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Responses of Soil Microbial Diversity to Forest Management Practices after Pine Wilt Disease Infection

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Abstract: Pine wilt disease (PWD) caused by the pine wood nematode (*Bursaphelenchus xylophilus*) is a serious threat to coniferous forests worldwide. However, little is known about how soil microbial diversity responds to PWD and associated management practices. We investigated the community composition and diversity of bacteria and fungi in bulk and rhizosphere soil of Masson pine (*Pinus massoniana* Lamb.) forests following 0, 1, and 5 year PWD, with the dead pine in a certain plot being either managed (logged and removed from the plot) or unmanaged (maintained as standing dead wood). Both bacterial and fungal alpha diversity decrease after 5 year PWD and logging, with response degree being different between site locations. Alpha diversity of rhizosphere fungi, rather than bacteria, significantly decreases with the disease and logging. We observe an increase in the relative amount of bacterial functional groups involved in carbohydrate and amino acid metabolism after PWD infection and logging practice. With the disease infection, the relative abundance of ectomycorrhizal fungi decreases, while the relative abundance of saprotrophic fungi increases. Compared with logging treatment, unmanaged practice had a weaker effect on soil microbial communities. Our findings provide new insights into the short-term responses of soil microbial diversity to management practices after PWD infection.

Keywords: *Pinus massoniana*; pine wilt disease; forest management; logging; bacteria and fungi; functional prediction

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1. Introduction

As a common pioneer community following anthropogenic disturbance, Masson pine (*Pinus massoniana* Lamb.) forests are widely distributed in southern China, providing critical ecosystem benefits to human society [1]. In recent decades, however, pine wilt disease (PWD) has become one of the most serious threats to coniferous forests across the world, and this disease causes huge economic and environmental losses [2,3]. PWD is mainly caused by the invasion of pine wood nematode [4]. Effective management, such as logging and removing the infected individuals, is often carried out to restrict PWD spread and maintain forest ecosystem functions [5,6]. Meanwhile, PWD infection and management practice may affect environmental conditions and forest biodiversity [7,8]. Soil microorganisms facilitate organic matter decomposition and nutrient cycling, and also aid in plant growth [9,10]. Therefore, the better we understand how soil microbial communities respond to PWD and forest management, the better we will be able to predict successional dynamics and functions of diseased forest ecosystems.

Soil microorganisms are closely associated with forest plants and abiotic environmental factors [11–13]. Disturbance of pine wood nematode can reshape the microbial communities via altered host plants and soil physicochemical properties [14]. With needle shedding after PWD, the microhabitat, such as light and temperature, can be varied in forest gap, which might lead to the changes in microorganism growth [15]. Fungi are the first consumers of belowground plant-derived carbon inputs, and large fungal groups are host-specific, thus, they are usually more sensitive to tree mortality and forest management than bacteria [11,16,17]. Differential sensitivity to tree mortality could also appear among different fungal groups. For instance, studies demonstrate that the mortality of trees inhibits their associated ectomycorrhizal (ECM) fungi but for the benefit of saprotrophic fungi [18–20]. Over the last decade, an increasing number of studies confirmed the long-lasting impacts of forest disturbance on soil microbial communities [8,21,22]. However, the number of studies on PWD is still not high enough to explore the mechanisms of soil microbial alteration in the disease region.

Logging and removing PWD-infected pine is helpful to control the disease [5,6]. Tree harvesting in coniferous forests can have a negative impact on soil microbial activity and organic matter mineralization due to the reduction in substrate availability [17,23,24]. In addition, changing microenvironments in logged patches shapes soil microbial diversity [19]. Changes in soil fungal community composition caused by harvesting or logging disturbance could persist for decades, with individual taxonomic groups responding differentially to the disturbances [7,22,25]. The short-term effects of management practice on soil microbial diversity after PWD are unknown. Moreover, the occurrence of PWD is likely to reduce the secretion of sugar and protein in the root [26]. After root exudates were depressed due to trees mortality, rhizosphere microorganisms were changed [27,28]. Most man-induced disturbance studies to date focus on anthropogenic activities, such as selective cutting or timber harvesting in planted forests, which is different from the process of PWD. Another study found lower relative abundance of beneficial microbes such as *Paraburkholderia*, *Bradyrhizobium*, and *Rhizobacter* was contained in the rhizosphere of nematode-inoculated seedlings [29]. To the best of our knowledge, no study has explored the alteration of microbial diversity as well as the potential function in the rhizosphere of mature pine after pine wood nematode infection.

In this study, we established the plots with different years of PWD infection and management practices in subtropical China. We aimed to explore the short-term effects of PWD and forest management on soil microbial diversity and potential function. We predicted the following: (1) soil microbial diversity would be varied after PWD infection, with fungi showing a much stronger response than bacteria, because of the strong host specificity of mycorrhizal fungi; (2) pine mortality would have a negative effect on ECM fungi but a positive effect on saprotrophic fungi; (3) there would be differences in soil microbial diversity responses between logging and unmanaged practice during PWD.

2. Methods

2.1. Study Area

The study was carried out in Zhejiang province, southeastern China. Masson pine forests are widely distributed in this region, and part of the area has been invaded by pine wood nematode. The study area was located from 27°22′06″ to 28°39′04″ N latitude, 119°16′49″ to 120°03′49″ E longitude (Figure 1a), has a mean annual temperature of 16.2–17.1 °C, and a mean annual rainfall of 1579–2047 mm (China Meteorological Data Service Centre, http://data.cma.cn/ (accessed on 10 June 2022)).

A total of 33 plots (30 m × 30 m each) were established in 2020 and 2021. The studied sites had previously been subjected to similar management practices: all these plots were developed from secondary succession of cut forests following logging in the 1950s, and now these plots were of similar forest age but different PWD-infected years, including 9 uninfected plots, 12 plots that were infected for 1 year, and 12 plots that were infected for 5 years. Meanwhile, these plots were either managed or left unmanaged with infected

individuals (Figure 1a). The managed treatment means the dead pine individuals were logged and removed out of the plot, with each infected year being of the same logging intensity and logging frequency. Unmanaged practice means the standing dead woods were kept in the plot. Taken together, among the 1 year infected plots, the wilted pine individuals of 9 plots were logged and removed, and the infected pine individuals of 3 plots were left unmanaged; among the 5 year infected plots, the infected pine individuals of 9 plots were logged and removed, and the infected pine individuals of 3 plots were left unmanaged (Table 1). All logging treatment plots within the same PWD year were of the same logging intensity and logging frequency.

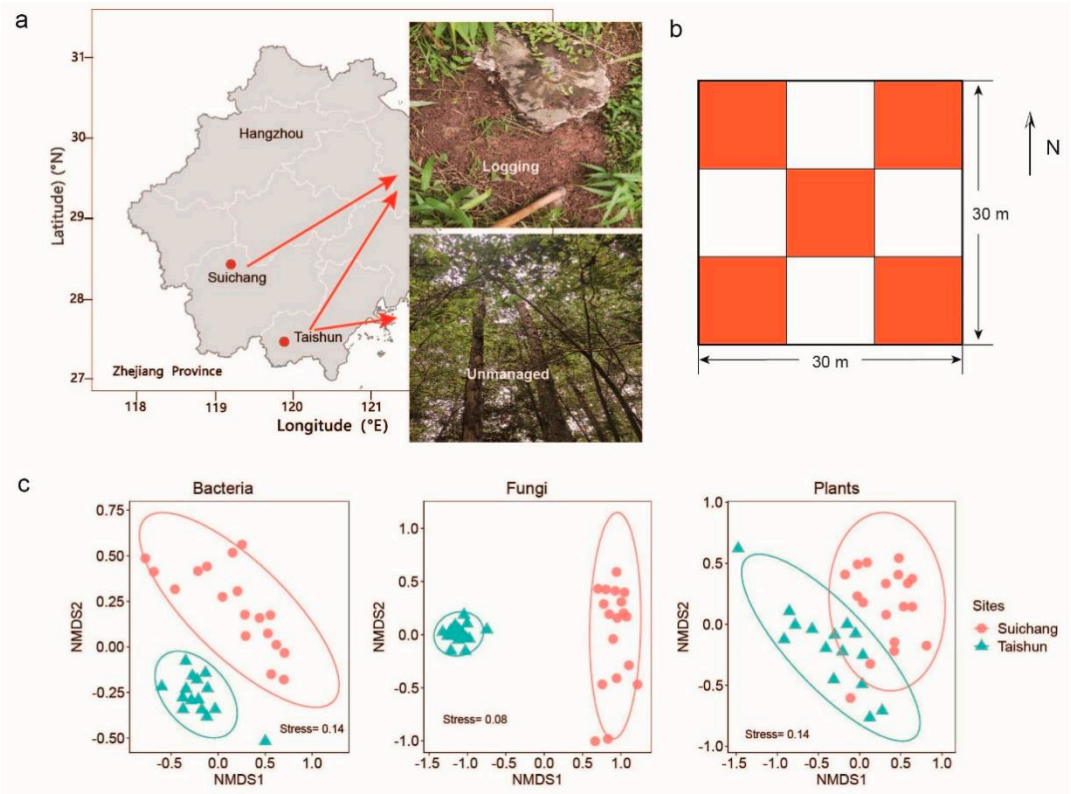

Figure 1. The location, sampling design, and ordination of the bacteria, fungi, and woody plant by non-metric multidimensional scaling (NMDS, based on the Bray–Curtis distance matrices) analysis at plot-level. (**a**) The locations of the 33 study plots in Zhejiang province, China. The dead pine were either logged and removed out of the plot (logging) or left unmanaged after pine wilt disease (PWD) infection. (**b**) Five 10 m × 10 m quadrats were investigated for bulk soil microbiomes at each plot. (**c**) Both soil microbiomes and woody plants were separable by two county sites. Samples were shaped according to the sites.

Table 1. The number of plots and pine individuals that were investigated under each treatment after PWD infection.

PWD Infected Years	Suichang Logging	Taishun Logging	Taishun Unmanaged
	PWD-infected plots		
Uninfected (0 year)	6	3	
1 year	6	3	3
5 years	6	3	3
	PWD-infected pine individuals		
Uninfected (0 year)	8	6	6
1 year	13	5	6
3 years	6	5	6

2.2. Soil Sampling

Five 10 m × 10 m quadrats (one in the center and four in each plot corner) were chosen at each plot for microbe investigation in bulk soil (Figure 1b). After removing the litter layer, three 0–10 cm deep soil cores around the center of quadrat were randomly collected, mixed, and passed through a sieve of 2 mm mesh size to form one soil sample. At each PWD plot, pine individuals that were healthy (uninfected), infected for 1 year and 3 years were selected for microbial community analysis in rhizosphere soil, with one or two individuals being selected for each infected stage. Pine root systems that had been dead for four years or more were decayed or had even disappeared. Therefore, we only analyzed rhizosphere microorganisms of pine individuals that had been infected for 0–3 years. After removing the surface litter and tracing the root extension in the 0–10 cm layer, samples at 0–4 mm distance from the root surface were collected as rhizosphere soil, and four directions for each tree were pooled together and mixed to form one soil sample. All the samples were stored at −80 °C before DNA extraction. In total, 165 bulk soil samples and 61 rhizosphere soil samples were collected (Table 1). After analysis of the soil samples, the replicates within each plot were averaged to obtain 30 m × 30 m plot-level estimates of microbial communities (Figure 1c). In addition, we investigated all free-standing woody plants in five 10 m × 10 m quadrats (one in the center and four in each plot corner) to estimate plant community composition.

2.3. DNA Extraction and Sequencing

We extracted soil DNA using the TGuide S96 Magnetic Soil/Stool DNA Kit (Tiangen Biotech (Beijing, China) Co., Ltd.). For bacteria, we targeted the V3–V4 region of 16S rRNA gene, using 338F (5′-ACTCCTACGGGAGGCAGCA-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′) primer pair [30,31]. For fungi, we targeted the internal transcribed spacer 1 (ITS1) region of rRNA gene using the ITS1F (5′-CTTGGTCATTTAGAGGAAGTAA-3′) and ITS2R (5′-GCTGCGTTCTTCATCGATGC-3′) primer pairs [31,32]. PCR amplification was performed with reactions containing 5–50 ng DNA template, 0.3 μL (10 μM) of each primer, 5 μL KOD FX Neo Buffer, 2 μL (2 mM each) dNTP, 0.2 μL KOD FX Neo, and up to 10 μL ddH2O. Thermal cycling conditions for the V3–V4 region of 16S rRNA gene were as follows: an initial denaturation at 95 °C for 5 min, followed by 25 cycles of denaturation at 95 °C for 30 s, annealing at 50 °C for 30 s, and extension at 72 °C for 40 s, and a final step at 72 °C for 7 min. For the ITS1 gene, thermal cycling conditions were as follows: an initial denaturation at 95 °C for 5 min, followed by 25 cycles of denaturation at 95 °C for 1 min, annealing at 50 °C for 30 s, and extension at 72 °C for 1 min, and a final step at 72 °C for 7 min. We purified all PCR amplicons using Agencourt AMPure XP Beads (Beckman Coulter, Indianapolis, IN, USA) and quantified amplicons using the Qubit dsDNA HS Assay Kit and Qubit 4.0 Fluorometer (Invitrogen, Thermo Fisher Scientific, Bend, OR, USA). The purified, pooled PCR products were subsequently sequenced on the Illumina novaseq 6000 (Illumina, Santiago, CA, USA) at Biomarker Technologies Corporation, Beijing, China.

2.4. Bioinformatics

We used Trimmomatic (Version 0.33, Golm, Germany) to filter raw data [33], and Cutadapt (Version 1.9.1, Dortmund, Germany) to identify and remove primer sequences [34]. We processed the remaining high-quality sequences using QIIME 2 (Version 2020.6, La Jolla, CA, USA) [35], and applied them to DADA2 pipeline for the assignment of amplicon sequence variants (ASVs) [36]. The sequences were classified to ASVs by naive Bayesian classifier-based method, with 0.005% conservative threshold for ASV filtration [37]. We searched the databases of SILVA 138.1 [38] and UNITE 8.0 [39] to determine the taxonomic classification of each ASV of bacteria and fungi, respectively. Before downstream analysis, bacterial samples were rarefied to 18,827 sequences per sample, and fungal samples were rarefied to 30,168 sequences per sample. We analyzed microbial community composition and diversity based on ASV tables. We assigned ecological functions of the soil bacteria

and fungi by PICRUSt2 (Version 2.3.0, Halifax, NS, Canada) [40] and FUNGuild (Version 1.0, St. Paul, MN, USA) [41], respectively.

2.5. Statistical Analyses

We used space-for-time substitution to study the effects of forest conversion on soil microbial communities and the associated functions. We averaged information from replicate samples to obtain plot-level estimates of microbial diversity and composition for further statistical analysis. All statistical analyses and visualizations were performed in the R software environment (Version 4.1.2, Vienna, Austria). All data were tested for normality and homogeneity of variance. The variations in bacterial and fungal community composition on differences by treatments (management practice) and year were analyzed using PERMANOVA (999 permutations, Adonis function) and visualized by non-metric multidimensional scaling (NMDS) using the "vegan" package [42], based on the Bray–Curtis distance matrices. The relative abundance of bacteria and fungi was assigned at both phylum and functional levels. One-way ANOVA was conducted to determine the significance of PWD and management practice on the responses of microbial diversity, and Tukey's HSD test was used for multiple comparisons across treatments. As plant community composition can influence soil microbial diversity (Guo et al. unpublished data), we used analysis of covariance to analyze effects of pine wood nematode infection and management practice on microbial diversity, with plant NMDS axes (NMDS1 and NMDS2) being a covariate. We also used Kruskal–Wallis test to assess the effects of PWD and management practice on the relative abundance of each phylum taxa, and used pairwise Wilcox test for their multiple comparisons across treatments.

3. Results

3.1. General Characterization of Soil Microbial Communities

A total of 9,462,481 high-quality bacterial reads were obtained by high-throughput sequencing of all bulk soil samples at the quadrat level. These reads were clustered into 2548 bacterial amplicon sequence variants (ASVs). At the plot level, the most dominant phylum in the bacterial community is *Acidobacteriota* (42.3% on average), followed by *Proteobacteria* (30.4%), *Actinobacteriota* (9.2%), *Verrucomicrobiota* (6.5%), and an unclassified phylum (4.3%) (Table S1). In terms of rhizosphere soil samples of Masson pine at individual levels, a total of 4,043,224 high-quality bacterial reads were clustered into 2522 ASVs. At the plot level, the most dominant phylum in the bacterial community is *Acidobacteriota* (41.2%), followed by *Proteobacteria* (34.1%), *Verrucomicrobiota* (6.4%), *Actinobacteriota* (5.3%), and an unclassified phylum (5.0%) (Table S1).

Regarding fungi, a total of 11,989,503 high-quality reads in bulk soil were clustered into 2073 fungal ASVs. Fungal communities are dominated by *Ascomycota* (49.4%) and *Basidiomycota* (43.1%), followed by an unclassified phylum (3.9%) (Table S2). In Masson pine rhizosphere soil, a total of 4,953,010 high-quality fungal reads were clustered into 2208 ASVs. Fungal communities in rhizosphere soil are dominated by *Ascomycota* (56.0%) and *Basidiomycota* (34.3%), followed by an unclassified phylum (5.4%) (Table S2).

3.2. Variations in Microbial Community Alpha Diversity

With pine wilt disease (PWD) occurrence and artificial management, significant variations in microbial alpha diversity appear in Suichang County: both bacterial and fungal richness in bulk soil are significantly lower in the plot with over 5 years of disease infection and logging (Slog5) than those in healthy (S0) or 1 year of disease infection and logged plots (Slog1) (Figures 2a and S1). However, no decrease in soil microbial diversity is observed in Taishun County (Figure 2a). Variations in microbial diversity is partly explained by woody plant composition. After removing the effect of NMDS axis from the woody plant community (NMDS1 and NMDS2), bacterial diversity did not change with disease infection, whereas significant shifts in fungal diversity caused by PWD and artificial management still persist (Table S3).

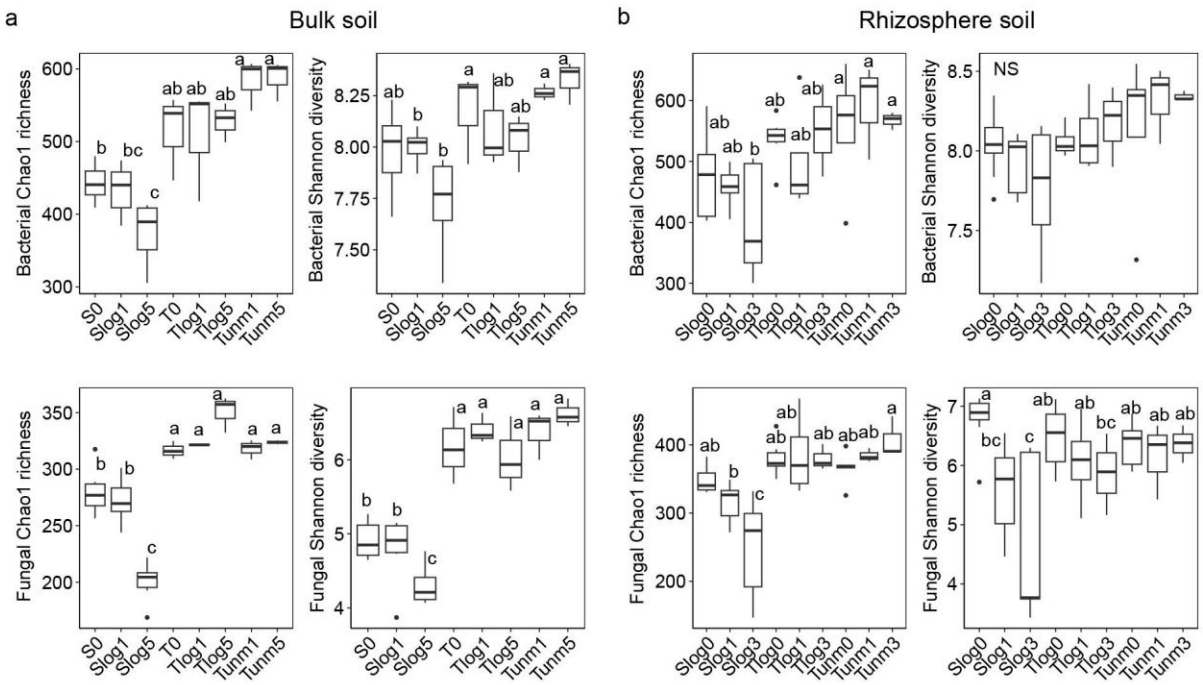

Figure 2. Alpha diversity measures in bulk and rhizosphere soil of Masson pine (*Pinus massoniana* Lamb.) forests. Letters represent difference among infected years and treatments at the significance level ($p < 0.05$). NS represent no significant difference among infected years or treatments. S, forest plots in Suichang County; T, forest plots in Taishun County; 0, uninfected plot or pine individuals; log1, logged plot or pine individuals after 1 year of disease infection; log5, logged plot after 5 years of disease infection; log3, logged pine individuals after 3 years of disease infection; unm1, unmanaged pine individuals after 1 year of disease infection; unm3, unmanaged pine individuals after 3 years of disease infection; unm5, unmanaged pine individuals after 5 years of disease infection.

In rhizosphere soil of infected Masson pine, bacterial diversity does not change with PWD or management practices. Fungal diversity, particularly saprotroph and pathogen diversity, has a stronger response to disease in rhizosphere than those in bulk soil. Fungal richness and Shannon diversity in rhizosphere soil of infected pine are significantly lower than those of healthy pine (Figure 2b). After PWD along with unmanaged practice, no significant variation in microbial diversity is found in either bulk soil or rhizosphere soil of infected Masson pine.

3.3. Variations in Microbial Community Composition

Bacterial community composition in bulk soil significantly varies with PWD-infected years, while in rhizosphere soil of Masson pine, it is mainly affected by management practices (logging vs. unmanaged) (Figure 3). Bacterial taxonomic groups show stronger responses to management practice in bulk soil than in rhizosphere soil. In forest bulk soil, *Acidobacteriota* becomes more abundant and some other bacterial phyla (including *Verrucomicrobiota*, *Chloroflexi*, and *Planctomycetota*) are less abundant in the infected plot (Slog5) (Figure 4a). In rhizosphere soil of Masson pine, only *Planctomycetota* tends to be decreased by PWD (Slog3) (Figure 4b).

Fungal community compositions in both bulk and rhizosphere soil have a significant response to PWD and management practice (Figure 3). Fungal taxonomic groups also have a small response to PWD along with logging in Taishun County, with less abundant *Ascomycota* and more abundant *Basidiomycota* in rhizosphere of infected pine than that of healthy pine (Figure 4c,d). Fungal taxonomic groups do not vary with unmanaged treatment. Only the relative abundance of *Planctomycetota* has a small decrease in the rhizosphere of infected pine (Slog3) (Figure 4d).

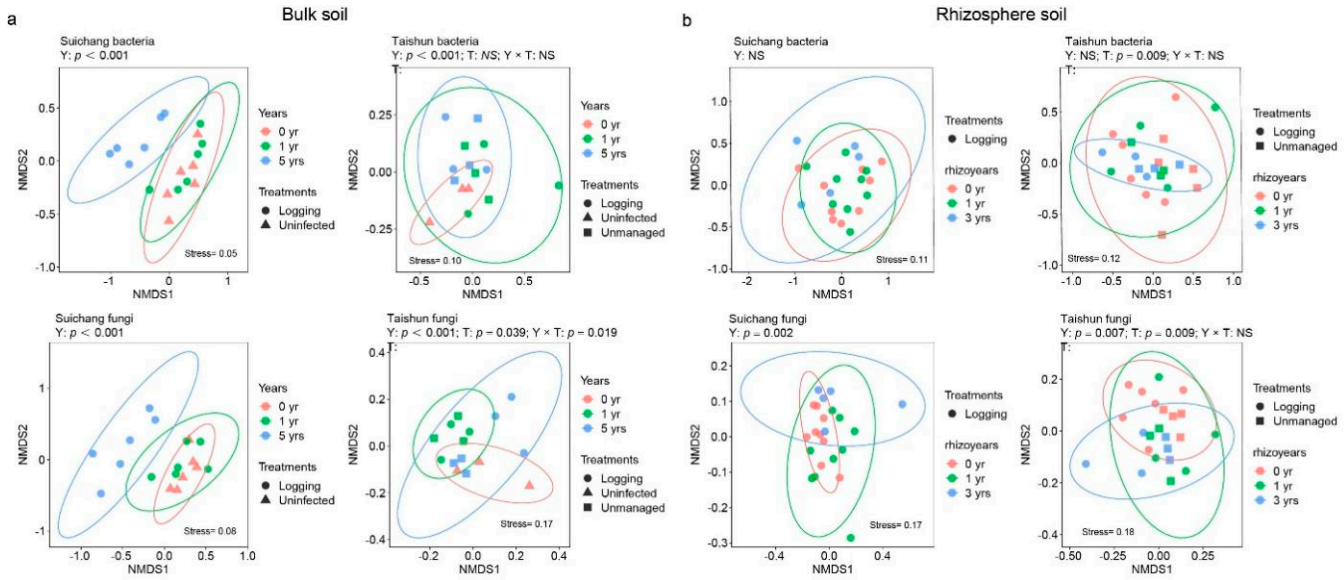

Figure 3. Non-metric multidimensional scaling (NMDS) analysis on soil microorganisms. The statistical results are of PERMANOVA, using the Adonis function and two-way ANOVA testing for effects of years and treatments on the communities of soil microorganisms. NS, $p > 0.05$, no significant difference. S, forest plots in Suichang County; T, forest plots in Taishun County; 0, uninfected plot or pine individuals; log1, logged plot or pine individuals after 1 year of disease infection; log5, logged plot after 5 years of disease infection; log3, logged pine individuals after 3 years of disease infection; unm1, unmanaged pine individuals after 1 year of disease infection; unm3, unmanaged pine individuals after 3 years of disease infection; unm5, unmanaged pine individuals after 5 years of disease infection.

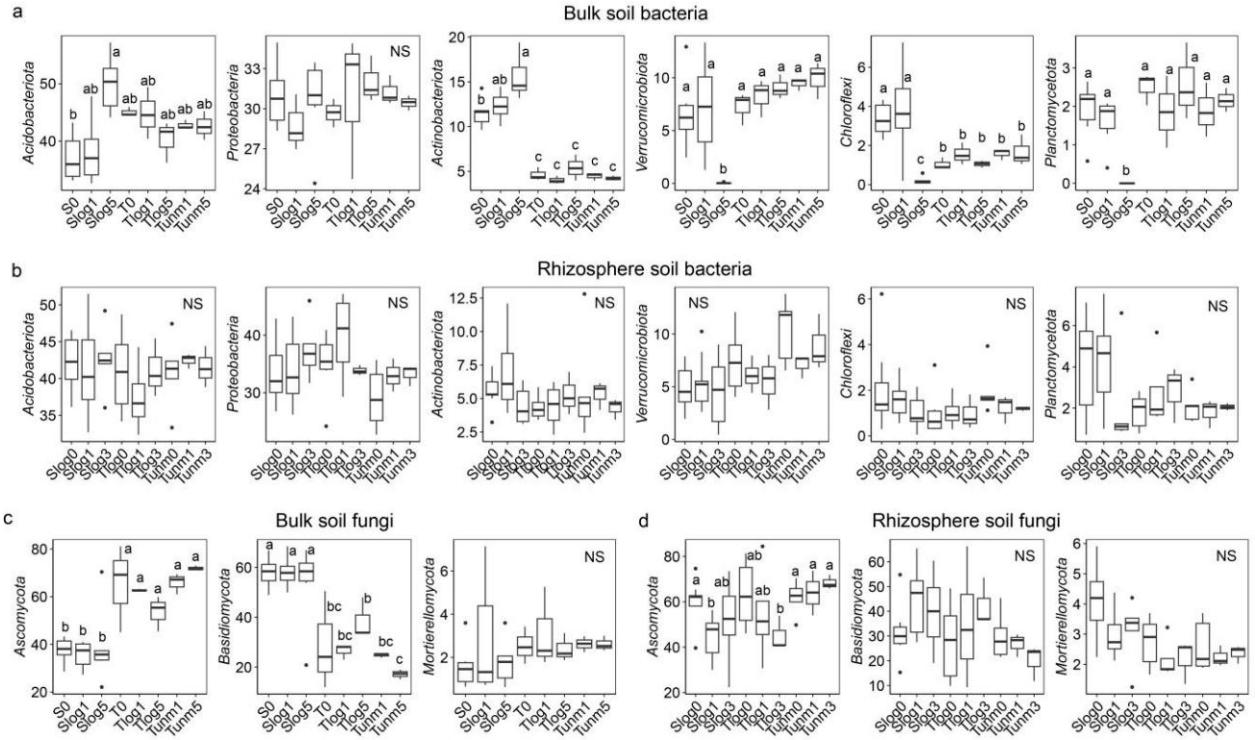

Figure 4. Relative abundance of the major phyla of soil microorganisms. Letters represent difference among infected years and treatments at the significance level ($p < 0.05$). NS represents no significant difference among infected years or treatments. S, forest plots in Suichang County; T, forest plots in Taishun County; 0, uninfected plot or pine individuals; log1, logged plot or pine individuals after 1 year

of disease infection; log5, logged plot after 5 years of disease infection; log3, logged pine individuals after 3 years of disease infection; unm1, unmanaged pine individuals after 1 year of disease infection; unm3, unmanaged pine individuals after 3 years of disease infection; unm5, unmanaged pine individuals after 5 years of disease infection. Outliers are represented by dots.

3.4. Variations in Microbial Community Functional Groups

Bacterial functional groups of amino acid, carbohydrate, and lipid metabolism tend to be more abundant after 5 year PWD along with logging (Slog5) (Figure 5a,b). Fungal guilds in rhizosphere soil of Masson pine have a strong response to PWD along with unmanaged practice (Tunm3). Specifically, the relative abundance of ECM fungi is lower while the relative abundance of saprotrophic fungi is higher under Tunm3 compared with uninfected plots (Figure 5d). There is no significant variation in fungal trophic groups in bulk soil or under logging treatment (Figure 5c,d).

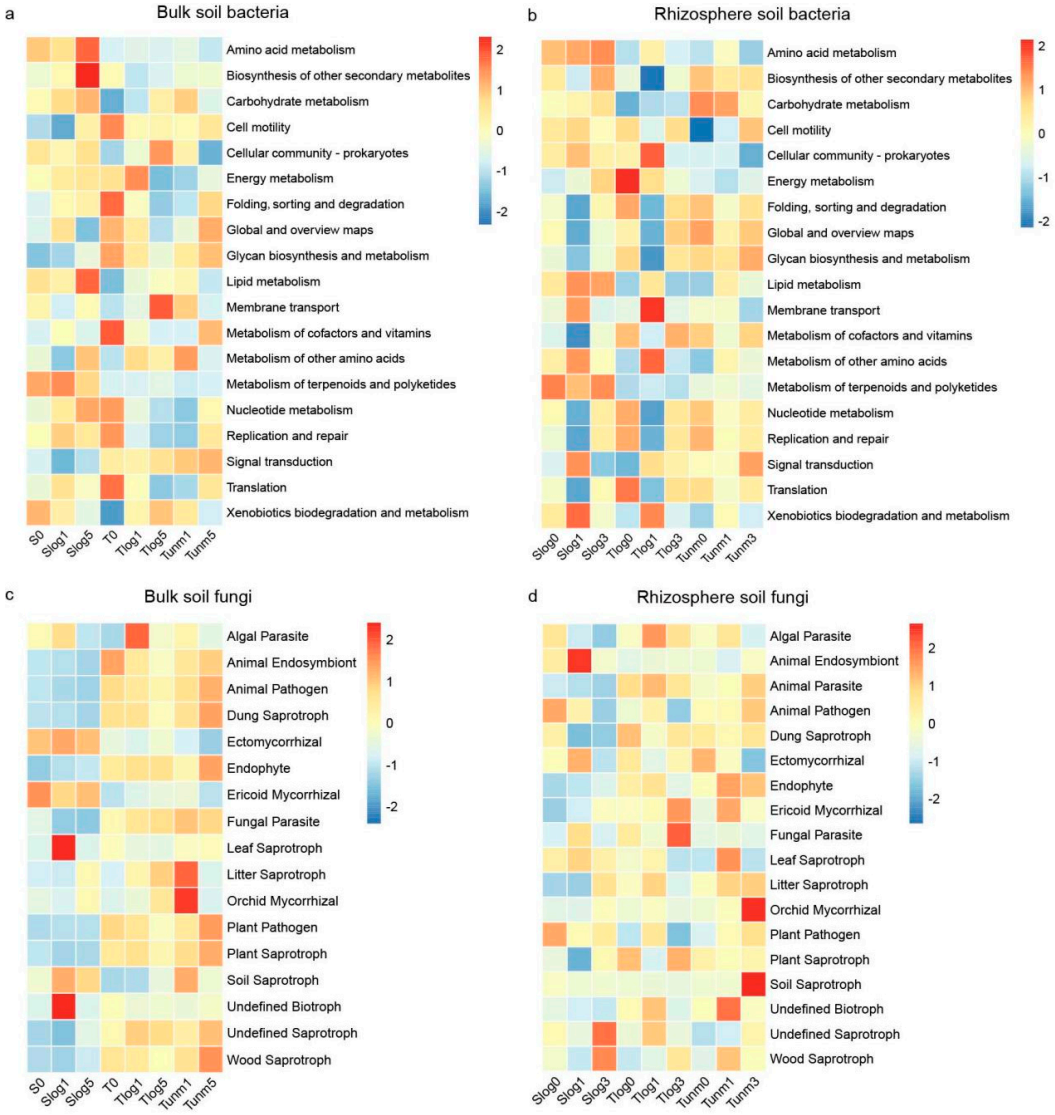

Figure 5. Relative abundance of the functional groups of soil microorganisms. S, forest plots in Suichang County; T, forest plots in Taishun County; 0, uninfected plot or pine individuals; log1, logged plot or pine individuals after 1 year of disease infection; log5, logged plot after 5 years of disease infection; log3, logged pine individuals after 3 years of disease infection; unm1, unmanaged pine individuals after 1 year of disease infection; unm3, unmanaged pine individuals after 3 years of disease infection; unm5, unmanaged pine individuals after 5 years of disease infection.

4. Discussion

4.1. Decrease in Soil Bacterial and Fungal Diversity to PWD

In Suichang County in this study, soil microbial communities show clear responses to PWD along with management practice. Microbial diversity in bulk soil is decreased after 5 years PWD along with logging (Figures 2 and S1), and this may be due to the reduction in the carbon pool [24] or variations in other abiotic conditions near surface soil after trees logging [43,44]. This finding is surprising because significant variation in microbial diversity has only been demonstrated after long-history disturbance [8,19,21,45]. Soil microbial diversity can be also affected by woody plant composition [46,47]. After removing the effect of woody plant composition, only fungal diversity maintains significant changes with the disease (Table S3). This result supports the findings of some other studies that fungi are responsive to management practices due to the strong association between plants and mycorrhizal fungi, whereas bacteria are relatively impervious [8,12,17,21]. As such, PWD and logging are likely to have a great effect on soil microbial communities and forest ecosystem functioning.

Fungal alpha diversity and the relative abundance of *Ascomycota* in bulk soil are increased at the early stage of PWD (Figures 2 and 4). These results are inconsistent with fungal variations during long-term succession from coniferous to broad-leaved forest caused by disease [8]. Therefore, time scale should be taken into consideration during forest disturbance research. In rhizosphere soil of Masson pine, alpha diversity of fungi rather than bacteria is decreased with disease infection (Figure 2b), and this can be matched with the strong sensitivity of fungal taxonomic composition to the disease. Similarly, Deng et al. [28] demonstrated that bacterial diversity did not change at the early stage of infected pines, and it only reduced at the late stages of disease. Our results reveal stronger effects of PWD and management practice on fungi than bacteria around pine roots. The composition of fungal communities may be determined by the different types of carbon substrates that exist in root exudates [48,49]. However, there are still some other opinions. For example, Ma et al. [50] only showed different microbial community structure in the main roots, and not in the surrounding soil, of healthy and diseased trees. The lower microbial diversity of the PWD-infected plot in this study mainly appears in Suichang but not Taishun County (Figures 2 and S1). Therefore, our results reveal that PWD along with management practice has negative impacts on microbial diversity, and the degree of impact is varied in different sites.

4.2. Response of Microbial Functional Groups to PWD

PICRUSt2 was used to predict bacterial gene abundance within metabolic pathways. PWD along with logging (Slog5) has a positive effect on bacterial functional groups of amino acid, carbohydrate, and lipid metabolism, and this functional response is consistent with the variation in taxonomic groups in bulk soil (Figures 4a and 5a), supporting the sensitivity of bacterial species to forest disturbance and associated environmental change [51]. Previous work concluded that bacterial metabolic function was mainly associated with carbohydrate metabolism for resource acquisition in infertile soil [52]. Forest disease along with logging may reduce soil nutrient availability, causing the increase in functional groups of carbohydrate metabolism.

Logging and timber harvest in pine forest has been shown to negatively affect soil ectomycorrhizal (ECM) fungi, and the dominance of fungal guilds shifted from ECM to saprotrophic fungi [19,25]. Based on the functional prediction of fungal guilds by FUNGuilds (http://www.funguild.org/, accessed on 24 August 2022) in this study, PWD decreases the relative abundance of ectomycorrhizal fungi, while it increases the relative abundance of saprotrophic fungi in rhizosphere soil of Masson pine under unmanaged treatment (Figure 5d). This result can be explained by the fact that ECM fungi are symbiotic organisms, and tree decline causes lower investment of carbohydrates in maintaining mycorrhizal associations [20]. A previous study found that removal of woody debris eliminated the habitat for many saprobic fungi [25]. In this study, although pine individuals

were dead and even logged, the growth of saprobes was not limited since the root system and associated organic substrate were kept belowground rather than removed from the plot. Our observations in pine forest soil, especially in rhizosphere soil (Figure 5d), imply that PWD has a negative effect on ECM fungi but a positive effect on saprotrophic fungi, which is probably due to the elimination of the energy source of symbiotic organisms after PWD infection.

4.3. Comparison between Logging and Unmanaged Practice

According to results in Taishun County, microbial diversity and functional groups tend to be affected by logging rather than unmanaged practice (Figures 2 and 5). This might be due to the decrease in canopy closure after tree logging, as forest gap can influence microbial communities via altered temperature, moisture, and nutrient availability in soil [53]. Logging and removing infected pines has a slight, negative effect on bacterial Shannon diversity in bulk soil and fungal Shannon diversity in Masson pine rhizosphere (Figure 2), and these patterns are not in line with microbial benefit from recalcitrant woody debris in another study [20]. Logging treatment tends to positively affect bacterial functional groups involved in metabolism, while unmanaged practice tends to affect fungal guilds in pine rhizosphere after disease (Figure 5a,d). No significant variation in microbial taxonomic groups is found in the PWD-infected forest of Taishun County (Figure 4). These results indicate that the responses of the microbial community to forest disturbance may differ at taxonomic and functional level because of microbial functional redundancy [45].

5. Conclusions

In this study, we demonstrate the negative responses of soil microbial diversity to pine wilt disease (PWD) along with logging treatment. In Masson pine rhizosphere, the community composition and alpha diversity of fungi, rather than bacteria, is significantly affected by the disease. In addition, PWD and management practice tend to affect microbial functional groups. With disease infection, bacterial functional groups involved in carbohydrate and amino acid metabolism increased; meanwhile, the dominance of fungal guilds shifts from ectomycorrhizal to saprotrophic fungi. Our results also suggest that logging treatment has a stronger impact on microbial communities than unmanaged practice. Our findings indicate the decrease in microbial diversity and variation in microbial potential functions following PWD and logging treatment. Therefore, the short-term effects of management practices on soil microorganism after PWD infection should be taken into consideration during forest management and the study of ecosystem functioning.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/f14050862/s1, Figure S1: Alpha diversity of trophic groups of soil fungi; Table S1: Relative abundance of bacterial phylum taxa in all samples of bulk soil and Masson pine rhizosphere soil; Table S2: Relative abundance of fungal phylum taxa in all samples of bulk soil and Masson pine rhizosphere soil; Table S3: Analysis of covariance on microbial alpha diversity in bulk soil, with PWD-infected years and management treatments (logging vs unmanaged) as fixed factor, and NMDS axis of woody plant community (NMDS1 and NMDS2) as covariate.

Author Contributions: Conceptualization, J.G., X.H. and M.Y.; methodology, J.G.; formal analysis, J.G.; investigation, J.G., B.W., L.C. and J.L.; resources, X.G., S.Y., X.H. and M.Y.; data curation, J.G. and L.C.; writing—original draft preparation, J.G.; writing—review and editing, J.G., X.G., S.Y., B.W., J.L., X.H. and M.Y.; visualization, J.G.; supervision, M.Y.; project administration, M.Y.; funding acquisition, M.Y., J.G. and J.L. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: The raw sequences of bacteria and fungi were submitted to the NCBI-SRA and are available under the accession number PRJNA940271 and PRJNA940305, respectively.

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