.* 2004, 2005; Schulz & Boyle 2005).

This study focuses on the order Hypocreales (Ascomycetes), a group whose taxonomy has been relatively well studied; identification aids are widely available (Rossman *et al.* 1999, Schroers 2001, Chaverri & Samuels 2003). In addition, the Hypocreales are ecologically and economically important. Hypocrealean fungi can also be saprobic, entomopathogenic, and mycoparasitic (Rossman *et al.* 1999). They are considered by some to be the most important regulators of insect and fungal populations, and for that reason they are also important for agriculture as biocontrol agents (Berger 1921, Brady 1981, Carruthers & Hural 1990, Esser & El-Gholl 1993, Samuels 1996a, Rossman *et al.* 1999). Anecdotal evidence and personal observations suggest that certain species of hypocrealean fungi are widespread in disturbed and old growth forests (*e.g.*, *Haematonectria haematococca* and *Ophionectria trichospora*), whereas other species, such as ones that parasitize insects (*e.g.*, *Cordyceps* spp.) and other fungi (*e.g.*, *Hypocrea* spp.) and that are economically important as biocontrol agents, are found in old growth forests and are rare or absent in disturbed areas. There is also evidence that some species of Hypocreales can enhance plant growth and induce resistance to pathogens (Inbar *et al.* 1994, Gromovich *et al.* 1998, Yedidia *et al.* 2001).

The objectives of this study were to determine how hypocrealean fungal diversity changes in different stages of forest succession and to compare the diversity of a forest fragment surrounded by timber plantations and second growth forest and a stand surrounded by old growth forest. The study was conducted in a lowland wet tropical forest in Northeastern Costa Rica. In Costa Rica, extensive deforestation of old growth forests from the 1940s to 1980s resulted in the elimination of approximately 50 percent of the original forest cover (Sader & Joyce 1988, Solórzano 1992). Since then, the rate of deforestation has been reduced and almost eliminated in Costa Rica thereby leading to a significant recuperation of forest cover due to the growth of secondary forests (Sánchez 1996). Therefore, we considered this particular forest in Costa Rica an appropriate site to conduct this study.

This study aims to provide an understanding of how deforestation and forest regrowth may impact the biodiversity of important organisms, such as microfungi. Hypocrealean fungi are an example of organisms that provide services to an ecosystem. If these fungi become extinct due to deforestation, then the ecosystem services that they provide and that natural areas and human landscapes depend on (*e.g.*, biological or natural control of plant pests) will also vanish. If an assessment of hypocrealean fungi, as well as other fungi, is not undertaken in remaining old growth forests, many fungal species that are important to humans and forest ecosystems may disappear before they are even discovered.

METHODS

STUDY SITES.—The study sites were located in Sarapiquí, Heredia, Costa Rica in a lowland tropical rain forest with a yearly average precipitation of 4000 mm and temperatures varying throughout the day between 21–31°C. The elevation of the sites varies between 20–50 m. Three forest stands that represent three ages (or successional stages) were selected for this study: 1–2 and 25–27 post-abandonment, and an old-growth forest (Table 1). These three stands are surrounded by old-growth forest. A 20-22-yr-old forest stand or fragment surrounded by *Gmelina* plantations and second growth forest was also evaluated and compared to the stand enclosed by old-growth forest. The area of the four stands range between 10 and >100 ha.

Clear-cutting in Costa Rica was reduced and almost eliminated in the 1980s and early 1990s; therefore, it was difficult to find a young forest stand. The 1- to 2-yr-old stand used in this study was established in La Selva Biological Station by other scientists to study vegetation changes in different stages of succession (*i.e.*, 0–1, 1–2, 2–3, and 3–4 yr old); stand area was *ca* 1 ha.

SAMPLING DESIGN.—Few studies have measured microfungal diversity; consequently, information on sampling protocols for ascomycetous fungi is sparse. Some authors recommend establishing small subplots along transects every 5–10 m to collect the greatest diversity of fungi, including Ascomycetes (Rossman *et al.* 1998, Cantrell 2004).

The available studies on fungal biodiversity have not determined the best sampling unit or subplot size for Ascomycetes inventories. In this study, we aimed to find an appropriate sampling unit size based on the premise that it should be large enough to obtain the greatest number of species and suitable for statistical

TABLE 1. *Characteristics of the study sites. Location: Sarapiquí, Heredia, Costa Rica.*

Forest stand	Age (yr)	Previous land-use	Surrounding area
"Sucesión"	1–2	Pasture	Old-growth forest
"Lindero El Peje"	25–27	Pasture	Old-growth forest
Old growth	>100	Old growth forest	Old-growth forest
"Tirimbina"	20–22	Pasture	Second-growth forest and timber plantation

analyses, but small enough as not to waste effort (Coddington *et al.* 1991). The sampling unit or subplot size was determined by collecting hypocrealean fungal specimens in overlapping subplots of three different areas: 1 m^2 (1 × 1 m), 9 m^2 (3 × 3 m), and 25 m^2 (5 × 5 m). First, a 1-m^2 subplot was established and all hypocrealean fungi were collected. Then, a 9-m^2 subplot was established symmetrically on top of the 1-m^2 subplot and fungi were collected from the non-overlapping area. A 25-m^2 subplot was placed symmetrically on top of the 9-m^2 subplot and fungi were collected in the non-overlapping area. This sampling scheme was repeated three times, 10 m apart along a randomly placed transect. The subplots were established in the 1- to 2-yr-old stand because we hypothesized that this site would have the largest number of species. The results showed that the 9-m^2 and the 25-m^2 subplots had statistically the same amount of species (Fig. 1); the 1-m^2 subplot had significantly less species than the 9-m^2 and 25-m^2 subplots. The 9-m^2 subplot took significantly less time to collect the specimens. Based on the three areas tested, we determined that the 9-m^2 subplot was the most efficient sampling unit size.

In this study, transects were placed longitudinally and randomly along 50 × 200 m plots that were randomly placed in each age-stand. Seventeen 9-m^2 subplots for each stand were established along transects every 10 m, as suggested by previous authors (Rossman *et al.* 1998, Cantrell 2004). It is better to establish subplots along transects than subplots within a plot because the distribution of species within the plots was not homogeneous in comparison to the distribution of a species along transects (Cantrell 2004).

COLLECTION AND IDENTIFICATION OF FUNGAL SPECIMENS.—Hypocreales were selected for this study because of their important roles in tropical forest ecosystems and their economic importance in agriculture. In addition, hypocrealean fungi are among the most common microfungi found in tropical forests and agricultural ecosystems, and are common in most substrates. Their identification is facilitated by relatively comprehensive taxonomic keys, which are available for most species.

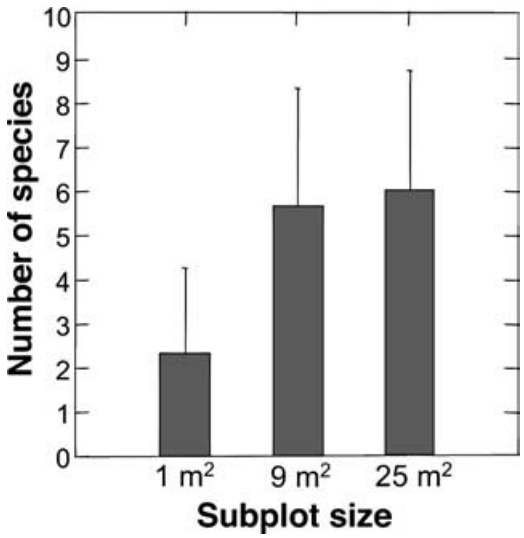

FIGURE 1. Determination of best size of sampling unit or subplot. Accumulated number of species per sampling unit size (1 m^2, 9 m^2, and 25 m^2). Lines with tick-marks represent standard error (95% confidence).

All hypocrealean fungal fruiting or spore bearing structures of teleomorphs (sexual stage) and anamorphs (asexual stage), such as stromata, perithecia, sporodochia, and synnemata, were collected in the subplots. If different forms of the species or genus were encountered, they were treated as being part of the same morph. For example, if the anamorph *Trichoderma harzianum* and the teleomorph *Hypocrea lixii* were found separately, for diversity calculations they will be considered as the same species.

The substrata examined were decaying and living leaves, wood, insects, and other fungi. All woody substratum units were sampled, as well as insects and other fungi at a maximum height of *ca* 2 m. Because the amount of decaying leaves on the ground was enormous, as many leaves as possible were sampled in a time span of 1.5–2 h. The time span within which sampling was completed for each 9-m^2 plot was *ca* 2.5–3 h. Fungi were recorded by using presence/absence data for each sampling unit.

Because fungi tend to produce fruiting bodies irregularly during the year due to variations in moisture, temperature, light, among other factors (Rossman *et al.* 1998, Cantrell 2004), sampling was done in June 2002, August 2003, and January 2004. The specimens collected were air-dried and then cultured in the laboratory using artificial media (see Chaverri & Samuels 2003, for details on isolation and cultivation of hypocrealean fungal spores). The identification of the species was done by microscopic and macroscopic examination of dried specimens and living cultures. Taxonomic keys and literature available at the Mycology Library of the Systematic Botany and Mycology Laboratory (USDA, Agricultural Research Service, Beltsville, Maryland, USA) were also used. Uncertain identifications were confirmed by sequencing a fragment of approximately 800–900 nucleotides of the large subunit nuclear ribosomal DNA (techniques used are detailed in Chaverri *et al.* 2005) and comparing it to existing personal and NCBI-GenBank databases.

DATA ANALYSIS.—The number of species and specimens in each subplot for each stand was recorded. Shannon–Wiener species diversity index (H) was calculated for each stand (Krebs 1998). The factors evaluated were: age of the stand, seasons, and plots. A two-way analysis of variance (ANOVA) using Tukey's Studentized Range test (HSD) for multiple comparisons between age-stands (*e.g.*, 1–2, 20–22 yr old, 25–27 yr old and old growth) was carried out using SAS version 8.0.2 (SAS Institute Inc., Cary, North Carolina, USA). ANOVA ($\alpha = 0.05$) was also performed to test differences between plots, between collecting seasons (*e.g.*, June 2002, August 2003, and January 2004), and between ages in each season (interaction age × season). An analysis of residuals was also performed in SAS to confirm the assumptions of the ANOVA.

Species accumulation curves were constructed to determine the minimum number of sampling units (9-m^2 subplots) needed. Randomization of subplots within each stand was done to obtain smoother curves.

RESULTS

SAMPLING INTENSITY.—The results shown in this study are based on 17 subplots per stand. Fungal species accumulation curves show that the sampling intensity did not reach the greatest number of species (Fig. 2); therefore, more subplots may be needed to determine the best sampling area for this group of fungi.

COMPARISON OF FUNGAL SPECIES DIVERSITY IN AGE-STANDS SURROUNDED BY OLD GROWTH FOREST.—Overall, 87 species in 27 genera from all hypocrealean families were found (Table 2). These included the families Bionectriaceae, Clavicipitaceae, Hypocreaceae, Nectriaceae, and Niessliaceae. The ANOVA found significant differences in species diversity between age-stands ($P = 0.0002$; Table 3). On the other hand, the ANOVA found no significant differences in species diversity (H) between plots within each stand ($P = 0.235$), collecting seasons ($P = 0.304$), or the interaction age × season ($P = 0.11$). These results indicate that there is no apparent effect of season on the species diversity (H) in each of the age-stands. Therefore, the data were combined and no distinction according to seasons was made. The analyses of residuals confirmed the reliability of the ANOVA assumptions.

Results show that overall hypocrealean fungal diversity was inversely proportional to the age of the forest stand (Table 3). Forty-two species were found in the Sucesión stand (1–2 yr old), 37 species in the Lindero El Peje stand (25–27 yr old) surrounded by old-growth forest, and 26 species in the old-growth forest. The total Shannon–Wiener indices ("H" in Table 3) for Sucesión, Lindero El Peje, and old-growth stands, were 1.402, 1.412, and 1.003, respectively. When Shannon–Wiener indices were averaged for each plot, and then compared ("*h*" in Table 3), the HSD test resulted in a significant difference between the Sucesión stand and the rest of the stands (HSD = 0.281). Diversity did not differ between Lindero El Peje and the old-growth stands.

A different trend was observed when families were analyzed separately (Fig. 3). The diversity of species in the Clavicipitaceae increased as the age of the forest stand increased. Eighteen species were found in the old-growth stand, and 12 species each in Lindero El Peje and Sucesión stands. In contrast, the diversity of Nectriaceae and Bionectriaceae decreased as the age of the stand increased. The Sucesión, Lindero El Peje, and the old-growth stands had 17, 12, and 4 species of Nectriaceae, respectively. Ten species of Bionectriaceae were found in Sucesión, six in Lindero El Peje, and one in the old-growth stand. No obvious trend was observed in the Hypocreaceae.

A trend can also be observed in the diversity of saprobic species, where the number of saprobic species decreases as the age of the forest increases (Fig. 4). The Sucesión, Lindero El Peje, and old growth stands had 27, 24, and 6 species, respectively. Fifty-three percent of the total number of saprobic species was found in the most disturbed area, viz. Sucesión, 47 percent in the Lindero El Peje, and 12 percent in the old-growth stand. Only 10 percent of the total number of species was found in the Tirimbina stand. Approximately 45 percent of the total number of saprobic species found in all subplots belongs in the family Nectriaceae, 27 percent in the Bionectriaceae, and 24 percent in the Hypocreaceae.

Hypocrealean fungi fruiting on necrotic tissue of dying or sick plants were more abundant in the Sucesión stand and decreased as

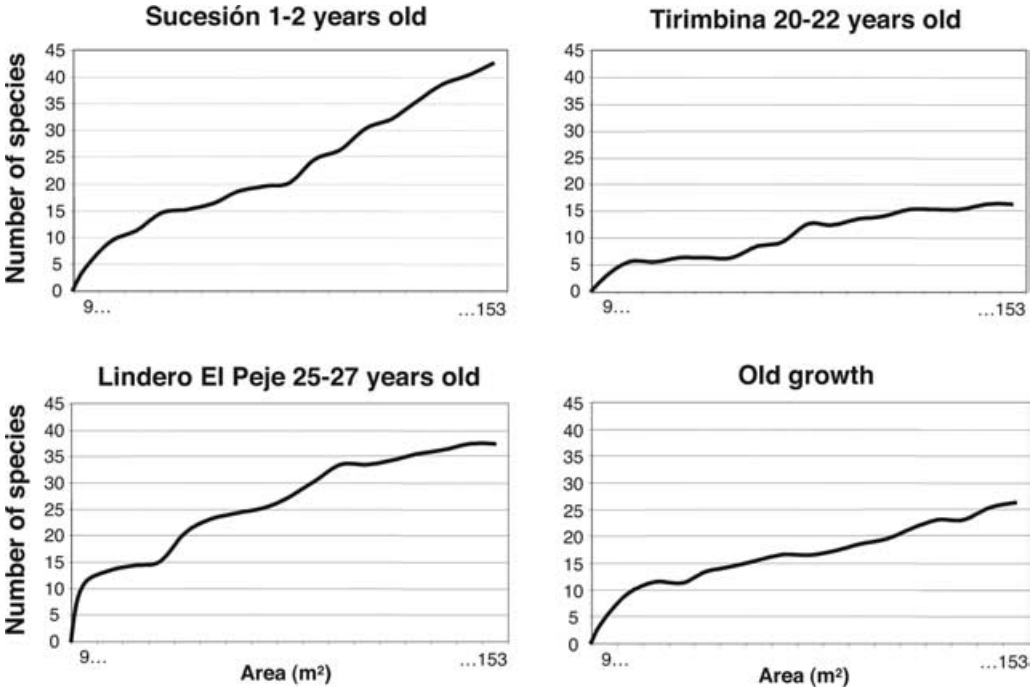

FIGURE 2. Species accumulation curves; area (m^2) versus number of species. Each bar represents a 3 × 3 m plot (9 m^2).

TABLE 2. *Hypocrealean fungi diversity (number of specimens) in four forest stands of different ages in Costa Rica.*

Species	Family[a]/genus[b]	Sub.[c]	Age of forest stand (yr)				
			Sucesión 1–2	*Tirimbina 20–22*[d]	El Peje *25–27*[e]	Old growth	Total
Albonectria rigidiuscula	N/*Albonectria*	s	0	0	2	0	2
Aschersonia aleyrodis	C/*Hypocrella*	i	4	0	2	2	8
A. andropogonis	C/*Hypocrella*	i	2	63	7	41	113
A. basicystis	C/*Hypocrella*	i	1	7	5	8	21
A. cubensis	C/*Hypocrella*	i	3	11	8	6	28
A. goldiana	C/*Hypocrella*	i	0	0	0	1	1
A. incrassata	C/*Hypocrella*	i	0	0	0	1	1
Aschersonia sp.[f]	C/*Hypocrella*	i	0	1	0	0	1
A. turbinata	C/*Hypocrella*	i	1	4	1	18	24
Ascopolyporus sp.[g]	C/*Ascopolyporus*	i	2	0	0	0	2
Balansia sp.[f]	C/*Balansia*	s	2	0	0	0	2
Bionectria byssicola	B/*Bionectria*	s	1	0	2	0	3
B. cf. *tornata*	B/*Bionectria*	s	0	0	1	0	1
B. ochroleuca	B/*Bionectria*	s/p	2	1	4	0	7
Bionectria sp.[f]	B/*Bionectria*	s	0	0	0	1	1
B. sporodoquialis	B/*Bionectria*	s	2	0	2	0	4
Calonectria colhounii	N/*Calonectria*	s	1	1	0	0	2
C. kyotensis	N/*Calonectria*	s	0	0	0	1	1
Chaetopsina cf. *polyblastiae*[g]	N/*Cosmospora*	s	1	0	0	0	1
Chaetopsina sp.[f]	N/*Cosmospora*	f	1	0	0	0	1
Clavicipitaceae[f]	C/?	i	0	0	0	1	1
Clonostachys sp.[f]	B/*Bionectria*	s	1	0	0	0	1
Corallomycetella repens	N/*Corallomycetella*	s/p	2	0	0	0	2
Cordyceps australis	C/*Cordyceps*	i	0	3	8	8	19
*C.*cf. *superficialis*[g]	C/*Cordyceps*	i	0	0	1	0	1
C. kniphofioides	C/*Cordyceps*	i	0	0	0	1	1
C. myrmecophila	C/*Cordyceps*	i	0	0	0	3	3
C. nutans	C/*Cordyceps*	i	1	0	1	0	2
C. owariensis	C/*Cordyceps*	i	0	0	0	2	2
Cordyceps sp.1[f]	C/*Cordyceps*	i	0	1	0	0	1
Cordyceps sp. 2[g]	C/*Cordyceps*	i	0	0	11	1	12
Cordyceps sp. 3[g]	C/*Cordyceps*	i	0	0	2	0	2
Cordyceps sp. 4[f]	C/*Cordyceps*	i	0	0	1	0	1
Cordyceps sp. 5[f]	C/*Cordyceps*	i	0	1	0	0	1
Cosmospora sp. 1[g]	N/*Cosmospora*	s	1	0	2	0	3
Cosmospora sp. 2[g]	N/*Cosmospora*	s	1	0	0	0	1
Cosmospora sp. 3[g]	N/*Cosmospora*	f	2	0	0	0	2
Cosmospora sp. 4[g]	N/*Cosmospora*	s	3	0	0	0	3
Cosmospora sp. 5[g]	N/*Cosmospora*	f	1	0	0	0	1
C. vilior	N/*Cosmospora*	f	0	0	0	1	1
Cylindrocladium sp.[f]	N/*Calonectria*	s	0	0	1	0	1
Didymostilbe cf. *capsici*[g]	B/*Didymostilbe*	s	0	0	2	0	2
Gibbellula cf. *pulchra*	C/*Torrubiella*	i	1	0	0	0	1
Gibberella sp.[f]	N/*Gibberella*	s	1	0	0	0	1
Gliocladium sp.[f]	H/*Sphaerostilbella*	f	0	0	0	1	1
Haematonectria haematococca	N/*Haematonectria*	s/p	17	1	8	1	27
Hirsutella sp.	C/*Cordyceps*	i	0	0	0	1	1
Hydropisphaeria peziza	B/*Hydropisphaeria*	s	0	0	1	0	1
H. suffulta	B/*Hydropisphaeria*	s	5	0	0	0	5

TABLE 2. *Continued.*

Species	Family[a]/genus[b]	Sub.[c]	Age of forest stand (yr)				
			Sucesión 1–2	*Tirimbina 20–22*[d]	El Peje *25–27*[e]	Old growth	Total
Hyperdermium bertonii	C/*Hyperdermium*	i	0	0	3	3	6
H. pulvinatum	C/*Hyperdermium*	i	1	0	0	0	1
Hypocrea cf. *capitata*[g]	H/*Hypocrea*	f	1	0	0	0	1
H. cf. *sulphurea*	H/*Hypocrea*	s	0	0	1	0	1
H. chlorospora	H/*Hypocrea*	s	0	0	0	1	1
H. koningii	H/*Hypocrea*	s	0	0	1	0	1
H. lixii/*T. harzianum*	H/*Hypocrea*	s/f	0	3	2	0	5
H. lutea	H/*Hypocrea*	s	0	0	3	0	3
H. rufa	H/*Hypocrea*	s	0	0	1	2	3
Hypocrea sp.[f]	H/*Hypocrea*	s	0	1	0	0	1
Hypocreaceae[h]	H/**	s	0	0	1	0	1
Hypocrella cf. *phyllogena*[g]	C/*Hypocrella*	i	0	0	0	1	1
Hypocrella sp. 1[g]	C/*Hypocrella*	i	0	0	0	1	1
Hypocrella sp. 2[g]	C/*Hypocrella*	i	1	0	0	2	3
Ijuhya cf. *paraparilis*[g]	B/*Ijuhya*	s	5	0	0	0	5
I. parilis	B/*Ijuhya*	s	1	0	0	0	1
Ijuhya sp.[g]	B/*Ijuhya*	s	1	0	0	0	1
Lanatonectria flocculenta	N/*Lanatonectria*	s	1	0	5	0	6
Metarrhizium anisopliae	C/*Cordyceps*	i	1	0	0	0	1
Nectria borneensis	N/*Nectria*	s	0	0	3	0	3
N. cf. *pseudocinnabarina*	N/*Nectria*	s	0	0	1	0	1
N. pseudotrichia	N/*Nectria*	s	0	0	5	0	5
Neonectria coronata	N/*Neonectria*	s	4	0	0	0	4
N. discophora	N/*Neonectria*	s	2	0	0	0	2
N. radicicola	N/*Neonectria*	s	0	0	2	0	2
Niesslia sp.[g]	Ni/*Niesslia*	s	2	0	0	0	2
Ophionectria trichospora	N/*Ophionectria*	s/p	11	0	1	0	12
Protocrea sp.[g]	H/*Protocrea*	s	3	0	0	0	3
Protocreopsis foliicola	B/*Protocrepsis*	s	5	0	0	0	5
P. fusigera	B/*Protocrepsis*	s	11	0	0	0	11
Sphaerostilbella aureonitens	H/*Sphaerostilbella*	f	0	1	0	0	1
Stilbella cf. *albocitrina*	N/*Stilbella*	s	0	0	0	1	1
S. minutissima	N/*Stilbella*	s	9	0	0	0	9
Stilbella sp.[f]	N/*Stilbella*	s	0	0	1	0	1
Trichoderma cf. *fertile*	H/*Hypocrea*	s	0	0	1	0	1
T. cf. *oblongisporum*	H/*Hypocrea*	s	0	1	0	0	1
T. hamatum	H/*Hypocrea*	s	0	1	0	0	1
Volutella sp.[g]	N/*Cosmospora*	s	1	0	1	0	2
Total individuals			119	101	104	110	434
Total species			42	16	37	26	87
Total new species/genus			12	0	7	2	19

[a]Family: B = Bionectriaceae; C = Clavicipitaceae; H = Hypocreaceae; N = Nectriaceae; Ni = Niessliaceae.

[b]Genus is indicated because in fungi the sexual and asexual forms have different names. If different forms of the species or genus were encountered, they were treated as being part of the same morph.

[c]Sub. = type of substratum; i = on insect; f = on fungus; s = on decaying plant material; p = on dying plant.

[d]Stand surrounded by second growth forest and plantation (forest fragment).

[e]Stand surrounded by old growth forest.

[f]Unidentifiable due to lack of diagnostic characters (*e.g.*, asexual stage, over or immature).

[g]New species

[h]New genus.

TABLE 3. *Fungal diversity summary of statistics in four forest stands of different ages in Costa Rica.*

Age-stand	# Plots (total area m^2)	# Species	# Individuals	H[ab]	h[ac]	HSD[d]
Sucesión 1–2 yr old	17 (153)	42	119	1.402	0.571	a
Lindero El Peje 25–27 yr	17 (153)	37	104	1.412	0.306	b
Old growth	17 (153)	26	110	1.003	0.317	b
Tirimbina 20–22 yr	17 (153)	16	101	0.657	0.216	c
Total	68 (612)	87	434			

[a]Shannon–Wiener diversity index. Maximum value: 3.5.

[b]H = Shannon–Wiener diversity index for each stand (all plots combined).

[c]h = Shannon–Wiener diversity index average per plot within each stand.

[d]Tukey's Studentized Range test, HSD = 0.281; ANOVA: $N = 17$, $\alpha = 0.05$, treatments = age-stands. Means with the same letter are not significantly different.

the forest aged. Sixteen individuals and three species were found in Sucesión and one individual in Lindero El Peje; no dying plants were found in the old-growth stand.

Of the 29 species on insects found in all subplots, the majority (18 spp.) was found in the old-growth stand and the least diversity (11 spp.) was in the Sucesión stand. In this case, the number of entomopathogenic fungal species increases as the age of the forest increases.

COMPARISON OF FUNGAL SPECIES DIVERSITY BETWEEN 25- TO 27-YR-OLD LINDERO EL PEJE AND TIRIMBINA.—The ANOVA resulted in a significant difference in species diversity (H) between Lindero El Peje stand surrounded by old-growth forest and Tirimbina stand surrounded by plantations and second-growth forest ($P = 0.0002$; Table 3). In addition, Tirimbina has a lower overall species diversity index than Lindero El Peje and the rest of the stands. Tirimbina resulted in a Shannon–Wiener index of 0.657, compared to 1.402, 1.412, and 1.003 in the 1- to 2-yr-old, Lindero El Peje, and the old-growth stands, respectively. Sixteen species and 101 individuals were found in the Tirimbina stand, compared to 37 species and 104 individuals in the Lindero El Peje stand and 26 species and 110 individuals in the old-growth stand (Table 3). While the number of individuals found was similar, the number of species was different.

When comparing families separately, Lindero El Peje always had a markedly higher number of species than Tirimbina (Fig. 3). In addition, the number of saprobic and entomopathogenic species were distinctly lower in Tirimbina than in Lindero El Peje and the old-growth stand (Fig. 4). Two individuals and two species fruiting

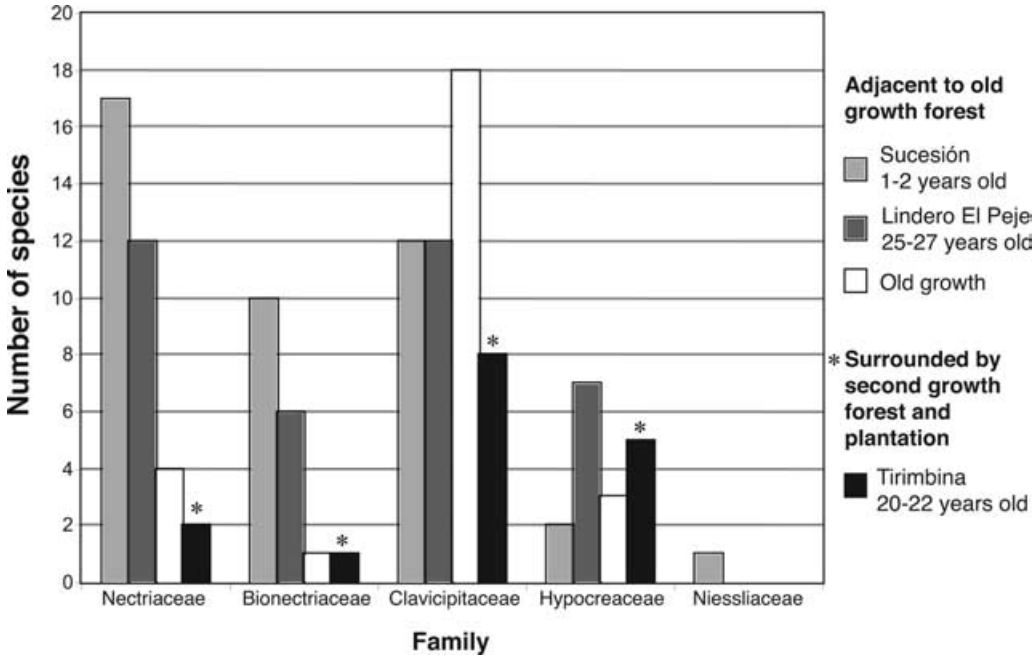

FIGURE 3. Number of species with respect to family and forest stand. This figure is based on data from Table 2.

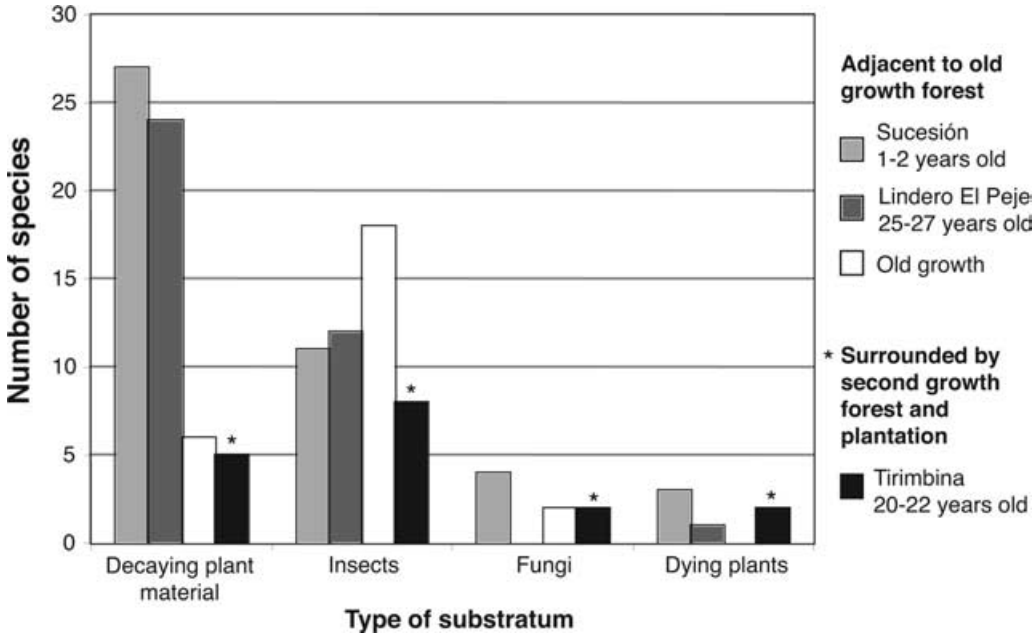

FIGURE 4. Number of species with respect to type of substratum and forest stand. This figure is based on data from Table 2.

on necrotic tissue of dying or sick plants were found in Tirimbina; only one individual was found in Lindero El Peje.

SPECIES COMPOSITION.—Eighty-seven hypocrealean species distributed in five families were found in this study (Table 2). In all the subplots, the genus *Cordyceps*, including all its anamorphs (*Hirsutella* and *Metarrhizium*), was the most diverse, with a total of 13 species, followed by *Hypocrella* (anamorph *Aschersonia*) and *Hypocrea* (anamorph *Trichoderma*) (Fig. 5) with 11 species each. *Cosmospora* (anamorph *Chaetopsina*) was the most diverse genus in the Sucesión stand, with eight species, followed by *Hypocrella,* with six species. *Cordyceps* and *Hypocrea* were the most diverse genera in Lindero El Peje stand, with six species each, followed by *Hypocrella,* with five species. In the old growth forest stand, *Hypocrella* was the most diverse genus (10 spp.) followed by *Cordyceps* (6 spp.). *Hypocrella* and *Hypocrea* were the most diverse genera in the Tirimbina stand, with five and four species, respectively.

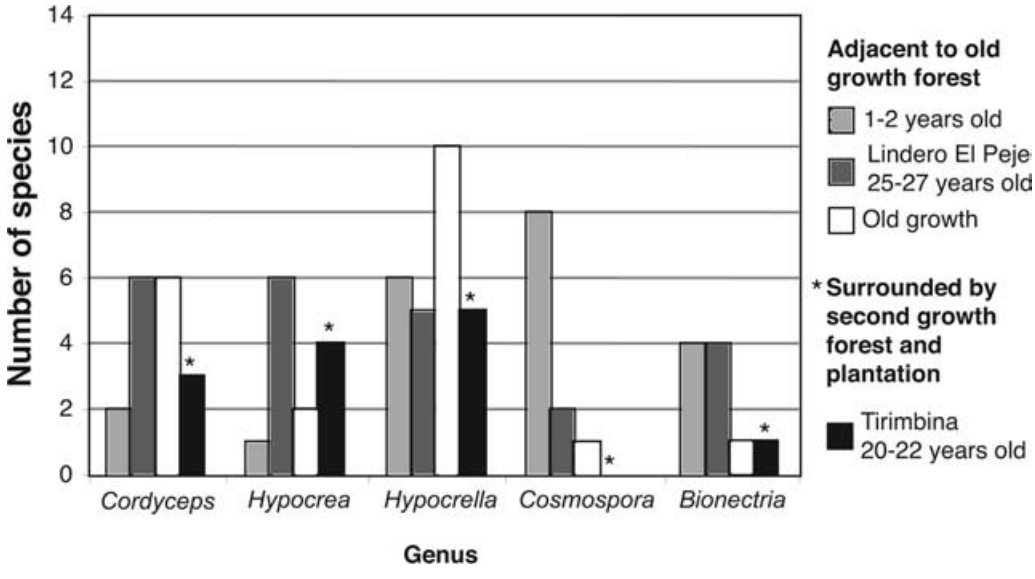

FIGURE 5. Numbers of species with respect to forest stand and the five most common genera. This figure is based on data from Table 2.

Overall, the family Clavicipitaceae was the most diverse with 31 species, followed by Nectriaceae with 30 species (Fig. 3). In addition, Hypocreaceae and Bionectriaceae had 15 and 13 species, respectively; only one species of Niessliaceae was found. Nectriaceae and Bionectriaceae were more diverse in the Sucesión stand, Clavicipitaceae had more species in the old-growth stand, and Hypocreaceae was more diverse in Lindero El Peje stand. Niessliaceae was found only in the Sucesión stand.

Although we found hypocrealean fungi on a variety of substrata, the results of our study confirm previous observations that the Hypocreales contains mostly saprobic species (Fig. 4). Fifty-nine percent of all the species found in all stands were on decaying plant material, such as wood, fruits, flowers, and leaves, 33 percent were on insects, 9 percent on other fungi, and 5 percent were on sick or dying plants. The most abundant saprophytes were *H. haematococca, O. trichospora*, and *Protocreopsis fusigera*.

The other hypocrealean species found were parasites of plants, insects, or other fungi. All of the fungi that were fruiting on the necrotic tissue of dying or sick plants were hypocrealean. These species included *Bionectria ochroleuca, Corallomycetella repens, H. haematococca*, and *O. trichospora*. The most abundant entomopathogenic species were *Aschersonia andropogonis, A. basicystis, A. cubensis, A. turbinata, Cordyceps australis*, and an unidentified species of *Cordyceps*. *Cosmospora* spp., *Hypocrea* spp. (anamorphs *Trichoderma* spp.), and *Sphaerostilbella* spp. (anamorphs *Gliocladium* spp.) were genera found growing on other fungi (mycoparasites).

There was little species overlap between stands (Table 4). In the Sucesión stand, 30 percent of the total number of species did not overlap with any of the other stands. In Lindero El Peje and the old-growth stands, 21 percent and 17 percent of the total number of species, respectively, did not overlap with any of the other stands. In the Sucesión stand, 26 species were not observed in any of the other stands; the majority (11 spp.) belong to the family Nectriaceae, which are mostly saprophytes or weak parasites of plants. In the old growth stand, 15 species were not found in any of the other stands; nine of these are members of the entomopathogenic family Clavicipitaceae. *Haematonectria haematococca, A. andropogonis*, *A. basicystis, A. cubensis,* and *A. turbinata* were found in all four stands.

Nineteen new species and one new genus were encountered in the stands (Table 2). Twelve species were found in the Sucesión stand, seven in Lindero El Peje, and two in the old-growth stand. The new genus was found in Lindero El Peje. The majority of the new species are in the genus *Cosmospora.*

DISCUSSION

FUNGAL DIVERSITY VS. SUCCESSIONAL STAGE OF THE FOREST STAND.—One objective of this study was to determine the relationship between hypocrealean fungal diversity and the different stages of forest succession. The data presented here suggest that a relationship between age of the stand and hypocrealean fungal species diversity exists in a tropical forest in Costa Rica. The results of this study demonstrate that overall hypocrealean species diversity decreased in later successional stages of a forest. Similar findings were reported in a study where wood-decaying basidiomycetous

TABLE 4. *Species overlap with respect to stand and family.*

			Family[b]				
	Stand[a]	Total number of species (percentage[c])	Bionect.	Clavicip.	Hypocr.	Nectri.	Niess.
No overlap	1	26 (30)	7	5	2	11	1
	2T	7 (8)	0	3	4	0	0
	2P	18 (21)	3	3	5	7	0
	3	15 (17)	1	9	2	3	0
Overlap	1, 2T	1	0	0	0	1	0
	1, 2P	7	2	1	0	4	0
	1, 3	1	0	1	0	0	0
	2T, 2P	1	0	0	1	0	0
	2T, 3	0	0	0	0	0	0
	2P, 3	3	0	2	1	0	0
	1, 2T, 2P	0	0	0	0	0	0
	1, 2T, 3	0	0	0	0	0	0
	1, 2P, 3	1	0	1	0	0	0
	2T, 2P, 3	1	0	1	0	0	0
	All	5	0	4	0	1	0

[a] 1: Sucesión stand, 2T: Tirimbina stand, 2P: Lindero El Peje stand, 3: Old growth stand.

[b] Number of species in each family, *i.e.*, Bionectriaceae, Clavicipitaceae, Hypocreaceae, Nectriaceae, and Niessliaceae, respectively.

[c] Percentage of the total number of species found in all stands ($N = 87$ spp.).

fungal species richness was negatively correlated to the age of forest stands (Norden & Paltto 2001). In contrast, most studies on fungal diversity have shown that fungal species richness was lower in disturbed forests than in the undisturbed sites (Albrecht 1991, Hagerman *et al.* 1999, Byrd *et al.* 2000). The reasons for these contradicting results are not clear, but it has been suggested that in older stands, increased competition due to the mycelial spread of fungi might lead to less colonization and, hence, lower diversity of fungal fruiting structures (Norden & Paltto 2001). Other authors also discussed that habitat disturbances may act on communities to prevent competitive exclusion, giving certain species better chances of finding patches free from competitors, especially in areas with relatively larger amounts of substrata (Palmer 1994, Lei & Hanski 1998).

One possible explanation for the higher hypocrealean fungal diversity in the younger stands is the larger amounts of decaying plant material compared to the older stands. In general, it was expected to find more species in the younger stands because there is more decaying plant material to colonize and the order Hypocreales includes mostly saprobes and weak parasites of plants (Samuels 1996b, Rossman *et al.* 1999). Even though the amount and type of decaying plant material was not measured in any of the stands established for the present study, we observed that there was more decaying plant material and sick or dying plants in the Sucesión (1–2 yr old) stand than in the older stands; the old-growth stand had smaller amounts of decaying plant material. In our study, we found that 59 percent of the total number of species found were on decaying plant material, 33 percent were on insects, 9 percent on other fungi, and 5 percent were on sick or dying plants. Therefore, there may be a positive correlation between quantity of decaying woody and leaf substrata and hypocrealean species diversity; this is supported by previous studies on other groups of fungi. These studies show that availability and abundance of substrata is an important factor in fungal biodiversity in forest ecosystems (Franklin *et al.* 1987, Esseen *et al.* 1992, Bader *et al.* 1995, Rossman *et al.* 1998, Norden & Paltto 2001). For example, species richness of basidiomycetous fungi was negatively affected by logging because there was a reduction of both substratum availability and quality; these factors led to a decrease in species richness (Bader *et al.* 1995). Other variables such as light, moisture, temperature, and plant and insect diversity, which were not evaluated in the present study, could have also influenced the production of fungal fruiting structures. The Sucesión stand was observed to have considerably more light than the rest of the stands, which may partly explain the higher abundance of fruiting structures. While light is not required for the vegetative growth of fungi, it has been reported that it is necessary in the induction of asexual and/or sexual fruiting structures (Alexopoulos *et al.* 1996). The evaluation of other environmental and ecological factors, in addition to forest age and degree of disturbance, may be necessary to further explain variation in hypocrealean fungal diversity.

Contradictory trends were observed when comparing the forest stands and the fungal species diversity in each family. Nectriaceae and Bionectriaceae, which include mostly saprobic and plant pathogenic species, were negatively related to the age of the stand (Fig. 3). In constrast, Clavicipitaceae, which contains mostly entomopathogenic species, had higher species diversity in the old growth stand than in the Sucesión stand. In the present study, we found that the majority of the entomopathogenic fungi (62%) were in the old-growth stand and their diversity was positively related to the age of the forest. These observations are important, for example, when looking for potential agents of biological control of plant pests, one is more likely to find them in old growth than in highly disturbed forests. Therefore, even though overall results indicate that fungal species diversity is negatively affected by forest age, this is not entirely accurate when analyzing taxonomically or ecologically different groups of fungi.

The diversity of plant disease-causing fungi also changed with the successional stage of the forest stand. Studies report that clear-cutting of forests, forest disturbance, and high abundance of decaying plant material may increase the incidence of plant diseases (Horne & Mackowski 1987, Horne & Hickey 1991, Hessburg *et al.* 2000, Thies 2001). Potentially plant pathogenic fungal species were more abundant in the recently cleared stand. *Haematonectria haematococca* (anamorph *Fusarium solani*), a well-known plant pathogen and saprobe in the family Nectriaceae, was more abundant on sick or dying plants in the Sucesión stand than in the rest of the stands, and was absent in the old-growth stand. Of all the potential plant pathogenic fungal species collected in all the subplots, 71 percent were found in the Sucesión stand (*i.e.*, *Balansia* sp., *Calonectria colhounii, C. repens, Gibberella* sp., and *H. haematococca*). These findings could have implications to agriculture or forestry, especially if the recently cut plant material is not eliminated from the area.

Some studies have suggested that singleton species be removed from the analyses (Polishook *et al.* 1996). This is based on the assumption that the detection of rare species is related to sampling intensity and their inclusion would probably underestimate the similarity between sites. Singleton species were not removed from the analyses to show a more comprehensive representation of the biodiversity sampled in this forest. In future studies that include a more exhaustive sampling of fungal diversity, analyses with and without singleton species are recommended.

FUNGAL SPECIES DIVERSITY IN FOREST FRAGMENTS.—The negative effects of forest fragmentation on plants and animals have been relatively studied, with the exception of a few studies related to fungi (Rukke 2000, Martinez-Garza & Howe 2003, Sverdrup-Thygeson & Lindenmayer 2003, de Castro & Fernandez 2004, Tabarelli *et al.* 2004, Zhu *et al.* 2004). Another objective of our study was to compare the fungal diversity of a forest fragment surrounded by plantations and second-growth forest (Tirimbina) and a stand surrounded by old-growth forest (Lindero El Peje). The Tirimbina stand, which is enclosed mainly by timber plantations and second-growth forest, had significantly lower fungal species diversity than the Lindero El Peje stand surrounded by old-growth forest and the rest of the stands. In this case, the reasons for a lower diversity are not apparent because no research has been done with fungi. However, other studies have reported that the effects of habitat fragmentation are being driven by several factors, one of them being the outside-patch influence of matrix habitat (Gascon & Lovejoy 1998, Chazdon 2003), for example, plant seed dispersal. Proximity

of disturbed areas to remnant forest patches promotes a more rapid recovery. Consequently, it is possible that one of the reasons for the low fungal species diversity in the forest fragment is caused by low influx of fungal spores from remnant old-growth forests. Low abundance of most species might also make tropical forest fragments especially susceptible to high rates of local extinction (Turner 1996, Maina & Howe 2000). As a result, fungal diversity may not fully recover in secondary succession forest stands or fragments that are surrounded by second-growth forests or cleared areas, and the probability of fungal species going extinct in second-growth forest fragments could be higher than in nonfragmented forests. Fungal diversity in old-growth tropical forests may not only be valuable for fungal recolonization in degraded or deforested areas, but also may contribute to the recovery of plant species. Fungi are major contributors to plant nutrition through decomposition, ammonification, nitrogen fixation, and solubilization of phosporus (Hoffman & Carroll 1995), and would have an important effect on the rapid colonization of plants and on forest succession.

Reestablishment of fungal diversity in second growth forests.—Fungal species diversity may be able to reestablish in some degree in second growth forests. Even though there are no studies with fungi, other studies with plants indicate that in secondary succession in tropical forests species can accumulate rapidly (Brown & Lugo 1990, Finegan 1996). It has also been reported that there is a large variation in the rate of recovery of diversity of plant species during secondary succession in the tropics, particularly if succession is taking place on highly degraded land (Finegan 1996). Additionally, other research indicates that recovery of forest structure, soil nutrients, and species richness is more rapid than recovery of species composition (Zou *et al.* 1995, Chazdon 2003). In our study, we found that fungal species diversity was slightly lower in Lindero El Peje (25–27 yr old) stand than in the old-growth stand; however, the ANOVA shows that there is no significant difference between the two stands. The fact that there was no significant difference in the species diversity between the Lindero El Peje and the old-growth stands suggests that some of the fungal diversity might be restored in secondary forests that are surrounded by old-growth forests.

Species composition was unique in each stand, suggesting that not all the original species composition may be restored. Overall, the majority of the species in the Sucesión stand, Lindero El Peje stand, and old-growth stand, did not overlap with any of the other stands (Table 4). In addition, only one species overlapped in the Sucesión, Lindero El Peje, and old-growth forest stands combined. However, there are other factors that could be related to the high complementarity (nonoverlap) of species. For example, the study was designed to detect differences in fungal diversity between sites. However, the incremental replacement of species (which may take place over a long time frame) may be difficult to detect and may require a different sampling design. Additionally, fungal distribution patterns could also play a role in the high complementarity. Limited knowledge is available about fungal distribution patterns and even similar sites that are a short distance apart may have a low percentage of species in common (Bills & Polishook 1994). The complexity of rain forest regeneration, including the rapid accumulation of plant species in secondary succession in tropical forests, it seems likely that stochastic factors also play a role in determining fungal species composition (Naeem & Wright 2003).

Interesting trends were observed in the species composition and complementarity of fungal species according to their substrate. The majority of the nonoverlapping entomopathogenic fungi found in the old-growth forest were not in any of the other stands. In contrast, the majority of the nonoverlapping saprobic species found in the Sucesión stand were not in any of the other stands and 27 percent of the nonoverlapping saprobic species found in the old-growth stand were not in any of the other stands (Table 4). These results suggest that as the forest ages and changes, fungal species composition also changes. Several studies have come to the conclusion that species replacement may have occurred in which some species are lost from certain stands and replaced by others (Smith *et al.* 2002, Peña-Claros 2003, Summerville 2004). Yet, there is no clear evidence that the fungal species composition in a severely disturbed area, such as a clearcut forest, would be restored to the original old-growth forest species composition.

Implications for conservation and agriculture.—Tropical forests are generally viewed as habitat to many endangered animals and plants and as a source of water and timber. Many conservation efforts have not succeeded because, among other things, they have failed to demonstrate the beneficial value of tropical forests to human activities and, therefore, stronger reasons to conserve it. Tropical forests, especially old-growth forests, should also be considered as sources of biodiversity for recolonization of degraded or deforested areas as well as sources of natural products (*e.g.*, biological control of pests and diseases). Fungi, especially those in the family Clavicipitaceae, not only maintain a balance of insect populations in natural forests, but also are the main source of economically important agents of biological control. If deforestation is causing their extinction, then ecosystem services, such as biological or natural control of plant pests in natural areas and agricultural landscapes, might also vanish.

Tropical forests may not only be a source of beneficial fungi, but can also harbor economically harmful species if the balance is broken. A consequence of deforestation is, in many cases, large amounts of leftover decaying plant material, which will be colonized by fungal saprobes. These fungal saprobes could then be dispersed to neighboring agricultural areas, become plant pathogens (*i.e.*, facultative parasites), and cause epidemics. This is supported by the fact that many fungi commonly found in natural forests can also cause important plant diseases (Crous 2002, Malaguti & Dereyes 1964, Nelson *et al.* 1981, Ploetz *et al.* 1996, Stover 1981).

Hawksworth (1991) has stated that only about 5 percent of the estimated 1,500,000 species of fungi have been classified. This statement indicates the great amount of undiscovered fungal biodiversity. The same report also acknowledged that most of these undiscovered fungi are in the tropics. In our study, we found that 22 percent of the species collected are new to science. If deforestation of tropical forests continues at the actual rate and an assessment of hypocrealean fungi, and for that matter, all fungi, is not undertaken in remaining forests, many fungal species that are important

to humans, agriculture, and forest ecosystems may disappear before they are discovered.

FUTURE WORK

To further elucidate how forest disturbance may affect hypocrealean fungal diversity, we emphasize the importance of (1) establishing more plots to determine the best sampling area that will recover the greatest diversity of species; (2) establishing plots in more young stands, especially between 2 and 20 yr olds; (3) collecting these fungi during different times of the year to recover a higher amount of fruiting structures; (4) evaluating hypocrealean endophytes and soil fungi because many of these fungi will not fruit and there is a high diversity of them based on results from previous studies; (5) measuring other variables that might correlate with hypocrealean species diversity, such as amount of decaying plant material, light, plant and insect diversity, among others; and (6) studying more in depth how forest fragmentation may affect the diversity of these fungi.

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