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Divergent Effects of Fertilizer Regimes on Taxonomic and Functional Compositions of Rhizosphere Bacteria and Fungi in *Phoebe bournei* Young Plantations Are Associated with Root Exudates

Zhong Luo [1,†], Xisha Yang [1,†], Jing Li [1], Shizhi Wen [1], Lili Yang [1], Li Ji [1,*] and Gongxiu He [2,3,*]

[1] School of Forestry, Central South University of Forestry and Technology, Changsha 410004, China
[2] Department of Soil and Water Conservation, Central South University of Forestry and Technology, Changsha 410004, China
[3] Key Laboratory of Soil and Water Conservation and Desertification Combating of Hunan Province, Changsha 410004, China
* Correspondence: jl917@csuft.edu.cn (L.J.); hegongxiu@csuft.edu.cn (G.H.); Tel.: +86-130-8681-2452 (L.J.)
† These authors contributed equally to this work.

Abstract: Fertilization is widely acknowledged as being an essential practice to improve forest productivity in forest ecosystems. However, too little consideration has been given to the taxonomic and functional compositions of rhizosphere soil microbes and their interactions with root exudates under different fertilizer regimes in forest plantations. Here, we investigated the effects of four typical fertilizer regimes (CK, no fertilizer; CF, compound fertilizer; OF, organic fertilizer; CMF, compound microbial fertilizer) on soil microbial communities and their potential functional groups in *Phoebe bournei* young plantations, as well as their associations with soil physicochemical properties and root exudates. These results showed that fertilizer regimes strikingly affected the rhizosphere soil microbial community compositions and alpha diversity indices. The pathotroph was the dominant fungal guild. With the applications of three fertilizations, the relative abundances of the plant pathogen and arbuscular mycorrhiza increased. The alpha diversity of soil bacteria was highest under the OF regime, and soil fungal diversity was more powerfully affected by the amendment of CMF. Additionally, while the fungal community was simultaneously influenced by soil physiochemical factors and root exudates, the bacterial community in the rhizosphere was mostly impacted by root exudates. More importantly, the application of OF and CF induced dramatic growths of *Fusarium*, while CMF treatment including *Bacillus* suppressed the development of *Fusarium* via adjusting bacterial species. Overall, our findings exhibit the divergent responses of rhizosphere bacteria and fungi to fertilizer regimes in *P. bournei* young plantations. The application of organic fertilizer provides benefits for rhizosphere bacteria, and microbial fertilizer can help alleviate inhibition through changing pathogens.

Keywords: organic fertilizer; compound microbial fertilizer; *Phoebe bournei* plantation; rhizosphere microbiome; *Bacillus*

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1. Introduction

In light of the increasing concerns about wood demands, fertilization has become one of the most constructive methods of boosting soil fertility and forest productivity [1,2]. Despite its capacity to effectively fulfill plant growth requirements [3], chemical fertilization is perceptibly contributing to environmental concerns (e.g., soil acidification [4] and water pollution [5]). Noteworthy is the fact that the application of organic fertilization improves soil nutrient status, aggregated stability, microbial metabolism, and co-operation between microbial communities and trees [6–9]. Additionally, due to environmental concerns and operating costs, researchers are currently evaluating the merits of substituting them

with bio-fertilizer in soil nutrient enrichment to promote a healthy and balanced soil microbial ecosystem [10,11]. Therefore, unraveling the microbial community composition and diversity under different fertilizer regimes is essential for the sustainable intensification strategies of forests.

Microorganisms in the soil play an important role in forest ecosystem functions, including soil productivity, nutrient cycling, decay of organic matter, and interaction with the host [12,13]. Generally, the amendment of organic fertilizers maintains [6] or improves [14] soil microbial diversity compared to without fertilization. The results contradict the application of chemical fertilizers on soil microbial diversity, which has either negative [7] or positive effects [15]. Growing evidence indicates that the soil microbial community differs noticeably under various fertilization regimes [16–18]. In general, bacteria are more responsive to environments with abundant nutrients and high C availability, whereas fungi seem to be more responsible for using sources of recalcitrant carbon [19–21]. Prior studies have demonstrated the contrasting results of organic and inorganic fertilization on varied soil microbial biomass [22,23]. The addition of organic fertilization did not change the soil microbial biomass, but it increased the soil organic carbon content and C/N ratio [24,25], and root exudates and plant growth were greatly promoted [26]. Several studies revealed that fungal communities are more susceptible to fertilization regimes than bacterial populations [16,27]. Conversely, Lazcano et al. [17] proposed that bacterial growth is particularly sensitive to fertilizer type, whereas fungal growth was influenced by the amount of fertilization. Pan et al. [18] suggested that in fluvo-aquic soils, organic and inorganic fertilizers predominate in altering bacterial and fungal community attributes, respectively. Nevertheless, there has been little discussion about the functional groups of soil microbiota depending on different fertilizer regimes. Li et al. [28] revealed that mineral fertilization strikingly affects the soil microbial functional and taxonomic structures. The revolutionized development of next-generation sequencing approaches and annotation tools provide new avenues for the functional composition of microbes [29–31]. Thus, disentangling the variation of functional groups of soil microbes under different fertilizer regimes can contribute to understanding potential mechanisms of boosting forest production.

Root exudates consist of a wide spectrum of carbon-containing metabolites [32]. They serve as carbon investments for the host plant as well as substrates, signaling molecules, and the driving forces behind intricate biogeochemical interactions between the microorganisms and the host plant [32,33]. In addition, root exudates can assist in resisting pests and maintaining the stability of the plant–soil–microbial ecosystem [34]. Numerous studies have demonstrated that plants can select and recruit inhabited/colonized micro-organisms by secreting unique root exudates [35–37]. Antibacterial activities of organic acids and amino acids from *Arabidopsis thaliana* root exudates have been demonstrated against soil bacteria and fungi, reducing *Arabidopsis thaliana* disease outbreaks [38]. Plants and nitrogen-fixing bacteria may interact symbiotically or neutrally, depending on soil nitrogen levels [39,40]. However, the effects of various fertilizer regimes on the soil microbial community and volatile organic compounds in root exudates in forest ecosystems have not received much attention.

Phoebe bournei is a large evergreen tree and precious timber species with high ornamental and economic values [41]. With high demand in the market and the over-harvesting of wild *P. bournei*, the available wild resources are becoming exhausted, which is becoming a major problem for sustainable *P. bournei* production [41,42]. Thus, the high-quality cultivation of *P. bournei* plantations is a matter of great urgency. In this study, we investigated the interactions between soil microbes and root exudates in a *P. bournei* young plantation, as well as the response of rhizosphere soil microbial communities to various fertilizer regimes, by high-throughput sequencing. The overall aim of this study was to elucidate the taxonomic and functional responses of the microbial community composition to fertilization and propose the optimal fertilizer regime during the early developmental stage of *P. bournei* forests. We hypothesized that: (1) Soil bacteria and fungi will exhibit divergent responses to different fertilizer regimes. Specifically, bacteria are more likely to be influenced by organic fertilizers, while fungi tend to be more sensitive to chemical fertilizer. (2) Except

for soil physiochemical variables, root exudates will have a strong association with soil bacterial and fungal communities. (3) Fertilization will strikingly change the potential functional groups of soil microbial community, and the functional compositions of soil microbes will exhibit more connections with soil properties and root exudates as compared with taxonomic composition.

2. Materials and Methods

2.1. Site Description and Sampling Design

The experiment was carried out at the Jindong Forest Farm in Hunan Province, China (112°04′30″E, 26°18′30″N). This area experiences a humid subtropical monsoon climate with average annual precipitation of 1745 mm and temperatures of 18.0 °C, with a 260–344 days frost-free period. The two extremes are 40 °C (in July) and 8 °C (in January), respectively. The forestry bureau's census reported that Jindong Forest Farm had 1557 plant species and an approximate 86% forest cover. The vegetation is mainly composed of *Phoebe bournei*, *Cunninghamia lanceolata*, *Ginkgo biloba* L., *Pinus massoniana*, *Taxus chinensis*, *Bretschneidera sinensis*, *Cinnamonum camphora*, *Pseudotsuga menziesii*, *Ormosia henryi Prain*, *Cephalotaxus oliveri*, and *Ilex chinensis* Sims. The soil type of this study area is mainly Acrisol.

In the spring of 2013, *P. bournei* seedlings that were five years old were planted with a planting density of 2.5 m × 2.5 m to establish a monoculture plantation. In August 2018, three replicated plots of *P. bournei* young plantations were established for each fertilization regime, including: (1) without fertilizer (CK); (2) chemical fertilizer (CF), application of 400 g N-P-K compound fertilizer (N:P_2O_5:K_2O = 15:15:15) per tree; (3) organic fertilizer (OF), which included organic matter 46%, 2.1% of N, 1.7% of P_2O_5 and 1.6% of K_2O; and 4) compound microbial fertilizers (CMF), an amendment of 20 g (*Bacillus* more than 2×10^{10} per gram fertilizer) per tree. The three typical fertilizers were provided from the forest farm. Fertilization was conducted in September 2018. The artificial ring application method was used to evenly distribute all nutrients in each plot into the 0–10 cm layer of soil after being precisely weighed.

2.2. Soil and Root Sampling

In March 2019, after 180 days of amendment of fertilization, eight trees of *P. bournei* were randomly selected for each plot. Empirically, it was considered that the soil attached to the *P. bournei* roots was rhizosphere soil, which was then shaken off the plant root systems. After removing surface litter, using a handheld auger, samples of rhizosphere soil and roots were taken from the 0–20 cm soil layer, which was close to the tree trunk (with a 1 m distance) using soil cores (approximately 10 cm in diameter). Notably, only live root samples were collected, and the dead roots were picked and discarded. To eliminate litter and root debris, the collected soil samples were sieved (2 mm mesh). Meanwhile, the root samples were flushed and rinsed using MilliQ water. We pooled and homogenized soil samples (or roots) from each plot. Immediately following the collection of the samples, these specimens were brought to the laboratory on ice. A soil sample was divided into two subsets: one for DNA extractions was stored at −80 °C, and another for soil physiochemical analyses was stored at 4 °C. All root samples were used for the profiling of root exudates.

2.3. Soil Physicochemical Properties Measurement

The pH of the soil–water suspension (1:5 *w/v*) was measured using a pH meter (PHS-3C, Leici, Shanghai, China) after shaking for 30 min. A UV spectrophotometer was used to measure the soil organic carbon (SOC) content based on potassium dichromate and sulfuric acidcolorimetry. An automatic discontinuous chemical analyzer was used to measure the total nitrogen (TN) in the soil. A UV spectrophotometer (TU-1901, Puxi Ltd., Beijing, China) was used to colorimetrically measure the total phosphorus (TP) content of soils after they had been wet-digested with $HClO_4$-H_2SO_4. A continuous flow analytical system was used to measure the amounts of available nitrogen (AN) and phosphorus (AP) in the soil (AA3, Seal Co., Norderstedt, Germany).

2.4. DNA Extraction and PCR Amplification

Using a MoBio PowerSoil DNA Isolation Kit (MoBio Laboratories Inc., Carsbad, CA, USA) in accordance with the manufacturer's protocols, genomic DNA was extracted from about 1 g (wet weight) of soil. DNA from the soil sample was extracted and measured using electrophoresis on 1% agarose gel. The primers ITS1-F and ITS2 (5′-CTTGGTCATTTAGAGG-AAGTAA-3′) were used to amplify the fungal universal ITS1 region (Zheng et al., 2015). The 16S rRNA gene from the universal V3-V4 region in bacteria was amplified using the primers 338F (5′-ACTCCTACGGGAGGCAGCAG-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′), respectively. The detailed PCR programs referred to the study of [43]. The original sequence information was submitted to the Sequence Read Archive (SRA) database at the National Center for Biotechnology Information (NCBI) under the BioProject ID: PRJNA889223.

Pooling, purification, and quantification of three PCR products were performed on each sample. Using an Illumina MiSeq platform, parallel-tagged sequencing was carried out. Subsequently, split readings were combined with FLASH V1.2.11 [44,45] and then sorted using QIIME (V1.7.0) according to its distinct barcodes [46].

After the screening, sequences with quality scores of less than 20 were initially removed from the raw data. These sequences included ambiguous bases, did not match primer sequences or barcode tags precisely, or both. Raw tags with less than 200 bp were removed using Mothur (V1.37.0) [47]. Chimeras were eliminated from the Gold and UNITE reference databases using USEARCH (V8.1.1861).

The UPARSE pipeline was used to aggregate high-quality sequences into operational taxonomic units (OTUs) with a 97% similarity [48]. To reduce the overprediction of rare OTUs, singletons that occurred just once were disregarded in subsequent studies. The Ribosomal Database Project (RDP) classifier was used to align and annotate the typical OTU sequences (V14). Quantitative diversity indicators (e.g., the observed species and Shannon diversity index) were calculated using QIIME (V1.7.0) [48].

2.5. Collection, Separation, Extraction, and Measurement of Root Exudates

The procedure for the collection of root exudates has been described by a previous study [49]. The roots were soaked in the newly made deionized water for 5 min and rinsed three times. The root samples were placed into a flask and 300 mL 0.5 mol·L^{-1} ethyl acetate was added and placed in the darkness for continuous ventilation for 24 h. After filtration and decompression, the concentrated liquid was root exudate.

The components of the root exudates were identified and analyzed using the Rtx-WAX elastic quartz capillary tube chromatographic column (30 m 0.35 mm 0.35 m; Restek Corporation, Bellefonte, PA, USA) and the Trace DSQ II gas chromatography–mass spectrometry (GC-MS) instrument. The working conditions of the GC–MS are as follows: the bombardment voltage was 70 eV, the scanning range was M/z1-600 amu, TR-50 ms column was used, and the injector port temperature and initial temperature of the cylinder were 250 °C and 25 °C, respectively. The increase in temperature was 20 °C per minute, and the temperature was sustained for 1 min. The temperature was maintained at 280 °C within 10 min. The flow rate and injection volume were 1 mL·min^{-1} and 1 µL, respectively, and helium was used as the carrier gas. The National Institute of Standards and Technology (NIST) mass spectrometry database was used to analyze the mass spectrometry and to determine the name and relative content of each component of root exudates.

2.6. Statistical Analysis

The Kolmogorov–Smirnov test was employed to confirm the normality of the data prior to any statistical analysis. One-way analysis of variance (ANOVA) was performed to examine how fertilizer regimens influenced the variety and composition of soil microorganisms using SPSS software (IBM SPSS Statistics, Chicago, IL, USA). Tukey's honestly significant test was employed to determine whether there was a difference in successional stages at the 0.05 level. Using the vegan package in R software, the principal coordinates analysis (PCoA) ordinations based on Bray–Curtis distance matrices were used to analyze

the soil bacterial and fungal community compositions (Version 4.0.5). Using the 'adonis' function of the 'vegan' package in the R program, an analysis of similarities (ANOSIM) was conducted to investigate the statistically significant differences in the community compositions under various fertilizer regimes (999 permutations). A Venn diagram produced by the 'VennDiagram' function in the R software was used to show the distribution of shared and unique OTUs among various fertilizer treatments. The partial Mantel test with 999 permutations was performed using the 'vegan' package in the R program to evaluate the primary drivers (root exudates and soil physiochemical parameters) that were significantly connected with the soil bacterial and fungal communities based on Spearman's correlation ($p < 0.05$). All data were shown with means and standard errors.

3. Results

3.1. Soil Properties and Root Exudates

Fertilization significantly changed SOC, TN, TP, TK, AN, and AP content. The application of the OF increased SOC content by 69.1% compared with CK (Table 1). In addition, soil TN, TP, TK, and AN contents under the CMF regime were markedly increased by 59.2%, 43.8%, 31.2%, 36.3%, and 31.0% compared to those in CK, respectively (Table 1). Notably, fertilization did not alter the soil pH in the *P. bournei* plantations.

Table 1. The soil physiochemical properties and root exudates under four fertilizer regimes.

Soil Variables		CK	CF	OF	CMF
Soil physiochemical property	pH	4.09 ± 0.01 a	4.04 ± 0.01 a	4.06 ± 0.03 a	4.06 ± 0.02 a
	SOC ($g \cdot kg^{-1}$)	12.52 ± 0.34 c	20.96 ± 0.39 ab	21.17 ± 0.54 a	18.88 ± 0.54 b
	TN ($g \cdot kg^{-1}$)	1.52 ± 0.01 b	1.87 ± 0.02 b	1.87 ± 0.02 b	2.42 ± 0.18 a
	TP ($g \cdot kg^{-1}$)	0.16 ± 0.01 c	0.21 ± 0.01 ab	0.20 ± 0.01 b	0.23 ± 0.01 a
	TK ($g \cdot kg^{-1}$)	6.50 ± 0.13 b	8.23 ± 0.03 a	8.23 ± 0.09 a	8.53 ± 0.11 a
	AN ($mg \cdot kg^{-1}$)	4.24 ± 0.08 c	5.62 ± 0.04 ab	5.51 ± 0.04 b	5.78 ± 0.06 a
	AP ($mg \cdot kg^{-1}$)	0.71 ± 0.01 c	0.89 ± 0.02 b	1.12 ± 0.04 a	0.93 ± 0.01 b
Root exudate	αCop (%)	4.85 ± 0.16 b	3.51 ± 0.09 c	6.74 ± 0.28 a	3.95 ± 0.06 c
	Cal (%)	22.60 ± 0.57 b	40.34 ± 2.50 a	29.45 ± 2.07 b	42.15 ± 1.66 a
	dLim (%)	5.41 ± 0.56 a	1.77 ± 0.13 bc	2.97 ± 0.15 b	0.97 ± 0.17 c
	αFen (%)	24.20 ± 0.03 a	11.53 ± 0.60 c	14.38 ± 0.06 b	11.07 ± 0.79 c
	Gua (%)	1.41 ± 0.14 c	3.72 ± 0.21 a	2.74 ± 0.15 b	4.10 ± 0.11 a
	αSan (%)	7.29 ± 0.82 b	14.24 ± 0.77 a	13.57 ± 1.04 a	13.63 ± 1.20 a
	Ses (%)	54.38 ± 3.85 b	74.84 ± 0.55 a	67.18 ± 1.84 a	76.29 ± 0.68 a
	Mon (%)	37.56 ± 2.98 a	15.38 ± 0.70 bc	22.06 ± 0.37 b	14.86 ± 0.46 c
	Fur (%)	——	0.57 ± 0.32 a	0.16 ± 0.16 a	0.50 ± 0.30 a
	Alc (%)	4.57 ± 0.85 a	6.08 ± 0.16 a	6.31 ± 0.59 a	6.77 ± 0.50 a
	Est (%)	3.40 ± 0.26 a	2.21 ± 0.55 a	4.29 ± 1.05 a	1.40 ± 0.75 a

CK, control; CF, compound fertilizer; OF, organic fertilizer; CMF, compound microbial fertilizer; SOC, soil organic carbon; TN, soil total nitrogen; TP, soil total phosphorus; TK, soil total potassium; AN, soil available nitrogen; AP, soil available phosphorus; αCop, α-Copaene; Cal, Calarene; dLim, D-Limonene; aFen, α-Fenchene; Gua, Guaiol; aSan, α-Santalene; Ses, sesquiterpenes; Mon, monoterpenes; Fur, furan; Alc, alcohols; Est, esters. Different lower letters indicate significant differences among the four fertilizer regimes ($p < 0.05$).

GC–MS analysis detected 53 volatile compounds in the root exudates collected from *P. bournei* plantations, including terpenes, alcohols, esters, and furans. Sesquiterpenes were the main components of the root exudates (ranging from 54.4% to 76.3%) (Table 1). CMF strikingly improved the relative content of sesquiterpene, but reduced the relative content of monoterpene. Calarene (ranging from 22.6% to 40.3%) and α-fenchene (ranging from 11.07% to 24.20%) were typical components under all fertilizer treatments, but their contents highly differed among the treatments (Table 1). Calarene and α-fenchene were the main sesquiterpenes and monoterpenes, respectively. The relative contents of calarene and guaiol increased significantly under CMF compared with CK, but the relative D-limonene and α-fenchene contents decreased significantly by 82.1% and 54.2%, respectively. Moreover,

CF significantly increased and delinked the relative content of α-santalene and α-copaene by 95.2% and 27.6%, respectively (Table 1).

3.2. Summary of Sequencing Information

Based on the Illumina MiSeq sequencing, after filtering reads by basal quality control, 594,750 soil bacterial and 858,864 fungal sequences were yielded across all soil samples. In total, 45,561–54,426 (mean = 49,562) bacterial and 64,744–74,360 (mean = 71,572) fungal sequences per sample were obtained. The average read length for 16S rRNA genes and ITS1 regions were 410 bp and 228 bp (>99% Good's coverage), respectively. As a result of 97% sequence similarity, rarefaction curves of OTUs tended to approach saturation (Figure S1), which suggested that the majority of the soil bacterial and fungal genes were recovered by the surveying effort.

3.3. Relative Abundance of Taxonomic and Functional Compositions of Soil Bacteria and Fungi

The high-quality sequences were assigned to 27 phyla and 71 classes of soil bacterial community, as well as 7 phyla and 27 classes of soil fungal community (Figure 1A–D). The soil bacterial community was predominated by Proteobacteria, Acidobacteria, and Chloroflexi, which accounted for over 70% of the bacterial sequences (Figure 1A). The relative abundance of Proteobacteria and Acidobacteria in three fertilizer regimes showed an increasing and decreasing trend compared with CK (Figure 1A). Four dominant fungal phyla were detected, including Ascomycota (28.7%–49.7%), unclassified_k_Fungi (16.6%–40.0%), Basidiomycota (16.9%–29.6%), and Zygomycota (6.1%–13.2%) (Figure 1C). The relative abundance of Ascomycota in OF treatment was 73.1% higher than that in CK (Figure 1C), and the relative abundance of Basidiomycota in CMF treatment declined by 42.8% compared with CK (Figure 1C). Fertilization significantly affected the relative abundance of dominant phyla and classes of soil fungi, not bacteria (Figure 1).

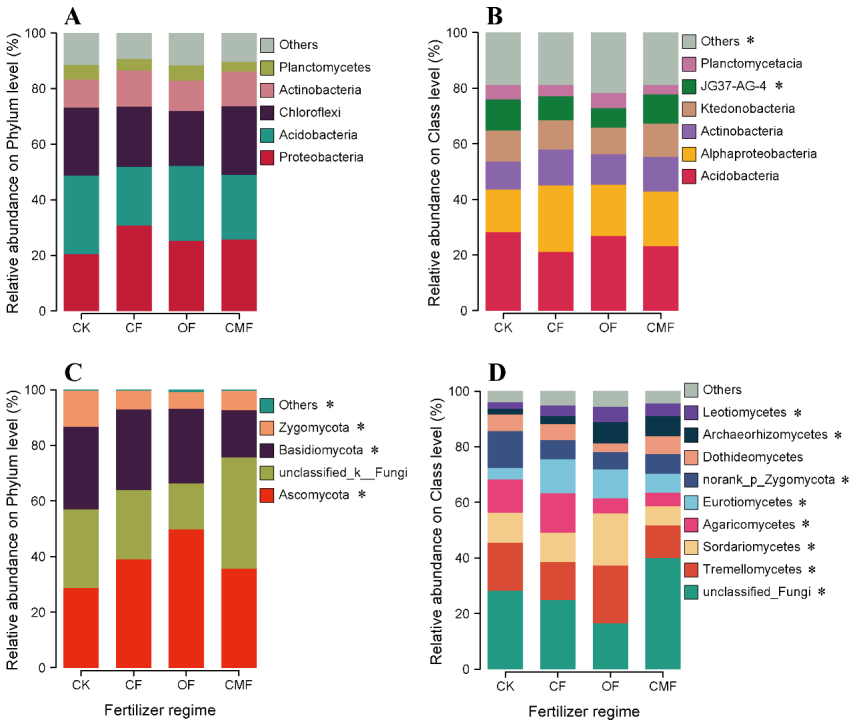

Figure 1. Relative abundances of dominant soil bacterial and fungal phyla (**A**,**C**) and classes (**B**,**D**) under four fertilizer regimes. CK, CF, OF, and CMF represent the control, compound fertilizer, organic fertilizer, and compound microbial fertilizer, respectively. Low-abundance phyla/classes with less than 0.5% of the total sequences across all samples are grouped into "Others". The asterisk indicates the significant differences among different fertilizer regimes.

The 49 functional groups from 2789 soil bacterial OTUs were assigned bacterial sequences related to C, N, and S cycling using the FRPROTAX database. The main ecological functions detected were chemoheterotrophy, aerobic_chemoheterotrophy, and cellulolysis, comprising over 80.8% of the bacterial sequences (Figure 2A). Notably, the relative abundance of cellulolysis showed an increasing and declining trend in CMF and CF (or OF) regimes, respectively (Figure 2A). Compared with CK, the relative abundance of nitrogen_fixation increased by 66.0%, 61.8%, and 10.8% in CF, OF, and CMF treatments, respectively (Figure 2A).

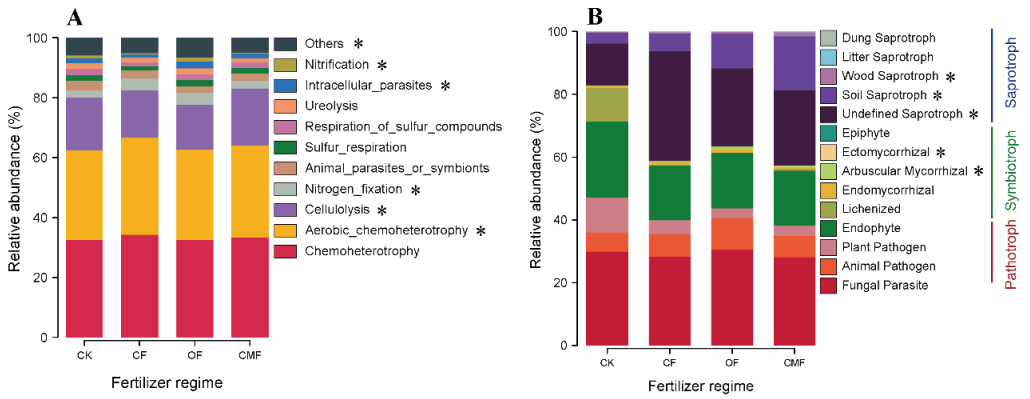

Figure 2. Relative abundances of dominant soil bacterial function (**A**) and fungal guilds (**B**) under four fertilizer regimes. CK, CF, OF, and CMF represent the control, compound fertilizer, organic fertilizer, and compound microbial fertilizer, respectively. The top 10 most abundant bacterial functions were identified and the rest of them were grouped into "Others". The asterisk indicates the significant differences among different fertilizer regimes.

The soil fungal community was assigned as trophic modes with pathotrophs, symbiotrophs, and saprotrophs using FUNGuild analyses (Figure 2B). Pathotrophs were the dominant fungal guild, accounting for approximately 42% of total sequences. Of these, the relative abundance of the plant pathogen and lichenized fungi significantly declined in three fertilizer regimes (lowest value in OF treatment) compared with CK, whereas the relative abundance of arbuscular mycorrhizal markedly increased after fertilization amendments (highest value in OF treatment) (Figure 2B). For saprotrophs, a higher proportion of undefined saprotrophs was observed in CF treatment (35.0%) in comparison to CK (13.3%) (Figure 2B). In contrast, under the CMF regime, the relative abundance of soil saprotroph, wood saprotroph, and litter saprotroph were 399.4%, 375.8, and 367.6% higher than those in CK treatment (Figure 2B). Additionally, after the amendment of CMF, the relative abundance of *Bacillus* significantly increased by 45.5%, 33.3%, and 128.6% compared to that in CK, CF, and OF, respectively (Table S1). The relative abundance of *Fusarium* showed the smallest value (0.06%) in CMF treatment (Figure S2).

3.4. Soil Microbial α and β Diversity under Different Fertilizer Regimes

Fertilization significantly affected the variation of three alpha diversity indices of soil bacteria and fungi. Compared with CK, the OF regime significantly improved the soil bacterial richness, Shannon, and Chao 1 indices by 11.5%, 4.8%, and 9.8%, respectively. For soil fungal community, richness, Shannon, and Chao 1 indices were highest in CMF treatment, which indicated the divergent responses of soil bacteria and fungi to OF and CMF regimes (Table 2).

Table 2. Richness, Shannon, and Chao 1 indices of the bacterial and fungal community under four fertilizer treatments.

Taxonomy	Parameter	CK	CF	OF	CMF
Bacteria	Richness	1589.33 ± 16.59 b	1646.33 ± 31.52 b	1772 ± 24.95 a	1605.33 ± 30.82 b
	Shannon	5.81 ± 0.02 b	5.88 ± 0.09 ab	6.09 ± 0.02 a	5.83 ± 0.04 b
	Chao 1	2002.65 ± 66.87 a	2084.03 ± 37.32 a	2198.76 ± 51.27 a	2065.84 ± 49.32 a
Fungi	Richness	851.33 ± 11.62 a	864.33 ± 73.95 a	819.67 ± 44.21 a	949.33 ± 12.25 a
	Shannon	4.28 ± 0.14 a	4.18 ± 0.26 a	3.99 ± 0.19 a	4.62 ± 0.03 a
	Chao 1	922.16 ± 15.06 a	944.2 ± 79.97 a	959.73 ± 53.92 a	1020.2 ± 18.92 a

CK, control; CF, compound fertilizer; OF, organic fertilizer; CMF, compound microbial fertilizer. Different lower letters indicate significant differences among the four fertilizer regimes ($p < 0.05$).

Principal coordinate analysis (PCoA) was used to assess differences in the bacterial and fungal community compositions of the soil under four different fertilizer regimes (Figure 3A,B). Both bacterial and fungal community compositions could be explained by more than 55% by the first two axes. Different fertilizer amendments caused distinct bacterial and fungal community separations in the PCoA plots. The result of ANOSIM showed that fertilization had a more profound effect on fungi (R^2 = 0.720) than bacteria (R^2 = 0.572) in the *P. bournei* plantations (Figure 3).

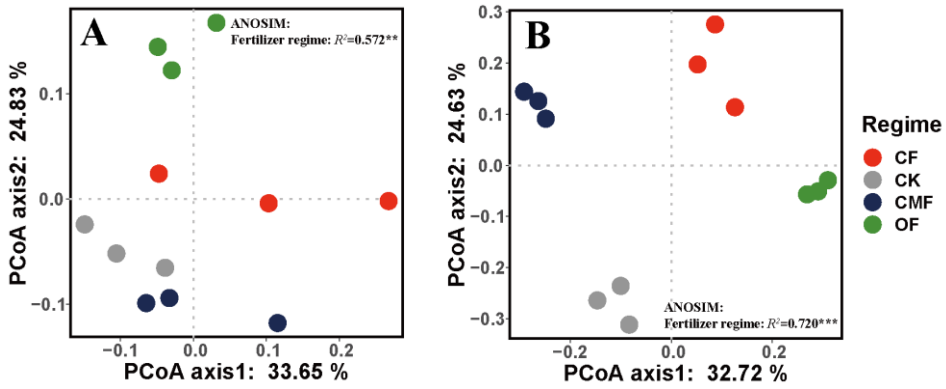

Figure 3. Principal coordinate analysis (PCoA) based on the Bray–Curtis dissimilarity matrices of bacterial (**A**) and fungal (**B**) communities. CK, control; CF, compound fertilizer; OF, organic fertilizer; CMF, compound microbial fertilizer. R^2 represents the variation in bacterial and fungal community composition that can be explained by the fertilizer regime, which was detected using ANOSIM. ** and *** denote the significant level at $p < 0.01$ and $p < 0.001$, respectively.

3.5. Shared and Unique OTUs

The shared and unique OTUs for different fertilizer regimes were assessed via a Venn diagram, which demonstrated that OTUs differed among the four regimes (Figure 4). The number of OTUs in the soil bacterial community shared was 1476 for the intersection among four fertilizer treatments, 1773 for that between CK and CF, 1788 for that between CK and OF, and 1792 for that between CK and CMF (Figure 4A). The trend of the number of unique OTUs was shown as: OF (135) > CF (86) > CMF (69) > CK (54). For the soil fungal community, there were 489 shared OTUs for intersection among four fertilizer regimes, 793 for that between CK and CF, 727 for that between CK and OF, and 868 for that between CK and CMF (Figure 4B).

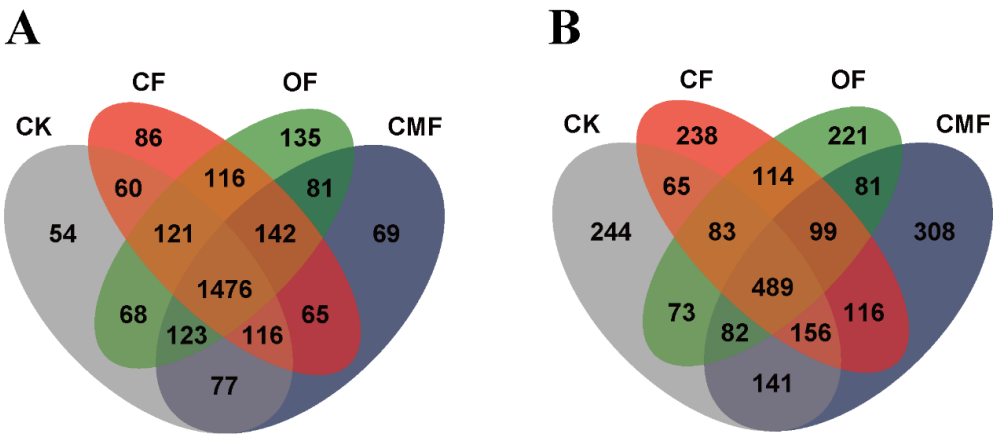

Figure 4. Venn diagram of the number of shared and unique OTUs under the different fertilization treatments in soil bacterial (**A**) and fungal communities (**B**). OTUs are defined at 97% sequence similarity. CK, control; CF, compound fertilizer; OF, organic fertilizer; CMF, compound microbial fertilizer.

3.6. Main Driving Factors of Taxonomic and Functional Compositions of Soil Microbial Community

Partial Mantel tests were performed to determine the distance-corrected differences between soil microbial community compositions and soil variables (Figure 5). Unexpectedly, root exudates (e.g., αCop, Cal, Fur.) had a strong effect on soil bacterial community composition instead of soil physiochemical properties (Figure 5). In contrast, soil fungal community composition was simultaneously driven by soil physiochemical properties and root exudates, and particularly affected by AP, αCop, and Gua (Figure 5).

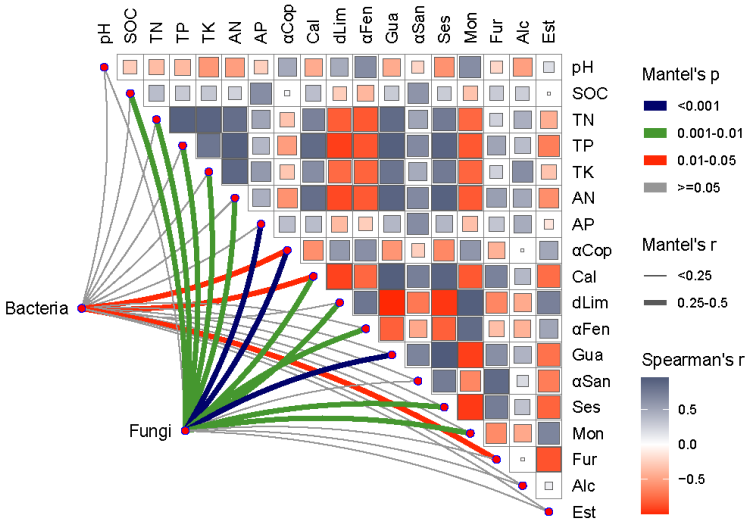

Figure 5. Soil physiochemical and root-exudate-related drivers of the soil bacterial and fungal community compositions. Soil microbial community composition was correlated to soil variables by partial Mantel tests based on the Bray–Curtis distance. Pairwise comparisons of soil physiochemical properties and root exudates are shown at the upper-right, with a color gradient representing Spearman's correlation coefficients. The edge width represents the partial Mantel's r statistic for the corresponding correlation, and the edge color denotes that significance was tested based on 999 permutations. SOC, soil organic carbon; TN, soil total nitrogen; TP, soil total phosphorus; TK, soil total potassium; AN, soil available nitrogen; AP, soil available phosphorus; αCop, α-Copaene; Cal, Calarene; dLim, D-Limonene; aFen, α-Fenchene; Gua, Guaiol; aSan, α-Santalene; Ses, sesquiterpenes; Mon, monoterpenes; Fur, furan; Alc, alcohols; Est, esters.

The drivers for the ecological function of bacterial and fungal functional guilds were further analyzed (Figure S3). Except for αCop, soil AP content strikingly affected the ecological functions of soil bacteria (Figure S3A). Although the relationship between the community composition of fungal guilds and soil variables exhibited a similar pattern to fungal community composition, there was a divergent response of three trophic modes to soil physiochemical properties and root exudates. It was observed that the saprotroph was primarily affected by the concentration of SOC and AP, whereas the symbiotroph was correlated with variations in αCop and Mon (Figure S3B).

4. Discussion

4.1. Soil Bacteria Were More Responsive to Organic Fertilizer, While Fungi Were More Sensitive to Compound Microbial Fertilizer

According to our results, our first hypothesis was partially supported; that is, soil bacteria and fungi were more responsive to organic and compound microbial fertilizers, respectively. This finding is consistent with the study of Pan et al. [18]. The alpha diversity of soil bacteria was highest under the OF regime, and soil fungal diversity was more powerfully affected by the amendment of CMF. This can be explained by soil microbes often exhibiting divergent substrate preferences [19]. In contrast, bacteria consume easily accessible carbon compounds, while fungi consume more complex ones [21]. In addition, the most abundant unique OTUs of soil bacteria and fungi were seen under the OF and CMF regimes, respectively. This result showed that to some extent, both bacterial and fungal richness in OF and CMF soils were significantly higher than those in CK.

Growing rapidly and adapting to diverse soil environments, Proteobacteria is abundant in nutrient-rich environments [50], due to its rapid response to unstable carbon and phosphorus nutrients [51,52]. A greater abundance of Proteobacteria was observed under the CF treatment, which indicated the use of chemical fertilizer enhances and benefits soil C storage, and the production of microbial mucilage and polysaccharides for stabilizing soil aggregates is also advantageous. [53]. This could also account for the growth-promoting of *P. bournei* followed by the CF. Nevertheless, the relative abundance of dominant bacterial phyla was not strikingly affected by the amendment of fertilization in this study. The variation of the responding taxa suggested that the amplitude of fungal communities following fertilization was significantly greater than that of bacterial communities, which is supported by the findings of Yao et al. [54]. As fungal communities have fewer species, they are more susceptible to fertilization than bacterial communities [55]. On the one hand, as a result of their larger size and higher cost of time, energy, and materials to grow, fungi have lower species diversity [56]. On the other hand, fungal communities have fewer redundant functions compared to bacterial communities [57], which implies that soil fungi are characterized by less resilience to environmental change in terms of maintaining both functional and species diversity [55]. Additionally, Ascomycota and Basidiomycota exhibited differential responses to fertilization regimes, which may be ascribed to their life strategies [58]. Taken together, our results validate that soil bacteria and fungi in *P. bournei* young plantations show a divergent pattern under different fertilizer regimes.

4.2. Root Exudates Had a Pronounced Association with Soil Microbial Community Than Soil Physiochemical Properties

Numerous studies have shown that soil physiochemical characteristics, particularly soil pH, organic carbon content, and nutrient levels, have a significant impact on the soil microbial community [48,59,60]. It is commonly acknowledged that soil pH affects the bacterial community structure and nutrient availability in the soil [61]. In our study, the addition of OF significantly improved the SOC content. Prior studies revealed that the use of OF can boost substrate availability, accelerate the cycle of organic matter, and release more soil organic carbon [62]. The amendment of compound microbial fertilizer can increase the soil enzyme activity and the number of micro-organisms, thereby promoting the decomposition of organic matter to release nutrients and some soluble substances in the soil, as well as improving the availability of nutrients [63].

Beneficial micro-organisms generate volatile organic compounds (VOCs) as a signal of interaction to encourage plant development and productivity [64]. However, a systematic study comparing volatile organic compound profiles for *P. bournei* roots exposed to different fertilizer regimes has not been reported. CF and CMF treatments reduced the relative content of α-copaene, which is a remote attractant of *Xyleborus glabratus*. A particular species of ambrosia beetle called *Xyleborus glabratus* transmits the symbiotic fungus that causes the lethal vascular disease known as laurel wilt in Lauraceae plants [65,66]. Therefore, the application of CMF and OF may reduce the risk of laurel wilt infection. Intriguingly, we detected an obvious effect of root exudates on soil microbial communities (supporting our second hypothesis). VOCs are considered to have an important contribution to soil fungistatic [67]. In this study, rhizosphere soil fungal community composition was strongly correlated with monoterpenes. Previous studies have shown that monoterpenes play crucial roles in the rhizosphere environment, where they can regulate the activities of microbes and the availability of nutrients [68]. We found that the soil bacterial community possessed significant correlations with calarene, and therefore speculated that the relative content of calarene can alter the structure of the soil microbial community and effectively regulate the soil nutrient cycles. Smolander et al. [69] revealed a strong relationship between terpenoids or phenolic compounds and the soil nutrient cycling in forest soils. We found that the volatile components in root exudates exhibited a profound effect on the fungal community compared with the bacterial community. Sesquiterpenes generally played a crucial role in plant capacity to defend themselves against harmful microbes [70]. Specifically, three fungal guilds had a significant correlation with sesquiterpenes. However, the volatile components in the root exudates may be affected by the fertilizer level, season, soil type, and other factors; thus, further research is required [71,72]. Overall, our results reveal that soil bacterial communities are mostly influenced by root exudates under different fertilization treatments, while soil fungi are jointly influenced by physiochemical factors and root exudates. These findings demonstrate that root exudates are more influential in affecting soil microbial communities than physiochemical properties under fertilization.

4.3. Functional Composition of Soil Bacteria and Fungi Exhibited Higher Resolution Information Than the Taxonomic Level

In the present work, following the amendment of three fertilizers, the relative abundance of pathogens significantly decreased, which suggests that fertilization improves the capacity of soil bacteria to suppress fungal pathogen growth. Similar results are reported in cropping systems [73,74]. A variety of crops and trees are susceptible to *Fusarium* wilt caused by soil-borne fungi called *Fusarium* [75,76]. Importantly, compared to CK, the relative abundance of *Fusarium* under CMF treatment was not significantly different, and the amendments of OF and CF tremendously promoted *Fusarium* growth, which implied that *Fusarium* growth was suppressed by changes in bacterial community assembly caused by compound microbial fertilizer application [74]. Raza et al. [77] revealed that *F. oxysporum* can be inhibited by several VOCs after the addition of organic fertilizer. Additionally, some secondary metabolites from Actinomycetes are antagonistic to pathogens [78]. Members of the genus *Nitrospira* are important participants in the biogeochemical cycle [79,80]. The relative abundance of *Nitrospira* decreased under CF and CMF, but increased under OF, which indicated the enhancement of nitrogen-cycling microbes in the *P. bournei* plantations and the effective promotion of this biogeochemical cycle. This result suggested that organic fertilizers could promote the N fixation pathways via assimilating nitrite to ammonium because *Nitrospira* is related to organic matter cycling and soil nitrification [81]. If excessive nitrification occurs, plants will be inhibited in absorption and utilization of the released NO_3^--N, thereby resulting in the loss of NO_3^--N [82]. In addition, we detected that fertilizer raised the relative abundance of nitrogen-fixing bacteria. Due to its ability to adapt to a variety of conditions, *Bradyrhizobium* is the predominant genus of nitrogen-fixing bacteria in soil ecosystems [83]. *Bacillus* is a phosphate-solubilizing bacteria, and numerous benefits can be derived from it in terms of plant growth and health [84]. In the present

study, *Bacillus* was more abundant in the CMF-amended soils, indicating that the fixed P in the soil minerals could be solubilized or transformed into the available P for plant uptake [85], which is consistent with the increased P content under the CMF regime. It has been reported that *Bacillus* produces specific VOCs (acetoin and 2, 3 butandiol) that suppress fungal pathogens and promote plant growth [86].

5. Conclusions

This study provides insights into the contrasting responses of soil bacterial and fungal and community compositions to different fertilization regimes in *P. bournei* young plantations (Figure 6). Firstly, our results highlighted the improvement of rhizosphere soil bacterial and fungal community structures upon the amendments of organic fertilizer and compound microbial fertilizer, respectively. Fungal community composition was more responsive to the amendment of fertilization than soil bacteria. Further, the functional composition of both bacteria and fungi under different fertilizer regimes showed a higher solution result than taxonomic composition. Fertilization promoted the proliferation of nitrogen-fixing bacteria and saprotroph, and compound microbial fertilizer significantly suppressed the growth of the pathogen. Except for the effect of soil physiochemical properties on fungi, root exudates had profound effects on rhizosphere microbial community compositions, especially functional compositions. Collectively, these findings strengthen our understanding of the optimal fertilizer regime in *P. bournei* young plantations. Future efforts to develop sustainable fertilization management must take into account the roles played by specific root exudates by micro-organisms at different phases of *P. bournei* plantation development.

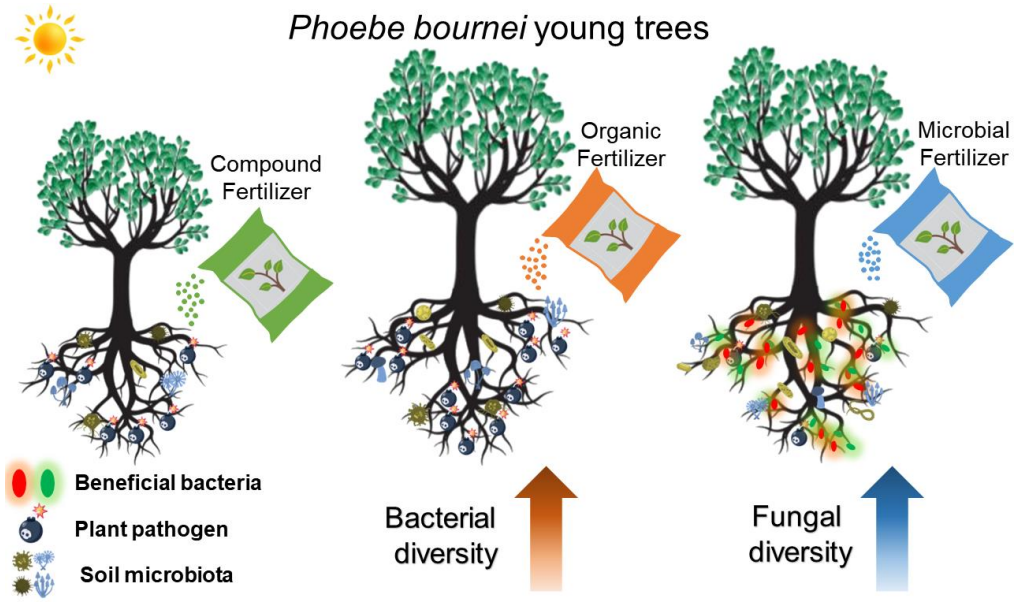

Figure 6. Conceptual diagram portraying the divergent strategy of rhizosphere bacteria and fungi under four typical fertilizer regimes.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/f14010126/s1, Figure S1: The rarefaction curves of the number of operational taxonomic units (OTUs) for soil bacterial (A) and fungal (B) communities under four fertilizer regimes; Figure S2: Relative abundance (%) of *Fusarium* under four fertilizer regimes. Different lower letters indicate the significant differences among four fertilizer regimes ($p < 0.05$); Figure S3: Soil physiochemical and root-exudate-related drivers of the ecological functions of soil bacteria and the fungal guilds. Both bacterial functions and fungal guilds were correlated to soil variables by partial Mantel tests based on the Bray–Curtis distance. Pairwise comparisons of soil physiochemical properties and root exudates are shown at the upper-right, with a color gradient representing Spearman's correlation coefficients. The edge width represents the partial Mantel's r statistic for the corresponding correlation, and the edge color denotes that significance was tested based on 999 permutations. SOC, soil organic carbon; TN, soil total nitrogen; TP, soil total phosphorus; TK, soil total potassium; AN, soil available nitrogen; AP, soil available phosphorus; αCop, α-Copaene; Cal, Calarene; dLim, D-Limonene; aFen, α-Fenchene; Gua, Guaiol; aSan, α-Santalene; Ses, sesquiterpenes; Mon, monoterpenes; Fur, furan; Alc, alcohols; Est, esters. Table S1: Relative abundance (%) of dominant genera of rhizosphere soil bacteria and fungi.

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