Fungal and bacterial communities in a forest relict of *Pinus pseudostrobus* var. *coatepecensis*

Yajaira Baeza-Guzmán (1), Sara Lucía Camargo-Ricalde (2), Dora Trejo Aguilar (3), Noé Manuel Montaño (2)

**Introduction**

*Pinus pseudostrobus* Lindl., a species known for its high genetic variation, is native to the Neotropical zone (Villegas-Jiménez et al. 2016). The majority of its populations are concentrated along the “Eje Neovolcánico Transversal” (Trans-Mexican Volcanic Belt, Central Mexico). Within the species, *P. pseudostrobus* var. *coatepecensis* Martinez holds particular importance due to the quality of its wood; however, it remains relatively understudied. Over the past few decades, there has been a noticeable decline in natural populations resulting in the emergence of forest relicts (Aceves-Rangel et al. 2018) in transformed forest or agricultural landscapes. This forest matrix serves as a valuable resource for the macro- and microbiota inhabiting these forest relicts, facilitating their dispersal and ensuring their survival (Byers et al. 2020).

Ectomycorrhizal fungi (EcM) play a crucial ecological role associated with pine roots, contributing to the survival of pines, by enhancing water uptake and nutrient acquisition. They also improve the resistance of host plants to drought, salinity, heavy metals, and pathogens (Bennett et al. 2017). These fungi play a fundamental role in the ecosystem functioning, inducing morphological changes at the root level, and expanding root exploration through the development of external mycelium and fungal mantle, which are influenced by bacteria (Reis et al. 2021). The bacterial community responds to changes in host photosynthetic activity, as well as drought conditions and precipitation levels (Reis et al. 2021).

Bacteria can act as growth promoters (PGPR) in the plant rhizosphere and as mycorhizal helper bacteria (MHB) in association with mycorrhizal plant roots. Bacteria-plant interactions play a vital role in the organic matter mineralization, nutrient acquisition, carbon (C) dynamics, and nitrogen (N) provision for plants, including biological nitrogen fixation or N-mineralization. This enhances resistance against pathogens and contributes to host survival (Rodríguez-Ramos et al. 2021). Changes in microbial community richness and diversity are closely linked to available habitat and forestry practices (Boeraeve et al. 2018).

Spiesman et al. (2018) demonstrated that patch isolation and the type of matrix habi-
tat increase bacterial richness and composition. This suggests that suitable feeding, shelter, or climatic conditions can be found across the matrix, allowing the dispersal and survival of biota inhabiting fragments (Jiménez et al. 2020). Dispersal mechanisms may limit the establishment of ECM fungi, such as host compatibility, spore germination capacity in response to the presence or absence of roots, and low abundance of spore-dispersing mammals or birds (Aguirre et al. 2021). Consequently, differences in mycorrhizal community assembly can occur between isolated or adjacent stands within different forest matrices. In current landscapes, these natural forest relics hold significant potential for conserving soil microorganisms (Gavito et al. 2019). Therefore, the objectives of this study were (i) to characterize fungal and bacterial communities associated with the roots of *P. pseudostrobus* var. coatepecensis, and (ii) to identify the soil physicochemical factors influencing microbial composition.

**Materials and methods**

**Study area and remnant description**

The study was conducted in the central-eastern part of Mexico, specifically in the eastern part of Mexico, specifically in the state of Veracruz (19° 26’ 04.12” N, 97° 04’ 19.5” W), at an altitude of 2209 m a.s.l. The study site covered an area of 25 hectares. The climate in this region is classified as humid temperate (Cf), with mean annual temperatures ranging from 12 to 18 °C. The coolest month experiences temperatures between -3 and 18 °C, while the hottest month remains below 22 °C. The driest month receives more than 40 mm of precipitation, and rainfall occurs throughout the year, with winter rainfall accounting for over 18% of the annual total (Kottke et al. 2006).

The soil in the study area is primarily Andisol, which develops on volcanic deposits rich in glass under various climatic conditions, except for hyper-arid habitats. However, Andisols can also form on other silicate-rich materials through acid weathering in humid and per-humid climates (IUS Working Group 2015). The primary vegetation in the area consists of *Pinus patula* Schl. et Cham and *P. pseudostrobus* var. coatepecensis, along with Montane Cloud Forest elements such as *Liquidambar styraciflua* L., *Carpinus caroliniana* Thomas Walter, *Clethra spp.*, *Cupressus lusitanica* Mill., and *Quercus* spp. The original forest has been fragmented due to agricultural and livestock activities. In the past two decades, reforestation with *P. patula* has led to the establishment of a pine forest plantation, resulting in the isolation of a forest remnant of *P. pseudostrobus* var. coatepecensis, which represents the only remaining stand within the forest plantation matrix (Fig. 1).

**Experimental design and sampling method**

Sampling was carried out along a 145 m transect, which was established from the area near the plantation to the edge of the remnant (Fig. 1). Roots were collected from a total of 18 soil samples, distributed as follows: (a) six samples at 30 cm intervals, (b) four samples at 1 m intervals, (c) four samples at 10 m intervals, and (d) four samples at 25 m intervals, according to the methodology described by Villarreal-Ruiz & Neri-Luna (2018). Soil samples were extracted as soil cores measuring 15 cm in length and 5 cm in diameter using a soil core sampler. Subsequently, the samples were carefully placed in sealed bags and transported in a primer for the laboratory. The roots were thoroughly washed with sterile distilled water and then transferred to a sieve series with mesh sizes of 1 mm and 2 mm. Once cleaned, the roots were preserved by storing them in liquid nitrogen in a freezer set at -20°C until further processing.

**DNA extraction and sequencing**

DNA extraction was carried out on root samples using DNeasy PowerSoil® Kit (Qiagen, Hilden, Germany). This kit is proposed for environmental samples with high humic acid or sediment content. Extractions were performed following the manufacturer’s instructions. Total DNA concentration was measured with Qubit® 3.0 Fluorometer (Thermo Fisher Scientific, Waltham, MA, USA) in the range of 60 ng μl⁻¹ to 100 ng μl⁻¹ for all samples before metagenomic analysis. PCR amplification was performed targeting the internal transcribed spacer ITS2 region and the conserved regions of 5.8S, and 28S rDNAs of the fungi, by using universal primers ITS3 (5′-GATCCTGATGAAGAA-CGGACG-3′) and ITS4 (5′-TCCCTCGCTATTGTATATC-3′) (Tedersoo et al. 2014). The 16S rRNA genes were amplified targeting the V3 and V4 regions, using the bacterial primers Bakt_341F (CCTACGGGNGGCWGCAG) and Bakt_805R (GACTACHVGGGTATCTAC- TAAATC) (Sinclair et al. 2015). DNA amplification conditions were performed as follows: 94°C for 5 min; 35 cycles at 94°C for 50 s, 58°C for 50 s, 72°C for 50 s; and a final extension of 72°C for 10 min. After amplification, each PCR product was confirmed using 1% agarose gel, and they were purified and sequenced by Macrogen Laboratory Seoul, Republic of Korea (https://dna.macrogen.com). Sequencing was performed on a MiSeq® Illumina (Illumina, Inc., San Diego, CA, USA).

**Bioinformatics**

Sequence processing and classification were performed in R studio ver. 1.3.1093. Primers were removed with “cut adapt” software, and low-quality nucleotides were removed using the DADA2 function to output representative sequences. Sequences were qualified, filtered, and trimmed using the “FilterAndTrim” method, for bacteria and fungal sequences (Callahan et al. 2017). We used sequences longer than 50 bp. Paired-ends sequences were merged, removing singletons and de novo chimera sequences using the “removeBimeraDenovo” function.
vo" method to denoise sequences into amplicon sequence variants (ASVs). The taxonomy of the ASVs was assigned with the Silva v132 database (Quast et al. 2013) for bacteria and UNITE dataset (Nilsson et al. 2019) for fungi. The “phyloseq” package was also used to explore the data, create an object, and generate a matrix of ASV abundances and the taxonomy matrix. The resulting ASV table was grouped at the species level.

Diversity analysis

Hill Diversity Indices were calculated for (α) diversity (Shannon diversity, Simpson diversity, richness, and effective number of species) and sample coverage for species richness using the INEXT package. To visualize taxa distribution along each distance point, we used heat maps at the phylum level and circos plots at the family level. The position of fungal communities in distance groups (d0 ≤ 0.20 m, d1 = 1 m, d10 = 10 m, d25 = 25 m). R values close to 1 indicate high dissimilarity. We used soil variables as dependent and separated into two components: species replacement (turnover) and species nestedness (Baselga 2010). Results were based on pairwise comparisons of each sample point, calculated as Sørensen’s Dissimilarity Index (Jsor) and, a dissimilarity analysis (ANOSIM) was performed to analyze the similarity of microbial communities in distance groups (d0 ≥ 0.20 cm, d1 = 1 m, d10 = 10 m, d25 = 25 m). R values close to 1 indicate high dissimilarity. We used indicator species analysis to assess whether ASVs occur in different sample distances.

Soil analysis

In the same way as the root sampling, 18 soil samples were taken along the 145 m transect, as follows: (a) six each 20 cm, (b) four each 1 m, (c) four each 10 m, (d) four each 25 m. We manually removed stones and litter before sampling. Each soil sample was stored, dried at room temperature, and used for analyzing soil organic matter (SOM) and organic C content by the oxidation method; pH was measured in a suspension of soil: deionized water (1: 2 w/v); total N, by micro-Kjeldahl method; the C:N ratio as the index determined by the organic C and total N content; ammonium (NH4-N) quantification was carried out using Nessler’s reagent (Bremner & Mulvaney 1982); Nitrate (NO3-N) were measured by the Cataldo’s method (Cataldo et al. 1995). Exchangeable acidity (Al+2-H+), exchangeable aluminum (Al+3), and total hydrogen (H+) according to Bremner & Mulvaney (1982); Bray-II determined available P (PO43-) in soil according to Bray & Kurtz (1945), and Fe2+ was quantified by the digestion of concentrated HCl (Chapman & Pratt 1962).

Statistical analysis

To infer the most influential variables affecting the composition of bacterial and fungal communities, we employed Nonmetric Multidimensional Scaling (NMDS) analysis in the R “vegan” package with Bray Curtis dissimilarity, using the “envfit” function which calculates multiple regression of environmental variables. Here, we used soil variables as dependent and selected ordination axes as an explanatory variable. Significance was tested by permutation test (p = 0.05). All statistical analyses were performed in R (R Core Team 2020).

Results

Fungal community composition

A total of 2.1 million paired-end raw reads were obtained for 17 samples. One fungal sample (point 2) was removed due to insufficient DNA. After applying quality filters, removing chimeras, and merging paired-end reads, we obtained 908,991 sequences, with an average of 49,471 sequences per sample. The DADA2 pipeline inferred 1233 fungal ASVs. After filtering out rare ASVs, we identified 995 fungal ASVs, including 212 ASVs belonging to Basidiomycota, 326 ASVs belonging to Ascomycota, and 20 ASVs belonging to Glomerastrum. Among the fungal sequences, 69 ASVs were attributed to ectomycorrhizal (ECM) fungi. The ECM families with the highest relative abundance were Thelephoraceae, Clavulinaceae, Russulaceae, Lactariaceae, Elaphomycetaceae, and Amanitaceae (Fig. S1 in Supplementary material).

Along the transect, we observed different functional fungal groups ranging from mutualistic to pathogenic (Fig. 2a). The dissimilarity of the total fungal community between samples at different distances was significant (p = 0.0392) with an R2 = 0.1984. Russula dissimulans Shaffer was associated with samples collected at short (20 cm) distances (p = 0.0275). Tomentellopsis zygodesmoids Ellis Hjortstam (p = 0.0165) and Lactarius chrysorrheus Fr. (p = 0.0188) were associated with samples taken at 1 m. Hyaloscypha variabilis (Hambl. & Sigler) Vohom. Feher & Reblova (p = 0.0263) were associated with samples taken at 10 m intervals, while Xenasmatella sp. (p = 0.0164) and Venturia sp. (p = 0.0085), a phytopathogenic genus, were both associated with samples spaced 25 m apart and located close to the remnant edge. Some phytopathogenic fungi, such as Armillaria and Lecanicillium, were found at the beginning of the transect (in the area close to the plantation matrix), but their association with these samples was not significant.

ASV abundance values show that the most abundant fungi were ectomycorrhizal (Fig. 2b), such as R. dissimulans (ASV1), with the highest relative abundance along the transect, 90.13% at the first sampling point. It was followed by Tomentella sp.1 (ASV2), Sulfur decipiens (Peck) Kuntze (ASV3), Elaphomycys granulatus Fr. (ASV4), Tomentella sp2 (ASVs), and Amanita sp.1 (ASVs). Eri- coid fungi and root endophytes were also observed along the entire transect with low relative abundance percentages.
The abundance curves of the fungal group exhibited a steep gradient at all sampling points, reflecting richness and evenness. The curves of short-distance samples showed similar slopes (Fig. S2 in Supplementary material). Specifically, the 20 cm sample had few species and low species evenness. The samples taken at a 1 m distance from each other displayed similar slopes and contained a greater number of fungal species, as observed at the 2 m and 4 m distance sample points. The range of ASVs at 5 m intervals was lower. The curves generated from the innermost zone of the remnant exhibited a long and shallow slope, indicating high uniformity. Near the edge of the remnant, with a greater distance between samples, a similar slope was observed at the beginning of the transect, with low evenness in all samples.

**Fungi, (α) and (β) diversity**

The study site had an effective richness (q=0) of 1333 fungal ASV. Diversity was determined with the Shannon index (q=1) with 53 virtual taxa and dominance (q=2) with 23 taxa; the sampling coverage was close to 100%, so it can be inferred that the sampling was sufficiently exhaustive for both fungi and bacteria (Tab. 1). Regarding β-diversity (βsor), species turnover (β-3) was the major component in dissimilarity among fungal assemblages (Fig. S3 in Supplementary material). Species turnover achieved high values, above 0.8 at sample points closer to the edge, and a lower turnover in the innermost part of the remnant. Overall, the sampling points along transects showed a low proportion of shared species yielding high dissimilarity values. The resulting nesting values (βnest) were shallow in all comparisons.

**Bacterial community composition**

A total of 2.2 million bacterial sequences were obtained from 18 samples. After filtering and removing chimeras, 876,010 sequences remained, resulting in a total of 16,233 ASVs. After filtering out rare ASVs, the number was reduced to 10,237. Taxonomically, these sequences were classified into different groups, including 3,996 Acidobacteria ASVs, 3,787 Proteobacteria, 1,306 Verrucomicrobia ASVs, 1,007 Actinobacteria ASVs, and 1,141 Bacteroidetes ASVs (Fig. S4 in Supplementary material). No significant differences were observed in bacterial community dissimilarity between sample distances (p = 0.1205), indicating a low dissimilarity among the communities (R2 = 0.1086). Twenty-six bacterial ASVs were found to be statistically significant in the 20 cm distance group, including Acidothermus sp., Bryobacter sp., Burkholderia-Paraburkholderia sp., Rhodospirillales spp., and Xanthomonadales spp. (p = 0.009). Acidibacter sp. (p = 0.0297) and Granulicella sp. (p = 0.0396) were also among the significant ASVs. In the 1 m distance group, 21 ASVs were associated with the samples, such as Acidobacteria sp., Acidobacterium sp., Solirubrobacterales spp. (p = 0.009), Rhizomicrobium sp. (p = 0.0297), and Bryobacter sp. (p = 0.0495). For samples spaced 10 m apart, 71 ASVs were found, with statistically significant ASVs including Xanthomonadales spp., Rhodospirillales spp., Rhodomicrobium sp., Chitinophagaceae spp., Dyella sp., Acidobacterium sp., Verrucomicrobia sp., and Candidatus methylicphilum sp. (p = 0.009). Some bacteria, such as Nevkia (p = 0.0297), Sorangium (p = 0.0396), and Gemmatisma (p = 0.0297), were exclusively present in this group. In the samples spaced 25 m apart, 18 bacterial ASVs were statistically associated, including Rhizomicrobium sp., Verrucomicrobia sp., and Granulicella spp. (p = 0.009), Bryobacter sp. (p = 0.019), Acidobacterium sp. (p = 0.0297), Acidothermus spp. (p = 0.0495), and Rudae sp. (p = 0.0495). At the family level, Acidobacteriaceae (18.6%), Solibacteriaceae (7.4%), and Burkholderiaceae (4.3%) were the most abundant along the transect, representing the major components of the bacterial community associated with the roots of P. pseudostrobus var. coatepecensis. Burkholderia, Bryobacter, Acidobacterium, and Acidothermus (Fig. 3a) were the dominant genera along the transect. As expected, numerous ASVs exhibited low abundance, while a few ASVs constituted most of the community (Fig. 3b), such as Bradyrhizobiurn sp.1 (ASVI) and Acidobacteriaceae sp.1 (ASV2).

The range-abundance curves showed that the first six sampling points (spaced every 20 cm) displayed similar steep slopes, indicating a higher number of ASVs compared to more distant sampling points and resulting in high evenness (Fig. S5 in Supplementary material). In the inner zone of the remnant, the slopes decreased and aligned with the graph, suggesting community uniformity and lower dominance.

---

**Tab. 1 - Estimation of (α) diversity using Hill numbers, evaluation of microbial representativeness and sample coverage.**

<table>
<thead>
<tr>
<th>Estimate</th>
<th>Fungal community</th>
<th>Bacterial community</th>
</tr>
</thead>
<tbody>
<tr>
<td>Effective richness</td>
<td>1,233</td>
<td>10,237</td>
</tr>
<tr>
<td>Shannon diversity</td>
<td>53</td>
<td>6,099</td>
</tr>
<tr>
<td>Simpson diversity</td>
<td>23</td>
<td>3,759</td>
</tr>
<tr>
<td>Sample coverage</td>
<td>99.9 %</td>
<td>99.8 %</td>
</tr>
</tbody>
</table>

---

**Fig. 3** - Taxonomic composition of bacterial communities in root samples of *Pinus pseudostrobus* var. coatepecensis. (a) Percentages indicate the relative values of paired reads at the genus level. (b) Relative values of the most abundant Amplicon Sequences Variant (ASV). The relative values <1 appear as 0; more than 80% of the relative values <1 were singleton representatives (data not shown).
**Discussion**

**Fungal community diversity and composition**

The results of this study demonstrate that the forest relict serves as a reservoir of rich fungal diversity. The ECM fungal community exhibited a higher relative abundance, consistent with previous findings reported by Gavito et al. (2019). Their study conducted in small pine forest fragments within an agricultural matrix revealed the presence of 60 to 109 ECM OTUs in patches ranging from 100 to 400 m². However, richness estimation should be carried out carefully, as the analysis method influences species richness and diversity.

The dominant fungal families identified in our study were Thelephoraceae, Clavulinaceae, Suillusaceae, and Russulaceae. These findings are consistent with previous studies carried out in Neotropical conifer forests. Argüelles-Moyao et al. (2016) reported similar dominance patterns in Abies-Pinus forests, where Russulaceae and Clavulinaceae, along with Inocybaceae and Atheliaceae, were dominant families. However, our results differ from those reported in Pinus montezumae Lamb., a species distributed in the same geographic area as P. pseudostrobus, where Atheliaceae, Cortinariaceae, and Sebacinaeae were found to be the most dominant (Reverchon et al. 2012). Notably, Atheliaceae has been identified as the primary family associated with P. hartwegii Lindl. in Neotropical alpine areas (Baëza-Guzmán et al. 2017). Interestingly, in our study, Atheliaceae was not dominant and was represented by only two genera: Tylospora and Toenisporas (anamorph).

Furthermore, our results highlight Thelephoraceae as the most dominant family, comprising four genera: Pseudotomentella, Thelephora, Tomentellopsis, and Tomentella. Among these, Tomentella exhibited the highest species diversity, including Tomentella stiposa (Link) Stalpers, Tomentella radiosa (P. Karst.) Rick, Tomentella coerulata Höhn. & Litsch., and two unidentified species. The Tomentella/Thelephora lineage is widely recognized as one of the most dominant in ECM communities worldwide, also in Neotropical ecosystems (Alvarez-Manjarrez et al. 2016).

It is important to consider the representation of the pathogenic fungal community in our study. We found that Armillaria gallica, a saprobic and facultative pathogenic species known to attack conifer roots, was present at the study site but in low abundance. Importantly, there was no evidence of damage to the trees caused by this pathogen. Previous studies, such as Viswanathan et al. (2019) have suggested that plant-fungal pathogen interactions tend to decrease in fragmented and smaller patches. In our results, we observed that the proportion of pathogens relative to the total fungal community was 21%, and for ectomycorrhizal (ECM) fungi specifically, it was 12%. This highlights the need for further research on the potential of these forest fragments to be integrated into forestry management plans to reduce and control potential diseases in adjacent plantations.

Regarding β-diversity, the fungal species composition showed a high degree of similarity even at short distances, such as 20 cm. This pattern can be explained by the different dispersal abilities of fungal species, which are influenced more by the forest matrix than by the distance between sampling points. Existing evidence suggests that the dispersal ability of fungal propagules plays a significant role in fungal community turnover. For instance, we observed a higher abundance of Russula species at sampling points closer to the forest matrix. These findings are consistent with the study by Boeraeve et al. (2018), which reported a higher abundance of Russula species in patches near natural forests, with a decrease in isolated patches. This pattern may be attributed to the contact exploration capacity of Russula species and the limited dispersal ability of some ectomycorrhizal fungi (Rosinger et al. 2018). Consequently, patches exhibiting a micro-environmental gradient between the forest edge and the interior of the main forest can show high turnover values in fungal communities.

The analysis of relative abundance revealed the dominance of species with short to medium-distance exploration types, includ-
ing Russula, Amanita, Thelephora, Tomentella, and Elaphomyces. This observation is in line with expectations considering the disturbance background of the study site. According to Correia et al. (2021), species with such exploration types are more resilient to disturbances because they can quickly regenerate their extraradical hyphal systems.

**Bacterial community diversity and composition**

The bacterial communities in acidic soils of reforested forests are often characterized by the dominance of common bacterial phyla, including Acidobacteria, Actinobacteria, Bacteroidetes, Verrucomicrobia, and Proteobacteria (Lladó et al. 2017). These same phyla were found to dominate the roots of P. pseudostrobus in our study. Notably, we observed the presence of Acidobacterium, Acidothermus, Burkholderia, and Bryobacter genera in all samples. This study represents the first report on the bacterial diversity associated with P. pseudostrobus. However, it is worth mentioning that certain bacterial genera such as Cohrella, Cupriavidus, Pseudomonas, Stenotrophomonas, and Rhodococcus have been reported as growth promoters in P. pseudostrobus seedlings, based on their isolation from the roots of Abies religiosa, P. halepensis, and P. montezumae (Heuvel-Abuña et al. 2018). It is worth noting that, in our study, the Pseudomonas genus was found to be present in low abundance in the 10 m distance group and did not show any statistically significant association.

To date, there have been limited reports on bacteria associated with Neotropical pines, their roots, and soils. For example, Rivera et al. (2022) reported that the dominant bacterial phyla in P. patula forests along a land-use gradient were Proteobacteria (40.13%), Actinobacteria (20.15%), and Acidobacteria (14.50%), which aligns with our findings. However, the bacterial community at the genus level differed from those associated with P. pseudostrobus. The aforementioned authors reported Halomonas, DA101, Bacillus, Streptomyces, Rhodoplanes, and Candidatus-Solibacter as the most abundant bacteria, with their abundance being influenced by land use, decreasing in arable soil and increasing in forest soil, irrespective of forest management. Another study identified 498 bacterial isolates from P. chiapensis rhizosphere, representing five genera: Bacillus, Paraburkholderia, Dyella, Luteimonas, and Enterobacter (Dominguez-Castillo et al. 2021). Some of these genera, such as Paraburkholderia and Dyella, were also found in our study.

Moreover, the dominant bacteria observed in P. pseudostrobus roots are known to be associated with host-pathogen resistance, such as Acidothermus, which exhibited high abundance along the transect. Zhang et al. (2022) recently reported a higher abundance of Acidothermus in the roots of three Pinus species (P. taeda, P. caribaea, and P. elliottii), which are known to be resistant to the nematode Bursaphelenchus xylophilus, in comparison to non-healthy pines such as P. massoniana. Another significant bacterial genus found in our study was Burkholderia-Paraburkholderia. These bacterial communities are predominantly found in Russula spp. sporocarps and act as mycorrhizal helper bacteria, promoting mycorrhizal colonization and hyphal growth (Yu et al. 2020). Our findings are consistent with this, as Burkholderia-Paraburkholderia dominated in short-distance samples. Several other studies have also identified this genus as dominant in forest soils, characterized by hydrophilicity and different adaptations in N acquisition (Clausing et al. 2021). ECm fungi, such as R. dissimilans, are involved in carbon cycling and transfer among plants, as they possess facultative saprotrophic abilities and rely on their host plants for carbon sources (Druebert et al. 2009). NMDS analysis further revealed that total C influenced the entire fungal community composition. In our study, R. dissimilans, T. zygodesmoides, and L. chrysorhoeus were statistically associated with samples at short distances. These ECm species, along with the abundant genera Tomentella, Elaphomyces, and Amanita, exhibit a short to medium-distance exploration type. This observation supports the findings of Rog et al. (2020), who proposed that ECm fungal species with contact exploration types dominate below-ground carbon networks. According to Santini et al. (2019), P. pseudostrobus forests have a high potential for soil carbon storage, with soil carbon reservoirs ranging from 42 to 145 Mg SOC ha⁻¹. Further analyses are needed to understand the environmental services provided by ectomycorrhizal networks in these forest relics within forest plantations.

Additionally, soil phosphorus concentrations (P Bray) influenced fungal communities, particularly ECm fungi (Zavišić et al. 2016). Different fungal species exhibit varying production of exoenzymes involved in phosphorus hydrolysis. For instance, Russula species exhibit high expression of acid phosphomonoesterases and phosphodiesterases, leading to increased phosphorus availability. As expected, we found that soil pH levels in the range of 4.05 to 4.80 strongly influenced soil bacterial composition. This finding is consistent with the study by Lammel et al. (2018), which demonstrated a direct correlation between low pH and the abundances of Acidibacter, Acidothermus, and Bradyrhizobiun. Ni et al. (2021) also reported a negative correlation between phyla Proteobacteria and Acidobacteria with soil pH and exchangeable Ca⁺, while Actinobacteria, Planctomycetes, Chloroflexi, Nitrospirae, and Gemmatimonadetes showed a positive correlation. Our results suggest that fungal and bacterial species may benefit from resources obtained from forest plantations adjacent to forest relics. Therefore, considering forest relics as reservoirs of native microbial diversity in forestry systems could represent...
iForest 16: 299-306

a potential alternative to enhance productivity and minimize diseases in forest plantations. Further investigations are needed to analyze the functional role of these natural relicts.

Conclusions

Neotropical forests are severely degraded and the potential of forest relicts as reservoirs of microbial diversity needs to be further explored and integrated into forest management and production programs. In this study, we identified a total of 212 ASVs (Basidiomycota), 326 ASVs (Ascomicota), 20 ASVs (Glomeromycota), 2,996 ASVs (Acidobacteria), 3,787 ASVs (Proteobacteria), 1,306 ASVs (Verrucomicrobia), 1,007 ASVs (Actinobacteria), and 1,141 ASVs (Bacteroidetes) associated with P. pseudostrobus var. coatepecensis. The EcM fungal communities were dominated by Amanita, Clavulina, Elaphomyces, Russula, and Tomentella genera, while the bacterial communities were dominated by Burkholderia, Paraburkholderia and Bryobacter genera. Our findings demonstrated the sensitivity of microbial communities to edaphic factors, particularly the C:N ratio, total C, available P, and total H. The β-diversity at small scales was strongly influenced by changes in soil properties and the dispersal ability of microorganisms across the adjacent matrix. The composition and structure of microbial communities can serve as early indicators of ecosystem health and resilience at local scales. Therefore, future research should evaluate the impact of native forest relicts on the conservation of microbial diversity within productive landscapes.

Declaration of competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This article is part of the requirements for YB-G to pursue a Doctoral degree by the Doctorado en Ciencias Biológicas y de la Salud from the Universidad Autónoma Metropolitana (UAM), Mexico City. YB-G gratefully acknowledges the financial support from CONACYT (753106). We thank the DNA Sequencing Center-MACROGENE (South Korea) for the metagenomic service. Special thanks to the Laboratorio de Organismos Benéficos de la Universidad Veracruzana for providing infrastructure, materials, and field support. We extend our gratitude to the people who facilitated the fieldwork in the forest plantation, and we are also grateful to the anonymous reviewers for their valuable comments and suggestions.

References

Baeza-Guzmán Y et al. - iForest 16: 299-306


Supplementary Material

Fig. S1 - Distribution and comparison of dominant fungi phyla along the transect.

Fig. S2 - Rank-abundance graphs for fungal communities within a transect.

Fig. S3 - Fungal β-diversity values between samples along the transect (145 m).

Fig. S4 - Distribution and comparison of dominant bacterial phyla along the transect.

Fig. S5 - Rank-abundance graphs for bacterial communities within a transect.

Fig. S6 - Bacterial β-diversity values between samples along the transect (145 m).

Link: Baeza_4284@suppl001.pdf