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Effects of Thinning of the Infected Trees and Cultivating of the Resistant Pines on Soil Microbial Diversity and Function

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Abstract: Pine wilt disease (PWD) poses a significant threat to pine forest health, making sanitation thinning of infected trees and cultivation of disease-resistant pine stands crucial measures for forest ecosystem restoration. To date, limited studies have systematically investigated how post-sanitation planting of pine-wilt-disease-resistant Pinus species affects soil microbiome, especially regarding bacterial and fungal diversity characteristics, functional succession patterns, and community assembly processes. In this study, we performed a comparative analysis of soil microbial community characteristics and biochemical properties between experimental plots subjected to sanitation thinning and those replanted with disease-resistant pine species. The results indicated that compared to the sanitation-thinned experimental plot, the disease-resistant experimental plots (*Pinus taeda* experimental plot and *Pinus thunbergii* experimental plot) exhibited significantly higher activities of β-glucosidase (S-β-GC), N-acetyl-β-D-glucosidase (S-NAG), and soil arylsulfatase (S-ASF). Compared with the sanitation logging stands, our analysis revealed that the *Pinus taeda* experimental plot and *Pinus thunbergii* experimental plot exhibited significantly higher fungal community evenness (OTUs), greater species abundance (OTUs), and more unique fungal taxa. Furthermore, the edaphic properties—specifically soil moisture content (SMC), pH levels, and total potassium (TK)—significantly influenced the structures of soil bacterial and fungal communities. Compared to the sanitation-thinned experimental plot, wood saprotrophic fungi and ectomycorrhizal fungi exhibited increased abundance in both the *P. taeda* experimental plot and *Pinus thunbergii* experimental plot. Furthermore, the null models indicated that both the *P. taeda* experimental plot and *P. thunbergii* experimental plot enhanced the undominated processes of bacteria and fungi. In summary, our data elucidate the differences in bacterial and fungal responses between pine forests undergoing thinning due to infected trees and those cultivated for disease resistance. This deepens our understanding of microbial functions and community assembly processes within these ecosystems.

Keywords: PWD; soil microorganisms; soil properties; sanitation cutting; *Pinus taeda*; *Pinus thunbergii*

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1. Introduction

Pine forests are important ecological treasures on the planet, providing a variety of ecological functions such as biodiversity conservation, carbon storage, soil and water conservation, and timber resources [1,2]. Pine wilt disease (PWD) caused by *Bursaphelenchus xylophilus* Steiner et Buhrer is devastating to pine trees, with infected specimens typically

exhibiting rapid wilting followed by death within months [3]. The disease spreads with alarming efficiency through its primary vector (*Monochamus alternatus* Hope), leading to explosive outbreaks that can decimate entire pine forests [4]. Sanitation cutting, as a critical management measure, effectively disrupts disease transmission by removing weakened, dead, and *B. xylophilus*-infested host trees, significantly reducing the risk of epidemic spread [5]. Timely sanitation felling can significantly reduce pine wilt disease transmission risk by 69.28% [6,7]. Cultivating pine resistant to *B. xylophilus* enhances forest resilience through genetic improvement. By selectively breeding resistant species such as *Pinus thunbergii* Parlatore and *Pinus taeda* L.—including resistant cultivars like *P. thunbergii* cv. Asuka—and integrating marker-assisted breeding techniques, sustainable nematode-resistant pine ecosystems can be established [8,9]. To improve the ecological benefits of pine forests, it is essential to rationally cultivate disease-resistant pine species and implement scientific thinning practices to enhance biodiversity. These measures will promote the formation of more stable forest communities and optimize their ecological functions [10].

Soil microorganisms serve as pivotal regulators in terrestrial ecosystems, playing indispensable roles in key ecological processes such as nutrient cycling, pathogen suppression, and plant productivity enhancement [11]. As essential drivers of ecosystem functioning, microbial communities sustain vital ecological services by facilitating nutrient cycling, supporting primary production, and participating in climate regulation [12]. Research demonstrates that alterations in soil biodiversity, particularly microbial diversity, profoundly influence ecosystem functioning [13]. This positive correlation primarily stems from niche complementarity, mutualistic interactions, and the suppression of pathogenic microorganisms [14]. These findings underscore the central importance of soil microorganisms in enhancing forest quality and restoring ecosystem vitality.

Generally, alterations in the soil microbial community structure reflect the ongoing ecological dynamics between the organisms and their environmental context. Key factors that significantly impact microbial communities encompass soil pH [15], soil moisture content [16], organic carbon [17], nutrient availability (including carbon, nitrogen, phosphorus, and sulfur) [18], and biotic elements like plant diversity and specific cultivars [19]. In addition, β-glucosidase (S-β-GC), N-acetyl-β-D-glucosidase (S-NAG), and arylsulfatase (S-ASF) serve as key functional enzymes in soil carbon, nitrogen, and sulfur cycling, whose activities directly regulate soil microbial community functions and plant nutrient availability [20]. The soil itself exhibits high diversity and heterogeneity (with variations in composition and properties across different regions), and its various characteristics (such as pH, nutrient content, moisture, organic matter, etc.) can directly or indirectly influence the composition and activity of microbial communities [21,22]. Due to the intricate and interconnected nature of these influencing factors, gaining a comprehensive understanding of microbial community structure and function remains a significant challenge [23].

Microbial community assembly or community structure is shaped by a combination of deterministic and stochastic ecological processes [24]. Deterministic processes (niche-based theory) result from the predictable filtering effects on species of ecological selection imposed by biotic and abiotic factors that affect the fitness of organisms and thus determine species composition and relative abundance [25]. In contrast, stochastic processes (neutral theory) involve ecological drift (birth, death, immigration, speciation, and limited dispersal) and are not the result of environmentally determined fitness [26,27]. However, current research has predominantly focused on characterizing microbial communities in specific ecosystems (e.g., marine, freshwater, and agricultural systems), while investigations into the evolutionary assembly processes of microbial communities within forest disease management regimes remain notably limited [28–31]. Therefore, gaining a more profound understanding

of how the thinning of pine trees infested with pine wood nematode and cultivation of disease-resistant pine trees impact the assembly and diversity of soil microbial communities could significantly contribute to the preservation and restoration of ecological biodiversity, particularly in the temperate forests of China.

The impact of current PWD control measures (selective thinning of infected trees and planting of disease-resistant pines) on soil microbial communities in this region remains poorly understood. To address this knowledge gap, this study collected soil profile samples from these forest stands and employed high-throughput gene sequencing to characterize the microbial community composition. This study sought to: (1) assess the effects of management measures for PWD on the diversity of soil bacterial and fungal communities; (2) identify the main environmental factors responsible for the diversity of soil bacteria and fungi in pine forests after the implementation of the management measures; (3) track the potential functions of soil bacterial and fungal communities after the implementation of the management measures; and (4) elucidate the processes of assembling soil bacterial and fungal communities after the implementation of the management measures.

2. Materials and Methods

2.1. Experimental Protocol and Sample Collection

The study was conducted in Jurong city, Jiangsu Province, located at 119°13′ E, 32°7′ N, with a forest area of 314.14 ha. The climate of the study area is characterized by a subtropical humid monsoon climate, featuring abundant sunlight, four distinct seasons, and obvious mountain climate characteristics. The average annual temperature is 15.2 °C, and the average annual precipitation is 1055.6 mm. The native forest type in this area was a mixed *Pinus massoniana* Lamb and *Larix principis-rupprechtii* Mayr forest. Following the invasion of *B. xylophilus* into the native forest, three experimental plots with similar site conditions were established. The elevation difference between plots was less than 50 m, the slope gradient difference was less than 10 degrees, and the soil type was yellow-brown earth. The following integrated management measures were implemented over the past 20 years: (1) sanitation felling to remove infected and weakened trees; and (2) replanting disease-resistant species separately—*P. taeda* and *P. thunbergii*—in the experimental plots after sanitation felling (detailed characteristics of the three plots are provided in Supplementary Table S1). Since the implementation of these control measures, a comprehensive protection system has been established in the study area, including disease monitoring and stand management practices.

2.2. Soil Sampling

Based on plot accessibility, soil samples were collected in April 2024 using a five-point sampling method. Specifically, within each of the three treatment plots—the sanitation cutting treatment group (PMLG), *P. taeda* replanting group (PTL), and *P. thunbergii* replanting group (PTP)—five biological replicates were established. At each sampling point, the surface cover (including litter, humus, and living ground vegetation) was removed before collecting soil samples at a 10 cm depth [32]. The obtained samples were evenly divided into two portions: one for soil microbial community analysis and the other for soil physicochemical property determination. For preservation, all samples were stored in an ultra-low-temperature freezer at −80 °C. High-throughput sequencing analysis was performed by Shanghai Majorbio Bio-Pharm Technology Co. (Shanghai, China) (www.majorbio.com (accessed on 7 May 2025)).

2.3. Determination of Enzymatic Activity and Physicochemical Properties of the Soil

The activities of aryl sulfatase (S-ASF), acid phosphatase (S-ACP), N-acetyl-β-D-glucosidase (S-NAG), and β-glucosidase (S-β-GC), which are associated with the cycling of sulfur, phosphorus, and carbon, were assessed using kits supplied by Suzhou Comin Biotechnology ((www.cominbio.com (accessed on 7 May 2025)) [33]. For comprehensive details regarding the methodology, please refer to the instructions available on the Suzhou Keming Biotechnology Co. website. The soil water content (SWC, %) was determined by drying at 105 °C, while the soil pH was measured with a pH meter after mixing the soil and water (2.5:1) [34]. The soil organic carbon (SOC) and total nitrogen (TN) contents were determined via an elemental analyzer (EA 3000, Vector, Redavalle, Italy), whereas the soil total phosphorus (TP), total potassium (TK), and total sulfur (TS) contents were quantified via an OLYMPUS XRF analyzer (made in Center Valley, PA, USA) [35,36]. All soil enzyme activity data reported in this study were measured using air-dried soil samples. Soil properties from the same sampling site (analyzed in the same project; see Supplementary Material, Table S1) were included solely as supplementary variables for correlation analyses with microbial communities and are not discussed in the main text.

2.4. DNA Extraction, PCR Amplification, and Illumina MiSeq Sequencing

A QIAamp kit was used for DNA extraction from different soil samples according to the manufacturer's instructions. The concentration and quality of the extracted DNA were determined via a Thermo Scientific NanoDrop2000 spectrophotometer (Thermo Fisher Scientific, Wilmington, DE, USA), and the integrity of the extracted DNA was tested via 1% agarose gel electrophoresis [37]. Using amplicon sequencing to identify fungi and bacteria in soil, the primer pairs 338F (5′-ACTCCTACGGGAGGCAGCAG-3′)/806R (5′-GGACTACHVGGGTWTCTAAT-3′) were employed to amplify the V3-V4 region of the 16S rRNA gene for bacterial community analysis, while the primer pair ITS1F (5′-CTTGGTCATTTAGAGAGGAAGTAA-3′)/ITS2R (5′-GCTGCGTTCTTCATCGATGC-3′) was used to amplify the ITS1 region (Internal Transcribed Spacer 1) for fungal community identification and analysis [38]. The PCR conditions consisted of denaturation at 95 °C for 2 min, followed by 25 cycles of 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 60 s; a 10 min extension at 72 °C for 10 min; a 10 min extension at 4 °C for 10 min; and an extension at 72 °C for 10 min, followed by holding at 4 °C. The PCR products were detected via electrophoresis on a 2% agarose gel and recovered by cutting the gel via an AxyPrepDNA Gel Recovery Kit (AXYGEN, Union City, CA, USA) [38]. The PCR products were quantified via the QuantiFluor™-ST Blue Fluorescence Quantification System (Promega, Madison, WI, USA), with reference to the preliminary quantification results of electrophoresis. PCR products were prepared with the NEXTFLEX Rapid DNA-Seq Kit (Bioo Scientific, Austin, TX, USA) and sequenced in paired-end mode (2 × 300 bp) on the Illumina MiSeq platform (Illumina, San Diego, CA, USA). The original sequences were submitted and stored in the NCBI Sequence Read Archive (SRA), with accession number SUB14777552.

2.5. Bioinformatics Analysis

First, we filtered fast for quality via FASTQ software (version 0.19.6) and merged it via Flash (version 1.2.11) to merge with the following criteria: (i) filter out bases with quality values below 20, set a window of 50 bp, and remove reads for bases containing N; (ii) merge sequences with overlap times greater than 10 bp and allow a maximum mismatch of 2 bp; and (iii) qiime (version 1.9.1) was used for denoising, removing sequences annotated to chloroplasts and mitochondria in the samples and thinning the resulting data via a minimum sample sequence levelling process. We subsequently used Uparse (version 11) to manipulate the classification units (OTUs) for annotation and finally used the RDP classifier

(version 2.13) to compare the measured microbial data with the Silicon Valley data. The measured microbial data were compared to the Silva 138 database (bacteria) and the Unite 9.0/its_fungi database (fungi), with a confidence threshold of 70% and an OTU sequence similarity of 97%.

2.6. Statistical Analyses

First, we employed one-way analysis of variance (ANOVA) combined with false discovery rate (FDR) correction to assess the significance of intergroup differences in soil enzyme activities, physicochemical properties, and microbial α-diversity indices (Coverage, Simpson, Shannoneven, Chao). Next, based on the Bray–Curtis distance matrix, we analyzed β-diversity differences at the OTU level using analysis of similarities (ANOSIM) and principal coordinate analysis (PCoA). To identify differentially abundant taxa, we applied linear discriminant analysis effect size (LEfSe) (LDA threshold > 2.5) and an all-against-all multi-group comparison strategy to screen for significantly different bacterial/fungal taxa (genus level) across the three sample types. To elucidate the influence of environmental factors on soil microbial communities, we first screened environmental parameters using variance inflation factor (VIF < 20). Subsequently, redundancy analysis (RDA/CCA) (default standardization) was performed in conjunction with the Bray–Curtis distance matrix to reveal microbial community–environmental factor relationships. The relative contributions of these factors were quantified using variance partitioning analysis (VPA). Based on the abundance data of the top 500 OTUs, we constructed a Spearman rank correlation network ($|R| > 0.8$, $p < 0.05$; implemented with the igraph package and visualized using Gephi 0.9.1). Functional annotation was conducted using FUNGuild (for fungi) and FAPROTAX (for bacteria) to identify indicator taxa significantly associated with composition/abundance [39,40]. We evaluated the roles of selection and dispersal in community turnover by employing null model approaches. In doing so, we determined phylogenetic and taxonomic beta diversity indices (β-nearest taxon index, βNTI) and utilized the Bray–Curtis-based Raup–Crick metric (RCbray) to assess variations in both phylogenetic and taxonomic diversity [41].

3. Results

3.1. Soil Enzyme Activities in Three Pine Plots

PMLG, PTL, and PTP showed significant differences in soil enzyme activities related to carbon, nitrogen, and sulfur cycling. Notably, S-β-GC and S-NAG esterase were significantly greater in PTL's soils and PTP's soils than in PMLG's soils. S-β-GC was higher in PTL's soils than in PTP's soils, and interestingly, the activity of S-NAG was the opposite in both. In addition, compared to PMLG's soils, S-ACP and S-ASF esterase activities in PTL's soil changed but not significantly (Table 1).

Table 1. Study of enzyme activities related to soil nutrient cycling in different *Pinus* plantations.

Foresty-Type	S-β-GC (umol/d/g)	S-NAG (umol/d/g)	S-ACP (umol/d/g)	S-ASF (umol/d/g)
Mixed pine forests	2.71 ± 1.87^{b}	7.96 ± 1.23^{c}	17.48 ± 1.70^{a}	0.66 ± 0.07^{b}
Pinus thunbergii forests	17.06 ± 3.36^{a}	18.83 ± 1.67^{b}	21.72 ± 8.99^{a}	1.99 ± 0.56^{a}
Pinus tada forests	12.12 ± 1.08^{b}	27.72 ± 7.26^{a}	21.52 ± 3.66^{a}	1.22 ± 0.166^{b}

Note: The difference identification symbols (such as a, b, c) are used to indicate significant differences between different treatment groups or samples. S-β-GC: β-glucosidase; S-NAG: N-acetyl-β-D-glucosidase; S-ACP: acid phosphatase; S-ASF: aryl sulfatase.

3.2. Bacterial and Fungal Community Composition and Diversity

A total of 8255 bacterial OTUs and 3913 fungal OTUs were obtained from the 15 soil samples, and the *P. taeda* replanting group (PTL) soil microorganisms had the highest abundance of fungi (61.03% of the total fungal OTUs) and bacteria (65.18% of the total bacterial OTUs) (Figure S1a,b). The minimum coverage index for each sample exceeded 99.80%, indicating sufficient sequencing depth and high coverage (Figure 1c). The fungal communities in the soil samples comprised Basidiomycota, Ascomycota, and Mortierellomycota (total abundance > 87%) (Figure 1a). Furthermore, Proteobacteria, Acidobacteria, Actinobacteria, and Chloroflexi (total abundance > 76%) were dominant in all samples (Figure 1b).

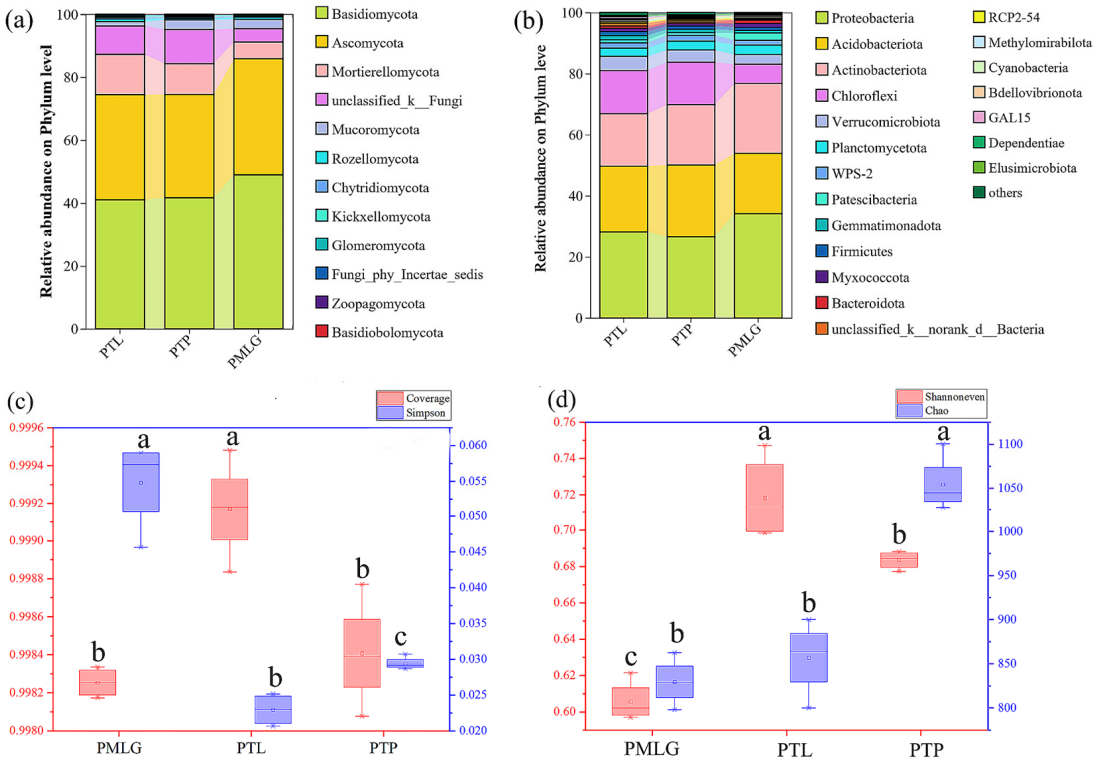


Figure 1. Phylum-level taxonomic structure and α-diversity (OTUs) of soil microbial communities across the three plantation plots. (**a**) Relative abundance of major taxa at the fungal level. (**b**) Relative abundance of major taxa at the bacterial level. (**c**) Simpson and Coverage indices in fungi. (**d**) Shannoneven and Chao indices in fungi. Notes: PMLG: sanitation cutting treatment group; PTL: *Pinus taeda* replanting group; PTP: *Pinus thunbergii* replanting group. Lowercase letters indicate significant differences ($p < 0.05$) between different plantations.

Replanting with *P. taeda* or *P. thunbergii* after sanitation cutting significantly enhances the richness and diversity of soil fungal communities. α-diversity analysis revealed that both the *P. taeda* replanting group (PTL) and *P. thunbergii* replanting group (PTP) exhibited significantly higher soil microbial Chao index (richness) and Shannoneven index (diversity) compared to the sanitation cutting treatment group (PMLG) (Figure 1d). Notably, however, the sanitation cutting treatment group (PMLG) displayed the highest Simpson index (dominance), with statistically significant differences ($p < 0.05$) from the other plots in soil. Although the Shannon index, Chao index, and Simpson index showed differences in bacterial abundance in the three plots, none reached statistical significance (Figure 1c,d) ($p < 0.05$).

3.3. Beta Diversity

The results of principal component analysis (PCA) (Figure 2a) showed that the fungal community structure differed significantly among the three habitats (R = 0.9902, p = 0.001), with axis 1 and axis 2 explaining 36.72% and 18.07% of the variance in the community structure, respectively. Bacterial community structure differed significantly among the three habitats (R = 0.4941, p = 0.001), with axis 1 and axis 2 explaining 43.84% and 13.55% of the variance in community structure (Figure 2b).

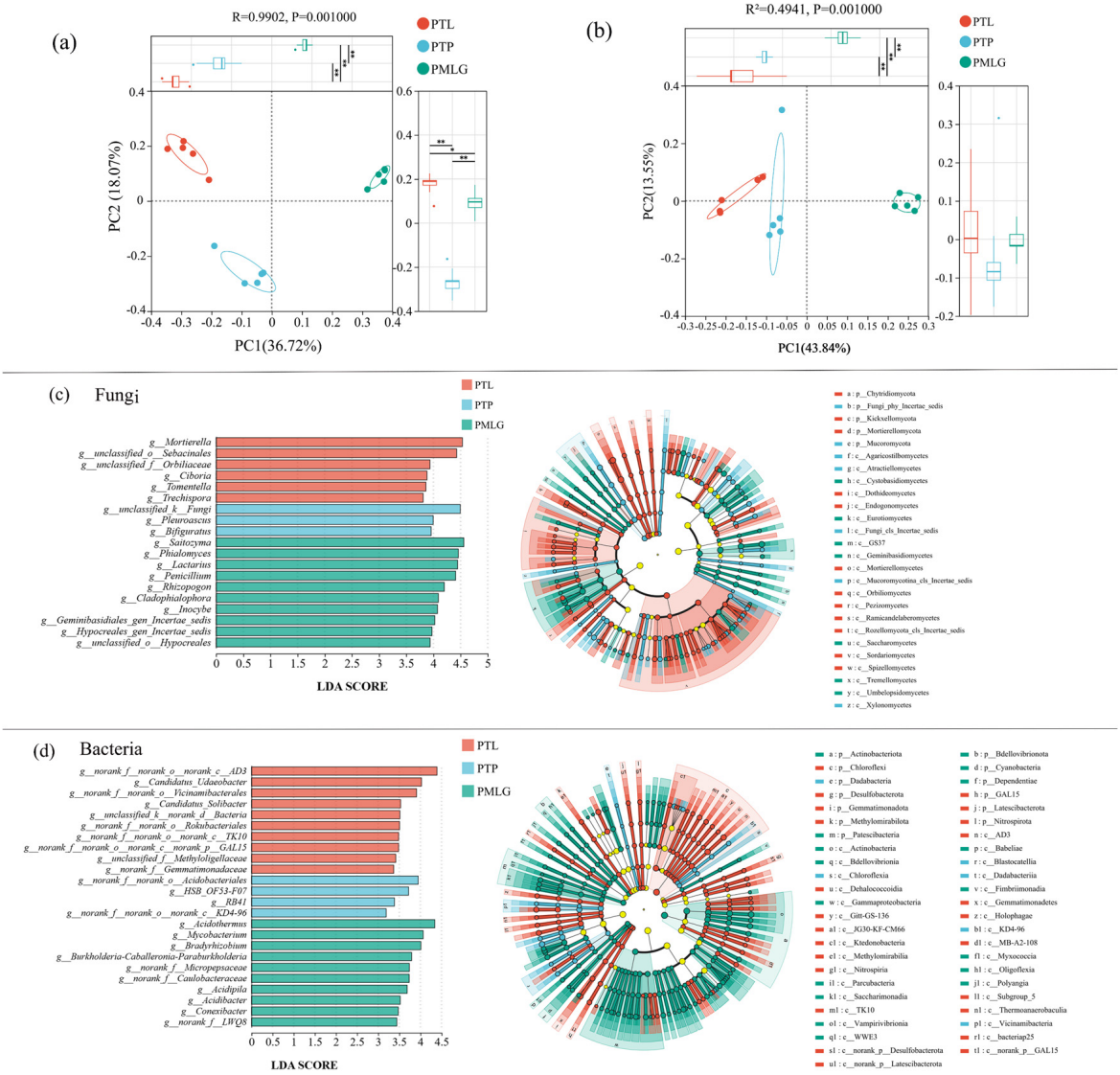


Figure 2. Changes in the soil microbial community structure in three pine plots. (**a**) PCoA of differences in fungal community structure. (**b**) PCoA of differences in bacterial community structure. Analysis of LEfSe species differences in fungal (**c**) and bacterial (**d**) communities. Note: * indicates a significance level of $p < 0.05$, ** indicates a significance level of $p < 0.01$, *** indicates a significance level of $p < 0.001$. PMLG: sanitation cutting treatment group; PTL: *Pinus taeda* replanting group; PTP: *Pinus thunbergii* replanting group.

The soil microbial community data were analyzed from the phylum to species level using LEfSe method to find the species that differed significantly in abundance between groups, of which there were 19 fungal genera with LDA scores greater than 2.5, as shown in Figure 2c. Among them, 6 genera were significantly enriched in the *P. taeda* replanting group (PTL), 3 genera were significantly enriched in the *P. thunbergii* replanting group (PTP), and 10 genera were significantly enriched in the sanitation cutting treatment group (PMLG). From the species evolution analysis, the genera significantly enriched in PTL were mainly from Chytridiomycota; the genera significantly enriched in PTP were mainly from Kickxellomycota and Mucoromycota. In terms of soil bacteria, there were 10, 4, and 10 genera significantly enriched in PMLG, PTP, and PTL respectively. From the species evolution analysis, the genera significantly enriched in PTL were mainly from Chloroflexi, Desulfobacterota, Gemmatimonadata, and Methylomirabilota; the genera significantly enriched in PTP were mainly from Dadabacteria; and the genera significantly enriched in PMLG were mainly from Actinobacteriota (Figure 2d). This suggests that the soil-enriched species-specific taxa differed significantly among the three habitats.

3.4. Correlations of Soil Microbial Communities with Environmental Variables

To investigate the effects of replanting disease-resistant *P. thunbergii* and *P. taeda* after sanitation cutting on soil microbial community structure, we performed redundancy analysis (RDA) to examine the relationships between microbial community composition and nine key environmental variables (including five soil physicochemical properties and four enzyme activity indicators) that were pre-selected through variance inflation factor (VIF) analysis (Tables S2 and S3).

Among the environmental variables, S-ASF exhibited the longest vector length in the RDA biplot, followed by SMC ($r^2 = 0.7213$, $p < 0.002$) and pH ($r^2 = 0.7638$, $p < 0.001$), indicating their most significant influence on soil fungal communities. In contrast, TP and S-β-GC showed the shortest vectors, demonstrating minimal impact on bacterial communities (Figure 3a). Variance partitioning analysis (VPA) revealed that soil enzyme activity was the primary driver of fungal community variation, accounting for 5.21% of the explained variance, while soil nutrients contributed 23.55% (Figure 3b). Notably, the three treatment groups formed distinct clusters in the ordination space, suggesting that replanting with disease-resistant pines significantly altered soil fungal communities, resulting in unique community characteristics for each group. In bacterial communities, SMC ($r^2 = 0.6525$, $p < 0.004$) displayed the longest vector, followed by TK ($r^2 = 0.5333$, $p < 0.009$), highlighting their strong influence. S-β-GC showed the shortest vector, indicating negligible effects (Figure 3c). Interestingly, in contrast to fungal communities, soil physicochemical properties emerged as the dominant factor explaining bacterial community variation, with enzyme activity and soil nutrients explaining 10.90% and 23.52% of the variance, respectively (Figure 3d).

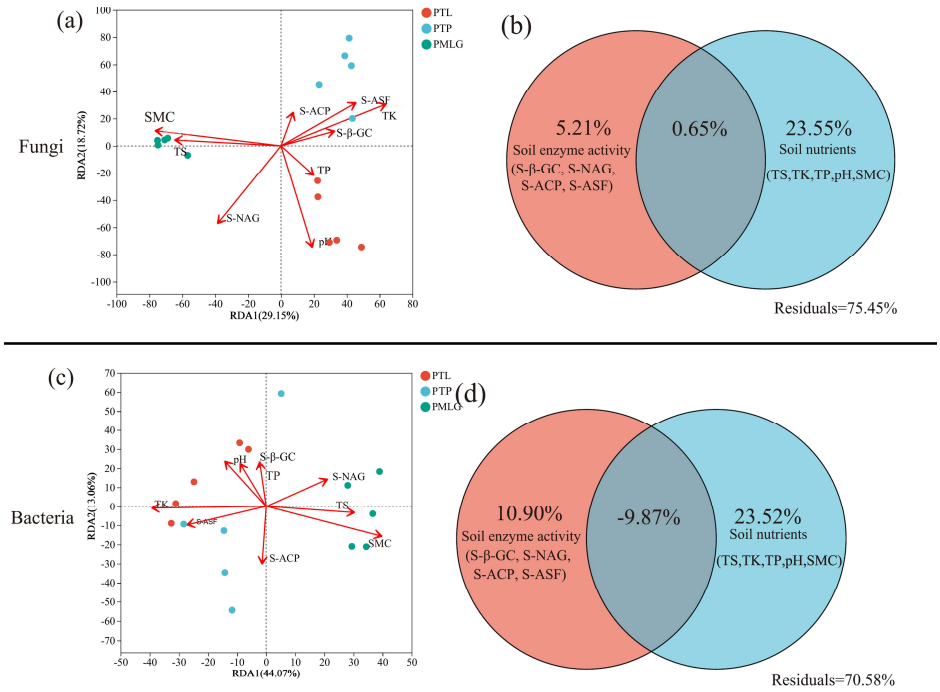


Figure 3. Associations between microbial communities and environmental factors. (**a**) Redundancy analysis of fungal community structure and its relationship with soil properties. (**b**) Variance partitioning analysis (VPA) of fungal communities showing the contributions of environmental factors to fungal communities. (**c**) Redundancy analysis of the bacterial community structure and its relationship with the soil properties. (**d**) Variance partitioning analysis (VPA) of the bacterial community showing the contributions of environmental factors to the bacterial community. PMLG: mixed pine forests; PTL: *Pinus tada* forests; PTP: *Pinus thunbergii* forests.

3.5. Potential Functional Structure of Different Microbial Communities

The three most abundant functional characteristics in the sanitation cutting treatment group (PMLG), *P. taeda* replanting group (PTL), and *P. thunbergii* replanting group (PTP) are undefined saprotroph, ectomycorrhizal, and endophyte–litter saprotroph–soil saprotroph–undefined saprotroph (Figure 4a). Compared to the PMLG group, the PTL and PTP groups showed a significant increase in the relative abundance of endophyte–litter saprotroph–soil saprotroph–undefined saprotroph (ELSSUS), wood saprotroph (WS), and ectomycorrhizal (EC) fungi. While the top five functions of soil bacterial relative abundance were not significantly different in the three experimental plots., we observed significant differences in the functions of the other bacterial communities (Figure 4b). Compared to PMLG, PTP had significantly higher functional taxa of bacterial denitrification, nitrous oxide denitrification, and nitrite denitrification in soil bacterial community function (Figure 4d). Only the anoxygenic_photoautotrophic were significantly increased in the bacterial community functions of PTL compared to PMLG (Figure 4c).

3.6. Deterministic and Stochastic Assembly of Soil Microbial Communities

The β-NTI and RCbray values, as determined between the samples, elucidate the ecological processes governing microbial communities.

Although the βNITs of the sanitation cutting treatment group (PMLG) soil microbial communities were not significantly different from those of the *P. taeda* replanting group (PTL) and *P. thunbergii* replanting group (PTP) ($p > 0.05$), their medians were all less than 2, suggesting that stochastic processes mainly control the formation of soil fungal communities (Figure 5a). Significant changes in soil bacterial community assembly occurred in PTP and PTL compared to PMLG. Although homogenizing dispersal contributed to the

bacterial community composition in PMLG, the bacterial community composition that determined PMLG remained dominated by stochastic processes (homogenizing dispersal and undominated) (Figure 5b). The magnitude of undominated soil increased in the order PTL > PTP > PMLG, and it played a significant role in the composition of bacterial communities.

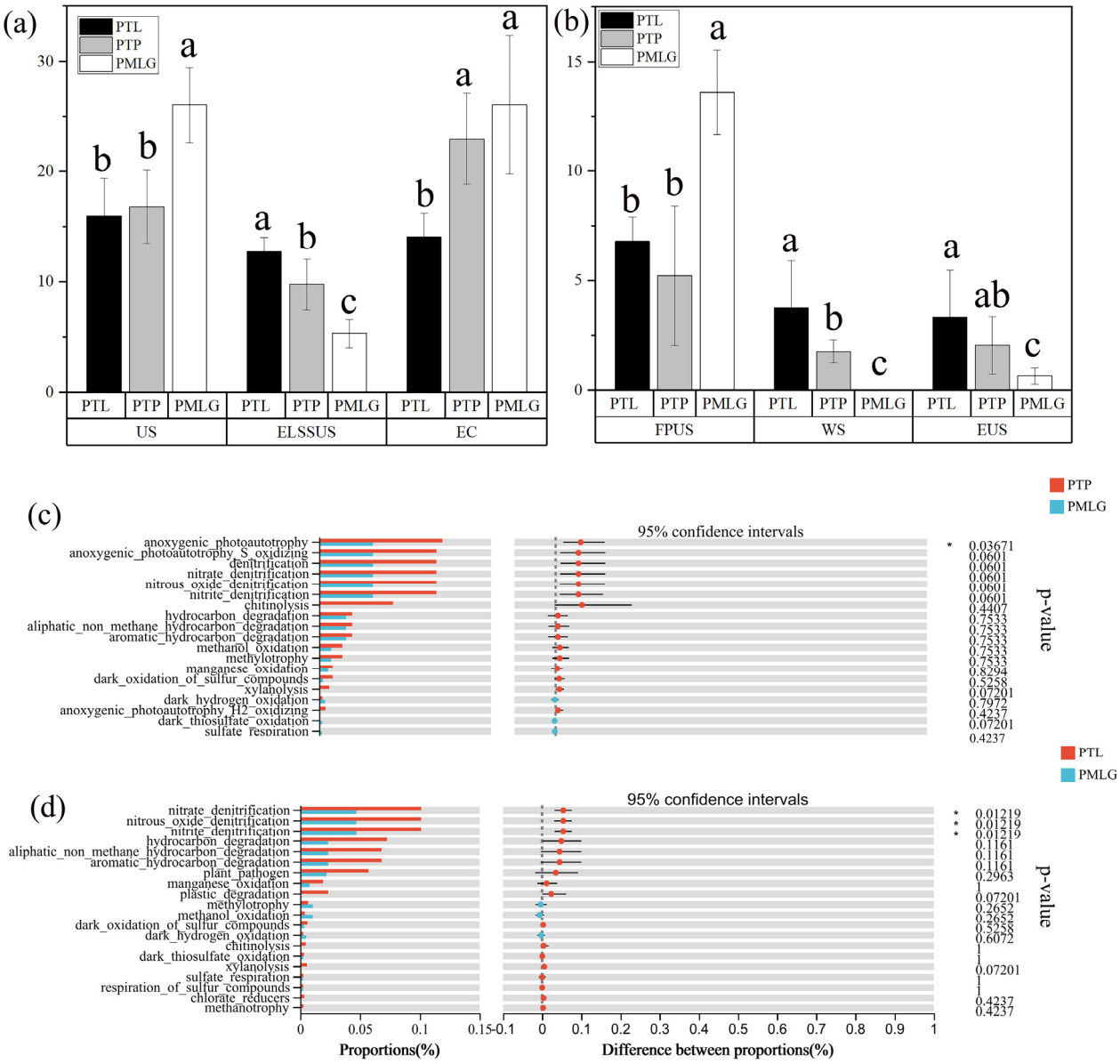


Figure 4. Functional predictive analyses of bacterial communities and bacterial communities. (**a**,**b**) FUNGuild functional prediction component difference tests for fungal communities. (**c**,**d**) FAPROTAX functional prediction component difference tests for bacterial communities. Notes: The difference notation symbols (such as a, b, c) are used to indicate significant differences among different treatment groups. * indicates a significance level of $p < 0.05$, ** indicates a significance level of $p < 0.01$, *** indicates a significance level of $p < 0.001$. US: undefined saprotroph; ELSSUS: endophyte–litter saprotroph–soil saprotroph–undefined saprotroph; EC: ectomycorrhizal; FPUS: fungal parasite–undefined saprotroph; WS: wood saprotroph; EUS: ectomycorrhizal–undefined saprotroph. PMLG: sanitation cutting treatment group; PTL: *Pinus taeda* replanting group; PTP: *Pinus thunbergii* replanting group.

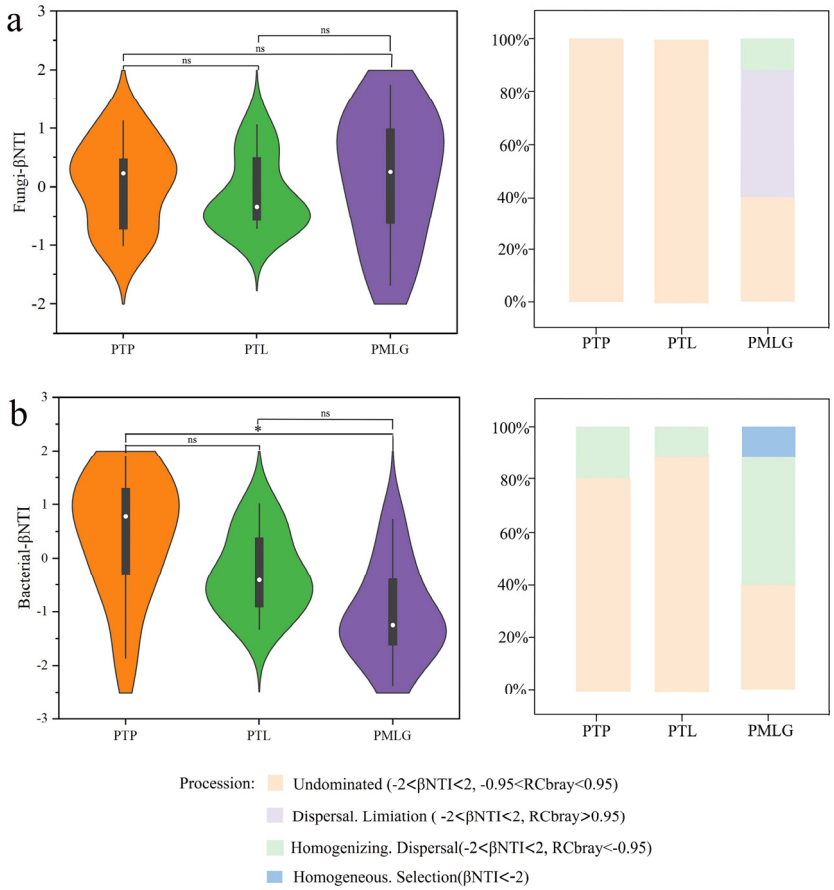


Figure 5. Distribution of β-NTI values for fungal (**a**) and bacterial (**b**) community comparisons. Notes: * indicates a significance level of $p < 0.05$. ns indicates a significance level of $p > 0.05$. PMLG: sanitation cutting treatment group; PTL: *Pinus taeda* replanting group; PTP: *Pinus thunbergii* replanting group.

4. Discussion

4.1. Effects of Infected Wood Harvesting and Planting of Disease-Resistant Pine Trees on Soil Enzyme Activity

Planting pines such as *P. thunbergii* and *P. taeda* has been shown to increase soil microbial activity and soil organic matter decomposition, which leads to a raise in soil enzyme activities [42,43]. This largely explains the increase in soil enzyme activities commonly observed after planting disease-resistant pine trees. β-Glucosidase catalyzes the hydrolysis and biodegradation of various β-glucosides present in plant residues and is a major source of carbon for the growth and activity of soil microorganisms [44]. The soil β-glucosidase (S-β-GC) activity was significantly greater in *P. thunbergii* plots and *P. taeda* plots than in sanitation cutting plots (Table 1), and planting *P. thunbergii* resulted in a 459% increase in the soil SOC content. This phenomenon may be attributed to the active participation of soil microorganisms in the decomposition and transformation of organic matter through a variety of metabolic pathways that contribute to the stabilization of organic carbon, thereby affecting soil carbon storage and turnover [45]. Soil S-NAG catalyzes the hydrolysis of N-acetyl-β-D-glucosamine to release nitrogen from the soil; however, high levels of SOC promote nitrogen release. Therefore, the activity of soil S-NAG in *P. taeda* plots increased [46]. The soil arylsulfatase activity decreased with increasing TS content after replanting pine trees after sanitation cutting. A lower TS content stimulates microbial communities to produce high-quality S-ASF to increase the soil S content, resulting in lower S-ASF activity in sanitation cutting plots than in *P. thunbergii* plots and *P. taeda* plots [47].

4.2. Microbial Communities and Their Diversity

Research indicates that planting *P. taeda* or *P. thunbergii* after sanitation logging can effectively restore the richness and diversity of soil fungal communities. This aligns with the findings of Lin et al., who observed that while thinning temporarily suppresses fungal diversity, reforestation facilitates its recovery [48]—a trend consistent with the observations in the *P. taeda* and *P. thunbergii* sample plots of this study. The soil fungal community was dominated by Basidiomycota, Ascomycota, and Mortierellomycota, while the bacterial community was primarily composed of Proteobacteria, Acidobacteria, Actinobacteria, and Chloroflexi. These findings align with global studies on forest soil microbiomes [49], where Basidiomycota and Ascomycota are recognized as core fungal phyla in forest ecosystems, playing key roles in lignin degradation and symbiotic relationships [50]. Meanwhile, the enrichment of Proteobacteria and Acidobacteria is typically associated with organic matter decomposition and acidic soil conditions [51]. Notably, the high abundance of Mortierellomycota may be linked to root exudates from *P. taeda* in our study sites, as this phylum is known to form mutualistic relationships with plant roots [52]. Similarly, the enrichment of Chloroflexi in the *P. thunbergii* replanting group (PTP) likely reflects their adaptation to pine litter decomposition.

4.3. Relationships Between Soil Properties and Microorganisms

Microbial and soil properties are intricately linked, and changes in soil nutrients and soil enzyme activities affect the abundance of microbial communities [53]. Compared with the sanitation cutting treatment group (PMLG), the soil enzyme activities of S-NAG and S-β-GC in the *Pinus taeda* replanting group (PTL) and *Pinus thunbergii* replanting group (PTP) were significantly increased, which was probably due to the relative increase in difficult-to-degrade organic carbon as a result of mesquite logging [54]. However, the higher moisture content in the sanitation cutting treatment group (PMLG) compared to the *Pinus taeda* replanting group (PTL) and *Pinus thunbergii* replanting group (PTP) indicates greater plant diversity and unfragmented litter content, which contributes to its ability to retain higher water levels [55].

The results of RDA suggested that the microbial community showed a significant correlation with SMC, pH, and TK. Changes in soil water content directly affect the composition and structure of microbial communities, and drought increases the abundance and diversity of fungal communities. SMC affects the community structure of soil microorganisms, and fungi gradually dominate the microbial community under drought conditions [56]. The study proved that SMC was the most important driver of soil fungal community changes in the region. The effect of TK on the bacterial community may be due to the ability of soil bacteria to promote the solubilization of insoluble potassium through the decomposition of minerals, so that soil bacteria have a more adequate supply of potassium, which in turn promotes the growth and reproduction of soil bacteria and enhances the biological activity of the soil [57]. Previous studies have established that soil pH is the most significant factor influencing the composition of the soil microbial community in the region [58]. In our findings, compared with the sanitation cutting treatment group (PMLG), the *Pinus taeda* replanting group (PTL) and *Pinus thunbergii* replanting group (PTP) showed an important change in pH. As a general rule, pH is a crucial factor influencing the structure of the soil fungal community; however, it exerts no significant influence on the bacterial community [59]. This may be due to the fact that the Acidobacteria gates in the soil affected the results of the experiment.

4.4. Functional Changes in Soil Microbial Communities

The findings of this study demonstrate a significant increase in the relative abundance of ectomycorrhizal fungi in both the *P. taeda* replanting group (PTL) and *P. thunbergii* replanting group (PTP). This phenomenon suggests that the replanting of disease-resistant pine trees may exert a positive influence on the structure of soil microbial communities. Ectomycorrhizal fungi form mutualistic symbiotic relationships with plant roots, substantially enhancing the host plant's efficiency in nutrient and water uptake [60]. Previous studies have confirmed a significant positive correlation between ectomycorrhizal fungi abundance and plant health status as well as growth indicators [61]. Notably, the abundance of the endophyte–litter saprotroph–soil saprotroph–undefined saprotroph functional guild also exhibited an increasing trend. This guild plays a pivotal role in litter decomposition and organic matter mineralization, and its elevated abundance may indicate that the replanting measures have enhanced soil organic matter breakdown and nutrient cycling efficiency [62]. Concurrently, the observed variation in wood saprotroph abundance could be linked to increased woody debris input following replanting. These taxa contribute to carbon cycling through lignin and cellulose decomposition [63]. Denitrifiers exhibited significantly higher abundance in the *P. thunbergii* replanting group (PTP). As crucial nitrogen-cycling agents, this increase likely reflects enhanced soil nitrogen transformation following replanting, potentially driven by elevated soil organic matter and improved microenvironments [64]. Anoxygenic phototrophs showed distinct enrichment in the *P. thunbergii* replanting group (PTP), suggesting potential adaptation to altered light availability and organic matter composition in surface soils post-replanting [65].

4.5. Soil Microbial Community Assembly

The processes influencing the composition of soil microbial communities are pivotal to the study of microbial ecology [24]. Microbial community assembly is governed by the processes affecting constituent species, such as selection, drift, speciation, and dispersal [66]. Dispersal and species formation are influenced by both stochastic and deterministic factors [67]. Our findings indicate that although undominated processes in microbial community assembly were attenuated in the *Pinus taeda* replanting group (PTL) and *Pinus thunbergii* replanting group (PTP) compared to the sanitation cutting treatment group (PMLG), bacterial homogenizing dispersal was significantly increased in the sanitation cutting treatment group (PMLG). Undominated processes played a significant role in the assembly of the microbial community compared with homogenizing dispersal. Moreover, bacterial communities, which tend to have broader niches, were more significantly influenced by homogenizing dispersal than were fungal communities [68]. Compared to fungi, bacterial community composition is relatively increased by homogenizing dispersal, which may be the result of decomposing difficult-to-degrade organic matter to provide substrate for symbiotic utilization by bacteria [69]. In addition, higher SMC suppresses fungal abundance and diversity, which may be why diffusion limitation in the sanitation cutting treatment group (PMLG) is important for microbial community assembly [70]. The stochastic nature and unpredictability of microbial composition are heightened by reduced resource competition, ecological niche selection, and amplified preferential attachment effects [71]. To better mechanistically understand the role of environmental factors in driving microbial communities in the intercropping of infected trees and planting of disease-resistant pine forests, future studies need to consider incorporating local factors into temporal sampling designs.

5. Conclusions

Our results showed that after pine forests were infested by pine wood nematode, by either inter-planting infected wood (sanitation-thinned experimental plot) or cultivating new pine forests resistant to the disease (*P. thunbergii* and *P. taeda*), there were significant differences in the structure and function of the soil microbial communities, especially the 468 fungi. The activities of enzymes such as β-glucosidase (S-β-GC), N-acetyl-β-D-gluco-sidase (S-NAG), and aryl sulfate (S-ASF) were significantly higher in the *Pinus taeda* experimental plot and *Pinus thunbergii* experimental plot compared to the sanitation-thinned experimental plot. In addition, the bacterial and fungal communities in the *Pinus taeda* experimental plot and *Pinus thunbergii* experimental plot significantly differed from the soil bacterial and fungal communities in sanitation-thinned experimental plot because of their significant β-diversity, different indicator groups, and unique functional properties. Soil moisture content, pH, and total potassium were the most important factors affecting the process of bacterial and fungal community assembly in soil. Wood saprotrophic fungi and ectomycorrhizal fungi exhibited increased abundance in both the *P. taeda* experimental plot and *Pinus thunbergii* experimental plot. Zero-modeling analyses indicated that undominated processes were among the major processes involved in microbial community assembly. Dispersal limitation and homogenizing dispersal played important roles in fungal and bacterial community assembly in the sanitation-thinned experimental plot, respectively, but their roles were diminished in the *P. taeda* experimental plot and *Pinus thunbergii* experimental plot. In the context of management measures taken after the occurrence of pine wood nematode disease, these results provide new insights into the understanding of soil microbial changes and contribute to the continued development of sustainable management of pine forest ecosystems.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/f16050813/s1, Table S1. Basic properties of the three different pinus plots. Significance indicators: a, ab, b. Table S2. Soil physicochemical properties of three pinus plots. Significance indicators: a, ab, b. Table S3. Soil factors of three pinus plots screened via variance inflation factor (VIF) analysis. Figure S1. Wayne analysis of microbial OTUs and α-diversity (OTUs) of soil microbial communities across the three plantation plots: (a) fungi; (b) bacteria. (c) Simpson and coverage indices in bacteria. (d) Shannoneven and Chao indices in bacteria. PMLG: sanitation cutting treatment group; PTL: *Pinus taeda* replanting group; PTP: *Pinus thunbergii* replanting group.

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