

Article

Silvicultural Practices Shape Fungal Diversity and Community Composition: Metabarcoding Study in a *Pinus* Forest in Central Mexico

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Abstract

Silvicultural practices significantly influence the diversity and composition of soil fungal communities, which play crucial roles in maintaining forest ecosystem functionality. This study evaluated the impact of three silvicultural treatments, consisting of liberation cutting, first thinning, and second thinning, on rhizospheric fungal and ectomycorrhizal (ECM) fungi communities in *Pinus* forests located in Puebla, Mexico. Using high-throughput metabarcoding of the internal transcribed spacer (ITS2) region, we identified 346 fungal genera across all treatments, with Ascomycota and Basidiomycota being the dominant phyla. Alpha diversity indices revealed a trend toward higher fungal richness for first thinning, followed by liberation cutting and lower values for second thinning. A beta diversity analysis demonstrated significant shifts in the fungal community composition across treatments, highlighting the influence of the thinning intensity. The proportions of different functional guilds were consistent across the treatments. However, compositional differences were observed, mainly in soil and wood saprotrophs and in pathogenic taxa. Liberation cutting showed enrichment in ECM taxa such as *Russula* and *Cenococcum*, whereas *Tuber*, *Humaria*, and *Tricholoma* were decreased for first thinning and *Russula* was decreased for second thinning. These findings underscore the need for sustainable forest management practices that balance productivity with the conservation of fungal biodiversity to ensure ecosystem stability and functionality.

Keywords: forest management; high-throughput sequencing; *Pinus*; soil fungi; thinning practices



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1. Introduction

Tree–fungi associations significantly impact the entire forest ecosystem by playing a crucial role in nutrient cycling, affecting plant recruitment, and supporting biodiversity.

These associations influence the spatial and genetic structure of forest communities [1,2]. Therefore, conserving fungal diversity is vital for maintaining ecosystem services that are essential for both forest functionality and human well-being [3,4].

Based on their ecological function, soil fungi are categorized as saprotrophic, pathogenic, or symbiotic. Saprotrophic fungi play a crucial role in nutrient cycling by decomposing soil organic matter and plant litter, contributing significantly to soil carbon resources that support plant growth [5]. In contrast, pathogenic fungi can negatively impact forest health by infecting roots or aboveground tissues, leading to reduced growth, increased mortality, and shifts in the community composition.

Symbiotic fungi, such as ectomycorrhizal (ECM) fungi, have attracted the most attention due to their role in providing their tree hosts with essential nutrients and protection against pathogens in exchange for photosynthetically derived carbon [6–8] and by transporting and storing carbon in the deep layers of the soil, playing a role in the regulation of C dynamics in ecosystems [9]. This relationship begins at the earliest stages of seedling establishment, integrating the plant and fungi into a mycorrhizal network that is critical for sapling survival, tree performance, and productivity [10–12]. Furthermore, ECM fungi influence the spatial and genetic structure of forest communities [1,2] by establishing physical connections among trees, even between different species [13].

Forest management influences the fungal diversity and composition by modifying stand structure attributes (such as tree density and species composition), altering soil and microclimatic conditions, and introducing other anthropogenic disturbances like mushroom harvesting, animal grazing, logging, controlled burning, and fertilization [14–16]. Thus, understanding the effect of silvicultural practices on mycological resources must be seen as an integral part of long-term forest management strategies [13,15,17].

In Mexico, one of the main methods for wood production is the “Silvicultural Development Method” (SDM), which has been used since the early 1970s [18]. The SDM is a high-intensity forest management system designed to maximize the productive potential of timber sites by applying various silvicultural treatments. These include regeneration cuts, which keep only selected trees (father trees or seed trees) with desirable physical traits, such as straight trunks, while removing the rest; liberation cutting, consisting of the removal of the seed trees; and thinning cuts, in which trees that do not meet specified commercial characteristics or other management criteria are selectively removed [19]. After the cut, the natural regeneration of a cohort of the harvestable species occurs in the open site, resulting in a predominantly even-aged forest at the end of the cycle [20]. The effect of the SDM on biodiversity is influenced by the cutting/thinning intensities. Therefore, from a sustainable forest management perspective, its implementation must consider the conservation of biological diversity, forest productivity, the regenerative capacity, and the maintenance of ecosystem services [18,19,21,22].

The effect of the SDM on the plant composition and biodiversity depends on factors such as the extent of the intervention, the original biodiversity of the area, and the number of cutting/thinning cycles [23–26]. Generally, ecosystems with higher biological complexity, such as tropical forests, experience more significant changes, especially when subjected to multiple cutting cycles [27–29]. Forest fungal communities are similarly affected by thinning and other silvicultural practices [15] that result from altering the local environment (soil properties, light availability, and humidity), as well as shifts in plant diversity and structures, to which mutualistic organisms, such as ECM fungi, are particularly susceptible [15,30–32]. The analysis of ECM mushroom production in Mediterranean forests in response to management intensity has shown that low to moderate thinning can increase yields [15,16], whereas heavy thinning or clear-cutting drastically reduces production, fungal richness, and diversity due to the removal of host trees and increased

soil temperature and dryness [15,16]. Furthermore, the intensity of silvicultural practices influences the abundance of particular species. Contrasting dynamics in belowground fungal communities, as evidenced by high-throughput sequencing, have been reported in *Pinus sylvestris* L. stands [33,34] and *P. pinaster* Ait. forests [15] managed under various thinning intensities. These studies reveal changes in the fungal composition, with ECM communities decreasing and saprotrophs increasing after treatments.

Although there is substantial evidence supporting the differential response of fungal communities, especially ECM, to silvicultural practices in temperate forests of the Northern Hemisphere, less is known about their impact on highly diverse tropical and subtropical montane forests [16]. In Mexico, evidence of the impact of different silvicultural practices is increasing, exhibiting both general patterns but also preliminary lists of the main taxa involved. In a managed *P. patula* Schiede ex Schltdl. & Cham. forest in Puebla in Central Mexico [14], as well as in a mixed stand of *Pinus* spp. in the southern Mexican state of Chiapas [26], a decline in ECM species richness and abundance, as evidenced by carpophore production, has been observed as the canopy cover decreases, with the most intensive management practices leading to significantly lower diversity compared to later successional stages. The ITS-RFLP analysis of ECM root tips in a *P. oaxacana* forest in the southern State of Oaxaca showed that although the fungal abundance remained similar under various silvicultural treatments, the species composition was altered, and the sustained presence of seed trees was key to preserving diversity [35]. The sequencing of mycorrhizal root tips was used to characterize ECM fungal taxa colonizing both young and mature *P. montezumae* Lamb. var. *montezumae* in central Mexico, revealing that the tree age is key in maintaining ECM fungal richness and diversity, as the fungal species composition varied with the growth stage [36].

These studies have provided insight into fungal diversity and its response to silvicultural practices in tropical and subtropical pine forests. However, significant gaps persist in our knowledge of how these communities are structured, maintained, and reshaped by forest management. The main purpose of this study was to evaluate the impact of different silvicultural practices, specifically liberation cutting (LC), first thinning (FT), and second thinning (ST), on soil fungal communities in a managed *Pinus* forest in central Mexico. We sought to answer the following questions: (1) Do different silvicultural practices affect soil fungal richness and diversity? (2) Do they alter the community composition? (3) Which sustains greater ECM richness and diversity? (4) Do they favor the presence of certain species? To address these questions, we employed a high-throughput sequencing approach targeting the internal transcribed spacer (ITS2) region of ribosomal DNA from mycorrhizal root tips. Assessing overall fungal biodiversity, identifying ECM associations, and examining their response to silvicultural treatments can provide indicators to support sustainable management strategies that better preserve or enhance plant–fungal symbioses, particularly in forests and plantations overseen by local communities.

2. Materials and Methods

2.1. Study Sites

This study was conducted at the Ejido Rancho Nuevo Nanacamila in the Zacatlán de las Manzanitas municipality in Puebla, Mexico, at coordinates 20°02′54.24″, 20°04′30.00″ N, and 98°04′42.24″, 98°06′38.88″ W. The ejido forest management program is certified by the Rainforest Alliance (FSC certificate, RA-FM/COC-006372) (Figure 1a). The tree mass under management by the local community covers 283.13 ha. The predominant vegetation consists of *Pinus patula* Schiede ex Schltdl. et Cham. forests with the presence, in smaller proportions, of *P. teocote* Schiede ex Schltdl. et Cham., *P. leiophylla* Schiede ex Schltdl. et Cham., *P. rudis* Endl., *P. pseudostrobus* Lindl., *Abies religiosa* (Kunth) Schltdl.

et Cham., *Quercus* spp., and *Arbutus xalapensis* Kunth. We selected three silvicultural treatments, aiming to keep the post-intervention times as close as possible across the sampled plots: liberation cutting (LC) with seven years (Figure 1b), first thinning (FT) with 9 years (Figure 1c), and second thinning (ST) with 7 years (Figure 1d). The sampled experimental plots measured 33×33 m for each management type in accordance with [14]; there were three for FT and two for LC and ST. Sampling was conducted in November 2016, at the end of the rainy season.

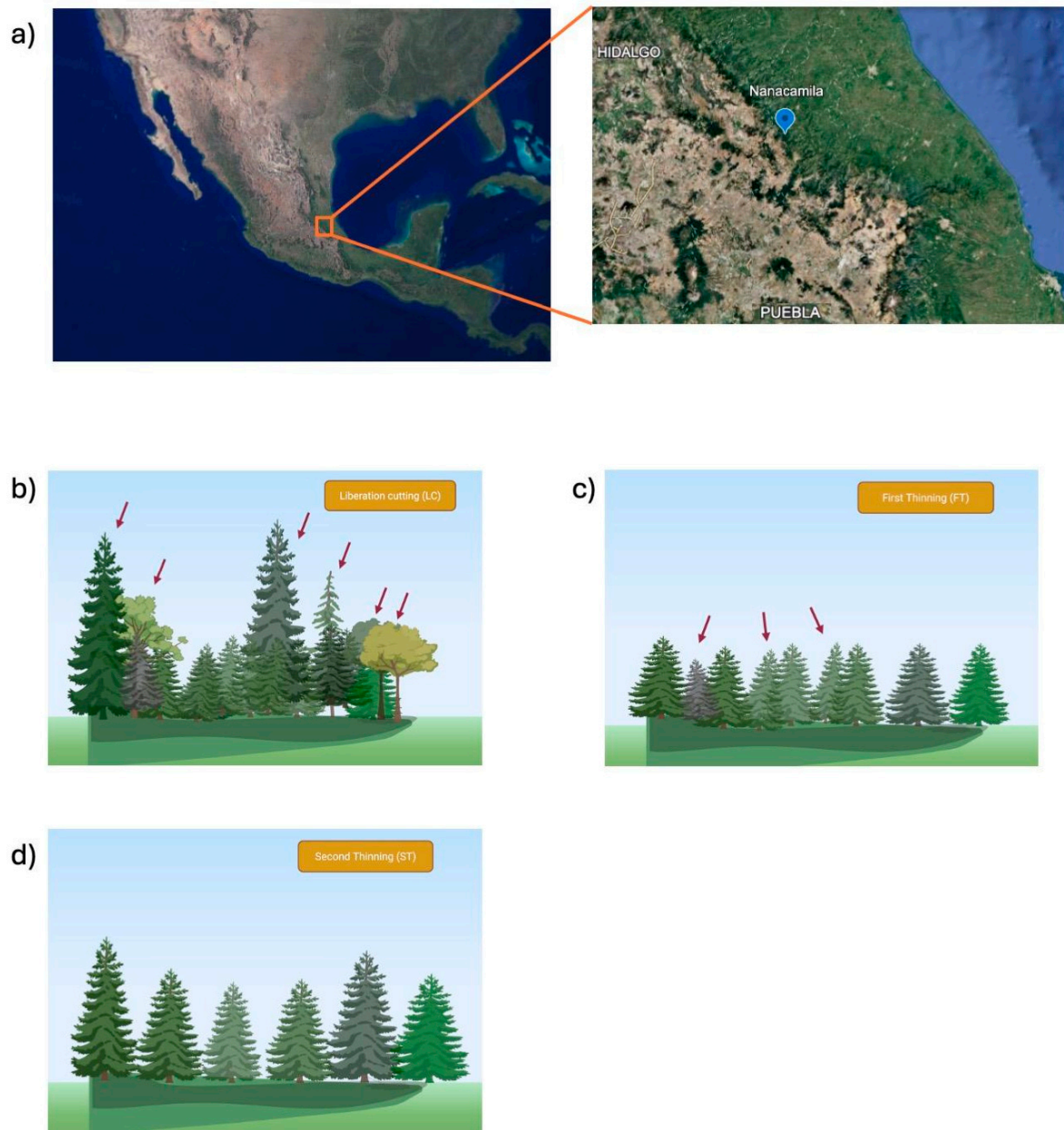


Figure 1. (a) Geographic location of the study area in ejido Nanacamila, Zacatlán de las Manzanillas municipality, Puebla, Mexico (Google Earth, 2025). Plots for the different treatments, (b) liberation cutting (LC), where seed trees (indicated by arrows) are removed; (c) first thinning (FC); and (d) second thinning (ST), the trees lacking specified commercial characteristics are selectively removed (indicated by arrows). Created with BioRender.com (accessed on 19 August 2025).

2.2. Sample Collection

For each treatment, nine soil cores (2.5×30 cm) were collected per experimental plot, maintaining a minimum distance of 15 m between them; to maintain a balanced number of

samples across treatments, additional cores were collected from one of the plots for both the LC and ST treatments. Litter was removed prior to sampling. A total of 81 soil samples were obtained, individually transferred into plastic storage bags, and refrigerated at 4 °C for a maximum of 21 days before processing. Each core sample was sieved individually and washed under cold running tap water, and mycorrhizal root tips were recovered using an optical microscope, placed in tubes, and stored at −80 °C until DNA extraction.

2.3. DNA Extraction, Amplification, and Sequencing

DNA was obtained from the pooled mycorrhizal root tips of each of the 81 cores (Supplementary Figure S1 illustrates the methodology). First, mycorrhizas were ground with liquid nitrogen, and DNA was isolated using XNAT (REDEExtract-N-Amp Tissue PCR Kit, Sigma-Aldrich, Darmstadt, Germany) Extraction and Dilution Solutions following the manufacturer's instructions. The resulting mix was then processed using PowerBead Soil DNA Isolation kit (MO BIO, Carlsbad, CA, USA), as indicated. The DNA pellets were resuspended in sterile deionized water, and the concentration and purity were assessed using a NanoDrop Spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA).

PCR amplification of the *ITS2* region was conducted using four primer combinations based on [37] (detailed Supplemental Figure S2), for a total of 324 reactions. Each reaction was carried out in 50 µL volume containing 0.5 U DNA polymerase (Promega, Madison, WI, USA), 0.4 µM of each primer, 200 µM dNTPs, and 2.5 µL of each DNA sample. The negative control consisted of a reaction with no DNA. The thermal cycling conditions included an initial denaturation and enzyme activation step of 5 min at 95 °C, followed by 35 cycles of amplification consisting of 30 s at 95 °C, 30 s at 55 °C, and 60 s at 72 °C, and then a final 10 min extension step at 72 °C. The PCR amplification products were visualized on an agarose gel.

Equal amounts of PCR products from each primer pair were combined for each core sample, and the resulting mix was purified using AMPure XP Beads (Beckman-Coulter Life Sciences, Indianapolis, IN, USA). Pools were quantified using a Qubit™ dsDNA HS Assay Kit and a Qubit 2.0 Fluorometer (Thermo Fisher Scientific). A final pooling step was performed to obtain a total of three sequencing pools per treatment (each representing a sampling site, for a total of nine pools) with a normalized concentration of 4 nM.

Libraries were constructed following the instructions and using the reagents indicated in the Fungal Metagenomic Sequencing Demonstrated Protocol (Illumina). Final pooling and library construction were performed in duplicate. Sequencing was conducted on an Illumina MiSeq (Illumina, San Diego, CA, USA) using a 2 × 300 cycle configuration.

2.4. Statistical and Bioinformatics Analyses

The statistical and bioinformatic analyses were performed following the protocol described by [38]. Briefly, data analysis was conducted using QIIME2 v2024.5 [39]. Sequences were merged using FLASH v1.2.11 [40], and quality filtering, denoising, dereplication, and chimera removal were performed using DADA2 v1.16 [41] to obtain amplicon sequence variants (ASVs). Taxonomic assignment was conducted from phylum to genus level using a naïve Bayesian classifier trained on the UNITE v8.99 database clustered at 97% similarity [42].

Multiple sequence alignment of ASV representative sequences was performed using MAFFT v7 [43], and a phylogenetic tree was constructed using FastTree v2 [44] with default parameters. The resulting rooted tree was incorporated into the QIIME2 framework for calculation of phylogeny-based alpha and beta diversity metrics. To assess the α - and β -diversities of the fungal communities and conduct corresponding statistical analyses, the samples were rarefied to the sequencing depth of the library with the lowest number

of reads. The α -diversity was estimated using the Chao1, Shannon, and Simpson indices. Differences in diversity between treatments were evaluated using the Kruskal–Wallis test, considering statistical significance at p -values < 0.05 . β -diversity was explored through a principal coordinate analysis (PCoA) using unweighted and weighted UniFrac distances, followed by permutational multivariate analysis of variance (PERMANOVA) with 999 permutations to evaluate community differences. To identify differences in the abundance of microbial taxa, differential abundance analysis was performed using Analysis of Compositions of Microbiomes with Bias Correction (ANCOM-BC [45]) in QIIME2 v2024.5. For each silvicultural treatment, taxa abundances were compared against those present in the other treatments. p -values were adjusted using the Benjamini–Hochberg false discovery rate (FDR), with statistical significance set at $\alpha = 0.05$. Fungal ecological functions were inferred using FUNGuild v1.0, which assigns functional guilds based on taxonomic information [46]. Finally, to assess the distribution of shared and unique ECM fungal genera among silvicultural treatments, a Venn diagram was generated based on presence/absence data at the genus level.

3. Results

3.1. Fungal ASV Taxonomic Composition

From a total of 6,502,616 raw ITS2 region sequences, 1,973,626 high-quality sequences were obtained and used for subsequent analyses, with an average of 109,646 sequences per library. The rarefaction curves from all treatments exhibited a tendency to reach the saturation plateau, suggesting that the sequencing effort was sufficient to capture most of the fungal diversity (Supplementary Figure S3). Following the quality control and rarefaction assessment, one low-depth library (LC1) was excluded due to its markedly low number of high-quality reads; downstream analyses were conducted on the remaining 17 libraries.

The analysis was conducted on sampled root tips to favor the detection of ECM fungi. However, the high-throughput metabarcoding revealed a wider spectrum of taxa, including fungi associated with diverse ecological roles. These encompassed other mutualistic associations (endomycorrhizae, ericoid mycorrhiza), non-mutualistic groups (saprophytic, parasitic, pathogenic, and endophytic taxa), mixed mutualistic/non-mutualistic groups, and taxa that remain insufficiently characterized. This approach enabled the identification of 400 ASVs distributed in 5 fungal phyla, 34 classes, 92 orders, 215 families, and 346 genera.

The most abundant phyla were *Ascomycota* (55.17%) and *Basidiomycota* (44.63%), with the remainder accounting for less than 1.0% of the overall relative abundance. The dominant fungal family was *Myxotrichaceae* (11.66%), followed by *Sebacinaceae* (9.75%), *Russulaceae* (9.41%), *Thelephoraceae* (8.39%), *Gloniaceae* (7.93%), *Inocybaceae* (7.67%), *Pyronemataceae* (5.17%), and *Arachnopezizaceae* (5.17%), with others contributing less than 5.0% of the overall relative abundance (Figure 2a). At the genus level, the prevalent taxa were *Oidiodendron* (12.22%), *Sebacina* (9.96%), *Russula* (9.76%), *Cenococcum* (8.30%), *Inocybe* (7.99%), *Tomentella* (6.68%), and *Arachnopeziza* (5.41%), with others representing less than 5.0% of the overall relative abundance (Figure 2b).

Focusing on ECM fungi showed 68 ASVs belonging to 25 families (including *Agaricales* gen. *incertae sedis* and *Agaricomycetes* gen. *incertae sedis*), collectively accounting for 51.4% of the overall relative abundance (Figure 2c). Of the 30 genera detected, 8 accounted for 94% of the total abundance in this group (Figure 2d): *Sebacina* (19.38%), *Russula* (18.9%), *Cenococcum* (16.14%), *Inocybe* (15.54%), *Tomentella* (12.99%), *Clavulina* (4.96%), *Amphinema* (4.94%), and *Amanita* (1.89%).

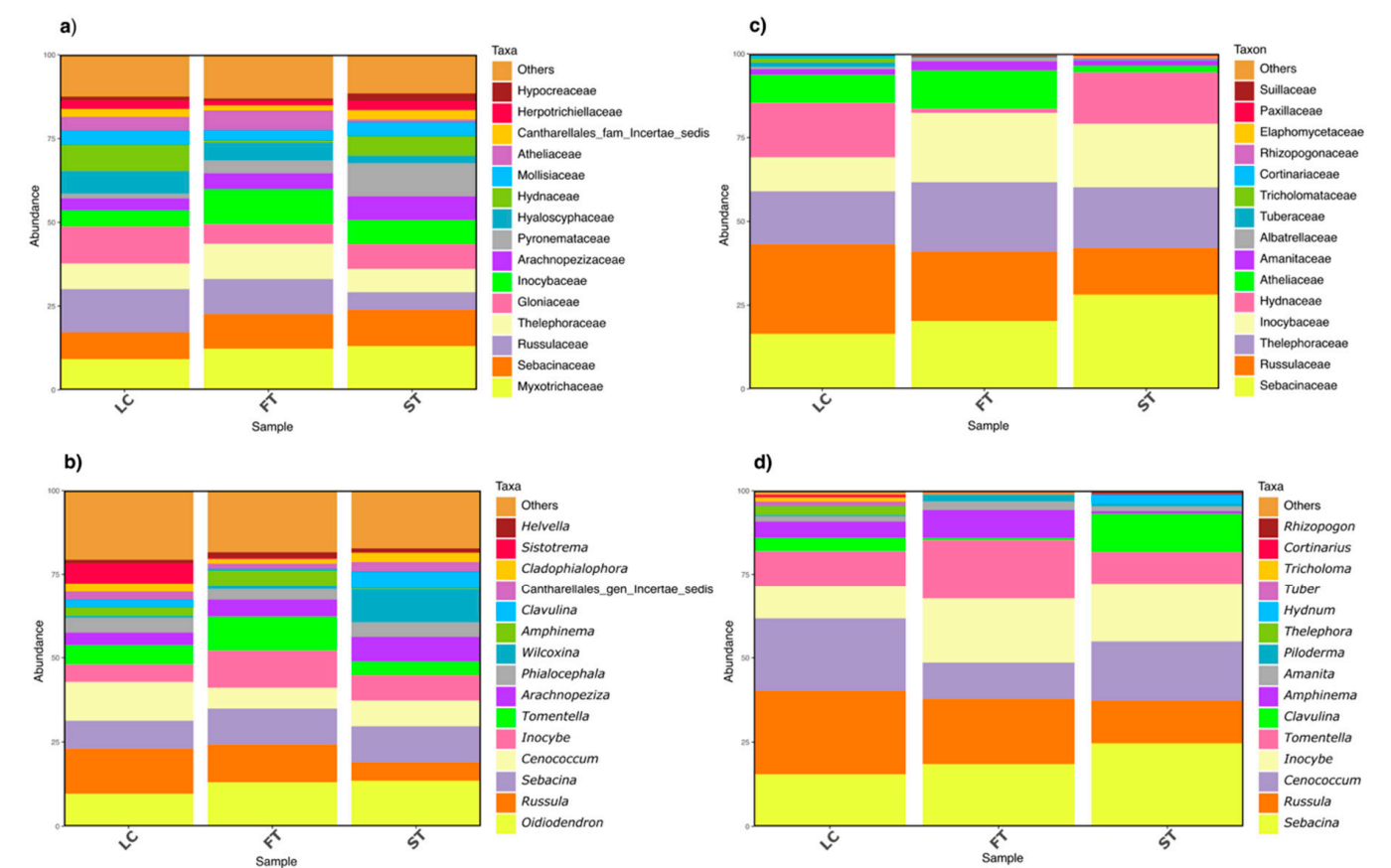


Figure 2. Bar plots depicting the relative abundance of whole fungal communities at (a) family and (b) genus and ECM fungi at (c) family and (d) genus levels. Liberation cutting (LC), first thinning (FT), and second thinning (ST).

3.2. Effect of Silvicultural Treatments on Fungal Diversity

The fungal α -diversity was similar across thinning treatments ($p > 0.05$), although both the richness (Chao1) and diversity (Shannon index) varied depending on the treatment and tended to show higher average values for the first thinning. Simpson’s diversity index values were similar across treatments, ranging between 0.98 and 0.99 (Table 1).

Table 1. Summary of sequence quality and α -diversity metrics.

Treatment	Raw Sequences	High-Quality Sequences	Chao1	Shannon	Simpson
Liberation Cutting (LC)	1,936,447	577,612	432.8 $\pm$ 39.52	7.2 $\pm$ 0.09	0.99 $\pm$ 0.002
First Thinning (FT)	2,367,577	703,262	459.17 $\pm$ 27.9	7.22 $\pm$ 0.06	0.99 $\pm$ 0.002
Second Thinning (ST)	2,198,592	692,752	382 $\pm$ 46.5	6.91 $\pm$ 0.17	0.98 $\pm$ 0.002

In terms of the β -diversity, the first three coordinates of the PCoA analysis, using the unweighted and weighted dissimilarity matrices, explained 46% and 75% of the total observed variation in the fungal community, respectively (Figure 3). Overall, the PCoA analyses revealed significant differences in the fungal community β -diversity ($p < 0.05$). However, in the case of the weighted analysis, accounting for species abundance, the first thinning and liberation cutting treatments did not differ significantly ($p = 0.074$).

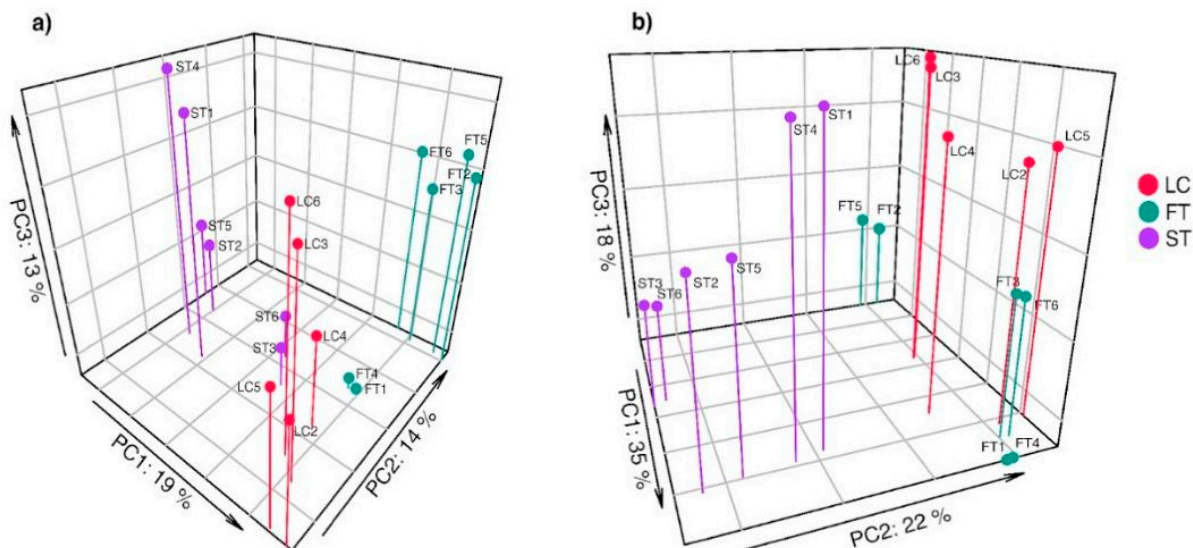


Figure 3. PCoA based on (a) unweighted and (b) weighted UniFrac distances showing the fungal community dissimilarity. Liberation cutting (LC), first thinning (FT), and second thinning (ST).

3.3. Effect of Silvicultural Treatments on Fungal Taxa Composition

The differential abundance analysis identified fungal taxa that were enriched in each silvicultural treatment. Most were Ascomycetes that belonged to the saprotroph, pathotroph–saprotroph–symbiotroph, or pathotroph–saprotroph fungal guilds. Functional assignments revealed treatment-specific shifts in the fungal guild composition. Liberation cutting (Figure 4a) showed the most pronounced changes in litter saprotrophs, with increases in *Sistotrema*, *Gorgomyces*, *Uwebraunia*, and members of *Sordariomycetes*, accompanied by declines in *Tetracladium* and the pathotrophic order *Hypocreales*. In contrast, first thinning (Figure 4b) exhibited only minor changes (increase in *Hypocreales*, decrease in *Gorgomyces*), while second thinning (Figure 4c) showed increases in *Thelonectria* and *Venturia*. Wood decomposers were significantly more abundant for liberation cutting (e.g., *Mytilinidiales* gen. incertae sedis, *Hyaloscyphaceae*; decrease in *Halokirschsteiniothelia*) and second thinning (*Halokirschsteiniothelia*, *Camposporium*), whereas for first thinning only a reduction was observed (members of *Auriculariales*). Soil saprobes showed the opposite pattern: first thinning supported the highest abundance (e.g., *Pyronemataceae*, *Pleotrichocladium*, *Archaeorhizomyces*), liberation cutting presented moderate shifts (increase in *Geminibasidium*, decrease in *Pleotrichocladium*), and second thinning showed a decline (decreases in several *Dothideomycetes* and *Pyronemataceae*, increase in *Teratosphaeriaceae*).

Likewise, ECM fungi showed differential presences. For liberation cutting, *Tricholoma*, *Ampinema*, *Russula*, and *Cenococcum* were significantly increased, whereas for first thinning the presence of various ECM fungi declined, including *Tricholoma*, *Agaricales* gen. incerta sedis, *Humaria*, and *Tuber*, while the mixed saprotroph–symbiotrophs *Entholomataceae* gen. incerta sedis increased. For second thinning, *Russula* exhibited a decreased abundance.

Changes in taxa that include tree pathogens were also detected, with increased presences of *Dothideomycetes* and *Mytilinidiales* for liberation cutting, *Chalara* and *Hypocreales* for first thinning, and *Dactylonectria* for second thinning.

Across all treatments, different taxa including endophytes increased: *Lasiodiplodia* for liberation cutting, *Pezoloma* for first thinning, and *Hypocreaceae* for second thinning.

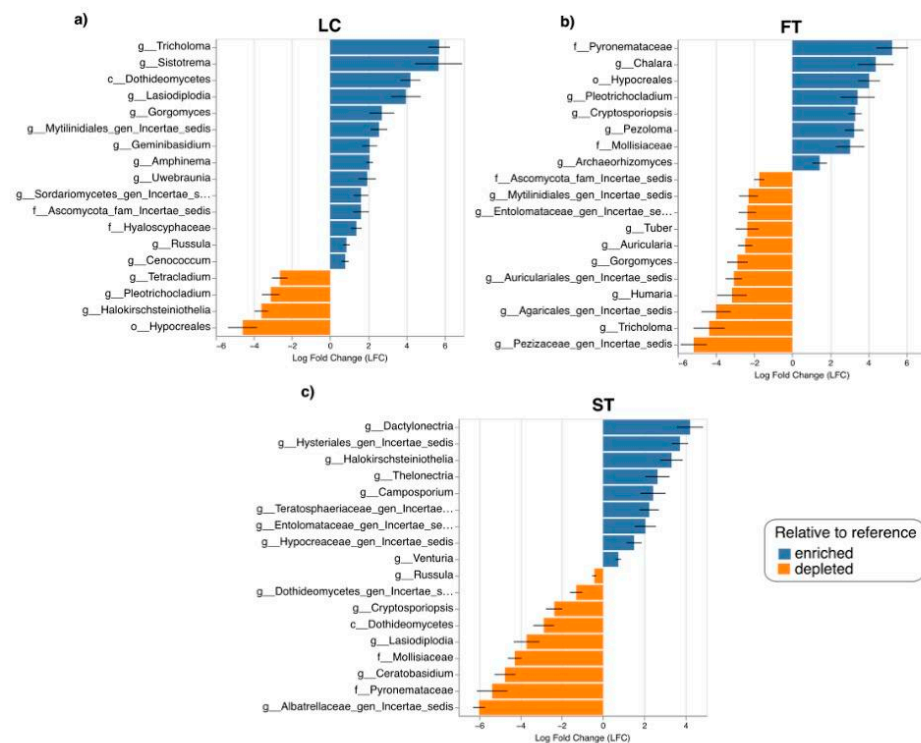


Figure 4. Differentially abundant taxa in (a) liberation cutting (LC) treatment, (b) first thinning (FT) treatment, and (c) second thinning (ST) treatment. Bars depict ANCOM-BC log fold change (LFC) values. Blue bars represent enriched taxa, whereas orange bars represent depleted taxa.

3.4. Changes in Fungal Guild Structure Across Treatments

Fungal ASVs were assigned to trophic groups using FUNGuild (Figure 5). Although there is no distinct trophic mode specifically associated with any particular treatment, symbiotrophs were the most abundant (52%–55%), followed by mixed pathotroph/saprotroph/symbiotrophic taxa (21%–25%) and saprotrophs (5%–16%). Between 7% and 18% were unidentified. The non-parametric Kruskal–Wallis test showed no significant differences among the silvicultural treatments, except for the artificial category “others” that collapses various underrepresented mixed categories of pathotrophs, pathotrophs–saprotrophs, and pathotrophs–ymbiotrophs (Supplementary Table S1).

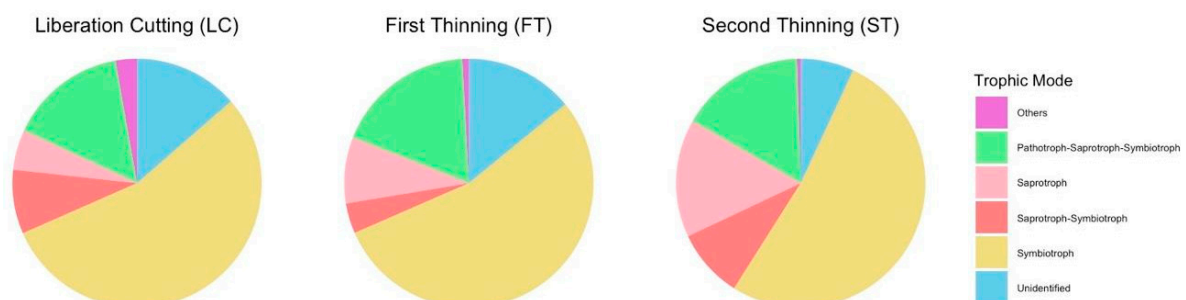


Figure 5. Plot of the trophic mode vs. silviculture treatment. The percentage for each treatment was plotted, grouped according to the trophic mode classification provided by FUNGuild.

Focusing on the ECM fungi revealed 30 genera, of which 14 were common to all silvicultural treatments (Figure 6), although their relative contributions varied: liberation cutting included *Lactarius*, *Geopora*, and *Lactifluus* as unique taxa, while *Hygrophorus*, *Croogomphus*, *Sarcodon*, and *Laccaria* were particular to first thinning, and *Strobilomyces* was only found for second thinning. Overall, second thinning exhibited the lowest number of detected genera.

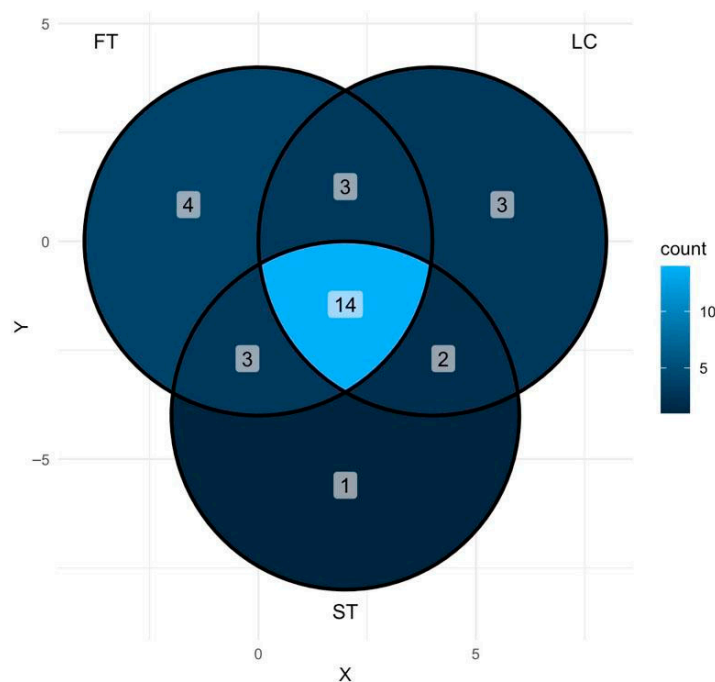


Figure 6. Venn diagram of ECM fungal genera showing the intersection of the three treatments—liberation cutting (LC), first thinning (FT), and second thinning (ST)—based on abundances.

4. Discussion

Sylvicultural practices represent ecological disturbances that affect forest dynamics by altering the stand composition, density, and spatial structure, as well as biotic interactions. These changes extend into the soil environment, where they influence fungal communities that play key roles in nutrient cycling, symbiosis, and decomposition [1–4]. The characterization of forest fungal communities has relied mostly on assessing the production of macroscopic carpophores [35,47,48]. However, due to the fluctuating seasonal occurrence of carpophores and the absence of visible fruiting bodies in many species, high-throughput sequencing has emerged as a powerful complementary approach that enables the exploration of belowground fungi, providing a more comprehensive assessment of their diversity, community composition, and functional responses to environmental disturbances [16,31,37].

To better capture potential changes in fungal taxa engaged in symbiotic interactions with trees in response to different silvicultural practices, for our high-throughput analysis, we focused our sampling colonized mycorrhizal roots rather than bulk soil. Thus, the strong representation of symbiotrophic taxa obtained in the analysis likely reflects the targeted nature of our sampling. ECM-forming Basidiomycota and Ascomycota fungi were the dominant trophic guilds, representing nearly half of the overall relative abundance, of which *Sebacina*, *Russula*, *Cenococcum*, *Inocybe*, *Tomentella*, *Clavulina*, *Amphinema*, and *Amanita* were the most represented genera and co-occurred in all treatment groups. These genera have also been found to be dominant in other forest ecosystems both in the Northern Hemisphere [49,50] and in the tropics [51], although much less is known about the species involved in the latter. Given that tropical and subtropical forests represent diversity hotspots for many plant and fungal groups, further research is warranted, particularly within the context of global climatic change [16,32].

Mexico, due to its geographic location, orography, and diverse vegetation, particularly *Pinus* and *Quercus*, exhibits a high ECM species richness and diversity in these genera [36,52,53]. This biodiversity underscores the high conservation value of Mexican tropical rainforests and woodlands [54]. Additionally, many of these genera include ed-

ible species that are collected and commercialized by local populations, representing an important part of their cultural heritage and an alternative source of food and income [52].

The ECM genera *Sebacina*, *Cenococcum*, and *Tomentella* are known for their efficient dispersal and low host specificity, whereas *Inocybe* is a widely distributed taxon that is considered to be stress-tolerant; it is abundant in harsh environments [36] and serves as an early colonizer of habitat patches [53], consistent with gaps created by silvicultural practices. *Russula* and *Clavulina* are usually found in older trees [36]. The generalized presence of both early and late colonizers suggests that the stands have reached an intermediate level of maturity [55] but may also reflect the dispersal of mycorrhizal inocula from surrounding forest areas.

However, the 400 ASVs that were recovered spanned multiple ecological guilds in addition to symbiotrophs, including saprotrophs and pathotrophs, highlighting the complexity and interconnectedness of rhizospheric fungal communities [49]. Saprotrophs and mixed saprotroph–symbiotrophs were also very highly represented. Members of these guilds play important roles in organic matter decomposition and carbon cycling in forest ecosystems, and the higher diversity in these taxa has been associated with early- to intermediate-stage forests under management [55]. In this respect, the most prevalent genus recovered across all treatments was *Oidiodendron* (family *Myxotrichaceae*), which includes both saprophytic and symbiotic species (e.g., erichoid mycorrhiza). High-throughput studies in temperate forests have revealed that *Oidiodendron* spp. are commonly found in acidic soils, in association with both ECM and arbuscular mycorrhizal roots, across many plant hosts, suggesting they play a role in the wider belowground symbiotic networks and nutrient exchange [56].

The genus *Arachnopeziza* was another prominent taxon recorded in our survey. Despite its widespread distribution, particularly in temperate regions, members of the order *Helotiales* remain understudied. There is growing recognition of their role in plant nutrition, including the mineralization of organic matter and their function as root symbionts (erichoid and ECM) or endophytes [57]. However, much remains to be understood about their interaction with tree roots, contributions to forest health and resilience, and responses to forest disturbances.

We found that the silvicultural treatments influenced both the α - and β -diversity indices of the belowground fungal communities. While no significant within-site differences were detected, a clear trend was observed in which fungal richness and diversity indices (Chao and Shannon) were highest for first thinning. A parallel study performed at the same study sites indicated differences in forest stand attributes [14]: the tree density was lower for liberation cutting (625 indiv.ha⁻¹) and for first thinning (672 indiv.ha⁻¹) than for secondary thinning (800 indiv.ha⁻¹), whereas the basal area was highest for first thinning (37.4 m²ha⁻¹), followed by secondary thinning (29.67 m²ha⁻¹), and was lowest for liberation cutting (17.55 m²ha⁻¹). When edaphic parameters were included in the comparisons, these revealed significant differences between treatments for various soil attributes. First thinning had the highest values for organic matter, phosphorus, and sodium, while registering the lowest values for the soil pH, potassium, magnesium, and calcium. With this context, the patterns observed in our research align with studies suggesting that early interventions, with fewer but larger mature trees and a moderate canopy opening, create moderate levels of disturbance that can enhance fungal diversity by improving root growth, reducing competition, and creating more heterogeneous microenvironments that support a broader range of fungal guilds [30–32,58,59]. In contrast, liberation cutting, despite similar tree densities, was associated with a lower basal area, resulting in a more open stand and a reduced biomass, likely consisting of smaller or younger trees with less-developed root systems that did not adequately support peak fungal richness. Secondary thinning,

which was associated with higher densities of smaller trees and reduced deadwood and decomposing matter [14], showed a shift in the fungal community composition, leading to a decline in the ECM fungal richness, diversity, and abundance, as well as an increase in saprotrophic fungi. The effects of soil attributes, mainly the increased organic matter and lower soil pH, also correlate with findings in other forest systems [60], especially for saprophytic and ECM taxa, respectively [55,61]. Similar patterns have been previously reported and have been shown to persist long after intervention [15,31].

Silvicultural treatments also influenced the prevalence of particular fungal taxa. Our findings show high Simpson index values (close to 1) across treatments, indicating that a few taxa are highly abundant: seven account for over 50% of the total registered abundance. This suggests that, while the richness and diversity may vary with the management intensity, the dominance by a few taxa remains consistent, showing a skewed community structure that may favor resilient groups under altered environmental conditions at the expense of less common taxa. This unevenness could impact ecosystem function, as fewer dominant taxa may fulfill a narrower range of ecological roles and decrease the capacity to respond to environmental stress [62]. Further studies considering fungal dynamics at the species level could provide valuable insights into their affinity for or vulnerability to environmental disturbances but also regarding the functional overlap and their contribution to overall forest resilience.

The FUNGuild analysis revealed that the proportions of the different functional guilds were consistent across treatments. Compositional differences among treatments indicate that certain fungal taxa responded differently, with some being favored, while the overall trophic function remained unchanged [48]. Changes were observed mostly in saprophytic and pathogenic Ascomycota genera. The artificial category of “others”, which includes pathotrophic, pathotrophic/saprotrophic, and pathotrophic/symbiotrophic taxa due to the low number of registers, was significantly increased in liberation cutting. Shifts in saprotrophic and pathotrophic guilds are mostly explained by variations in microclimatic and edaphic conditions among the stands [37,60], particularly by the accumulation of the organic matter substrates, pH, and soil texture for saprotrophs, as these factors influence the availability of the decomposable litter, nutrient supply, and soil moisture [16,55,60]. In the case of pathotrophs, fluctuations in taxa have been related to changes in the canopy cover that create illumination, temperature, and humidity gradients resulting from increased exposure [51].

The functional assignments of the differentially abundant taxa [63] showed that liberation cutting, which creates the most open stands and reduces the basal area, was associated with stronger shifts in litter saprotrophs and wood decomposers. This is likely due to the increased input of both fine litter and coarse woody debris due to biomass removal, as reported both in temperate and boreal forests [64,65]. In contrast, first thinning, characterized by fewer but larger trees and moderate canopy opening, seems to have favored soil saprobes (*Pyronemataceae*, *Archaeorhizomyces*), consistent with studies showing that moderate disturbances enhance soil microbial diversity through improved root growth and more heterogeneous microhabitats [66,67].

Second thinning was associated with a pattern of increased wood and litter decomposers, although the associated taxa differed (*Halokirschsteiniotelia* and *Camposporium* for wood and *Thelonectria* and *Venturia* for litter), which is consistent with a report that the availability and quality of woody substrates strongly regulate the decomposer community composition [65]. Together, these findings indicate that the thinning intensity mediates the balance among litter, soil, and wood saprotrophs, with liberation cutting exerting the strongest effect on decomposer guilds, while first thinning supports higher soil fungal activity.

Given the crucial role that mycorrhizal fungi diversity plays in the functioning and resilience of forest ecosystems, assessing its variability is important to understand how management practice influences its dynamics. Our results indicate that the richness and β -diversity of belowground ECMs reflected the same overall pattern (first thinning > liberation cutting > second thinning), with most taxa being shared across the different silvicultural treatments, despite variations in their relative prevalence.

The production of ECM fungi fruiting bodies at the treated plots during a previous and concurrent season [14] followed a similar pattern, although the species richness remained consistent among treatments. Interestingly, fruiting bodies of *Lactarius indigo* (Schwein.) Fr. were only found with liberation cutting, consistent with our metabarcoding data on the presence of the genus, suggesting that this taxon is sensitive to microhabitat changes. This highlights how—by altering the tree biomass and canopy openness, as well as the accumulation of organic matter, the nutrient status, the soil pH, and the microbial community composition and structure—silvicultural practices shape both belowground diversity and the emergence of fruiting bodies. Future studies that focus on the precise identification of ECM fungal species could be instrumental in guiding the selection of the most suitable host–mycobiont combinations adapted to local conditions for use in ectomycorrhization, thereby enhancing nursery production, plantation survival, and productivity, as well as supporting reforestation and restoration efforts [49,68].

5. Conclusions

These results emphasize the value of alternative and complementary approaches, such as metabarcoding, for exploring fungal community dynamics and provide insights into ecosystem functioning. Particularly, they contribute to our knowledge of fungal diversity in subtropical pine forests in central Mexico, highlighting the importance of silvicultural practices for soil fungal communities. Regarding α -diversity indices, the Chao1, Shannon, and Simpson values showed variations among the silvicultural treatments, with a tendency toward higher richness and diversity for first thinning, followed by liberation cutting, and lower values for second thinning. Likewise, the β -diversity analysis showed that the treatments significantly influenced the fungal community composition, although the dominant taxa remain similar, especially between the first thinning and liberation cutting. Thus, moderate intervention practices that support a more heterogeneous environment, light penetration, and root development (as in stands with intermediate basal area and tree densities), as seen for first thinning, may serve as a basis for integrating strategies that minimize severe disturbances.

Ultimately, recognizing the ecological role of fungal communities and integrating their conservation into forest management not only enhances ecosystem resilience, but also supports sustainable forestry practices that reconcile productivity with biodiversity conservation.

Supplementary Materials: The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/f16091397/s1>: Figure S1: Diagram of the workflow. LC: Liberation Cutting; FT: First Thinning; ST: Second Thinning. Figure S2: Primers used for amplifying an ITS2 region. (a) Position of the primers used in this study. (b) Primer sequences. Created with BioRender.com (accessed on 25 July 2025). Figure S3: Rarefaction curves depicting the number of species rarefied as a function of sample size. The dashed vertical line represents the lowest sample size. Table S1: Summary of Kruskal–Wallis Test Results for Silvicultural Treatments.

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Data Availability Statement: The datasets generated and/or analyzed during the current study are available upon request in the NCBI Bioproject database under the accession number PRJNA1299065 (metagenomic sequences).

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Abbreviations

The following abbreviations are used in this manuscript:

SDM	Silvicultural development method
ECM	Ectomycorrhizal
ITS	Internal transcribed spacer
LC	Liberation cutting
FT	First thinning
ST	Second thinning
PCoA	Principal coordinate analysis

References

1. Delavaux, C.S.; LaManna, J.A.; Myers, J.A.; Phillips, R.P.; Aguilar, S.; Allen, D.; Alonso, A.; Anderson-Teixeira, K.J.; Baker, M.E.; Baltzer, J.L.; et al. Mycorrhizal Feedbacks Influence Global Forest Structure and Diversity. *Commun. Biol.* **2023**, *6*, 1066. [[CrossRef](#)] [[PubMed](#)]
2. Dyshko, V.; Hilszczańska, D.; Davydenko, K.; Matic, S.; Moser, W.K.; Borowik, P.; Oszako, T. An Overview of Mycorrhiza in Pines: Research, Species, and Applications. *Plants* **2024**, *13*, 506. [[CrossRef](#)]
3. Millennium Ecosystem Assessment. *Ecosystems and Human Well-Being: Synthesis*; Island Press: Washington, DC, USA, 2005; ISBN 1597260401.
4. Heilmann-Clausen, J.; Barron, E.; Boddy, L.; Dahlberg, A.; Griffith, G.W.; Nordén, J.; Ovaskainen, O.; Perini, C.; Senn-Irlet, B.; Halme, P. A Fungal Perspective on Conservation Biology. *Conserv. Biol.* **2015**, *29*, 61–68. [[CrossRef](#)] [[PubMed](#)]
5. Talbot, J.M.; Bruns, T.D.; Smith, D.P.; Branco, S.; Glassman, S.I.; Erlandson, S.; Vilgalys, R.; Peay, K.G. Independent Roles of Ectomycorrhizal and Saprotrophic Communities in Soil Organic Matter Decomposition. *Soil. Biol. Biochem.* **2013**, *57*, 282–291. [[CrossRef](#)]
6. Buscot, F.; Weber, G.; Oberwinkler, F. Interactions Between *Cylindrocarpon destructans* and Ectomycorrhizas of *Picea abies* with *Laccaria laccata* and *Paxillus involutus*. *Trees* **1992**, *6*, 83–90. [[CrossRef](#)]
7. Smith, J.E. Mycorrhizal Symbiosis (Third Edition). *Soil Sci. Soc. Am. J.* **2009**, *73*, 694. [[CrossRef](#)]
8. Morin, C.; Samson, J.; Dessureault, M. Protection of Black Spruce Seedlings against *Cylindrocladium* Root Rot with Ectomycorrhizal Fungi. *Can. J. Bot.* **1999**, *77*, 169–174.
9. Niego, G.A.T.; Lambert, C.; Mortimer, P.; Thongklang, N.; Rapior, S.; Grosse, M.; Schrey, H.; Charria-Girón, E.; Walker, A.; Hyde, K. The Contribution of Fungi to the Global Economy. *Fungal Divers.* **2023**, *121*, 95–137. [[CrossRef](#)]
10. Bai, C.; He, X.; Tang, H.; Shan, B.; Zhao, L. Spatial Distribution of Arbuscular Mycorrhizal Fungi, Glomalin and Soil Enzymes under the Canopy of *Astragalus adsurgens* Pall. in the Mu Us Sandland, China. *Soil. Biol. Biochem.* **2009**, *41*, 941–947. [[CrossRef](#)]

11. McGuire, K.L. Common Ectomycorrhizal Networks May Maintain Monodominance in a Tropical Rain Forest. *Ecology* **2007**, *88*, 567–574. [CrossRef] [PubMed]
12. Nara, K. Ectomycorrhizal Networks and Seedling Establishment during Early Primary Succession. *New Phytol.* **2006**, *169*, 169–178. [CrossRef]
13. Koide, R.T.; Dickie, I.A. Effects of Mycorrhizal Fungi on Plant Populations. In *Diversity and Integration in Mycorrhizas*; Smith, S.E., Smith, F.A., Eds.; Springer: Dordrecht, The Netherlands, 2002; Volume 244. [CrossRef]
14. Zamora Morales, B.P.; Zamora-Martínez, M.C.; de Pascual Pola, M.C.d.C.N.; García Campusano, F.T.A. Condiciones Edáficas, Abundancia y Riqueza de Hongos Ectomicorizógenos Comestibles. *Rev. Mex. Cienc. For.* **2018**, *9*, 226–251. [CrossRef]
15. Collado, E.; Castaño, C.; Bonet, J.A.; Hagenbo, A.; Martínez de Aragón, J.; de-Miguel, S. Divergent Above- and below-Ground Responses of Fungal Functional Groups to Forest Thinning. *Soil. Biol. Biochem.* **2020**, *150*, 108010. [CrossRef]
16. Tomao, A.; Antonio Bonet, J.; Castaño, C.; de-Miguel, S. How Does Forest Management Affect Fungal Diversity and Community Composition? Current Knowledge and Future Perspectives for the Conservation of Forest Fungi. *For. Ecol. Manag.* **2020**, *457*, 117678. [CrossRef]
17. Dove, N.C.; Keeton, W.S. Structural Complexity Enhancement Increases Fungal Species Richness in Northern Hardwood Forests. *Fungal Ecol.* **2015**, *13*, 181–192. [CrossRef]
18. Aguirre-Calderón, O.A. Manejo forestal en el siglo XXI. *Madera Y Bosques.* **2015**, *21*, 17–28. [CrossRef]
19. *FAO Soils for Nutrition: State of the Art*; FAO: Rome, Italy, 2022.
20. Pérez-Rodríguez, F.; Vargas-Larreta, B.; Aguirre-Calderón, O.A.; Corral-Rivas, J.J.; Rojo-Alboreca, A. Proceso analítico jerárquico para seleccionar métodos de manejo forestal en Durango. *Rev. Mex. Cienc. For.* **2012**, *4*, 55–72.
21. Quijada, G.E.M.; Balderas, J.M.M.; Garza, E.J.T.; Calderón, Ó.A.A.; Rodríguez, E.A.; Yamallel, J.I.Y. Diversity, Structure and Floristic Composition of Temperate Forests of Southern Nuevo León State. *Rev. Mex. Cienc. For.* **2020**, *11*, 94–123. [CrossRef]
22. British Columbia. Ministry of Forests. Forest Practices Branch. Silvicultural Systems Handbook for British Columbia. For. Pract. Br., BC. Min. For., Victoria, BC. 2003. Available online: <https://www2.gov.bc.ca/assets/gov/farming-natural-resources-and-industry/forestry/stand-tending/silvsystemshdbk-web.pdf> (accessed on 19 August 2025).
23. Hernández-Salas, J.; Aguirre-Calderón, Ó.A.; Alanís-Rodríguez, E.; Jiménez-Pérez, J.; Treviño-Garza, E.J.; González-Tagle, M.A.; Luján-Álvarez, C.; Olivas-García, J.M.; Domínguez-Pereda, L.A. Efecto Del Manejo Forestal En La Diversidad y Composición Arbórea de Un Bosque Templado Del Noroeste de México. *Rev. Chapingo Ser. Cienc. For. Ambiente* **2013**, *19*, 189–199. [CrossRef]
24. Bautista, L.J.; Damon, A.; Ochoa-Gaona, S.; Tapia, R.C. Impact of Silvicultural Methods on Vascular Epiphytes (Ferns, Bromeliads and Orchids) in a Temperate Forest in Oaxaca, Mexico. *For. Ecol. Manag.* **2014**, *329*, 10–20. [CrossRef]
25. López-Reyes, A.; de la Rosa, J.P.; Ortiz, E.; Gernandt, D.S. Morphological, Molecular, and Ecological Divergence in *Pinus Douglasiana* and *P. maximinoi*. *Syst. Bot.* **2015**, *40*, 658–670. [CrossRef]
26. Pérez-López, R.I.; González-Espinosa, M.; Ramírez-Marcial, N.; Pérez-Moreno, J.; Toledo-Aceves, T. Forest Management Effects on the Ectomycorrhizal Macromycete Community in Tropical Montane Forests in Mexico. *For. Ecol. Manag.* **2021**, *501*, 119670. [CrossRef]
27. Abbasi, U.A.; Mattsson, E.; Nissanka, S.P.; Ali, A. Biological, Structural and Functional Responses of Tropical Forests to Environmental Factors. *Biol. Conserv.* **2022**, *276*, 109792. [CrossRef]
28. Rosen, A.; Jörg Fischer, F.; Coomes, D.A.; Jackson, T.D.; Asner, G.P.; Jucker, T. Tracking Shifts in Forest Structural Complexity through Space and Time in Human-Modified Tropical Landscapes. *Ecography* **2024**, *2024*, e07377. [CrossRef]
29. Sabogal, C.C.J.K.W. *Silviculture in Natural Forests*; FAO: Roma, Italy, 2017.
30. Tedersoo, L.; Bahram, M.; Zobel, M. How Mycorrhizal Associations Drive Plant Population and Community Biology. *Science* **2020**, *367*, eaba1223. [CrossRef] [PubMed]
31. Hartmann, M.; Howes, C.G.; Vaninsberghe, D.; Yu, H.; Bachar, D.; Christen, R.; Henrik Nilsson, R.; Hallam, S.J.; Mohn, W.W. Significant and Persistent Impact of Timber Harvesting on Soil Microbial Communities in Northern Coniferous Forests. *ISME J.* **2012**, *6*, 2199–2218. [CrossRef]
32. Collado, E.; Bonet, J.A.; Alday, J.G.; Martínez de Aragón, J.; de-Miguel, S. Impact of Forest Thinning on Aboveground Macrofungal Community Composition and Diversity in Mediterranean Pine Stands. *Ecol. Indic.* **2021**, *133*, 108340. [CrossRef]
33. Parladé, J.; Queralt, M.; Pera, J.; Bonet, J.A.; Castaño, C.; Martínez-Peña, F.; Piñol, J.; Senar, M.A.; De Miguel, A.M. Temporal Dynamics of Soil Fungal Communities after Partial and Total Clear-Cutting in a Managed *Pinus Sylvestris* Stand. *For. Ecol. Manag.* **2019**, *449*, 117456. [CrossRef]
34. Kujawska, M.B.; Rudawska, M.; Wilgan, R.; Leski, T. Similarities and Differences among Soil Fungal Assemblages in Managed Forests and Formerly Managed Forest Reserves. *Forests* **2021**, *12*, 353. [CrossRef]
35. Valdés, M.; Pereda, V.; Ramírez, P.; Valenzuela, R.; Pineda, R.M. The Ectomycorrhizal Community In a *Pinus Oaxacana* Forest Under Different Silvicultural Treatments. *J. Trop. For. Sci.* **2009**, *21*, 88–97.

36. Reverchon, F.; del Pilar Ortega-Larrocea, M.; Bonilla-Rosso, G.; Pérez-Moreno, J. Structure and Species Composition of Ectomycorrhizal Fungal Communities Colonizing Seedlings and Adult Trees of *Pinus Montezumae* in Mexican Neotropical Forests. *FEMS Microbiol. Ecol.* **2012**, *80*, 479–487. [\[CrossRef\]](#) [\[PubMed\]](#)
37. Tedersoo, L.; Bahram, M.; Pölme, S.; Kõljalg, U.; Yorou, N.S.; Wijesundera, R.; Ruiz, L.V.; Vasco-Palacios, A.M.; Thu, P.Q.; Suija, A.; et al. Global Diversity and Geography of Soil Fungi. *Science* **2014**, *346*, 1256688. [\[CrossRef\]](#)
38. Muñoz-Castellanos, L.N.; Avila-Quezada, G.D.; Sáenz-De La Riva, G.; Salas, E.; Muñoz-Ramírez, Z.Y.; González-Escobedo, R. Revealing Microbial Patterns in the Rhizosphere of Pecan Trees Asymptomatic and Symptomatic for Texas Root Rot Using a High-Throughput Sequencing Approach. *Rhizosphere* **2024**, *29*, 100833. [\[CrossRef\]](#)
39. Bolyen, E.; Rideout, J.R.; Dillon, M.R.; Bokulich, N.A.; Abnet, C.C.; Al-Ghalith, G.A.; Alexander, H.; Alm, E.J.; Arumugam, M.; Asnicar, F.; et al. Reproducible, Interactive, Scalable and Extensible Microbiome Data Science Using QIIME 2. *Nat. Biotechnol.* **2019**, *37*, 852–857. [\[CrossRef\]](#) [\[PubMed\]](#)
40. Magoč, T.; Salzberg, S.L. FLASH: Fast Length Adjustment of Short Reads to Improve Genome Assemblies. *Bioinformatics* **2011**, *27*, 2957–2963. [\[CrossRef\]](#) [\[PubMed\]](#)
41. Callahan, B.J.; McMurdie, P.J.; Rosen, M.J.; Han, A.W.; Johnson, A.J.A.; Holmes, S.P. DADA2: High-Resolution Sample Inference from Illumina Amplicon Data. *Nat. Methods* **2016**, *13*, 581–583. [\[CrossRef\]](#)
42. Nilsson, R.H.; Larsson, K.H.; Taylor, A.F.S.; Bengtsson-Palme, J.; Jeppesen, T.S.; Schigel, D.; Kennedy, P.; Picard, K.; Glöckner, F.O.; Tedersoo, L.; et al. The UNITE Database for Molecular Identification of Fungi: Handling Dark Taxa and Parallel Taxonomic Classifications. *Nucleic Acids Res.* **2019**, *47*, D259–D264. [\[CrossRef\]](#)
43. Katoh, K.; Standley, D.M. MAFFT Multiple Sequence Alignment Software Version 7: Improvements in Performance and Usability. *Mol. Biol. Evol.* **2013**, *30*, 772–780. [\[CrossRef\]](#)
44. Price, M.N.; Dehal, P.S.; Arkin, A.P. FastTree 2—Approximately Maximum-Likelihood Trees for Large Alignments. *PLoS ONE* **2010**, *5*, e9490. [\[CrossRef\]](#)
45. Lin, H.; Peddada, S.D. Analysis of Compositions of Microbiomes with Bias Correction. *Nat. Commun.* **2020**, *11*, 3514. [\[CrossRef\]](#)
46. Nguyen, N.H.; Song, Z.; Bates, S.T.; Branco, S.; Tedersoo, L.; Menke, J.; Schilling, J.S.; Kennedy, P.G. FUNGuild: An Open Annotation Tool for Parsing Fungal Community Datasets by Ecological Guild. *Fungal Ecol.* **2016**, *20*, 241–248. [\[CrossRef\]](#)
47. Valdés, M.; Córdova, J.; Gómez, M.; Fierros, A.M. Understory Vegetation and Ectomycorrhizal Sporocarp Diversity Response to Pine Regeneration Methods in Oaxaca, Mexico. *West. J. Appl. For.* **2003**, *18*, 101–108. [\[CrossRef\]](#)
48. Martínez-Peña, F.; de-Miguel, S.; Pukkala, T.; Bonet, J.A.; Ortega-Martínez, P.; Aldea, J.; Martínez de Aragón, J. Yield Models for Ectomycorrhizal Mushrooms in *Pinus Sylvestris* Forests with Special Focus on *Boletus Edulis* and *Lactarius Group Deliciosus*. *For. Ecol. Manag.* **2012**, *282*, 63–69. [\[CrossRef\]](#)
49. Assad, R.; Reshi, Z.A.; Rashid, I.; Wali, D.C.; Bashir, I.; Rafiq, I. Metabarcoding of Root-Associated Ectomycorrhizal Fungi of Himalayan Pindrow Fir through Morphotyping and Next Generation Sequencing. *Trees For. People* **2021**, *6*, 100153. [\[CrossRef\]](#)
50. Katrevičs, J.; Bitenieks, K.; Jansons, Ā.; Jansone, B.; Ruņģis, D.E. Forest Soil Fungal Diversity in Stands of Norway Spruce (*Picea abies* (L.) Karst.) of Different Ages. *Forests* **2025**, *16*, 500. [\[CrossRef\]](#)
51. Tedersoo, L.; May, T.W.; Smith, M.E. Ectomycorrhizal Lifestyle in Fungi: Global Diversity, Distribution, and Evolution of Phylogenetic Lineages. *Mycorrhiza* **2010**, *20*, 217–263. [\[CrossRef\]](#) [\[PubMed\]](#)
52. Garibay-Orijel, R.; Argüelles-Moyao, A.; Álvarez-Manjarrez, J.; Ángeles-Argáiz, R.E.; García-Guzmán, O.M.; Hernández-Yáñez, H. Diversity and Importance of Edible Mushrooms in Ectomycorrhizal Communities in Mexican Neotropics. In *Mushrooms, Humans and Nature in a Changing World: Perspectives from Ecological, Agricultural and Social Sciences*; Springer International Publishing: Cham, Switzerland, 2020; pp. 407–424, ISBN 9783030373788.
53. Boeraeve, M.; Honnay, O.; Jacquemyn, H. Effects of Host Species, Environmental Filtering and Forest Age on Community Assembly of Ectomycorrhizal Fungi in Fragmented Forests. *Fungal Ecol.* **2018**, *36*, 89–98. [\[CrossRef\]](#)
54. Tedersoo, L.; Mikryukov, V.; Zizka, A.; Bahram, M.; Hagh-Doust, N.; Anslan, S.; Prylutskyi, O.; Delgado-Baquerizo, M.; Maestre, F.T.; Pärn, J.; et al. Global Patterns in Endemicity and Vulnerability of Soil Fungi. *Glob. Change Biol.* **2022**, *28*, 6696–6710. [\[CrossRef\]](#)
55. Gómez-Hernández, M.; Ramírez-Antonio, K.G.; Gándara, E. Ectomycorrhizal and Wood-Decay Macromycete Communities along Development Stages of Managed *Pinus Patula* Stands in Southwest Mexico. *Fungal Ecol.* **2019**, *39*, 109–116. [\[CrossRef\]](#)
56. Toju, H.; Sato, H. Root-Associated Fungi Shared between Arbuscular Mycorrhizal and Ectomycorrhizal Conifers in a Temperate Forest. *Front. Microbiol.* **2018**, *9*, 433. [\[CrossRef\]](#)
57. Bruyant, P.; Moëgne-Loccoz, Y.; Almario, J. Root-Associated Helotiales Fungi: Overlooked Players in Plant Nutrition. *Soil. Biol. Biochem.* **2024**, *191*, 109363. [\[CrossRef\]](#)
58. Teste, F.P.; Lieffers, V.J.; Strelkov, S.E. Ectomycorrhizal Community Responses to Intensive Forest Management: Thinning Alters Impacts of Fertilization. *Plant Soil* **2012**, *360*, 333–347. [\[CrossRef\]](#)
59. Goldmann, K.; Schöning, I.; Buscot, F.; Wubet, T. Forest Management Type Influences Diversity and Community Composition of Soil Fungi across Temperate Forest Ecosystems. *Front. Microbiol.* **2015**, *6*, 1300. [\[CrossRef\]](#)

60. Alem, D.; Dejene, T.; Oria-de-Rueda, J.A.; Geml, J.; Martín-Pinto, P. Soil Fungal Communities under *Pinus Patula* Schiede Ex Schltdl. & Cham. Plantation Forests of Different Ages in Ethiopia. *Forests* **2020**, *11*, 1109. [[CrossRef](#)]
61. Tedersoo, L.; Anslan, S.; Bahram, M.; Pölme, S.; Riit, T.; Liiv, I.; Kõljalg, U.; Kisand, V.; Nilsson, R.H.; Hildebrand, F.; et al. Shotgun Metagenomes and Multiple Primer Pair-Barcode Combinations of Amplicons Reveal Biases in Metabarcoding Analyses of Fungi. *MycoKeys* **2015**, *10*, 1–43. [[CrossRef](#)]
62. Wittebolle, L.; Marzorati, M.; Clement, L.; Balloi, A.; Daffonchio, D.; Heylen, K.; De Vos, P.; Verstraete, W.; Boon, N. Initial Community Evenness Favours Functionality under Selective Stress. *Nature* **2009**, *458*, 623–626. [[CrossRef](#)]
63. Pölme, S.; Abarenkov, K.; Henrik Nilsson, R.; Lindahl, B.D.; Clemmensen, K.E.; Kauserud, H.; Nguyen, N.; Kjoller, R.; Bates, S.T.; Baldrian, P.; et al. FungalTraits: A User-Friendly Traits Database of Fungi and Fungus-like Stramenopiles. *Fungal Divers.* **2020**, *105*, 1–16. [[CrossRef](#)]
64. Baldrian, P. Forest Microbiome: Diversity, Complexity and Dynamics. *FEMS Microbiol. Rev.* **2017**, *41*, 109–130. [[CrossRef](#)]
65. Purahong, W.; Kahl, T.; Schloter, M.; Bauhus, J.; Buscot, F.; Krüger, D. Comparing Fungal Richness and Community Composition in Coarse Woody Debris in Central European Beech Forests under Three Types of Management. *Mycol. Prog.* **2014**, *13*, 959–964. [[CrossRef](#)]
66. Tanney, J.B.; Seifert, K.A. Mollisiaceae: An Overlooked Lineage of Diverse Endophytes. *Stud. Mycol.* **2020**, *95*, 293–380. [[CrossRef](#)]
67. Koukol, O. New Species of *Chalara* Occupying Coniferous Needles. *Fungal Divers.* **2011**, *49*, 75–91. [[CrossRef](#)]
68. Flores-Rentería, D.; Barradas, V.L.; Álvarez-Sánchez, J. Ectomycorrhizal Pre-Inoculation of *Pinus Hartwegii* and *Abies Religiosa* Is Replaced by Native Fungi in a Temperate Forest of Central Mexico. *Symbiosis* **2018**, *74*, 131–144. [[CrossRef](#)]

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