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The Resilient and the Vulnerable: Root-Associated Fungal Communities in the Regeneration of *Nothofagus pumilio* Forest Eight Years After Fire

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Abstract

Wildfires are increasing in frequency and severity worldwide, with long-lasting effects on forest vegetation and fungal communities essential for seedling establishment and forest resilience. *Nothofagus pumilio*, the dominant tree of South American temperate forests, forms key fungal symbioses but shows limited post-fire regeneration. Root-fungal community responses in seedlings under long-term post-fire conditions remain unknown. We characterized root-associated fungal communities in *N. pumilio* seedlings growing in burned (eight years post-fire) and unburned forest of the southern Andes, using ITS1 metabarcoding. We assessed diversity, composition, and abundance of root-associated fungi, including ectomycorrhizal fungi and dark septate endophytes. Diversity and evenness were significantly lower in burned than in unburned forest, with no significant differences in richness or composition. The ectomycorrhizal genus *Cortinarius* was less abundant in burned-forest seedlings. In seedlings from burned forest, the dark septate endophyte *Cadophora* was significantly more abundant, whereas *Exophiala* and *Cladophialophora* were less abundant than in unburned forest. Despite these shifts, burned areas retained root-associated fungal taxa linked to plant-beneficial functions and shared most OTUs with unburned forest, indicating an incomplete but ongoing recovery. Further research should evaluate how these shifts affect early seedling performance, and assess the role of persistent beneficial taxa for restoration after wildfires.

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1. Introduction

Wildfires can profoundly alter forest vegetation and the biological composition of soils, including fungal communities that are essential for plant recruitment [1,2]. Fungal composition recovery may remain negatively affected even a decade after fire disturbances [3]. As wildfires increase in frequency and severity worldwide in response to rising temperatures and prolonged droughts [4], understanding how fungal communities respond to long-term post-fire conditions becomes increasingly relevant, given their influence over seedling growth [5] and plant distribution patterns after fires [6]. Moreover, the reduced canopy cover and drier conditions that are characteristic of burned forests, and may persist over long periods [1], can affect plant development [7], which in turn can shape the composition and structure of root fungal communities [8].

Root-associated fungi may contribute positively to the resilience of forests affected by wildfires [9]. An increasing number of studies indicate that fungi inhabiting or closely associated with plant tissues play a fundamental role in influencing host fitness and resilience [10]. These microbes can be beneficial by participating in nutrient cycling, facilitating resource acquisition, and mitigating stress caused by pathogens or environmental pressures such as drought and contaminants [11]. Diverse, complex, and abundant fungal communities may enhance the capacity of soils to support multiple ecosystem functions, thereby contributing to ecosystem functioning and stability [12,13].

Temperate forests in the Southern Hemisphere are Gondwanan relics with high endemism, especially in South America [14], where *Nothofagus pumilio* [Poepp. & Endl.] Krasser (commonly known as austral beech or *lenga*) exhibits one of the broadest latitudinal distributions among temperate trees [15]. This endemic species of the Southern cone coexists with diverse fungal communities encompassing a wide range of ecological functions [16]. *N. pumilio* maintains close symbiotic relationships with fungi, primarily through ectomycorrhizal associations [17], although dark septate endophytes have also been reported [18]. Ectomycorrhizal fungi are symbiotic organisms that associate with plant roots, enhancing nutrient and water uptake [19], and their persistence is tied to the presence of established tree presence and cover [19,20]. Dark septate endophytes are root-colonizing fungi characterized by pigmented-septate hyphae, which are commonly found in extreme environments [21], and at high proportion in Andean plant species [10]. Both ectomycorrhizal fungi and dark septate endophytes are predominant in high-latitude and high-altitude forests and can provide important benefits to plants [18,22].

Wildfires, intensified by long-term drought conditions [4], have impacted *N. pumilio* forests, where post-fire recruitment occurs only in low-severity burned sites located near unburned forest, whereas it is absent in sites affected by high-severity fires [23]. Moreover, recruitment is further constrained by a sharp decline in the germinable seed bank beyond approximately 20 m from remnant forest edges [24]. As a key structural species of Andean forests, the decline of *N. pumilio* may compromise forest resilience and recovery potential [25]. Although there is growing evidence that climate change is intensifying the frequency and severity of wildfires, and that fungi can play a key role in post-fire plant recruitment, research on specific plant–microorganism interactions, including microbial responses to post-fire conditions, remains largely underrepresented in the literature [26]. Wildfires are a key driver of fungal community dynamics in *N. pumilio* forests [27]. In particular, knowledge of fungal communities associated with *N. pumilio* in fire-affected

forests is limited, and has focused mostly on mature trees [2] or soil compartments (i.e., bulk and rhizosphere) [16,27]. However, fungi associated with root tissues establish direct physiological interactions with their host plant, representing a functionally distinct assemblage from those of the surrounding soil [28]. Thus, there is a knowledge gap regarding the root-associated fungal communities of *N. pumilio* seedlings in burned forests, including ectomycorrhiza and dark septate endophytes.

The main goal of this study was to characterize the root-associated fungal communities of *N. pumilio* seedlings under long-term post-fire conditions (i.e., eight years after severe fire) using DNA metabarcoding. Given that (i) *N. pumilio* recruitment currently occurs in low- to moderate-severity burned areas adjacent to unburned forests [23], which may act as fungal inoculum sources [29]; (ii) ectomycorrhizal fungi depend on tree cover, whereas dark septate endophytes do not [10,20]; and (iii) considering the time elapsed since the fire, we hypothesized that, compared with seedlings growing under unburned forest, *N. pumilio* seedlings in burned areas would harbor root-associated fungal communities of similar richness and composition but lower diversity. Within functional groups with plant-beneficial potential, we further predicted lower diversity and abundance of ectomycorrhizal fungi, but similar diversity and abundance of dark septate endophytes. To the best of our knowledge, this is the first study to characterize the diversity of root-associated fungal communities in *N. pumilio* seedlings using DNA metabarcoding, which is a novel contribution for gaining insights into forest recovery processes and ecosystem resilience. This knowledge can better inform forest management practices and conservation strategies in burned temperate forests.

2. Materials and Methods

2.1. Study Area and Wildfire Description

This study was conducted in a National Reserve, located in La Araucanía Region of south-central Chile. The study sites were located within the Andean range, and lie between 759 and 2000 m above sea level. The area is situated within the latitudinal and longitudinal coordinates of approximately 38°40′–38°50′ S and 71°25′–71°37′ W [30]. The region has a temperate climate characterized by warm, dry summers and snowy winters. Between 1990 and 2025 [31], the area recorded an average annual precipitation of 2390 mm. The mean monthly temperature during summer (December to February) was 15.9 °C, while in winter (June to August), it averaged −0.3 °C [31]. The soils originate from volcanic ash deposits and are classified as Andisols and Histosols. They are well-stratified, dark brown in color, and exhibit a coarse texture and high permeability throughout the profile [32]. Vegetation in the reserve is characterized by high-Andean deciduous forests dominated by *N. pumilio* and *Araucaria araucana* (Molina) K. Koch, two endemic species of the temperate forests of South America, occurring in either pure or mixed stands [30].

A major disturbance event in the reserve occurred in 2015, when a large wildfire burned approximately 3765 hectares of native *Araucaria–Nothofagus* forest within the National Reserve. According to the Chilean Forest Service (CONAF), the fire was ignited by an inadequately extinguished campfire that rapidly spread across the landscape [33], exacerbated by several preceding years of drought that has affected south-central Chile since 2010 [34]. The fire was of mixed severity [35], damaging the forest with low (i.e., surface fire, greater damage to the understory), moderate (i.e., partially burned trees, canopy with living and dead trees), and high severity (i.e., completely charred canopy and understory). Burn severity assessment of the study area was previously conducted by [33], performing the delta normalized burn ratio (dNBR) analysis. Over time, ground truthing has been conducted to specifically define areas of moderate and high fire severity and refuges of unburned forests [36].

2.2. Field Sampling, DNA Extraction, Sequencing and Bioinformatics

Root samples were collected from *N. pumilio* seedlings located in both unburned and low severity burned forests within the National Reserve, at elevations ranging from 1455 to 1713 m a.s.l. Sampling was constrained by the natural occurrence of *N. pumilio* regeneration, which depends on seed rain from surviving adult trees. Because *N. pumilio* has low fire resistance and adult trees rarely survive burning [1], post-fire seedling establishment is restricted to burned areas adjacent to unburned forest [24]. Seedlings in burned sites were therefore sampled close to unburned forest edges, as no regeneration was found in the interior of the burned area (Figure 1D). At each sampling point, five seedlings of up to 10 cm in height were selected and their root systems were excised. Roots from the five seedlings were pooled into a single 50 mL conical centrifuge tube to form one composite sample which was considered the experimental unit for downstream analyses. Five composite samples were obtained per condition (unburned and burned forest), each pooling root material from five seedlings (25 seedlings per condition). Composite samples were treated as the unit of replication, giving $n = 5$ per condition for all analyses. Sampling points were spaced at least 50 m apart to ensure spatial independence. Samples were stored in a cooler with ice packs immediately after collection, transported to the laboratory, and maintained at 4 °C until DNA extraction, which was performed within one week of field sampling. Adhering soil particles were carefully removed from the roots, and no surface sterilization was performed, as the aim was to capture the full community of root-associated fungi, including both endophytic and rhizoplane-associated taxa, which together represent the fungal assemblage in direct contact with root tissues. Accordingly, all taxonomic and functional inferences reported here apply to the root-associated assemblage as a whole rather than to a strictly endophytic subset. Discriminating true endophytes from rhizoplane colonizers would require complementary microscopic or cultivation-based approaches, which were outside the scope of this study. Total DNA was then extracted from cleaned root tissues using the DNeasy Plant Pro Kit (Qiagen, Valencia, CA, USA), following the manufacturer's instructions. DNA concentration was quantified using a Qubit® 2.0 Fluorometer (Thermo Fisher Scientific, Waltham, MA, USA). Amplicon sequencing of the fungal Internal Transcribed Spacer 1 (ITS1) region of rDNA was performed by Novogene Co. (Sacramento, USA). Amplification was carried out using primers ITS1-1F-F (5′ CTTGGTCATTTAGAGGAAGTAA 3′) and ITS1-1F-R (5′ GCTGCGTTCTTCATCGATGC 3′). Sequencing was conducted on the Illumina NovaSeq 6000 platform (San Diego, USA) using 250-cycle paired-end mode. Raw sequence reads were processed using the USEARCH-UNOISE3 v11.0.667 pipeline [37]. Paired reads were merged with a minimum overlap of 50 bp, and sequences with expected error rates greater than 1 (EE = 1) were removed. Reads were then dereplicated, and chimeras were filtered out. Denoising was performed using the UNOISE3 algorithm to generate zero-radius Operational Taxonomic Units (OTUs). Taxonomic classification was conducted using a Naïve Bayes classifier (scikit-learn v1.4.2) trained on the UNITE v8 database [38].

2.3. Statistical Analysis

All the statistical analyses were conducted in the statistical software R version 4.5.0 [39] and RStudio version 2024.12.1.563 [40]. To improve the reliability of community composition data, operational taxonomic units (OTUs) with fewer than 10 reads were removed prior to analysis (i.e., 109 OTUs), following the procedures recommended by Nikodemova et al. [41]. To standardize sequencing depth across samples, the dataset was rarefied once to 102,988 reads, the number of reads in the sample with the lowest sequencing depth, using the '*rrarefy*' function from the *vegan* package [42]. To assess sequencing depth and richness saturation, rarefaction curves were generated using the '*rarecurve*' function. A Mantel test

was conducted to validate the preservation of community structure after the filtering and rarefaction procedures. Alpha diversity indices were calculated using the *vegan* package, and differences in diversity between burned forest (BF) and unburned forest (UF) samples were assessed using analysis of variance (ANOVA). Normality and homogeneity of variances were tested using the Shapiro–Wilk test and Levene's test, respectively, with the latter implemented via the *car* package [43]. To examine differences in fungal community structure, a non-metric multidimensional scaling (NMDS) analysis was performed using Bray–Curtis dissimilarity matrices derived from the rarefied OTU abundance data. NMDS ordination was conducted using the '*metaMDS*' function in the *vegan* package, with two dimensions (k = 2), a maximum of 100 iterations, and a random seed set for reproducibility. Permutational multivariate analysis of variance (PERMANOVA; 9999 permutations) was performed using the 'adonis2' function in the *vegan* package to test for significant differences in community composition between the two forest conditions. To assess whether multivariate dispersion differed between conditions, we applied the PERMDISP procedure using the *vegan* package. The 'betadisper' function was used to compute the distance from each observation (sample) to its group's spatial median based on the Bray–Curtis dissimilarity matrix described above. Group means were then compared with a permutation test for homogeneity of multivariate dispersions with 'permutest' function also from the *vegan* package. We used a Venn diagram to determine the number of OTUs that are exclusive to each forest condition as well as those shared between them. Additionally, we visualized the taxonomic composition and abundance of exclusive genera for each condition. To focus on taxa representative of each forest condition, only genera detected in at least two samples were included in this analysis, as single-sample occurrences cannot be reliably distinguished from transient colonization events.

We compared functional fungal groups with plant-beneficial potential (i.e., ectomycorrhizal fungi and root-associated dark septate endophytes) between unburned and burned forest conditions. Functional groups were assigned to each OTU using the FungalTraits database [44]. Among these, we focused on those classified as ectomycorrhizal (EMF) fungi and dark septate endophytes (DSEs), as they are considered to have direct plant-beneficial potential [45,46]. In addition, to complement the functional classification provided by the FungalTraits database, we manually assigned the following taxa as DSE based on the previous literature: the genera *Cadophora* [47], *Cyphellophora* [48], *Exophiala* [49], *Knufia* [50], *Leptodontidium* [51] and the species *Pleotrichocladium opacum* [52]. Similarly, we classified the following taxa as ectomycorrhizal fungi: the families *Endogonaceae* [53], *Pezizaceae* [54], *Hyaloscyphaceae* [55], and the genera *Entoloma* [53] and *Sistotrema* [54]. EMF and DSE OTUs were selected from the filtered, non-rarefied dataset described above. For the comparison of alpha diversity indices between conditions, each guild-specific table was then rarefied to the lowest guild-specific read count observed among samples (8313 reads for EMF; 498 reads for DSE) to equalize sequencing effort across samples, using the 'rrarefy' function from the *vegan* package. A Mantel test was conducted to validate the preservation of community structure between the pre- and post-rarefaction functional group datasets. We then calculated alpha diversity indices using the *vegan* R package, and compared these metrics with analysis of variance (ANOVA; assumptions of normality and homogeneity of variances were tested using the Shapiro–Wilk and Levene's tests, respectively). Comparisons of genus abundance between conditions were performed on the filtered, non-rarefied dataset. For each genus, read counts of all constituent OTUs were summed within each sample (n = 5 per condition), and a separate generalized linear model was fitted using the *glmmTMB* package [56]. The response variable was genus abundance (read counts), the predictor variable was forest condition, and an offset term, the log of the total number of reads in the whole fungal community of each sample, was included. The same offset

was applied to ectomycorrhizal (EMF) and dark septate endophyte (DSE) genus-level models. Three negative binomial parameterizations were considered for each genus: linear (family = nbinom1), quadratic (family = nbinom2), and mixed linear-quadratic (family = nbinom12). Model adequacy was assessed with the *DHARMa* R package [57]: scaled residuals were simulated with the 'simulateResiduals' function and inspected through QQ and residual-versus-predicted plots, which reveal deviations from the expected distribution and report formal tests for uniformity, dispersion, and outliers. For each model that met the assumptions, the *p*-value of the condition coefficient was extracted, and *p*-values were grouped by guild (EMF or DSE). For each group separately, *p*-values were adjusted for multiple comparisons using the Benjamini–Hochberg procedure selected in the 'p.adjust' function of *stats* package [39]. Data processing and cleaning were carried out using the *dplyr* [58], *stringr* [59] *and tidyr* [60] R packages. Data visualization was performed with *ggplot2* [61], *ggpubr* [62] and *ggvenn* [63] R packages.

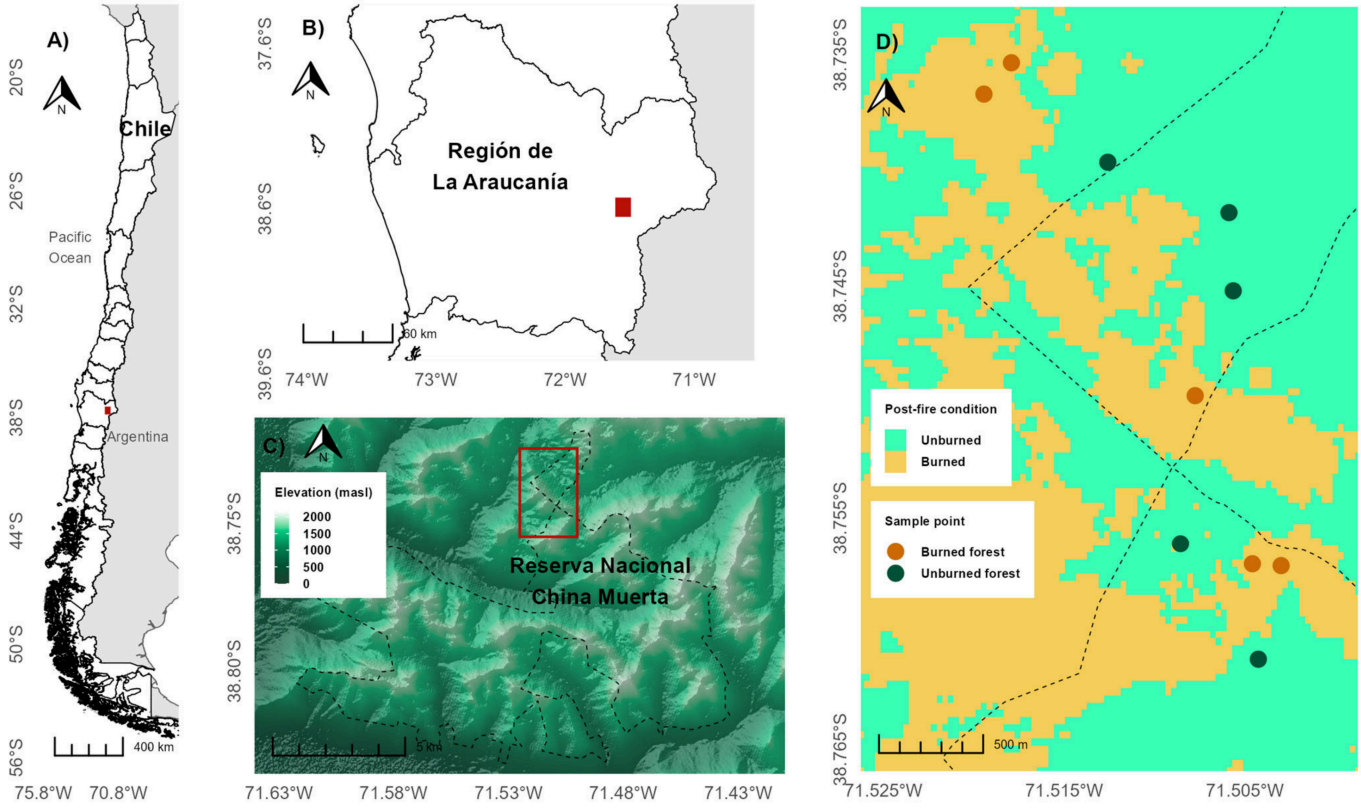

Figure 1. (**A**) Geographic location of Chile in South America, highlighting the study region. (**B**) La Araucanía Region in southern Chile, with the red square indicating the location of the study site. (**C**) China Muerta National Reserve, showing elevation gradients, the official boundary of the reserve (dashed line), and the study section (red box). (**D**) Detailed map of the study section, showing post-fire conditions classified as burned (yellow) and unburned forest (light green). For visualization purposes only, burned areas were identified using the Normalized Burn Ratio (NBR) index calculated from pre- and post-fire Landsat 8 imagery. Sampling points for root-associated fungal communities are marked as dots, with orange indicating burned forest and green indicating unburned forest.

3. Results

Our analyses indicate that both the sample size and sequencing depth were sufficient to capture the majority of taxa present in the two study conditions (i.e., unburned and burned forests). Moreover, the filtering applied to improve representativeness and the rarefaction procedure to equalize read numbers across samples did not alter the original community structure. The Mantel test revealed a strong and statistically significant correlation between

the original and filtered community distance matrices (r = 0.9997, $p = 0.001$), confirming that the data filtering and rarefaction procedures preserved the overall structure of the fungal community. Rarefaction curves reached a clear asymptote for all samples, indicating adequate sequencing depth and sampling completeness (Figure A1 in Appendix A).

3.1. Diversity of Root-Associated Fungal Communities

Eight years after the fire, root-associated fungal communities associated with *N. pumilio* seedlings in unburned forests exhibit higher diversity and evenness than those in burned forest areas (Figure 2). For all four diversity indices, model residuals met the assumptions of normality and homogeneity of variance between conditions (Table A1 in Appendix A). Across all indices, unburned forest showed higher mean values and lower standard errors than burned forest, although the confidence intervals of both conditions overlapped (Table A1 in Appendix A). While species richness did not differ significantly between conditions ($p = 0.36$), unburned forest samples showed significantly higher Shannon diversity ($p < 0.05$), Inverse Simpson index ($p < 0.05$), and Pielou's evenness ($p < 0.05$), indicating a more diverse and evenly distributed fungal community with lower dominance by few taxa.

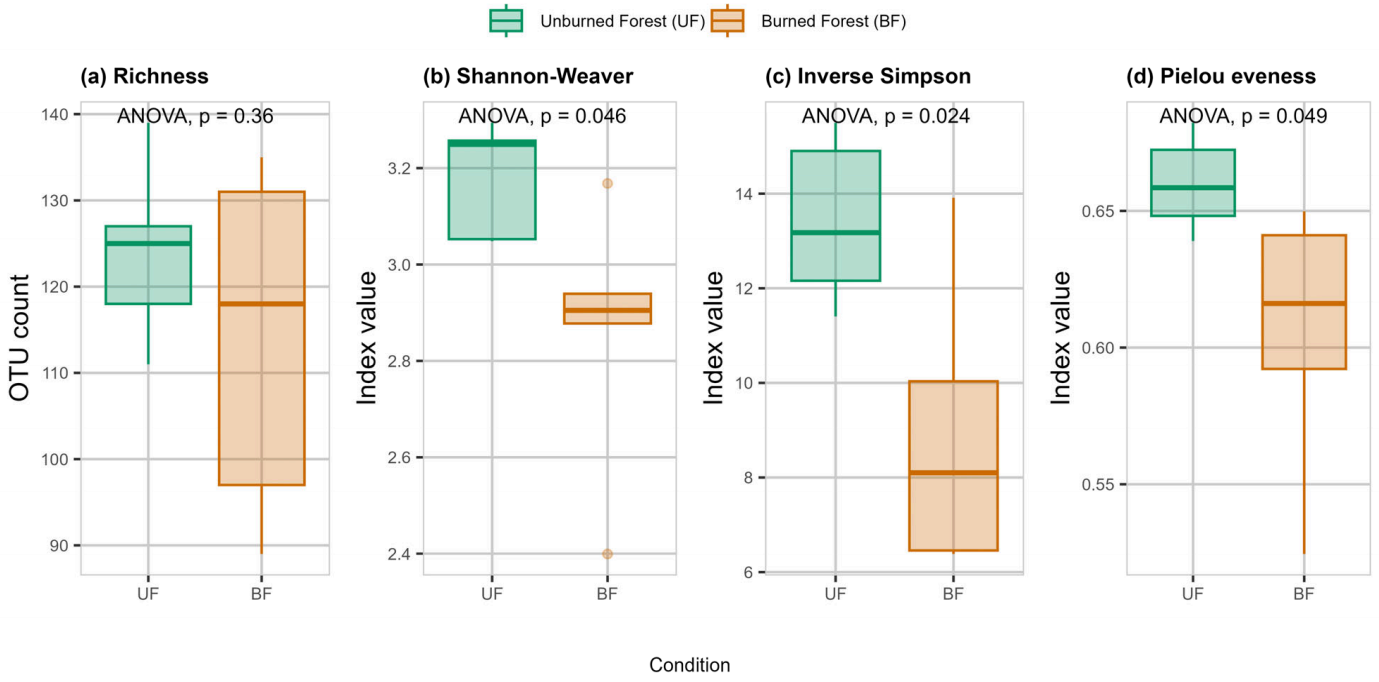

Figure 2. Alpha diversity indices of root-associated fungal communities in *N. pumilio* seedlings growing in unburned forest (UF; $n = 5$ composite samples) and burned forest (BF; $n = 5$ composite samples) sites. Boxplots show values for (**a**) OTU count as fungal richness, (**b**) Shannon–Weaver diversity, (**c**) Inverse Simpson index, and (**d**) Pielou's evenness.

The NMDS analysis showed a good fit (stress = 0.092) and we did not detect a statistically significant difference in the composition of root-associated fungal communities between burned and unburned forests (PERMANOVA, $p = 0.279$). Multivariate dispersion did not differ significantly between conditions either (PERMDISP, $p = 0.25$), although the mean distance to the spatial median was higher in burned (0.50) than in unburned sites (0.45), as reflected in the ellipses in Figure 3. The absence of a dispersion effect indicates that the non-significant PERMANOVA result is not confounded by heterogeneous within-group variability.

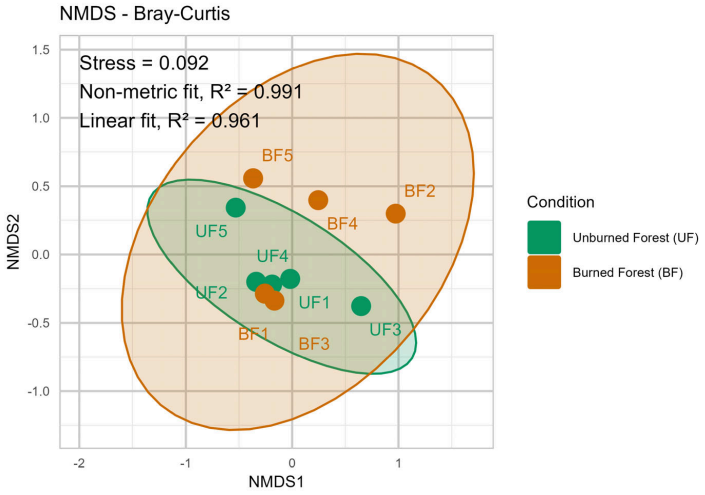

Figure 3. Non-metric multidimensional scaling (NMDS) ordination based on Bray–Curtis dissimilarity, showing root-associated fungal community composition of *N. pumilio* seedlings growing in unburned (green) and burned (orange) forest. Each point represents a sample (n = 5 per condition), and ellipses represent 95% confidence regions based on the multivariate t-distribution and are included for visualization purposes to illustrate the dispersion of samples within each group.

Regarding richness, a total of 294 OTUs were identified, of which 67 were exclusive to unburned forest, 64 were exclusive to burned forest, and 163 were shared between both conditions, representing 22.8%, 21.8%, and 55.4% of the total, respectively (Figure 4a). The most abundant fungal genera found exclusively in the roots of *N. pumilio* seedlings growing in unburned forest were *Meliniomyces*, *Cryptodiscus*, *Phialocephala*, and *Zasmidium* (Figure 4b). In contrast, the most abundant genera found exclusively in roots of seedlings growing in burned forest conditions were *Paraphoma*, *Morchella* and *Tausonia* (Figure 4c).

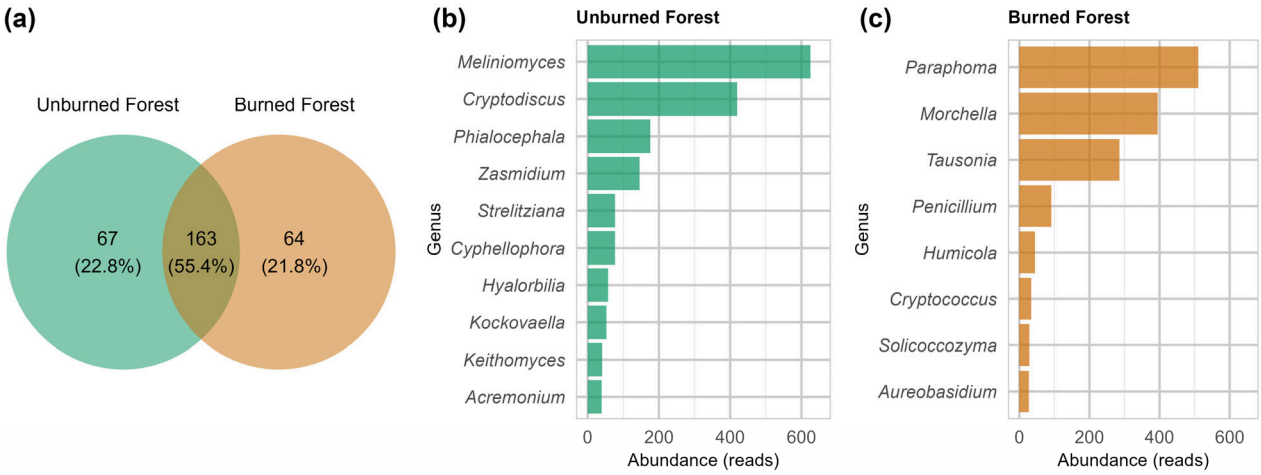

Figure 4. Shared and exclusive root-associated fungal OTUs and genera of *N. pumilio* seedlings growing in unburned and burned forest sites. (**a**) Venn diagram showing the number and percentage of fungal OTUs exclusive to unburned forest (green), burned forest (orange), and those shared between both conditions (overlap). (**b**) Bar plot showing the most abundant exclusive genera detected in seedlings growing in unburned forest (green). (**c**) Bar plot showing the most abundant exclusive genera detected in seedlings growing in burned forest (orange). Abundances in bar plots (**b**,**c**) represent the total number of sequence reads detected for each genus.

3.2. Putative Plant-Beneficial Functions Groups of Root-Associated Fungi

EMF fungi samples were rarefied to 8313 reads, with pre- and post-rarefaction Bray–Curtis dissimilarity matrices showing a strong correlation (r = 0.913, p = 0.001). For

DSE, samples were rarefied to 498 reads, yielding a moderate but significant correlation between pre- and post-rarefaction matrices (r = 0.689, $p = 0.002$), indicating that rarefaction adequately preserved the overall community structure in both functional groups.

For all diversity indices, model residuals met the assumptions of normality and homogeneity of variance (Table A2 in Appendix A). Across all indices, unburned forest showed higher mean values than burned forest in both functional groups, and the confidence intervals of the two conditions overlapped. Variability differed between groups: for EMF, burned forest showed higher standard errors, whereas for DSE the higher standard errors occurred in unburned forest (Table A2 in Appendix A). Although alpha diversity indices of EMF did not show statistically significant differences between conditions (Figure 5a–d), the unburned forest consistently exhibited slightly higher values for richness, diversity (Shannon–Weaver and Inverse Simpson), and evenness (Pielou), along with lower dominance of specific taxa (as reflected by the Inverse Simpson index). Similarly, although DSE communities in the unburned forest tended to be slightly more diverse, no significant differences were detected between forest conditions in any of the alpha diversity metrics (richness, $p = 0.24$; Shannon–Weaver, $p = 0.51$; Inverse Simpson, $p = 0.41$; Pielou's evenness, $p = 0.68$; Figure 5e–h).

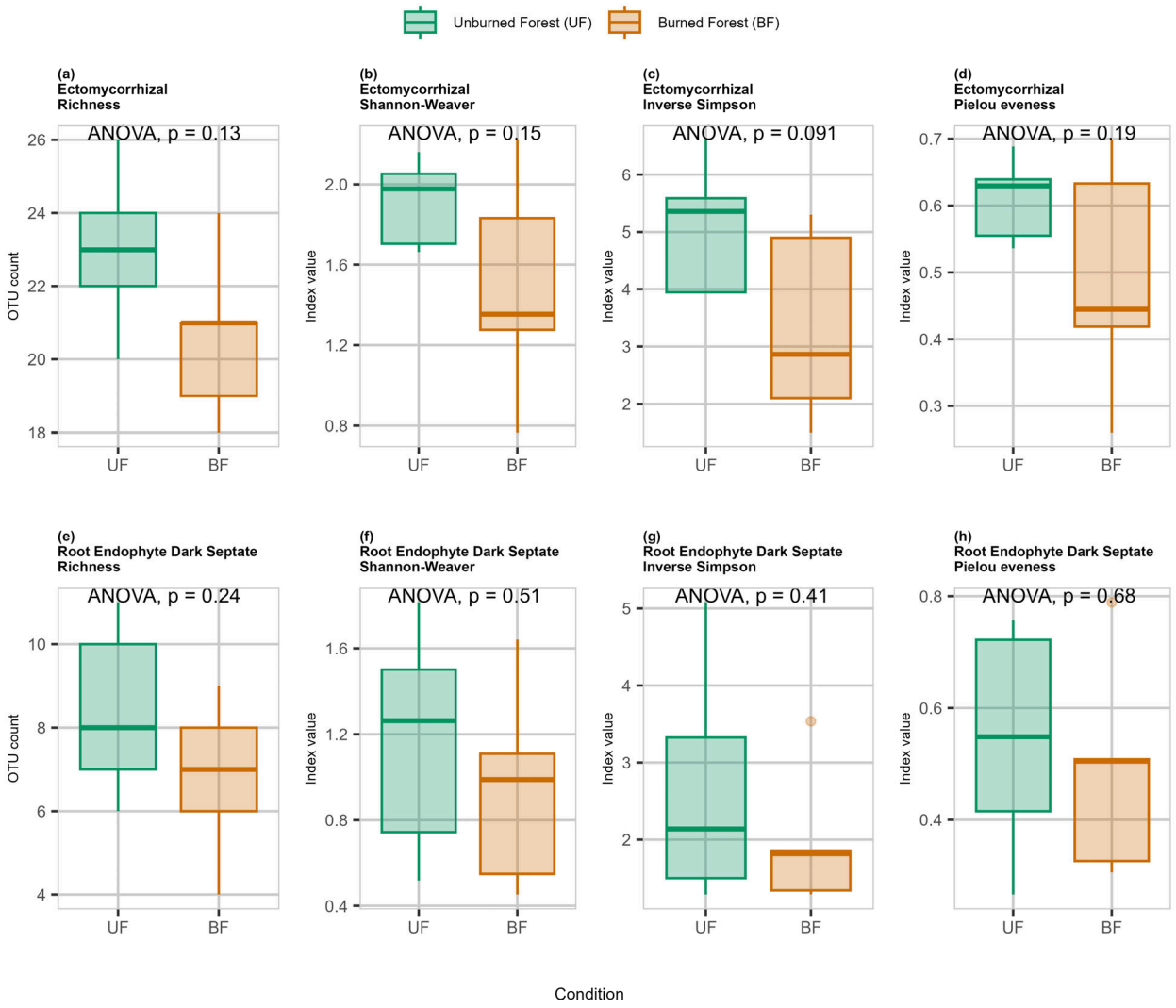

Figure 5. Comparison of alpha diversity indices (Richness, Shannon–Weaver, Inverse Simpson, and Pielou's evenness) for the functional groups ectomycorrhizal fungi (**a**–**d**) and root-associated dark septate endophytes (**e**–**h**) associated with *N. pumilio* seedlings growing in unburned (green) and burned (orange) forest conditions.

Among the 10 EMF genera tested, only *Cortinarius* differed significantly in abundance between forest conditions (adjusted $p < 0.05$; Figure 6a), being higher in unburned forest. Among the 8 DSE genera tested, *Cadophora* was more abundant in burned forest, whereas *Exophiala* and *Cladophialophora* were more abundant in unburned forest (adjusted $p < 0.05$; Figure 6b). All genus-level models met the residual diagnostics of the DHARMa package, with no departures from uniformity (Kolmogorov–Smirnov), dispersion, or outlier (Tables A3 and A4, Appendix A.3).

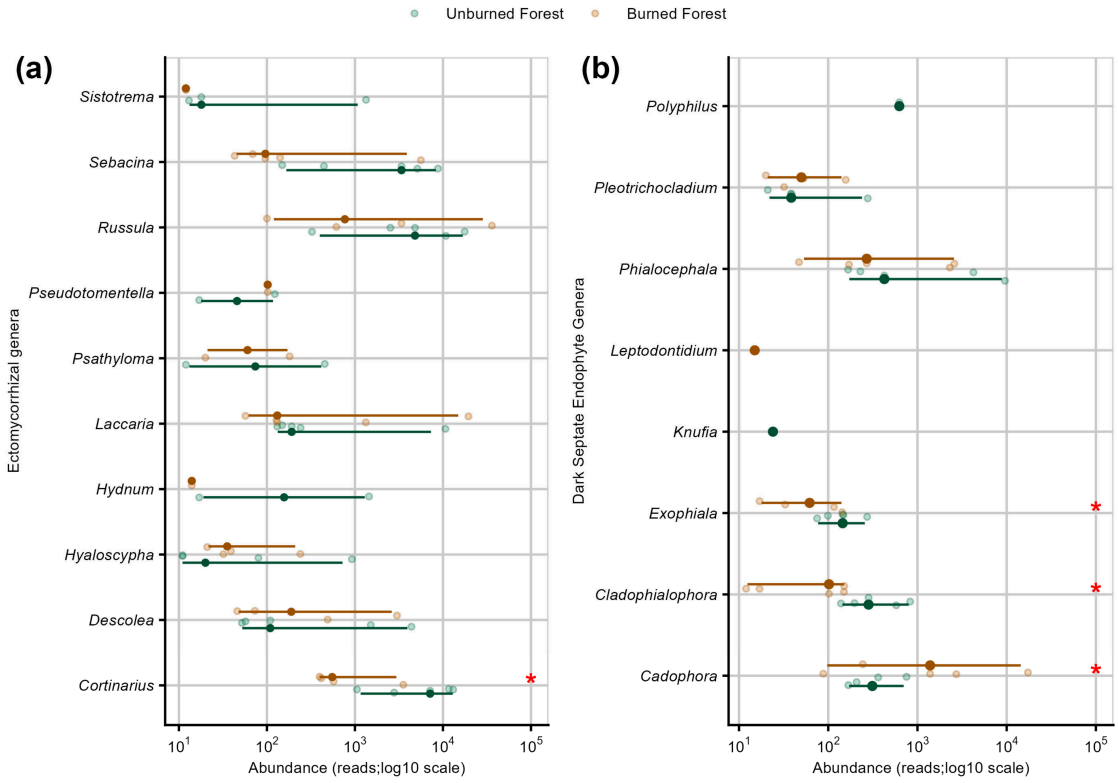

Figure 6. Abundance (reads) of (**a**) ectomycorrhizal fungal genera and (**b**) dark septate endophytes genera detected in roots of *N. pumilio* seedlings growing in unburned (green) and burned (orange) forest conditions. Each small point represents the abundance ($\log_{10}$ scale) of the genus within each sample. Horizontal lines indicate the range of observed abundances, and the larger solid-colored points represent the mean abundance per condition. A red asterisk (*) denotes a statistically significant difference (p adjusted < 0.05) in abundance between forest conditions.

4. Discussion

4.1. Burned Forests Shape Root-Associated Fungal Diversity

Our results are consistent with our hypothesis that *N. pumilio* seedlings in burned areas would harbor root-associated fungal communities of similar richness and composition but lower diversity. While species richness and overall community composition did not differ significantly between unburned and burned forests, fungal diversity, evenness, and dominance structure, as indicated by the Shannon index, Pielou's evenness, and the Inverse Simpson index, respectively, remained significantly different in burned areas, indicating that recovery is incomplete despite the time elapsed since the disturbance. The overlap in their confidence intervals indicates a consistent but limited reduction in diversity rather than a pronounced loss. Evidence suggests that root-associated fungal community responses to fire in *Nothofagus* forests are highly variable [2,64,65]. A previous study in Tierra del Fuego (Argentina) found that soil fungal communities can vary according to microsite conditions such as temperature, precipitation, soil pH, and nitrogen content [27]. Since soil nutrients and vegetation composition influence fungal communities [66], the decline in soil

nitrogen levels [67] and the post-fire shifts in vegetation structure [23] previously reported for the study area may contribute to reduced diversity of fungi associated with the roots of *N. pumilio* seedlings, even over the long term.

4.2. Exclusive Fungal Taxa Suggest Potential Functional Shifts After Fire

The functional roles reported for condition-exclusive genus differed between forest conditions, with unburned forest harboring genera commonly reported as mutualistic or plant-associated, whereas burned forests included genera more frequently associated with saprotrophy, disturbance, or biomass degradation. This pattern is similar to previous findings indicating that post-fire environments, characterized by reduced canopy cover and increased dryness [1], may promote long-term reductions in fungal biomass due to the high drought sensitivity of fungi [68], shifting fungal community composition in favor of saprotrophs over ectomycorrhizal fungi [69]. Such shifts could limit resource acquisition by host seedlings.

Among the fungal taxa found exclusively in seedlings from unburned forests, several genera with documented plant-beneficial traits were particularly prominent. The most abundant genus, *Meliniomyces*, is a widespread ectomycorrhizal taxon known to associate with diverse tree seedlings and enhance drought tolerance [70–72]. The third most abundant genus, *Phialocephala*, has been reported to promote seedling growth in the other host [73,74], increase plant nutrient concentrations [74], and suppress root pathogens in conifers [75,76]. Although less abundant, other taxa such as *Strelitziana* have been associated with enhanced drought resistance [77], and *Cyphellophora* exhibits plant-growth-promoting traits including auxin production and phosphate solubilization [47]. Some of these fungi may also provide indirect benefits via saprotrophic functions. For instance, *Cryptodiscus* (primarily lichen-associated; [78]), *Zasmidium* [79], *Hyalorbilia* (also fungicolous; [80]), and *Acremonium* [81]). At the species level, a taxon was identified as *Zasmidium citrigriseum* (Appendix A.4), a known pathogen of *Citrus sinensis* [L.] Osbeck [82]. Further research is needed to determine whether it also acts as a pathogen of *N. pumilio*, or whether it promotes plant growth and enhances defence against biotic stress, as reported for other *Zasmidium* species [83]. Similarly, *Acremonium sclerotigenum*, found exclusively in seedlings growing in unburned forest (Appendix A.4), has been shown to suppress fungal diseases and promote growth in agriculturally important plants [84,85]. Other genera such as *Kockovaella* and *Keithomyces* remain poorly characterized ecologically [86], although some representatives have been isolated from forest soils and woody substrates [87,88].

In seedling growing in burned forest, the exclusive fungal taxa included genera frequently associated with disturbance or post-fire environments. This aligns with the extreme environmental conditions characteristic of burned areas, such as snow during winter, increased dryness, higher solar radiation in summer, and a greater presence of dead plant biomass [1,23]. The most abundant exclusive genus in burned forests was *Paraphoma*, a group known for its plant-pathogenic species of agricultural relevance. Previous studies have reported increases in *Paraphoma* abundance following fire events [89]. The OTU count identified that *Paraphoma fimeti* remains poorly studied (Appendix A.4). It may be non-pathogenic and could potentially confer beneficial traits to seedlings. However, one study reports its ability to enhance grass growth [90]. The occurrence of *P. fimeti* may be related to the presence of livestock and introduced pasture species in the reserve, which were observed during field expeditions [91]. The second most abundant genus, *Morchella*, includes species that can be both saprotrophic and ectomycorrhizal. Several *Morchella* species are known to be pyrophilous, with reproduction stimulated by fire [92]. In our study, *Morchella arbutiphila* was found exclusively in burned forest, consistent with prior reports from mountainous and xerophytic habitats with high solar exposure [93]. Fungi

from the third most abundant genus, *Tausonia*, are associated with lignin and hemicellulose degradation [94]. The yeast *Tausonia pullulans*, often isolated from cold or extreme environments [95,96], is capable of degrading plant material at low temperatures [97], suggesting a possible role in decomposing post-fire biomass. The genus *Penicillium*, though less abundant, includes pyrophilous species [89]. In our case, *Penicillium angulare* was detected, and it has previously been described as a fungicolous species occurring on woody substrates [98]. *Humicola nigrescens* is recognized as a thermophilic fungus [99], consistent with the drier and more sun-exposed conditions of burned sites. Likewise, species from the genus *Solicoccozyma* are saprotrophic and have been reported as dominant taxa in post-fire mountain forest ecosystems [100]. Interestingly, several fungi exclusively found in burned sites may also provide potential plant benefits. Genera such as *Cryptococcus*, *Solicoccozyma*, and *Aureobasidium pullulans* have been shown to promote plant growth and enhance tolerance to abiotic stress [101–104]. However, it is worth noting that some strains of *A. pullulans* may also act as plant pathogens [105,106]. Finally, *Cryptococcus watticus* has been previously isolated from glacier environments [107] and the Antarctic region [108], which may be consistent with the snowy conditions present in our study area during winter.

4.3. Stable Diversity of Ectomycorrhizal and Dark Septate Endophytes

Contrary to our expectations, EMF showed no statistically significant differences in richness, diversity, or evenness between unburned and burned forests, which may suggest that seedlings in burned areas recruit EMF without detectable reductions relative to unburned conditions, eight years post-fire. However, a slight trend toward lower diversity was observed in burned areas, as indicated by the Inverse Simpson index, which suggests a mild tendency toward community dominance under post-fire conditions (Figure 5). The overall comparability of EMF between conditions could be explained by the influence of nearby unburned forest patches as inoculum sources and the time elapsed since the disturbance. This interpretation is supported by a temporal pattern emerging from previous studies: ten months after fire, ectomycorrhizal communities in *Nothofagus* forests were negatively affected in terms of colonization rates, richness, diversity, and community composition [65]; two years post-fire, *Nothofagus alpina* (Poepp. & Endl.) Oerst. seedlings growing in burned conditions exhibited a slight reduction in EMF richness and a significant dominance of a single morphotype [64]; and in the long term, ectomycorrhizal morphotype diversity associated with the roots of *N. pumilio* appeared unaffected by post-fire conditions, showing no significant differences compared to unburned conditions [2]. Our results at eight years post-fire are consistent with this trajectory of progressive recovery.

As hypothesized, DSE communities showed a similar, although non-significant, tendency. Seedlings growing in burned forests exhibited slightly lower richness, diversity, and evenness than those growing in unburned forests, suggesting that DSE communities may be relatively stable under long-term post-fire conditions. Alternatively, DSE communities may have been initially affected by fire but recovered within the eight-year period examined in this study, a possibility that warrants further investigation through temporal monitoring. DSE fungi may show similar richness and diversity across fire conditions due to their high melanin levels, which improve tolerance to both heat and cold [29]. DSE are commonly found in harsh environments due to their high tolerance to abiotic stress [109]. We found no other assessments of the response of root-associated DSE communities to forest fires. Existing literature primarily focuses on their occurrence in plant species [10] and the agricultural applications [110]. Specifically in *N. pumilio* forests, post-fire fungal research has primarily focused on EMF communities [2,27,65].

4.4. Genus-Level Variation in Putative Plant-Beneficial Fungi

Contrary to our expectation of a broad reduction in ectomycorrhizal abundance, only *Cortinarius* declined significantly among the ten EMF genera tested, being less abundant in burned forests. Changes in vegetation cover following fire may partially explain these shifts, as canopy structure has been shown to influence the abundance of rhizosphere-associated fungi in *Nothofagus*-dominated forests [20]. The lower abundance of *Cortinarius* in burned forests is unexpected from the perspective of soil nutrient relations, as lower nitrogen availability, such as that reported for the study area [67], would typically be expected to increase rather than reduce its abundance [111]. However, post-fire conditions may impair the ability of ectomycorrhizal fungi to colonize roots, even six to seven years after the disturbance [2], and previous studies have shown that some *Cortinarius* taxa display a marked affinity for unburned forests, whereas others tend to prefer post-fire environments [27]. The specific taxa detected in unburned forests may be particularly sensitive to burned environments.

Contrary to our hypothesis of no differences in DSE abundance between conditions, three DSE genera differed significantly. Among 8 DSE genera tested, *Cadophora* showed a statistically significant difference in abundance, with higher values in burned forests, whereas *Exophiala* and *Cladophialophora* were more abundant in unburned forest *Cadophora* possesses a strong saprotrophic capacity and has been reported in semiarid environments [112], which may explain its higher abundance in burned forests, where greater amounts of dead plant material and drier soil conditions were observed during field visits compared to the unburned forest floor. How *Exophiala* and *Cladophialophora* respond to disturbance remains poorly studied; existing work has focused mainly on their distribution and applied uses [113–115].

4.5. Potential Implications for N. pumilio Forest Recovery

Despite the time elapsed since the disturbance, burned forests still exert a detectable influence on the root-associated fungal communities of *N. pumilio*. In the context of increasing wildfire frequency and severity driven by climate extremes (e.g., drought) [4], the resilience of these fungal communities may become a critical factor limiting seedling establishment and shaping post-disturbance forest dynamics. Lower fungal diversity and biomass may negatively impact multiple ecosystem functions by reducing both functional redundancy (fewer fungi performing similar roles) and the capacity for unique functional contributions [12,13]. Furthermore, the richness and abundance of fungal communities may shape the spatial distribution of plant species across landscapes [6]. This, in turn, could influence the recovery of burned *N. pumilio* forests. Fungal genera with reported plant-beneficial functions were less abundant in the roots of seedlings growing in burned forest, which may have implications for early performance. Members of the *Cortinariaceae* are typically dominant ectomycorrhizal associates of *Nothofagus* [54] and possess enzymatic capabilities that allow them to mobilize organic nitrogen [111]. A reduction in their abundance may therefore limit nutrient mobilization to seedlings. A similar rationale applies to two dark septate endophyte genera that were likewise less abundant in burned forest: *Exophiala*, whose species can promote plant growth, phosphorus uptake, and photosynthesis [114,115], and *Cladophialophora*, reported to promote growth and, together with *Exophiala*, to suppress the severity of diseases caused by other pathogenic fungi [116].

4.6. Insights into Assisted Regeneration of N. pumilio Forests

Although our results show that the root-associated fungal community of *N. pumilio* seedlings in burned forest still differs from that of seedlings in unburned forest, functionally relevant groups such as EMF and DSE remained present in burned areas. Some genera

were less abundant in burned forests, but these functional groups were still detectable in association with seedling roots, suggesting that a baseline of plant-beneficial fungal potential persists in post-fire conditions. A similar pattern was observed in the bulk soil fungal communities of burned forests, which closely resembled those of unburned forests in the study area [16], potentially enhancing seedling establishment and increasing the success of reforestation efforts [117].

Given that *N. pumilio* is characterized by limited seed dispersal and low germination capacity, with natural regeneration rarely occurring beyond approximately 20 m from intact forest edges and being absent in areas affected by high-severity fires [1,24], the spatial context of seedling establishment becomes particularly relevant. Based on our results, planting seedlings in proximity to unburned forests could potentially facilitate the colonization of beneficial fungi in burned areas where no seed trees have survived, as unburned forests may serve as fungal inoculum sources [29]. Supporting this idea, the root-associated fungal community of *N. pumilio* seedlings in unburned forests was found to be highly diverse, despite roots generally being the plant compartment with the lowest expected fungal diversity [8], highlighting the inoculum potential of intact forest patches for assisted regeneration efforts.

4.7. Study Limitations and Research Perspectives

These results should be interpreted with caution owing to methodological limitations: a small sample size (five composite samples per condition) may increase the risk of type II error, rarefying to a single random subsample, moderate but significant Mantel test in DSE community, and reliance on a single fire event. Functional interpretations in this study were based on taxonomic identity, published trait databases, and previous literature. Accordingly, the classification of taxa as ectomycorrhizal fungi, dark septate endophytes, saprotrophs, or fungi with potential plant-beneficial roles should be understood as ecological inference rather than confirmed functional roles. A key limitation is that most of the plant-beneficial functions attributed to these fungi come from their application to other plant species rather than to *N. pumilio.* To our knowledge, direct evidence for this topic remains scarce. Of the 294 OTUs detected, 207 were assigned only to the genus level, limiting our ability to fully understand the ecological roles of specific taxa and their interactions with the environment, including host plants. While DNA sequencing enables detailed community profiling, both the technology and reference databases remain under continuous development [118], and important taxonomic gaps persist, particularly for fungi identification in *Nothofagaceae* forests [54]. Additionally, soil chemical properties were not measured in this study. Post-fire changes in soil chemistry are known to influence fungal community composition [27], and differences in soil conditions between unburned and burned areas could partially explain the observed patterns in root-associated fungal communities. Including soil chemical analyses in future studies would allow for a more comprehensive understanding of the factors driving fungal community differences under post-fire conditions. Despite these limitations, our approach provides a useful framework for interpreting broad patterns in root-associated fungal communities under post-fire conditions. Future studies combining metabarcoding with direct assessments of root colonization, enzymatic activity, nutrient transfer, and seedling physiological responses would help clarify the functional contribution of these fungal groups to *Nothofagus* regeneration. Improving species-level identification would enhance our capacity to assess the ecological roles of understudied taxa and to explore potential interactions between native and non-native fungi. For example, Hewitt et al. [119] reported that *Cortinarius amoenus* was negatively correlated with mean seedling biomass in *N. pumilio*, although this species was not detected in the present study, suggesting that the functional role of *Cortinarius* may vary depending

on the species present. Consistent with this, some *Cortinarius* taxa show a marked affinity for unburned forests whereas others prefer post-fire environments [27], and the specific taxa we detected may be particularly sensitive to burned conditions. Additionally, we detected the exotic ectomycorrhizal species *Hebeloma mesophaeum* in both unburned and burned conditions (Appendix A.4), a species introduced from the Northern Hemisphere through *Pinus* plantations [120]. Non-native fungi may compete with native taxa and facilitate co-invasion processes involving plants, potentially altering successional trajectories [121].

Such research is particularly relevant given the key role of fungi in supporting soil multifunctionality [122], their influence on plant distribution patterns [6] and their faster response compared to vascular plants to climate-disturbance [26], fungi should receive greater attention in the context of increasing wildfire frequency, whose severity is being amplified by both climatic and anthropogenic pressures.

5. Conclusions

This study provides a comprehensive characterization of root-associated fungal communities in *N. pumilio* seedlings eight years post-fire in Andean forests of southern Chile, using DNA metabarcoding techniques. To the best of our knowledge, this is the first study characterizing the diversity and composition of dark septate endophytes in response to long-term post-fire conditions. We hypothesized that, compared with seedlings growing under unburned forest, *N. pumilio* seedlings in burned areas would harbor root-associated fungal communities of similar richness and composition but lower diversity. Within functional groups with plant-beneficial potential, we further predicted lower diversity and abundance of ectomycorrhizal fungi, but similar diversity and abundance of dark septate endophytes. Our results are partially consistent with this hypothesis. First, root-associated fungal communities in seedlings growing in burned forests exhibit significantly lower overall diversity and evenness despite maintaining similar OTU richness and community composition. Second, while both forest conditions shared 55.4% of OTUs, fungi found exclusively in unburned forests are predominantly taxa with plant-beneficial functions, whereas those exclusive to burned forests tend to be associated with biomass degradation and adaptation to harsher environmental conditions. Third, neither ectomycorrhizal fungi nor dark septate endophytes differed significantly between forest conditions in any alpha diversity index. Fourth, significantly lower abundances of *Cortinarius* (EMF), *Exophiala* (DSE), and *Cladophialophora* (DSE) were detected in burned forest, but a higher abundance of *Cadophora* (DSE).

Eight years after fire, the root-associated fungal communities of *N. pumilio* seedlings in burned forest remain associated with detectable differences, including lower diversity and evenness and reduced abundance of specific taxa such as *Cortinarius*, *Exophiala*, and *Cladophialophora*. Nevertheless, these communities retain their main functional groups (EMF and DSE) and share most OTUs with unburned forest, indicating an incomplete but ongoing recovery. These findings contribute to filling the knowledge gap in fungal responses to post-fire conditions, which remain largely underrepresented in the literature, and have implications for forest restoration strategies in South American temperate forests. The contrasting patterns observed across diversity metrics, community composition, and genus-level abundance suggest complex fungal community trajectories that extend beyond what classic diversity indices can capture. These results underscore the importance of preserving unburned forest remnants within fire-affected landscapes, as they may serve as critical sources of fungal inoculum for seedling establishment in adjacent burned areas, and suggest that restoration efforts could benefit from establishing *N. pumilio* seedlings in burned areas adjacent to intact forests where no seed trees have survived.

Future research should investigate how these contrasting patterns may influence early seedling performance of *N. pumilio* in post-fire environments, considering both the potential negative effects of the observed community shifts and the potential beneficial role that persistent ectomycorrhizal fungi and dark septate endophytes may play in supporting seedling establishment, which could inform future restoration strategies. Long-term monitoring beyond the eight-year timeframe of this study would help determine whether these patterns represent a transitional state or a stable alternative community configuration in fire-affected *N. pumilio* forests.

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Appendix A

Appendix A.1. Rarefaction Curves

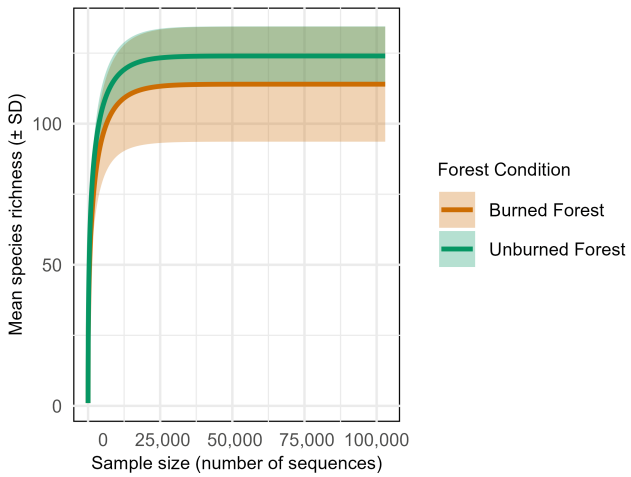

Figure A1. Rarefaction curves showing mean species richness (solid lines) ± standard deviation (shaded ribbons) for root-associated fungal communities of *N. pumilio* seedlings in burned (orange) and unburned (green) forest conditions. Curves were generated by rarefying OTU counts to a standardized sequencing depth of 102,988 reads per sample.

Appendix A.2. Descriptive Statistics and Verification of ANOVA Assumptions

Table A1. Descriptive statistics (mean, standard error, 95% confidence intervals) and ANOVA assumption tests (Shapiro–Wilk, Levene's) for alpha diversity indices of the total fungal community.

Index	Mean		Standard Error		Confidence Intervals 95%		Shapiro–Wilk (*p*-Value)	Levene's Test (*p*-Value)
	UF	BF	UF	BF	UF	BF		
Richness	124	114	4.69	9.11	[111, 137]	[88.7, 139]	0.778	0.164
Shannon diversity	3.18	2.86	0.05	0.12	[3.03, 3.33]	[2.51, 3.21]	0.149	0.497
Inverse Simpson index	13.4	8.98	0.78	1.40	[11.3, 15.6]	[5.08, 12.9]	0.364	0.436
Pielou's evenness	0.66	0.60	0.007	0.02	[0.63, 0.68]	[0.54, 0.66]	0.316	0.213

Table A2. Descriptive statistics (mean, standard error, 95% confidence intervals) and ANOVA assumption tests (Shapiro–Wilk, Levene's) for alpha diversity indices of the ectomycorrhizal (EMF) and dark septate endophyte (DSE) functional groups.

Functional Group	Index	Mean		Standard Error		Confidence Intervals 95%		Shapiro–Wilk (*p*-Value)	Levene's Test (*p*-Value)
		UF	BF	UF	BF	UF	BF		
EMF	Richness	23	20.6	1.001	1.03	[20.2, 25.8]	[17.7, 23.5]	0.679	1
	Shannon diversity	1.91	1.49	0.09	0.24	[1.64, 2.18]	[0.80, 2.18]	0.906	0.211
	Inverse Simpson index	5.08	3.33	0.51	0.75	[3.66, 6.50]	[1.24, 5.43]	0.308	0.416
	Pielou's evenness	0.61	0.49	0.02	0.07	[0.53, 0.68]	[0.27, 0.71]	0.869	0.154
DSE	Richness	8.4	6.8	0.92	0.86	[5.83, 11]	[4.41, 9.19]	0.710	0.788
	Shannon diversity	1.17	0.94	0.23	0.21	[0.59, 1.83]	[0.35, 1,54]	0.417	0.744
	Inverse Simpson index	2.66	1.97	0.70	0.40	[0.72, 4.61]	[0.83, 3.10]	0.223	0.356
	Pielou's evenness	0.54	0.48	0.09	0.08	[0.28, 0.79]	[0.24, 0.72]	0.705	0.727

Appendix A.3. Model Specification and Residual Diagnostics for Genus-Level Negative Binomial Models

Table A3. Residual diagnostics for EMF genus-level negative binomial models. For each genus, the selected negative binomial parameterization is shown, together with *p*-values from DHARMa tests of uniformity (Kolmogorov–Smirnov), dispersion, and outliers.

Genus	Parametrization	Kolmogorov–Smirnov Test (*p*-Value)	Dispersion Test (*p*-Value)	Outlier Test (*p*-Value)	*p*-Value	*p*-Value Adjust
Cortinarius	nbinom12	0.707	0.796	1	0.0003	0.003
Descolea	nbinom1	0.963	0.708	1	0.321	0.642
Hyaloscypha	nbinom2	0.581	0.414	1	0.233	0.583
Hydnum	nbinom1	0.991	0.564	1	0.425	0.665
Laccaria	nbinom2	0.187	0.416	1	0.604	0.755
Psathyloma	nbinom1	0.594	0.712	1	0.956	0.956
Pseudotomentella	nbinom1	0.381	0.798	1	0.466	0.665

Table A3. *Cont.*

Genus	Parametrization	Kolmogorov–Smirnov Test (*p*-Value)	Dispersion Test (*p*-Value)	Outlier Test (*p*-Value)	*p*-Value	*p*-Value Adjust
Russula	nbinom12	0.873	0.57	1	0.915	0.956
Sebacina	nbinom1	0.428	0.790	1	0.115	0.386
Sistotrema	nbinom12	0.711	0.498	1	0.028	0.142

Table A4. Residual diagnostics for DSE genus-level negative binomial models. For each genus, the selected negative binomial parameterization is shown, together with *p*-values from DHARMa tests of uniformity (Kolmogorov–Smirnov), dispersion, and outliers.

Genus	Parametrization	Kolmogorov–Smirnov Test (*p*-Value)	Dispersion Test (*p*-Value)	Outlier Test (*p*-Value)	*p*-Value	*p*-Value Adjust
Cadophora	nbinom2	0.912	0.258	1	0.0006	0.005
Cladophialophora	nbinom2	0.881	0.812	1	0.001	0.004
Exophiala	nbinom1	0.882	0.918	1	0.007	0.031
Knufia	nbinom1	0.302	0.428	1	0.999	0.999
Leptodontidium	nbinom2	0.577	0.452	1	0.999	0.999
Phialocephala	nbinom1	0.402	0.566	1	0.480	0.769
Pleotrichocladium	nbinom1	0.854	0.472	1	0.374	0.748
Polyphilus	nbinom1	0.986	0.400	1	0.999	0.999

Appendix A.4. List of Fungi Identified at the Species Level

Table A5. Fungal species identified in root samples from seedlings growing exclusively in unburned forest, exclusively in burned forest, and species shared between both conditions.

Unburned Forest	Shared Species	Burned Forest
Acremonium sclerotigenum	*Acremonium alternatum*	*Acaulospora alpina*
Apiotrichum dehoogii	*Aleurina Argentina*	*Alternaria subcucurbitae*
Beauveria varroae	*Babjevia anomala*	*Aureobasidium pullulans*
Colletotrichum feijoicola	*Cladophialophora chaetospira*	*Chalara pseudoaffinis*
Cyphellophora sessilis	*Cortinarius dibaphoides*	*Cladophialophora minutissima*
Fusarium proliferatum	*Cortinarius mustella*	*Coniochaeta decumbens*
Fusarium solani	*Descolea antarctica*	*Cryptococcus watticus*
Ganoderma australe	*Exophiala equina*	*Diaporthe kochmanii*
Geomyces auratus	*Gremmenia infestans*	*Humicola nigrescens*
Hannaella luteola	*Halomyces littoreus*	*Hyphodiscus hymeniophilus*
Keithomyces carneus	*Hebeloma mesophaeum*	*Ilyonectria robusta*
Knufia peltigerae	*Hyphozyma roseonigra*	*Inocybe neuquenensis*
Kockovaella prillingeri	*Lecanicillium fusisporum*	*Melanomma japonicum*
Lasionectria hilhorstii	*Linnemannia amoeboidea*	*Microcera coccophila*
Mycena stylobates	*Melanodiplodia tianschanica*	*Morchella arbutiphila*
Ruhlandiella patagonica	*Metapochonia bulbillosa*	*Paraphoma fimeti*
Strelitziana sarbhoyi	*Mortierella pseudozygospora*	*Penicillium angulare*
Talaromyces infraolivaceus	*Mycena metata*	*Pezicula ericae*
Trichoderma oblongisporum	*Mycena strobilicola*	*Phacidium lacerum*
Vanrija humicola	*Odontia ferruginea*	*Phanerochaete alnea*
Zasmidium citrigriseum	*Paludomyces mangrovei*	*Solicoccozyma terrea*
	Penicillago nodositata	*Solicoccozyma terricola*
	Penicillium aquadulcis	*Sporobolomyces patagonicus*

Table A5. *Cont.*

Unburned Forest	Shared Species	Burned Forest
	Penicillium bialowiezense	*Sterigmatomyces halophilus*
	Penicillium miczynskii	*Tausonia pullulans*
	Penicillium sanguifluum	*Vishniacozyma victoriae*
	Peristomialis corynospora	
	Phaeosphaeria oryzae	
	Phialocephala fortinii	
	Pleotrichocladium opacum	
	Zasmidium fructigenum	
	Podila humilis	
	Pseudogymnoascus roseus	
	Ramularia armoraciae	
	Rickenella minuta	
	Russula echidna	
	Russula fuegiana	
	Russula nothofagi	
	Saitozyma podzolica	
	Sphaerostilbella toxica	
	Trichoderma stellatum	
	Trichothecium crotocinigenum	
	Zasmidium fructigenum	

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