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Tree Cover Species Modify the Diversity of Rhizosphere-Associated Microorganisms in *Nothofagus obliqua* (Mirb.) Oerst Temperate Forests in South-Central Chile

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Abstract: Chilean native forests have been subjected to several types of disturbances, with one of them being the replacement by exotic species. *Pinus radiata* D. Don is a widespread exotic tree that forms extensive plantations in southern Chile. It covers extended areas, affecting the landscape, biodiversity, and ecosystem services associated with native forest ecosystems. Although advances in assessing the impact of exotic plant species have been conducted, few studies have focused on the alteration of soil microorganisms. This study aimed to characterize the rhizosphere bacterial and fungal communities associated with the tree species *Nothofagus obliqua* inside a native forest stand and within a *P. radiata* plantation growing nearby. We used a 16S rRNA gene and ITS region metabarcoding approach. Using bioinformatics, diversity indices, relative abundance, preferential taxa, and predicted functions and guilds were estimated. The β-diversity analysis showed that both factors, the type of soil (rhizosphere or bulk soil) and the type of site (native forest or *P. radiata* plantation), were significant, with the site explaining most of the variation among bacterial and fungal communities. Proteobacteria and Basidiomycota were the most abundant bacterial and fungal phyla in both types of soil and sites. Similarly, bacteria showed similar abundant taxa at the family level, independent of the soil type or the site. The main fungal taxa associated with native forests were Tricholomataceae and Cantharellales, whereas in *P. radiata* plantations, Russulaceae and Hyaloscyphaceae were the most abundant families. The main bacteria functional groups were chemoheterotrophy and aerobic chemoheterotrophy, without significant differences between the type of soil or sites. Overall, these results demonstrate that the composition and diversity of bacterial and fungal communities associated with native *N. obliqua* forest are influenced by the surrounding forest, and mainly depend on the site's characteristics, such as the lignin-rich wood source. These results improve our understanding of the impact of native forest replacement on soil microbial communities, which can alter microbial-related soil ecosystem services.

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1. Introduction

The sustainability in natural ecosystems depends on multiple factors that promote the balance in the soil system and sustain the associated ecological services [1]. In the soil, microorganisms directly influence the formation and aggregation, nutrient cycling, and plant health [2]. The plants provide a specific niche for the proliferation of symbiotic microorganisms, forming complex interactions that often result in beneficial effects on plant growth and health [3]. The essential role of these microorganisms in the growth, nutrition, and development of plants is a widely accepted fact, especially those microbial taxa inhabiting the rhizosphere—the soil section in the close vicinity of the roots under the direct influence of plants [4]. The rhizosphere is a complex environment and is considered a hotspot of microbial activity and diversity [5]. Therefore, the characterization of microbial taxa associated with the rhizosphere can provide clues about the productivity and health of the soil.

Bacteria and fungi can promote the growth and increase the survival of plants through several mechanisms, including phytohormone production, enhanced nutrient acquisition, tolerance to abiotic stresses, biocontrol against phytopathogens, and induced systemic resistance [6,7]. Moreover, soil microorganisms can regulate gene expression in plants, enhancing the physiological responses to severe environmental conditions [8,9]. Additionally, symbiosis with soil microorganisms has been described as a bottleneck in the lifecycle of plants, even being necessary to promote specific lifecycle stages such as seed germination and plantlet establishment [10]. Therefore, microorganisms are essential components in the lifecycle of plants and must be preserved and integrated into management strategies of native forest ecosystems worldwide.

Chile has more than half of the temperate forests in the southern hemisphere, distributed between the Coastal and Andes Cordillera [11]. These forests have high endemism levels and are considered a biodiversity hotspot [12]. Historically, the native temperate forests of south-central Chile have been highly fragmented, mainly due to their conversion to agriculture and its replacement by fast-growing forest plantations [13,14]. Chilean temperate forest has a wide variety of tree species [15]. Among these, *Nothofagus obliqua* (Mirb.) Oerst (Nothofagaceae) is a deciduous, moderately shade-tolerant or even shade-intolerant tree that is considered a pioneer species that dominates the structure and composition after disturbances [16,17].

Exotic plantations, particularly those of *Pinus radiata* D. Don. (Pinaceae), showed a great edaphoclimatic adaptation and performance since the beginning of the 20th century in Chile, when they were tested for erosion control [18]. In Chile, native forests were then cut down and replaced by exotic tree plantations from the early 1970s until the early 1990s [14]. The loss of species associated with native forests for plantations, added to other adverse facts such as biological invasions [19], extensive clearcutting, and associated water deficit, has promoted increased threats to biodiversity conservation in the native ecosystems of the southern Andes [20,21].

Interactions between plants and microorganisms are influenced by several factors, including the environment, soil characteristics (chemical, physical, and biological features), metabolism of plant roots (i.e., carbohydrates, ions, and low molecular weight organic acids), environmental stress, and biological interactions [22,23].

Within a forest, plants influence the input of organic matter by the leaf litter's contribution that enters into the soil, which is transformed by soil microbial communities, improving nutrient availability [24,25]. These inputs can modulate microbial diversity, especially when different species are compared [26]. The distinct quality of leaf litter can modify chemical and physical parameters of soil, such as nutrients and cations content and pH [27–29]. Site characteristics such as precipitation or temperature also strongly influence

soil microbial diversity in forest ecosystems [30]. On the other hand, plants can change their root exudates under biotic and abiotic stress conditions to stimulate specific microbial taxa, with critical roles in establishing plants [3,31]. These changes may underline the loss of particular microbes with critical roles in promoting plant growth and soil-related ecosystem services.

The interactions between soil microorganisms and plants that allow the growth and adaptations of native plants within disturbed ecosystems are mainly unknown, especially when native ecosystems have been subjected to the encroachment of exotic trees, such as pine species. On the other hand, the effect of exotic tree plantation on native soil microorganisms associated with native trees has been scarcely explored. Recently, advancements in molecular biology have strengthened the knowledge of plant–microbe interactions [32]. These approaches offer an integrative view of microbial processes in the soil, especially in the rhizosphere [33]. In this context, soil metabarcoding provides valuable information about the abundances of microorganisms, even about taxa that cannot be cultured using standard methods and their possible contribution to basic and essential soil processes [30].

This study aimed to characterize the rhizosphere-associated fungal and bacterial communities of *N. obliqua* growing within a native forest and exotic plantations of *P. radiata*. The information about the microbial communities associated with native habitats will provide essential information to identify specific taxa absent in the altered forest and understand the effects of tree plantations on soil microbial communities. On the other hand, knowing the microbial diversity associated with native trees will help design better management strategies to improve the establishment, growth, and survival of native species using compatible rhizosphere-associated microbial communities.

2. Materials and Methods

2.1. Study Area

This study was conducted within a protected area in south-central Chile: the Parque Ecológico y Cultural Rucamanque (38°39′33.4″ S 72°36′19.4″ W; hereafter termed as Rucamanque), Temuco, Araucanía Region. The climate is temperate, with an average annual precipitation of 1400 mm and a mean temperature of 12 °C. The average altitude is 376 m.a.s.l and 62% of its surface is between 201 and 400 m.a.s.l. Rucamanque preserves the original vegetation that covered the valley in south-central Chile before the Spanish colonization, being mostly covered by old-growth forest, and is mainly composed of *N. obliqua*, *Laurelia sempervirens* (Ruiz & Pav.) Tul., *Persea lingue* (Ruiz & Pav.) Nees, *Aextoxicon punctatum* Ruiz & Pav., and *Eucryphia cordifolia* Cav. Some of these tree species can be aged > 460 years old, with the forest stand averaging ca. 350 years old [34]. The intermediate and shrub layer is dominated by *Rhamnus diffusus* Clos, *Pseudopanax valdiviensis* (Gay), and *Lomatia dentata* (Ruiz & Pav.) R.Br, whereas on the forest floor, *Blechnum hastatum* Kaulf., *Luzuriaga radicans* Ruiz & Pav., and *Lapageria rosea* Ruiz & Pav. are frequent.

2.2. Sampling

Three *N. obliqua* trees from a native forest stand (38°39′29.87″ S, 72°36′9.59″ W) and another three from *N. obliqua* trees growing within a *P. radiata* plantation of ~40 years old, >23 m height (38°39′38.55″ S, 72°36′16.53″ W), were randomly selected within the study area. Each sampling condition (i.e., native forest or plantation) was located not further than 300 m apart. Each of the *N. obliqua* individuals were at least 15 m in height and 20 cm in diameter at 1.3 m. From each tree, composite rhizosphere soil samples were collected from different sections of the roots at a depth between 5 and 15 cm. The soil was removed by gently shaking the roots. The samples were pooled together, obtaining three samples (of 20 g each) of rhizosphere soil (i.e., one sample per tree). Additionally, 3 samples of bulk soils were collected at each site at least 20 m away from the focal trees selected above. All samples were obtained using a sterile spatula and placed in sterile Falcon tubes. Afterward, the soil samples were stored in a cooler with ice and transported to the laboratory for

further processing. The samples were sieved (2 mm) to remove roots and organic matter traces. Finally, all samples were stored at −80 °C until chemical analysis.

2.3. Chemical Characterization of Soil

The content of carbon (C) and nitrogen (N) was determined according to the Dumas method procedures using a Leco TruSpec Macro (Leco, St. Joseph, MI, USA) [35]. Whereas phosphorus (P), potassium (K), calcium (Ca), sulfur (S), magnesium (Mg), manganese (Mn), sodium (Na), silicon (Si), titanium (Ti), chrome (Cr), zinc (Zn), vanadium (V), iron (Fe), lithium (Li), copper (Cu), lead (Pb), nickel (Ni), aluminum (Al), and strontium (Sr) of rhizosphere and bulk soils were determined by inductively coupled plasma-optical emission spectrometry using an ICP-OES 720-ES instrument (Agilent, Santa Clara, CA, USA). The pH was measured with a glass electrode meter in a 1:2.5 suspension of soil in water and electrical conductivity in a 1:5 suspension of soil in water [36]. All analyses were performed in triplicate.

2.4. Sample Preparation and Sequencing

Total DNA was extracted from: (i) three composite samples of the rhizosphere of *N. obliqua* growing inside a native forest stand (RSN), (ii) three composite samples from the rhizosphere of *N. obliqua* growing inside a *P. radiata* plantation (RSP), (iii) three composite samples of bulk soils from a native forest (BSN), and (iv) three composite samples of bulk soils from a *P. radiata* plantation (BSP). For this, 0.5 g of soil was subjected to DNA isolation using the PowerSoil® DNA Isolation Kit (MoBio Inc.,Carlsbad, CA, USA), following the manufacturer's recommendations, and stored at −20 °C until further processing. The DNA concentration was estimated with the Qubit dsDNA HS Assay Kit on the Qubit 4.0 fluorimeter (Invitrogen, Carlsbad, CA, USA).

For the elaboration of the libraries, a protocol based on the "Illumina 16S Metagenomic Sequencing Library Preparation" was followed for the amplicons obtained after amplification with the primers 16SF (5′CCTACGGGNGGCWGCAG3′) and 16SR (5′GACTACHVGG GTATCTAATCC3′) [37] for the V3–V4 region of the 16S rRNA gene, and the primers ITS3_2024F (5′GCATCGATGAAGAACGCAGC3′) and ITS4_2409R (5 ′TCCTCCGCTTATT GATATGC 3′) [38] of the internal transcribed spacer (ITS) region. The amplicons were sequenced in an Illumina platform for 300 cycles in a paired-end mode for the 16S rRNA region and 250 cycles paired-end for the ITS2 region by Novogene Co Ltd. (Beijing, China). The sequences obtained in this study were submitted to the NCBI Sequence Read Archive (http://trace.ncbi.nlm.nih.gov/Traces/sra, accessed on 22 November 2021) under the bio-project PRJNA782644.

2.5. Sequence Analysis

The quality of resulting raw reads was explored and analyzed using QIIME2 v2019.10 (https://qiime2.org/), accessed on 26 July 2021 [39] built-in applications, as described below. The denoising step was performed using the DADA2 algorithm [40]. The first 18 bases of reads were removed to improve the identification of chimeric sequences. The read lengths in the 16S rRNA amplicon were truncated at positions 250 and 230 for forward and reverse, respectively. Meanwhile, they were truncated at position 220 for the ITS2 amplicon. These trimming steps improved quality filtering, merging, and chimeric sequences' removal output. The amplicon sequence variants (ASV) were called from the trimmed reads. The identification of amplicons was performed using a Naïve Bayes classifier, trained with sequences from the SILVA v138.1 [41] and UNITE v8.3 [42] databases for 16S rRNA and ITS2 amplicons, respectively. The 16S rRNA amplicons assigned as mitochondria or chloroplast were removed for further analyses. A principal coordinate analysis (PCoA) and hierarchical clustering were constructed based on the Bray–Curtis dissimilarity matrix to further explore the bacterial and fungal communities' overall trends among samples.

The bacterial samples were rarified at a sampling depth of 15,153 and the fungal samples at a depth of 46,098. The diversity indices (Shannon's diversity index, Faith's

phylogenetic diversity, observed features, and Pielou's evenness) were calculated using the QIIME v2019.10 (https://qiime2.org/, accessed on 28 July 2021) core-metrics-phylogenetic function. The resulting values were compared among sites by the Kruskal–Wallis test, and the Dunn test was applied where significant differences were found. The statistical determination of differences between samples was evaluated by permutational multivariate analysis of variance (PERMANOVA) [43], with 999 random permutations on the Bray–Curtis dissimilarity matrix. Statistical analysis and visualization were performed using functions from the vegan package [44] contained in the R software [45]. The distribution of genera in the samples was also explored using Venn Diagrams drawn with Venny v2.1 (https://bioinfogp.cnb.csic.es/tools/venny/, accessed on 28 July 2021) [46]. Microbial differences were further explored using LEfSe (https://github.com/SegataLab/lefse, accesed on 28 July 2021) [47] and IndicSpecies R package [48]. The functional group of bacteria was predicted from the 16S rRNA marker gene using FAPROTAX database [49], whereas for fungi, it was predicted using FUNGuild database [50].

3. Results

The soil chemical analyses showed statistical differences among them ($p < 0.05$), especially in the bulk soil of the *Pinus radiata* plantation, which showed the lowest values of C, N, and P and the highest values of Cr, Cu, and Fe (see details in Table S1).

The sequencing analysis showed 1,552,085 raw reads across 12 input libraries. After the quality filtering step, 648,929 sequences were retained, ranging from 12,130 to 81,803 amplicons, and had, on average, 54,077 amplicons per sample (Table S2). The sequencing analysis showed 2,334,973 raw reads across 12 input libraries for fungal taxa. After the quality filtering step, 1,101,259 amplicons were retained, ranging from 46,224 to 112,601, and had, on average, 91,771 amplicons per sample (Table S3).

Significant differences in α diversity were observed in bacteria (Shannon Index, H = 9.6667, $p = 0.02162$). RSN has a less diverse community showing significant differences ($p < 0.1$) with BSN and with the bacteria from RSP (Figure S1A). On the other hand, no significant differences in α fungal diversity were observed (Shannon Index, H = 0.0256, $p = 0.8728$) among samples (Figure S1B).

β-diversity showed that the structures of bacterial communities were significantly different, with the site (native forest or pine plantation) being the most influential variable ($p = 0.001$), explaining 31.11% of communities' variance. Meanwhile, the type of soil (rhizosphere or bulk soil) was also significant ($p = 0.006$), but only explained 17.47% of the variance. In the fungal communities, only the site was significant ($p = 0.002$), explaining 35.22% of fungal communities' variation (Figure 1).

Proteobacteria and Acidobacteria were the most abundant phyla in the samples, with relative abundances of 33.66% ± 2.58% and 24.62% ± 4.59%, respectively. The remaining abundance was composed of Verrucomicrobia 13.23% ± 2.07%, Bacteroidetes 9.15% ± 3.93%, Planctomycetes 8.84% ± 2.03%, and other less abundant phyla (Figure 2A). At the family level, the results were similar, showing the same representative groups with similar abundances (Figure 2B). For fungal communities, the taxonomic assignment showed that most of the ASV found belonged to the phylum Basidiomycota and Ascomycota, with relative mean abundance of 67.97% ± 12.67% and 31.35% ± 12.30%, respectively, both in native forest and the *P. radiata* plantation. The remaining phyla had relative abundances of less than 1% in the samples (Figure 2C). In soil from the native forest, the main families were Tricholomataceae and Cantharellales, whereas in pine plantations, they were Russulaceae and Hyaloscyphaceae (Figure 2D).

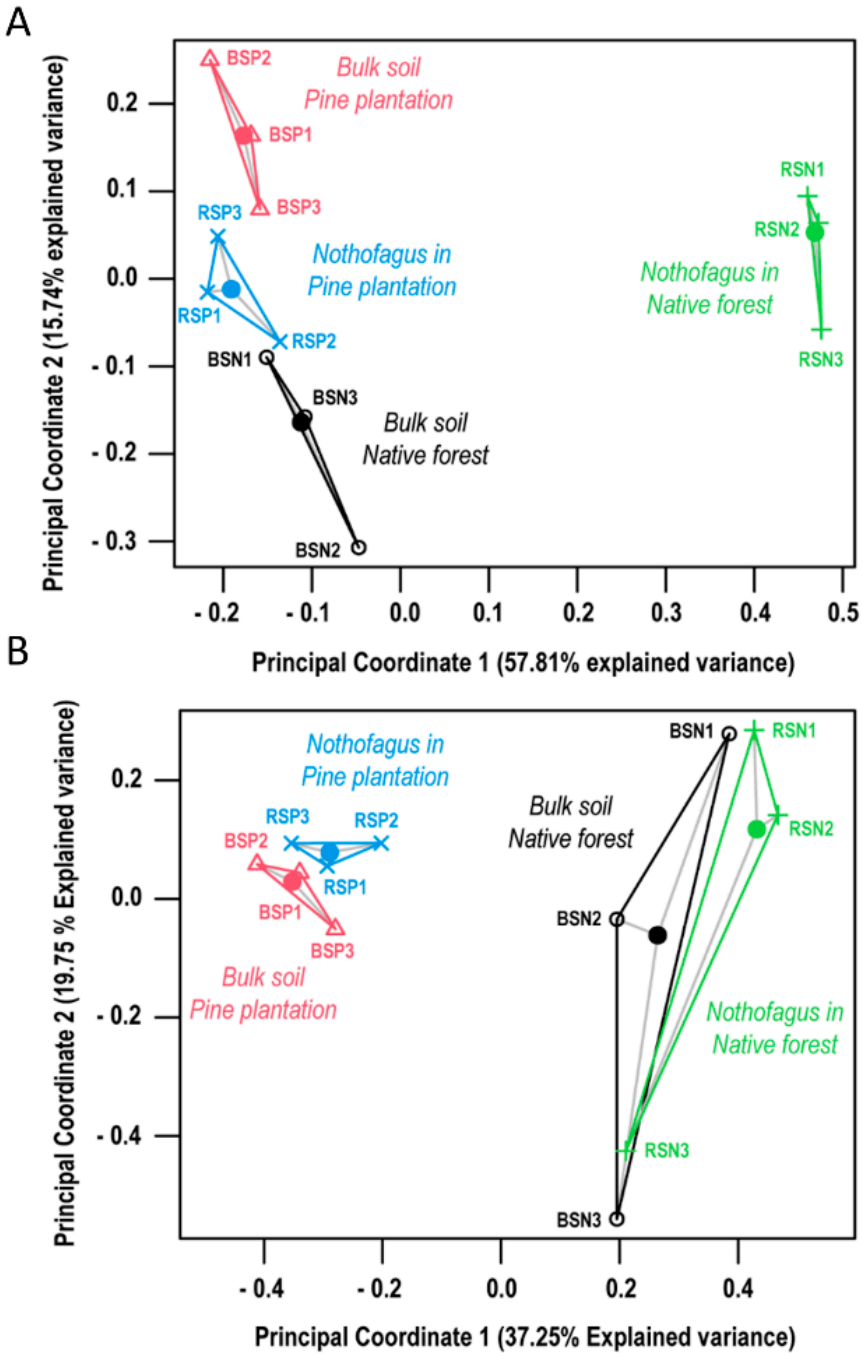

Figure 1. Principal coordinates analysis (PCoA) of bacterial (**A**) and fungal (**B**) communities. RSN: Microbial communities from rhizosphere soil of *N. obliqua* growing in native forest. RSP: Microbial communities from rhizosphere soil of *N. obliqua* growing in pine plantation. BSN: Microbial communities from bulk soil of native forest. BSP: Microbial communities from bulk soil of pine plantation.

For bacterial communities, the Venn diagrams showed that the sum of the total observed ASV in the 4 soil conditions was 712 ASV, from which 200 ASV were common in all soil samples. The highest percentage of unique ASV was found in RSP (80%), followed by BSN (70%) (Figure 3A). Whereas for fungal communities, the Venn diagrams showed that the sum of the total observed ASV in the 4 soil conditions was 115, of which 44 were common in all the soil samples. The highest percentage of unique ASV was found in RSN (16%), followed by RSP (10%) (Figure 3B).

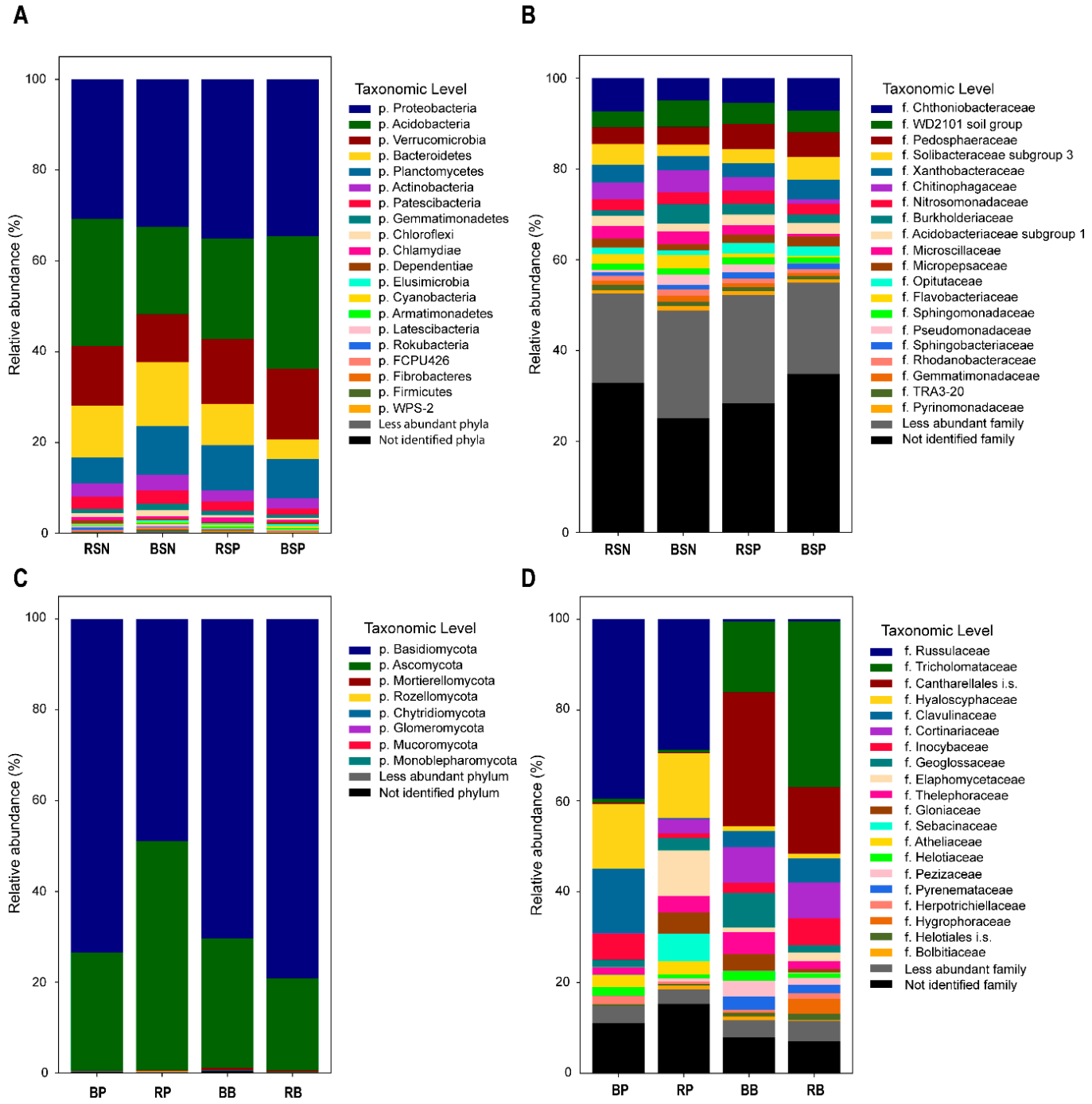

Figure 2. The taxonomic structure of communities showed the relative abundance of the most abundant bacterial phyla (**A**), bacterial families (**B**), fungal phyla (**C**), and fungal families (**D**). RSN: Microbial communities from rhizosphere soil of *N. obliqua* growing in native forest. RSP: Microbial communities from rhizosphere soil of *N. obliqua* growing in pine plantation. BSN: Microbial communities from bulk soil of native forest. BSP: Microbial communities from bulk soil of pine plantation.

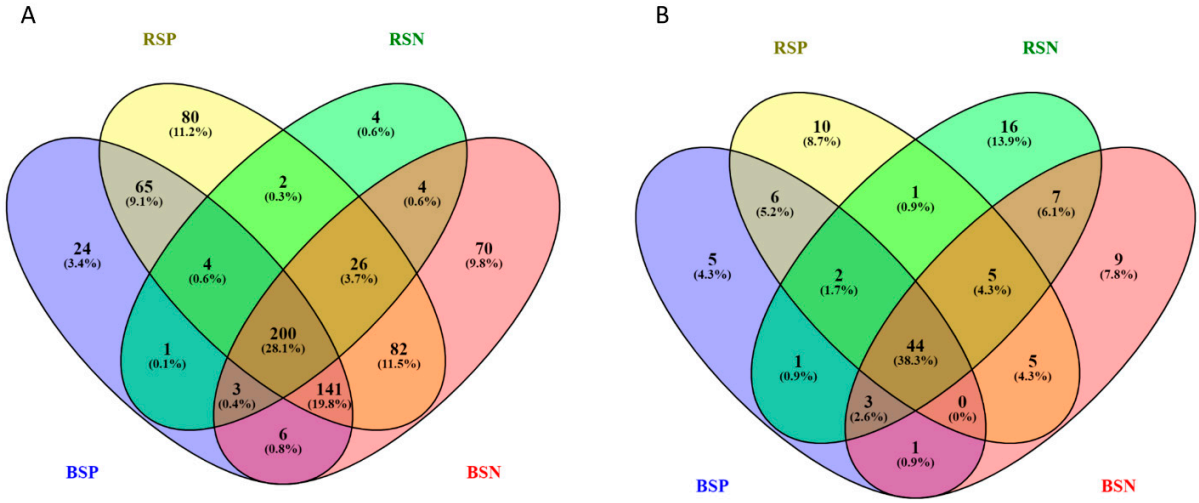

Figure 3. Venn diagram showing the number of unique ASV of (**A**) bacteria and (**B**) fungi. RSN: Microbial communities from rhizosphere soil of *N. obliqua* growing in native forest. RSP: Microbial communities from rhizosphere soil of *N. obliqua* growing in pine plantation. BSN: Microbial communities from bulk soil of native forest. BSP: Microbial communities from bulk soil of pine plantation.

FAPROTAX analysis was performed to predict the functions of the soil bacterial community. A total of 42 functional groups were obtained, including chemoheterotrophy (29%), aerobic chemoheterotrophy (25%), intracellular parasites (7.8%), nitrification (3.4%), nitrate reduction (3.4%), aerobic ammonia oxidation (3%), fermentation (2.7%), and predatory or exoparasitic (2.4%), among others, not showing significant differences among sites or type of soil (Figure 4A). On the other hand, the FUNGuild analysis showed that ectomycorrhizal fungi were the most abundant group, especially in samples from rhizosphere (RSP = 62.6%) and bulk soil (BSP = 53%) of *N. obliqua* growing in the *P. radiata* plantation, followed by undefined saprotroph (RSN = 12%, BSN = 13.4%, RSP = 19.3%, BSP = 21.3%) and soil saprotroph. The last group was especially abundant in samples from rhizosphere (RSN = 36.7%) and bulk soil (BSN = 15.8%) of *N. obliqua* growing under native forest (Figure 4B).

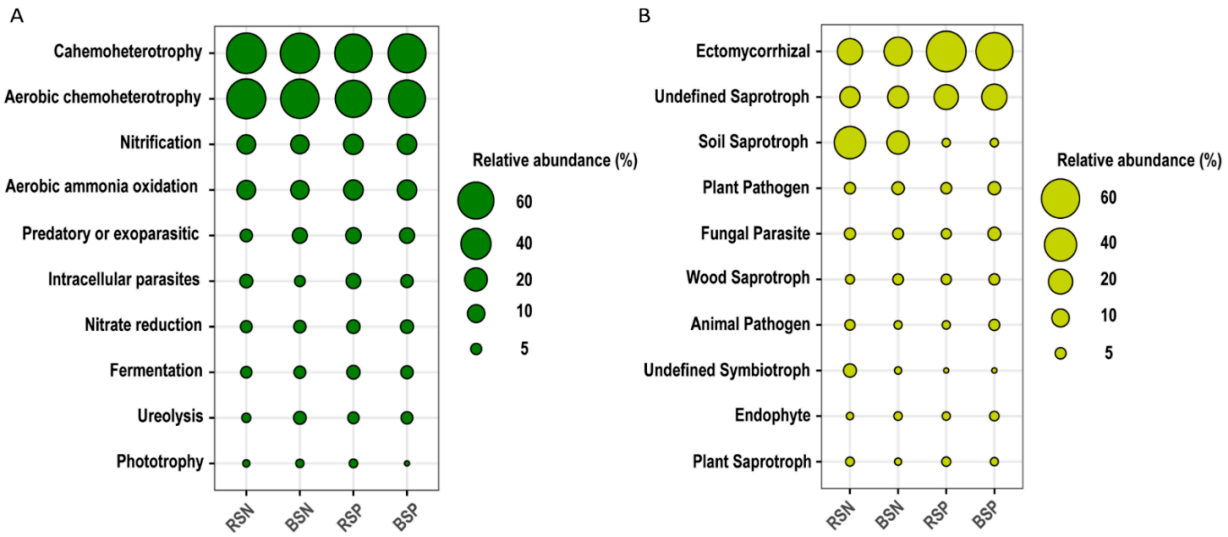

Figure 4. (**A**) Functional groups of bacteria (Faprotax) and (**B**) functional groups of fungi (FunGuild). RSN: Bacterial communities from rhizosphere soil of *N. obliqua* growing in native forest. RSP: Bacterial communities from rhizosphere soil of *N. obliqua* growing in pine plantation. BSN: Bacterial communities from bulk soil of native forest. BSP: Bacterial communities from bulk soil of pine plantation.

The linear discriminant analysis effect size (LEfSe) showed that three bacterial families were predominant in RSN, five in BSN, six in RSP, and seven in BSP (Figure 5A). Similarly, no predominant families of fungi were found in RSN, four were found in BSN, two in RSP, and three in BSP (Figure 5B).

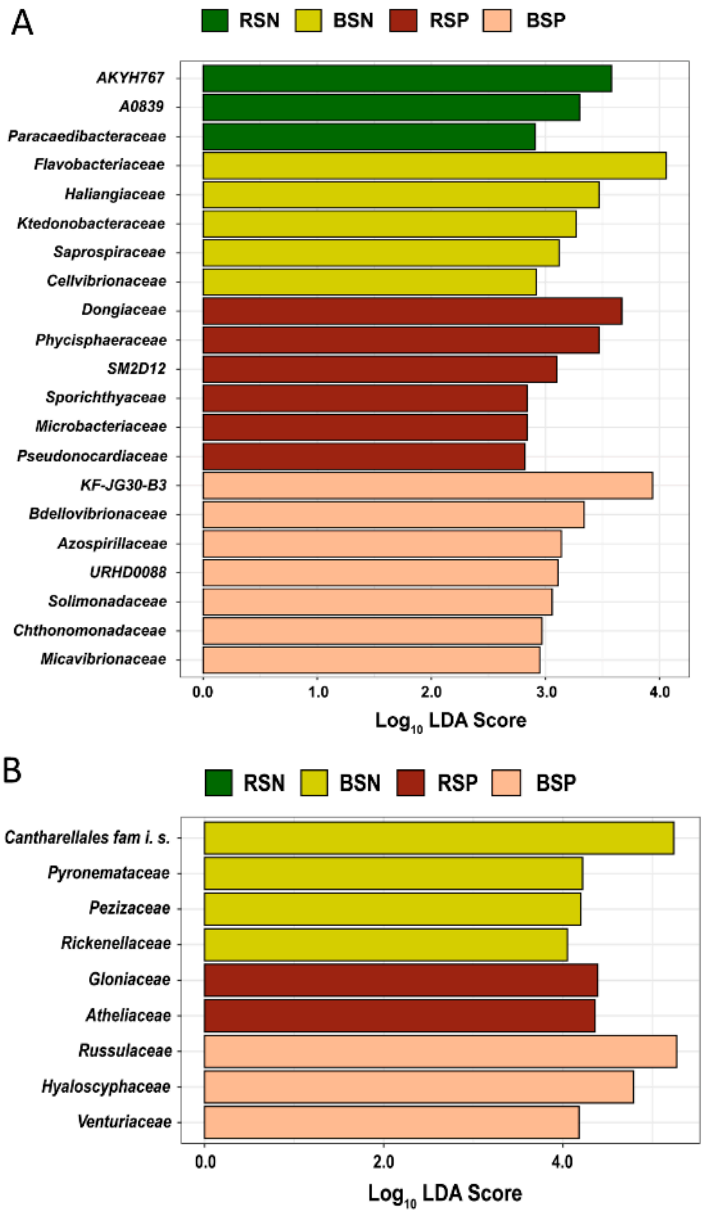

Figure 5. LEfSe analysis by (**A**) bacterial and (**B**) fungal communities. RSN: Bacterial communities from rhizosphere soil of *N. obliqua* growing in native forest. RSP: Bacterial communities from rhizosphere soil of *N. obliqua* growing in pine plantation. BSN: Bacterial communities from bulk soil of native forest. BSP: Bacterial communities from bulk soil of pine plantation.

Principal component analysis (PCA) was used to identify relationships among soil properties across the samples (Figure 6). We observed clustering of the two sampling sites, and two principal components accounted for 60.3% of the total variance. The first axis of the PCA (PC1, which explained 38.7% of the variation) was positively correlated mainly with Fe, Cu, Li, V, Zn, Cr, and Mg, and negatively correlated with C, N, Ca, S, Ti, and Sr (Figure 6). Similarly, the second axis of the PCA (PC2, which explained 21.6% of the variation) was positively correlated mainly with Mn and Na (Figure 6). On the other hand, RSN and BSN showed high pH values and high P concentration, whereas RSP showed

high concentrations of C, and BSP had high concentrations of microelements such as Fe, Cu, Cr, and Mg.

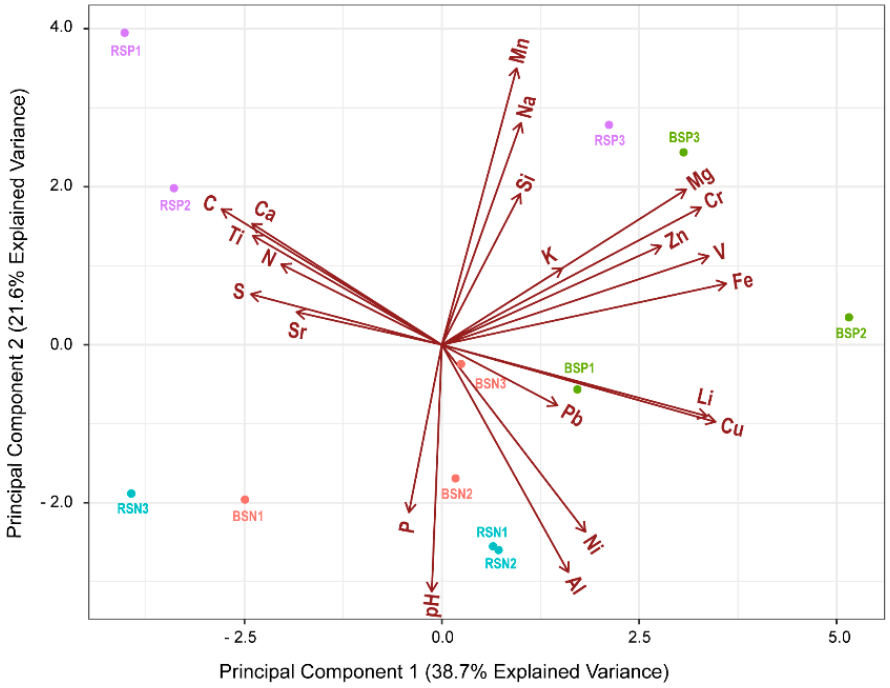

Figure 6. Principal component analysis (PCA) of sites (RSN, BSN, RSP, BSP) and soil parameters (vectors). RSN: Rhizosphere soil of *N. obliqua* growing in native forest. RSP: Rhizosphere soil of *N. obliqua* growing in pine plantation BSN: Bulk soil of native forest. BSP: Bulk soil of *N. obliqua* growing in pine plantation. Values between brackets indicate the percentage of the variation in the original dataset that is explained by axes PC1 and PC2.

4. Discussion

This study showed that bacterial and fungal communities associated with *N. obliqua* plants are greatly affected by the tree cover species, which can also change the biological, chemical, and physical properties of the soil and the site. It is common to find studies that report differences between the rhizosphere and the bulk soil, especially considering that the rhizosphere represents a unique habitat that is highly specific and rich in abundance and activity of microorganisms [51]. On the other hand, microorganisms are affected by their immediate surrounding micro-niches [52], which may differ from the properties of their environmental matrix [53]. This study was carried out on a site dominated by native broadleaf forest and a *P. radiata* plantation, where the differences in plant communities directly affect litter and litter layer composition, as well as the nutritional quality of the soil [54,55]. This explains that both bacterial and fungal communities were different, with the site explaining most of the variation among communities. According to the PCA, microbial communities from the native forest were correlated with pH and P concentration, whereas rhizosphere soil of *N. obliqua* growing in the pine plantation was correlated with C, and their corresponding bulk soil was correlated with microelements such as Fe, Cu, Cr, and Mg. This is related to Table S1, which showed that the bulk soil of the *P. radiata* plantation showed the lowest values of C, N, and P attributable to the low nutritional quality of the needles compared to the leaves of the native forest [1].

Proteobacteria and Acidobacteria were the most abundant bacterial phyla identified in the study. Proteobacteria is a very common bacterial phylum in the soil and is related to various functions involved in the C, N, and S cycles [56]. Similar results were found by Castañeda and Barbosa [57], who identified Proteobacteria, Acidobacteria, and Planctomycetes in sites near native forests. The same was shown by Muñoz-Arenas [58], where Proteobacteria was the most abundant phylum in high-altitude temperate forests and arable

soils. Similar results were found by Meng et al. [59] in four forest types (native forest, mixed forest, Chinese fir forest, and bamboo forest) in China. Acidobacteria is a group with high efficiency in the degradation of polysaccharides and presented the broadest spectrum of C source utilization (low molecular mass oligosaccharides, amino acids, amines/amides, and carboxylic acids) [60]. Therefore, the high abundance of both types of bacteria responds to their ability to develop in sites with high levels of organic matter, such as the soil of native forests and exotic plantations, playing an important ecological role, especially in organic matter degradation in these ecosystems [60].

The most abundant fungi identified were basidiomycetes, such as Tricholomataceae, Cortinariaceae, Russulaceae, and saprobe Ascomycetes belonging to the family Hyaloscyphaceae. Several studies have shown that Basidiomycota and Ascomycota are the predominant phyla in soil samples [3,61,62]. Fernández et al. [62] showed that the main macrofungal species identified in a transect in a Mediterranean forest belong to Tricholomataceae, Cortinariaceae, and Russulaceae. The highest abundance of Ascomycetes was found in RSP, while the lowest amount was found in RSN. These results are similar to those reported by Fernández et al. [62], who found a greater abundance of Ascomycetes in *Pinus ponderosa* plantations and Basidiomycetes in *Nothofagus* native forest. These results relate to the organic matter's nutritional quality present in both sites. At the family level, in the pine plantation, the most abundant family of fungi was Russulales, and this was observed as related to the high amounts of carpophores of fungi such as *Lactarius quieticolor* and *Russula sardonia* that colonize the study site. The Venn diagrams showed many unique bacteria ASVs present in RSP concerning soil from the native forest. The high presence of ectomycorrhizal fungi can influence these results, which was also shown in the FUNGuild analysis (Figure 4B). Ectomycorrhizal fungi are capable of secreting large amounts of compounds such as carbohydrates or organic acids, which can be used as a food source by bacteria [26]. This is related to the high concentration of carbon present in RSP, even higher than that found in RSN and BSN (Table S1). Similarly, microbial community compositions in the rhizosphere and bulk soil differ, mainly due to the selective environment of rhizospheres [63]. Bacterial recruitment could occur due to the differences between rhizosphere soil (from *N. obliqua*) and bulk soil that *P. radiata* influences. In the native forest, more ASVs were observed in bulk soil, which is explained because this soil is also influenced by other native species colonizing the same site, which would share a similar microbiome.

The Faprotax analyses showed a higher abundance of bacteria involved in chemoheterotrophy and aerobic chemoheterotrophy, showing that many microbes obtain carbon and energy from the oxidation of preformed organic compounds [64]. Similarly, FunGuild showed that ectomycorrhizal fungi were the most abundant group in samples from rhizosphere soil of *N. obliqua* growing in the *P. radiata* plantation and the bulk soil. In contrast, saprophytic soil was especially abundant in samples from the rhizosphere of *N. obliqua* growing under native forest and its corresponding bulk soil (Figure 4B). Similar results were found by Jiang et al. [65], who linked this fact to competition between symbiotic and free-living fungi species. On the other hand, the reduction in the number of saprophytic fungi in the *Pinus radiata* plantation can respond to the nutritional quality of the leaf litter because the soil of pines was covered by needles, which are more resistant to decomposition than the litter derived from *Nothofagus* species [66]. This is related to the result from the LEfSe, which showed, in the case of bacteria, the presence of Saprospiracea, which are bacteria associated with the degradation of organic matter [67]. In BSP, we identified Solimonadacea, which can decompose compounds such as atrazine and Azospirillaceae, members of which are free-living nitrogen-fixing bacteria. While in RSP, families such as Microbacteriaceae and Sporichthyaceae were found, which belong to the phylum actinobacteria and Pseudonocardiaceae involved in metabolism of cellulose, and in this sample, a greater amount of carbon was found, compared to bulk (Table S1). Similarly, the LEfSe analysis shows Russulaceae family's presence as a biomarker in the *P. radiata* plantation, and families such as *Cantharellales* and *Pezizacea* in native forest.

The microorganisms associated with the rhizosphere of plants are influenced by environmental factors, including the plant species established in each site, which also strongly affects the soil's nutritional quality. Knowing the main taxa associated with each type of forest (native or planted) can be considered a helpful tool to assess the conservation status of soil via their essential roles in specific soil-related ecosystem services. Even some of these microorganisms could be isolated and used as inoculants to preserve the native soil microbiome, as they can directly influence critical lifecycle stages of native species [68,69].

5. Conclusions

This study demonstrated that the composition of bacterial and fungal communities associated with native *N. obliqua* differs between the sites, dependent on the conditions of each location, with the chemical composition of soil being the main factor that regulates microbial composition. Proteobacteria and Basidiomycota were the most abundant phyla of bacteria and fungi, respectively, in both types of soil and sites. The main fungal taxa associated with native forest were Tricholomataceae and Cantharellales, whereas in the *Pinus radiata* plantation, Russulaceae and Hyaloscyphaceae were more abundant. On the other hand, rhizosphere soil of *N. obliqua* showed several specific bacterial taxa despite growing inside a pine plantation. Studying soil microorganisms associated with forest plants improves our understanding of the impact that native forest replacement has on soil microbial communities, and changes that can alter essential soil-related ecosystem services.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/f13050756/s1, Table S1: Soil chemical analysis; Table S2: Trimming summary, showing the number of initial bacterial reads, reads that pass quality control, reads after denoising step, number of sequences after merging them by their 3′ ends, and resulting non-chimeric sequences; Table S3: Trimming summary, showing the number of initial fungal reads, reads that pass quality control, number of fungal sequences after merging them by their 3′ ends, and resulting non-chimeric amplicons; Figure S1: α-diversity measurements of (A) bacterial and (B) fungal: Shannon diversity index, Pielou's evenness, and observed features as boxplots.

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