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Article

Morfotipos de ectomicorrizas en una cronosecuencia de rodales de *Pinus patula* Schiede ex Schltdl. & Cham. en la zona este de México

Ectomycorrhizae morphotypes in a *Pinus patula* Schiede ex Schltdl. & Cham. stand chronosequence in eastern Mexico

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Resumen

La actividad forestal en México se sustenta en el aprovechamiento de diversas especies de pinos; una de las más utilizados es *Pinus patula*. Los hongos ectomicorrízicos (HECM) incrementan la supervivencia de las plántulas, por lo que su uso asegura el éxito de las plantaciones; además, la reforestación con taxa nativos de pino aumenta la diversidad de HECM. Los objetivos de este trabajo fueron comparar la diversidad de HECM asociados a la edad de los rodales y determinar los cambios en la comunidad fúngica a nivel de morfotipos. El estudio se realizó en una plantación con rodales de 2, 5, 7, 9, 14 a 25 y >50 años. Se recolectaron raíces micorrizadas al inicio de la temporada de lluvias, se agruparon de acuerdo con su morfología y se calculó el índice de diversidad, porcentaje de abundancia y frecuencia relativa. Se determinaron 28 morfotipos, tres a nivel de género: *Laccaria* (2), *Tomentella* (2) y *Cortinarius* (1). Se obtuvo una correspondencia positiva entre el número de morfotipos y la edad del arbolado. Los rodales maduros (14-25 y >50 años) fueron más diversos, con respecto a los jóvenes (2 a 9 años); a excepción del de 7 años, cuyos valores coincidieron con los rodales maduros. Los morfotipos probables de *Laccaria* spp. fueron más abundantes y frecuentes en el rodal más joven (33 %). A través de la cronosecuencia se registraron morfotipos únicos y compartidos. Se concluye que la edad del rodal es un factor importante en la composición de la comunidad HECM, elementos clave en los planes de manejo forestal.

Palabras clave: Morfotipos, *Pinus*, plantación, reforestación, simbiosis ectomicorrízica, sucesión fúngica.

Abstract

Forest activity for timber purposes in Mexico is based on the harvesting of various pine species, one of the mostly used is *Pinus patula*. Ectomycorrhizal (ECM) fungi increase seedling survival, and, therefore, their use ensures the success of forest plantations. Reforestation with native pine species increases the diversity of ectomycorrhizal fungi. The aim of this work was to compare species diversity associated with stand age and to determine the changes in the fungal community at the morphotype level. The study was carried out in a commercial plantation, with stands aged 2, 5, 7, 9, 14 to 25 and > 50 years. Mycorrhizal roots were collected at the beginning of the rainy season and grouped according to their morphology, and the diversity index, percentage of abundance and relative frequency were determined. Twenty-eight morphotypes were determined, including two of the *Laccaria* genus, two of *Tomentella* and one of *Cortinarius.* There is a positive correspondence between the number of morphotypes and the age of the pines. Mature stands (14-25 and >50 yr-old) were more diverse than young (2 to 9 yr-old) stands, with the exception of the 7 year-old stand, which showed similar diversity values to the mature stands. Probable morphotypes of *Laccaria* spp. were more abundant and frequent in the youngest stand (33 %). Throughout the chronosequence, unique and shared morphotypes were recorded. This work requires further research; however, it shows that stand age is an important factor in the composition of the ECM fungal communities and a key element in forest management plans.

Key words: Morphotypes, *Pinus*, plantation, reforestation, ectomycorrhizal symbiosis, fungal succession.

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Introduction

Pinus patula Schiede ex Schltdl. & Cham. is one of the most commonly used subtropical species for reforestation in Mexico, with a high commercial value due to its fast growth and wood quality (Aparicio-Rentería, 2014). Forest activity in the country is based on the harvesting of some pine species in commercial forestry plantations, which represents 5% of timber production at the national level (López-López and Caballero *et al*., 2018). Due to this utilization, these plantations have short intervals for rotations, and these changes in the age classes (Spake *et al.,* 2015) reduce the richness of ectomycorrhizal (ECM) fungi (Kranabetter *et al.,* 2005).

Ectomycorrhizal symbiosis plays an important role in the acquisition of water and nutrients, mainly in species of the Pinaceae family, which facilitates the establishment and survival of seedlings (Guo *et al.,* 2020). ECM fungal communities are made up of assemblages of species that change in space and time (Peay *et al.,* 2010). Changes in the composition of ECM fungal communities are related to stand development; thus, stand age is a key factor in the dynamics of ECM fungi (Bonet *et al.,* 2004).

Fungal taxa are classified into two succession patterns under a model of adaptive strategies. The first consists of early-stage species that thrive in areas with high disturbance and low stress intensity, such as *Hebeloma* (Fr.) P. Kumm*, Lactarius* Pers. e *Inocybe* (Fr.) Fr*.*, which colonize the seedlings by means of spores that remain in the form of resistant propagules in the ground (Nara, 2008). The second includes late stage taxa, found in the roots closer to the trunk, associated with older and larger individuals (Nara *et al*., 2003a); belonging to such genera as *Boletus*, *Russula* and *Cortinarius*, which have the ability to colonize new hosts through extraradical mycelium; in addition, some Amanita species have been recorded in mature sites as cointroduced (Taylor and Bruns, 1999; Vlk *et al.,* 2020).

These succession patterns have been evaluated as a function of both sporomes (Jones *et al.,* 2003; Nara *et al.,* 2003a; Fernández-Toirán *et al.,* 2006; Dejene *et al.,* 2017; Gómez-Hernández *et al.,* 2019), as well as mycorrhizal root morphotypes (Nara *et al.,* 2003b; Horton *et al.,* 2005; Palfner *et al.,* 2005; Ashkannejhad and Horton, 2006;

Peay *et al.,* 2010; Reverchon *et al.,* 2010; Ma *et al.,* 2012, Palacios *et al.,* 2012). For example, an increase in morphotype diversity was observed in 30 to 60 year old stands in *Picea* A. Dietr. (Palfner *et al.,* 2005).

Other authors report an increase in the diversity of ECM fungi at the morphotype level in *Pseudotsuga menziesii* (Mirb.) Franco in 40 and 400 year old stands (Horton *et al.,* 2005) and in *Pinus densiflora* Siebold & Zucc*.,* in 55 and 80 year-old trees (Ma *et al.,* 2012) over a chronosequence. Guo *et al.* (2020) indicate that diversity of ECM fungi increased significantly with stand age, and the structure of the ECM fungal communities differs between age groups in 26, 33, and 43 year-old *Pinus sylvestris* var. *mongolica* Litv. stands, in all of which the *Wilcoxina* genus was the most dominant.

Species belonging to the Russulaceae and Thelephoraceae families are dominant in late successional stages of temperate ecosystems, where they play a critical role in nutrient cycling and are more adapted to the particular climatic conditions of the site (Erland y Taylor, 2002; Koizumi *et al.,* 2018).

As a result of reforestation with *Pinus* spp. in regions outside their range, some species of ECM fungi have been introduced into areas where their native host is not present, or where the existing native ECM propagules are incompatible with the introduced host. According to the review by Vellinga *et al.* (2009) and Vlk *et al.* (2020), around 300 introduced ECM species have been identified worldwide; with at least 54 genera recorded in pine plantations, among them: *Amanita, Astraeus, Boletus, Clavulina, Cortinarius, Gautieria, Gomphidius, Hebeloma, Inocybe, Laccaria, Lactarius, Pisolithus, Ramaria, Russula, Scleroderma, Suillus, Thelephora,* and *Tricholoma*. Taxa belonging to *Wicoxina, Suillus, Rhizopogon, Laccaria, Pisolithus*, and *Scleroderma* have ecological adaptations (*e.g.,* increased germination rate and spore longevity) that allow them to establish in early stages associated with hosts of the Pinaceaceae family, primarily.

Plantations with introduced hosts show a low ECM fungi richness (<50 taxa) in regard to the plantations established with native hosts (Ning *et al.,* 2020). Within this context, Alem *et al.*

(2020) document that, in a *Pinus patula* plantation in Ethiopia with different age classes (5, 11 and 36 years), 41 % of the operational taxonomic units (OTU) of the fungi identified were saprobes, 7 % were pathogens, and 2 % were ECM fungi.

In Mexico, although *P. patula* is a native species, fungal succession patterns in forest plantations have been little studied. Gómez-Hernández *et al.* (2019) identified a total of 63 taxa of EMF fungi and 43 saprobes in 1-, 11-, and 60-year-old stands, based solely on sporome sequencing, and Ramírez-Miguel *et al.* (2021) cited 14 ECM morphotypes in *P. patula and Q. crassifolia* roots; of these, *Lactarius* sp., *Cenococcum geophilum* Fr. and *Tomentella radiosa* (P. Karst.) Rick were the most frequent in both hosts.

Analyses based on both morphotype and sporome sequencing and morphological characterization of morphotypes and sporomes provide insight into the adaptive strategies of ECM fungi to establish in areas and hosts outside and within their distribution area. Although the morphological characterization of mycorrhizal roots has some methodological disadvantages, such as sample evaluation time and low identification to species level, in contrast to the advantages offered by molecular techniques on the latter aspect. This technique is used as a key strategy in nursery plant production, when controlled inoculations of ECM fungi are applied (Galindo-Flores *et al.,* 2015), and also as an important complement in studies on ectomycorrhizal symbiosis.

The objective of this study was to determine the changes in the ECM fungal community at the ectomycorrhizal level in stands of different ages in an established plantation of *P. patula*. Furthermore, this study highlights the importance of forest plantations as refuges of great fungal diversity and the high potential of these fungi to be selected in future research as forest inoculants of *P. patula*, which will favor a greater success in the establishment of its plantations in sites within and outside its natural distribution area.

Materials and Methods

Sampling site

Sampling was carried out in a *Pinus patula* plantation in *Coxmatla, Xico* municipality, *Veracruz* State (19°25'53.29" N, 97°04'47.91" W); at 2 209 masl, with 25 h (Figure 1). The plantation was established in 2010 with seeds from the region and plants produced in local nurseries. These sites correspond to the stand that had reached the age of 9 years by the sampling date, in 2019. The 7-year-old site was planted in 2012, the 5-year-old, site in 2014, and the 2-year-old site, in 2017. The 14-25 and 50+ year old areas were established as permanent measurement and observation sites to collect data on wood increments and volumes. All stands are pure *P. patula* and have a density of 1 100 plants per hectare at 3 × 3 m.

Figure 1. Central area of the state of *Veracruz*, location of the sampling site.

The 5, 7 and 9 year old sites for 2019 had a 30 % thinning. Only dry, malformed trees, and some suppressed ones, have been extracted from older stands. The total area of the plantation is characterized by natural relicts of *P. patula* and *P. pseudostrobus* Lindl. with a history of land use for cattle ranching. The soil exhibits compaction and degradation issues; however, during the last decade the plantation has been dedicated for conservation and research.

Sampling of roots

Six age categories were established: 2, 5, 7, 9, 14-25 and >50 years, and roots were collected from 10 randomly selected trees in each stand (Table 1); sampling was carried out during July 2018. For each individual, three replications were obtained, in the shape of a triangle, at a distance of 25 cm from the trunk (Guo *et al.,* 2020 modified); organic matter was removed and a metal nucleator (AMS) with a 5 cm diameter and a depth of 20 cm was used (Figure 2).

Table 1. Description of the sampled stands.

Stand	Age of the stand (years)	Altitude (masl)	Cordinates
S1	2	2 022	19°25'53.64120" N 97°04'47.77320" O
S2	5	2 008	19°25'52.32720" N 97°04'48.11880" O
S3	7	2 005	19°25'55.62480" N 97°04'42.85920" O
S4	9	2 018	19°25'50.71080" N 97°04'48.01800" O
S5	14-25	2 028	19°25'58.03680" N 97°04'37.15680" O
S6	>50	2 014	19°25'53.90400" N 97°04'41.40120" O

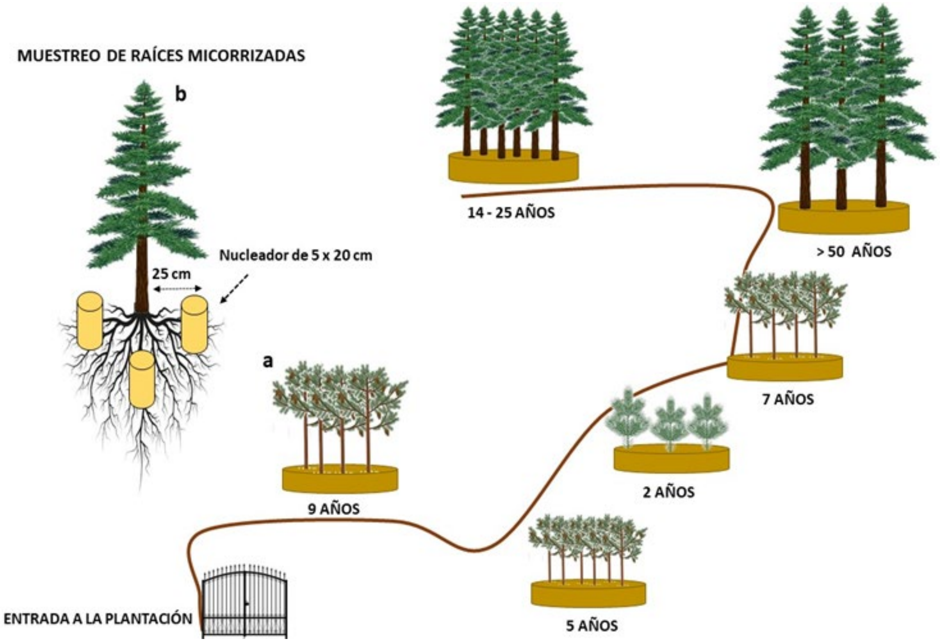

Figure 2. Distribution of stands within the *Pinus patula* Schiede ex Schltdl. & Cham. plantation (a); sampling (b).

The mycorrhizal tips were extracted from each sample. The largest roots were separated, and the remaining roots were washed and sieved with a 1 mm and a 2 mm sieve. These were dissected under a Leica EZ4 stereoscopic microscope, and roots with turgid mycorrhizae and with mantle were separated from the dead or non-mycorrhizal ones. They were carefully cleaned with distilled water. The morphological characters of mycorrhizae were described macroscopically in order to divide them into morphotypes according to their morphological characters, using the standard methodology of Agerer (1991): type of branching, color, tip branching, mantle color, rhizomorphs, and emanant hyphae. Each morphotype was photographed with a Motorola G8 camera with a 40X magnification.

Statistical analyses

The Shannon-Wiener (*H*') and Simpson (*D*') richness and diversity indices were estimated. These variables were compared among the different ages of the stands using a generalized linear model, LSD Fisher comparison test with a significance level of 95 %, after checking the assumptions of normality and heterogeneity of variance in the data. The Statistica 9.0 (StatSoft, Inc.), *EstimateS* 9.1.0, *PAST* softwares were used (Hammer *et al.*, 2001).

Relative abundance was calculated by dividing the number of mycorrhizal roots per morphotype by the total number of mycorrhizal roots for each stand. Relative frequency was estimated as the frequency of each morphotype divided by the sum of the frequencies of all morphotypes present per stand. The influence of stand age on the distribution of morphotypes according to their presence and abundance was determined by means of a detrended correspondence analysis (DCA). A Venn diagram was constructed to compare the shared and exclusive morphotypes per stand.

Results

A total of 1 849 mycorrhizal tips were assessed; from a total of 30 samples per stand studied, which morphologically corresponded to 28 morphotypes. Five morphotypes were tentatively identified at the genus level: *Cortinarius* sp., *Laccaria* spp., and *Tomentella* spp. (Figure 3); the remaining ones could not be identified due to the absence of diagnostic characteristics.

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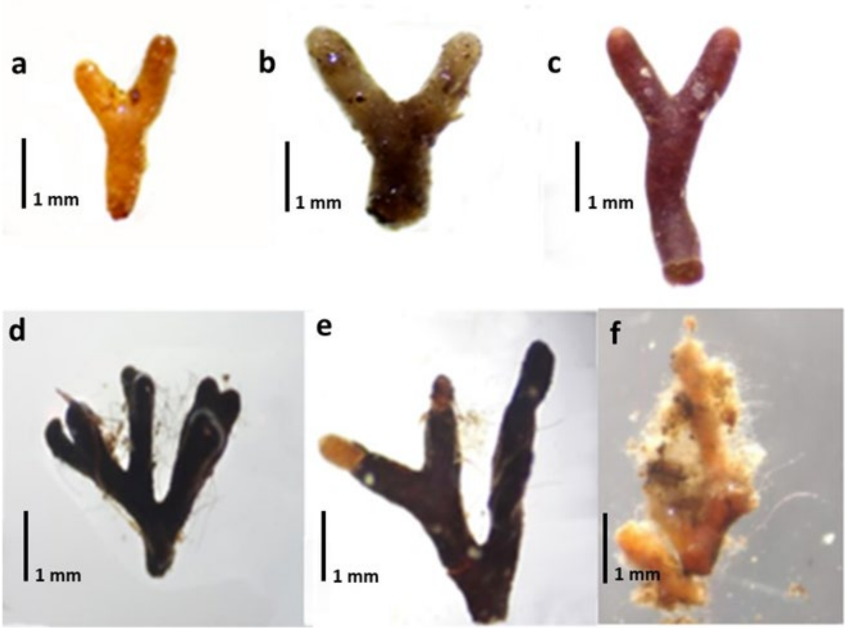

(a) *Laccaria* spp.; (b, c) Unidentified morphotype (M6, M7); (d, e) *Tomentella* spp., (f) *Cortinarius* sp. (scale bar = 1 mm).

Figure 3. Morphology of the most frequent ectomycorrhizae and their probable identification in a chronosequence of *Pinus patula* Schiede ex Schltdl. & Cham.

The stand with the highest richness of morphotypes was S5 with 14, which corresponded to 50 % of the total recorded; followed by S6, with 9 morphotypes (32.1 %); S3 with 8 morphotypes (28.5 %); S2 with 7 morphotypes (25 %); S4 with 6 morphotypes (21.4 %), and S1 with 5 morphotypes (17.8 %) (Figure 4, Table 2).

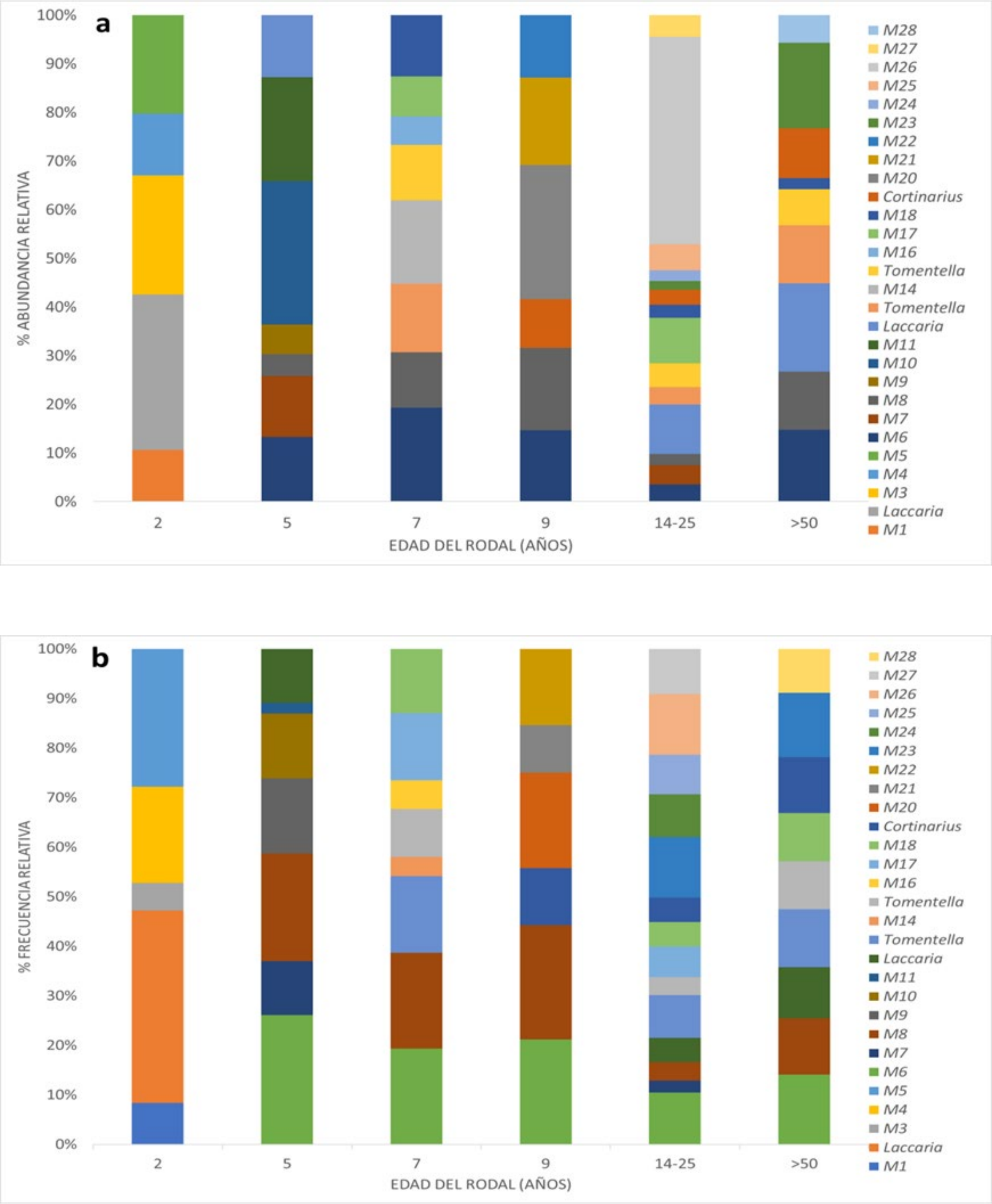

Figure 4. Relative abundance (a) and relative frequency (b) of morphotypes of EcM fungi in six stands of *Pinus patula* Schiede ex Schltdl. & Cham. of different ages

Table 2. EMF community ɑ-diversity indices (based on morphotypes) in *Pinus patula* Schiede ex Schltdl. & Cham. stands of different ages.

Stand	**Number of morphotypes**	**Total number of mycorrhizal tips analyzed**	**Morphotypes**	***Simpson* (D´)**	***Shannon Wiener* (H')**
S1	5	94_{c}	M1, *Laccaria* spp., M3, M4, M5	0.76±0.04	$1.53\pm0.07_{d}$
S2	7	360_{b}	M6, M7, M8, M9, M10, M11, *Laccaria* spp.	0.81±0.06	$1.79\pm0.02_{c}$
S3	8	326_{bc}	M6, M8, *Tomentella* spp., M14, M16, M17, M18	0.86±0.08	$2.02\pm0.05_{b}$
S4	6	668_{a}	M6, M8, *Cortinarius* sp., M20, M21, M22	0.81±0.05	$1.74\pm0.02_{c}$
S5	14	225_{bc}	M6, M7, M8, *Laccaria* spp., *Tomentella* spp., M17, M18, *Cortinarius* sp., M23, M24, M25, M26, M27	0.78±0.04	$2.07\pm0.06_{a}$
S6	9	176_{bc}	M6, M8, *Laccaria* spp., *Tomentella* spp., M18, *Cortinarius* sp., M23, M28	0.86±0.06	$2.08\pm0.05_{a}$

The ɑ-diversity values represent the averages per 30 samples per stand (three replicates per tree per stand). Letters represent significant differences ($P < 0.05$).

Significant differences were observed in the abundance of ECM fungi with respect to stand age ($P = 0.002$). The highest total abundance of mycorrhizal apices was found in S4, with 36 % (668); followed by S2, with 19.4 % (360); S3, with 17.6 % (326); S5, with 12.16 % (225); S6, with 9.5 % (176), and S1, with 5.08 % (94) (Figure 4).

The probable morphotype of *Laccaria* spp. exhibited a relative abundance (*Ai*) of 33.9 % in the 2-year old stand (S1) and a relative frequency (*Fi*) of 38.8 %. In S2, morphotype M10 showed the highest values for *Ai* (29.44 %) and Fi (13.04 %). Morphotypes M6 (*Ai*=19.3 %) and M14 (*Ai*=17.1 %) had the highest values in S3; however, M14 exhibited a relative frequency of 3.8 %, the lowest for this stand. In S4, M20 had a high Ai value (27.5 %), with a *Fi* of 19.23 %. Stands S5 and S6 shared a large number of morphotypes, with a dominance of morphotype M26 in S5, whose Ai was 41.9 % and *Fi* of 12.28 %. In S6, the relative abundance and frequency for each morphotype was similar. Morphotypes M6 and M8 were identified in all stands, except in S1 (2 years), where they contributed 23.4 % of the total abundance (Figure 4).

The α-diversity indices (Table 2) indicated an increase in diversity in relation to stand age; the older stands S5 and S6 (*H*' = 2.07±0.06, *H*' = 2.08±0.05) exhibited higher values compared to the younger ones, with the exception of S3, which had a similar *H'* index to the one estimated for stands S5 and S6.

The detrended correspondence analysis (DCA) shows the distribution of all morphotypes of ECM fungi in the different ages of the stands, and explains 78 % of the variability of the data in axes 2 (64 %) and 3 (14 %) (Figure 5). The graph shows the distribution of the morphotypes and divides them into four groups: G I consists of the morphotypes shared in S3, S5 and S6, among which many intermingled units are observed, suggesting a high similarity between their EMF morphotype assemblages. The second group (G II) is composed of M1, *Laccaria* spp., M3, M4 and M5 in the 2-year-old stand, which were not found in the other stands. A third group (G III), with M7, M9, M10 and M11, present only in the 5-year old stand; and a fourth group (G IV) of morphotypes distributed in the 14-25 year-old stand (S5).

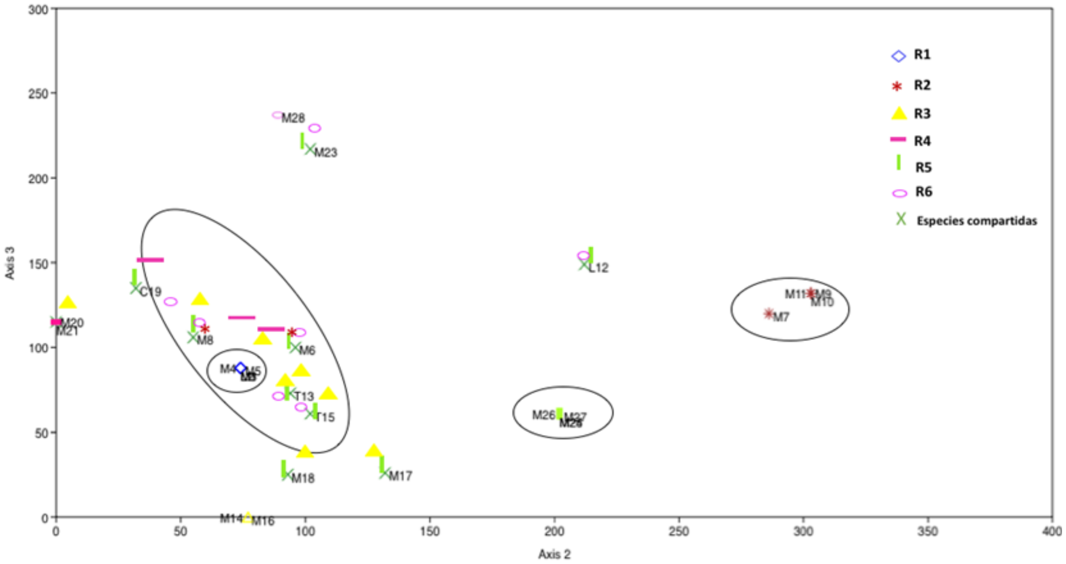

Figure 5. Detrended correspondence analysis (DCA) for EMF morphotypes present in six stands of different ages in a *Pinus patula* Schiede ex Schltdl. & Cham. plantation.

Throughout the chronosequence, unique and shared morphotypes were registered. Morphotypes M6 and M8 were observed in all stands, with the exception of S1, which had no morphotypes shared with the rest of the stands. The largest number of single morphotypes (4) was obtained in S5, while S6 included only one single morphotype (M28). Older stands (S5 and S6) shared a larger number of morphotypes (Figure 6).

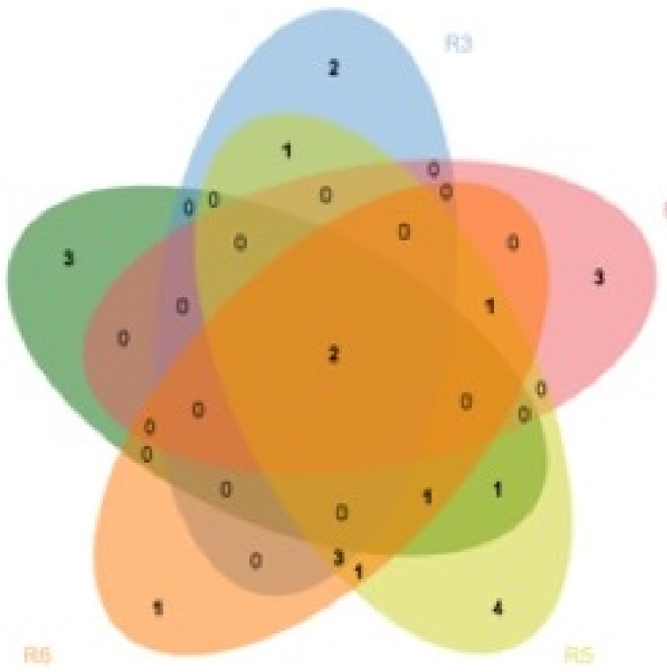

Figure 6. Shared morphotypes among *Pinus patula* Schiede ex Schltdl. & Cham. stands of different ages. The 2-year-old stand (S1) is excluded because it does not share any morphotype with the rest of the stands.

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Discussion

The ɑ-diversity indexes showed differences between stand ages. The diversity at the morphotype level was expected to increase with stand age; although there was indeed an increasing trend in the 14-25 and >50 year-old stands, the results showed similar diversity values in the 7 year-old stand. This can be explained by the fact that the 7-year-old stand has had a 30 % thinning, and, thus, more light was allowed to enter the understory plants, which contribute to improve the availability of water and nutrients in the soil (Dang *et al*., 2018).

The 2-year-old stand has not yet received the first thinning and has a higher density of trees and, therefore, less light input, which may have resulted in the low number of morphotypes. Gómez Hernández *et al.* (2019) observed a similar pattern in intermediate stages of development in 11 year-old plantations of *P. patula* and *Quercus rugosa* Neé, where the diversity of ECM fungal species was similar to that of mature (60-year-old) stands with a mixed tree composition *(P. patula, P. douglasiana* Martínez*, P. teocote* Schiede ex Schltdl. & Cham., *Q. rugosa*, and *Q. laurina* Bonpl*.*), while it decreased in young stands (1 year) consisting exclusively of *P. patula*. The authors point out that canopy development and tree density are important factors in sporome production and in the increase in the diversity of ECM fungi.

Although the present study is exploratory, its results show that succession processes directly influence ECM fungi and that diversity is modulated by the time of plantation establishment. Older stands tend to provide better habitats for ECM fungal species than younger, heterogeneous stands. Unlike cited by Dejene *et al.* (2017) in sites where the *Pinus* genus is an introduced species, few species of ECM fungi and low diversity associated with *P. patula* are registered, due to the small amount of compatible propagules in the soil. These authors note that they found no ECM fungal species in 5-year-old stands, but cite an increase in sporomes of ECM fungi in 11 to 36-year-old stands.

Alem *et al*. (2020) suggest that thinning and previous land use are the main factors modulating edaphic ECM fungal communities in *P. patula*. These authors suggest that 5 and 36 year-old stands are more diverse in terms of ECM fungal species than 11 year-old intermediate stands, due to the poor development of the canopy and to the possibility that the site was previously used for agricultural purposes. For this reason, it has a high number of propagules associated with young stages, from the spore bank of adjacent plantations. The same has been observed in other conifers planted in areas outside their natural distribution range, such as *Pinus radiata* D. Don. This species has been studied in 20-year-old stands, which included a total of 11 morphotypes and exhibited a low diversity of ECM fungi, compared to 5 and 10 year-old stands, the latter of which has a lower canopy density (Palacios *et al.,* 2012).

The highest values for diversity of ECM fungal species were found in the 14-25 years old and >50 years old stands, which reflects the difference between the forestry interventions applied. Parladé *et al.* (2017) and Tomao *et al.* (2017) mention that the intensity of partial thinning is a factor that favors the appearance of ECM fungi sporomes but does not necessarily affect species diversity at the root level (Castaño *et al.,* 2018). Although this parameter was not directly evaluated in the study, it should be considered under different soil and climatic conditions in order to model trends regarding its effect on other functional groups of fungal diversity.

Figure 5 shows that, in the 7, 14-25, and >50 year old stands, most of the morphotypes are shared, which implies that the age of the stand does not necessarily determine either the diversity or the composition of the ECM fungal community. Castaño *et al.* (2019) point out that the diversity and abundance of ECM fungi species increase with tree age, but are directly affected by the availability of N and P, as well as by the increase in the C/N ratio.

In the present work, a high number of morphotypes of ECM fungi associated with a plantation of *P. patula* were registered through the use of morphological characterization of ectomycorrhizae. This method has limitations due to the difficulty in differentiating between species, which generates the underestimation or overestimation of the wealth of species associated with a host. Undoubtedly, molecular methods are useful in the identification of these ECM fungal communities, as they allow to clarify the identity of fungal taxa. However, morphological methods are a first approach to the differentiation of replacement and dominance patterns of species associated with hosts, about which —it should be noted— little or no information is available. Such is the case of *P. patula*, a taxon with a high potential for forest exploitation within and outside its natural distribution range.

Some morphotypes have been described in Mexico, at early stages of *P. patula*: *Boletus edullis* Bull., *Laccaria bicolor* (Maire) P.D. Orton*, L. proxima* (Boud.) Pat.*, Hebeloma alpinum* (J. Favre) Bruchet*, H. leucosarx* P.D. Orton*, H. mesophaeum* (Pers.) Quél, and *Suillus pseudobrevipes* A.H. Sm. & Thiers (Carrera-Nieva and López-Ríos, 2004; Carrasco-Hernández *et al.,* 2010; Jiménez*,* 2011), mainly in experiments with controlled inoculation. However, this is one of the first studies to explore mycorrhizal roots at the morphotype level, within a chronosequence in stands of the same host. Yet, further research, at the molecular level, is still required (Ramírez-Miguel *et al*., 2021).

Ectomycorrhizal fungi play a structural role in determining the conditions for the establishment and development of different forest species (Pérez-Moreno *et al.,* 2020). Therefore, it is essential to assess fungal diversity in plantation forests from the perspective of understanding such forestry practices as partial logging, low intensity thinning (30-50 %), mixing of native plant varieties, and the development of stands that promote interconnectivity within the forest matrix and ensure the establishment of pioneer ECM fungal species.

Conclusions

The diversity of morphotypes of ECM fungi is related to stand age, although it is not the only factor. The highest diversity values were found in 7, 14-25 and >50 year old stands. The composition of ECM fungal communities is clustered into single morphotypes in the youngest stand and similar ectomycorrhizas among the 7, 9, 14-25 and >50 year old sites. The most abundant and frequent morphotypes of ECM fungi in roots of *P. patula* were determined, describing *Laccaria* as one of the most abundant in the youngest sites. This work highlights the importance of forest plantations as refuges of fungal diversity.

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Conflict of interest

The authors declare no conflict of interest.

Contribution by author

Yajaira Baeza Guzmán and Dora Trejo Aguilar: study design, dissection of roots, statistical analyses and interpretation of results; Jesús Dorantes López: establishment of the *Pinus patula* plantations, which made this study possible by creating a natural laboratory. Yajaira Baeza Guzmán, Rosario Medel Ortiz and Dora Trejo Aguilar: contribution to the drafting and review of the final manuscript.

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