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Excluding Roots or Mycorrhizal Hyphae Alters the Microbial Community and Function by Decreasing Available C and N in a Subtropical Chinese Fir Forest

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Abstract: Carbon (C) inputs, primarily from roots and associated mycorrhizal hyphae, serve as crucial energy sources for microbial-driven C and nitrogen (N) cycling in the soil. However, our understanding of how soil microbial diversity, function, and associated soil properties respond to the exclusion of roots and their associated mycorrhizal hyphae remains limited. In our study, we conducted an experiment with no exclusion of roots or mycorrhizal hyphae (Control), exclusion of roots and retention of mycorrhizal hyphae (NR), and exclusion of roots and mycorrhizal hyphae (NRH) in a Chinese fir (*Cunninghamia lanceolata*) forest, the most important plantation in China. The soil properties, microbial community diversity and composition, and microbial function were investigated after 2 years of experiment exclusion. We found that exclusion of roots and hyphae significantly decreased DOC, DON, NH_4^+-N, and NO_3^--N, but not SOC, TN, and TP, indicating that the exclusion of roots and mycorrhizal hyphae mainly reduced available C and N concentrations. Meanwhile, the species richness and Chao1 of bacteria and fungi were significantly reduced, primarily due to the decrease in available C and N levels. These findings suggest that the removal of roots and mycorrhizal hyphae results in a decrease in C and N availability, subsequently leading to a loss of microbial diversity. Compared to after the CT treatment, the relative abundances of *Proteobacteria* and *Actinobacteria* phyla were reduced after exclusion of roots and hyphae. However, the relative abundances of the phyla *Acidobacteria*, *WPS2*, *Rozellomycota*, and *Glomeromycota* showed an increase in exclusion treatments. Furthermore, the relative abundances of genes for C degradation (e.g., *malQ*, *malZ*, *chi*, *rfbB*, *bglX*, and *ablA*), C fixation (e.g., *accA*, *icd*, *korA*, and *korB*), and N fixation (*nifS*) were increased; conversely, the N degradation genes (e.g., *nasA*, *nirB*, *ureC*, and *gdh2*) were decreased in treatments involving excluding roots and hyphae. These results, in conjunction with the strong relationships between functional genes and DOC, DON, NH_4^+-N, and NO_3^--N, suggest that microorganisms regulate functional genes to enhance C and N fixation or organic matter decomposition in response to C or N limitation resulting from root and mycorrhizal hypha exclusion. Collectively, our study revealed that the changes in roots-derived C directly altered available C and N in soil, which influenced the microbial community and function, and, in turn, regulated microbial-driven nutrient cycling in forest soils.

Keywords: forest soil; microbial diversity; mycorrhizal hypha; degradation gene; fixation gene

Citation: Lian, P.; Xu, L.; Yue, K.; Yang, L. Excluding Roots or Mycorrhizal Hyphae Alters the Microbial Community and Function by Decreasing Available C and N in a Subtropical Chinese Fir Forest. *Forests* **2023**, *14*, 1847. https://doi.org/10.3390/f14091847

Academic Editors: Yanlong Jia, Robert G. Qualls, Jūratė Aleinikovienė, Zhongqi Xu, Zhidong Zhang, Li Xu and Yue Pang

Received: 14 August 2023
Revised: 5 September 2023
Accepted: 8 September 2023
Published: 11 September 2023

1. Introduction

Soil microbes are predominantly responsible for governing soil biogeochemical processes, such as carbon (C) and nitrogen (N) cycling and their availability in terrestrial ecosystem soils [1–3]. However, the performance of microbial functions primarily depends on soil C availability [4], as most soil microbes are primarily limited by C or energy [5].

Compared to aboveground C input, such as that from leaf litter decomposition, and belowground C, particularly dissolved organic C (DOC) originating from plant root exudates is considered a more significant microbial source of C [1,6], which plays a fundamental role in sustaining microbial growth and activity [7]. Therefore, placing emphasis on studying the relationships between root-derived C and microbes is crucial for a mechanistic understanding of the changes in microbial functional traits and their impact on C and N cycling in soils.

Roots and mycorrhizal hyphae are recognized as the two main pathways through which plants transfer photosynthetic C inputs below the surface of the soil [8,9]. The exclusion of roots and mycorrhizal hyphae is anticipated to result in a decrease in available C in the soil, particularly dissolved organic carbon (DOC), as it originates from plant photosynthate [10]. However, the potential impacts of reduced available C on available N have received less attention; even the changes in photosynthetic C input can affect N release from soil organic matter decomposition by altering priming effects, thereby potentially affecting soil N availability [6,11]. In contrast to the availability of C or N, the influence of C input on SOC remains debated. On one hand, C derived from roots and mycorrhizal hyphae can be efficiently assimilated by microbes, thereby contributing to the accumulation of SOC [1,7]. On the other hand, plant-derived C input may also stimulate the decomposition of native SOC, which can be unfavorable for soil C sequestration [6,12]. This discrepancy highlights the uncertainty regarding the response of soil C and N to root and mycorrhizal hypha C input, and further research is needed to clarify this matter.

Microbial community diversity and composition, as the typical microbial traits, are primarily influenced by the availability of C or N in soil [13,14]. According to the stress gradient hypothesis, microbial diversity tends to be high in conditions of low C or N availability. This is because in resource-stressed environments, cooperation among species becomes more important than competition [13,15]. However, experimental studies and meta-analyses have found that low C or N availability is often associated with a decline in microbial biomass, leading to a subsequent decrease in microbial diversity [13,16,17]. These contrasting findings suggest that the mechanisms influencing microbial diversity are complex, and the critical factors may vary in different soils.

Elucidating specific microbial compositions in response to changes in soil C or N availability is challenging due to the tremendous complexity of microbial species in soils [12,18]. Previous studies have identified dominant bacterial phyla (e.g., *Acidobacteria*, *Proteobacteria*, and *Actinobacteria*), as well as fungal phyla (e.g., *Ascomycota*, *Mortierellomycota*, and *Rozellomycota*) in subtropical forest soils [14,19]. These microbial species exhibit different trophic strategies, which in turn influence their abundances in soils. For instance, *Actinobacteria* is a phylum of copiotrophic species that thrive in C-rich soils, while *Acidobacteria* is a phylum of oligotrophic species that are dominant in soils with low available C [12,20]. Consequently, the changes in available C resulting from the exclusion of roots and mycorrhizal hyphae are expected to alter the microbial composition in the soil.

Given the diverse capabilities of different microbial species in mediating C or N turnover in soils [21,22], changes in microbial diversity and composition have the potential to alter the function of microbially driven soil nutrient availability [22]. When microbial growth is limited by energy or nutrients, one possible strategy for microbes is to upregulate C or N degradation genes, thereby accelerating the decomposition of soil organic matter and releasing available C or N [23,24]. Additionally, certain microbial species with C or N fixation genes have the ability to obtain available C or N through biological C or N fixation [25,26]. Therefore, insights gained from changes in gene abundances can improve our understanding and prediction of microbial function in response to environmental changes. However, our knowledge regarding how and which functional genes respond to the exclusion of roots and mycorrhizal hyphae, as well as their key influencing factors in subtropical forest soils, is still far from complete.

As the most important plantation species in China, Chinese fir (*Cunninghamia lanceolata*) accounts for 6% of the global plantation area. It plays a crucial role in meeting the

growing demand for timber and serves as a carbon sink [27]. In this study, we selected a Chinese fir plantation forest and conducted an experiment that included three treatments: (1) no exclusion of roots or mycorrhizal hyphae (Control), (2) exclusion of roots only (NR), and (3) exclusion of both roots and mycorrhizal hyphae (NRH). The objectives were as follows: (1) to investigate the impact of root and/or mycorrhizal hypha exclusion on soil C and N availability, (2) to examine the response of microbial community diversity and function to root and/or mycorrhizal hypha exclusion, and (3) to identify the critical factors that regulate microbial community diversity and function following root and/or mycorrhizal hypha exclusion.

2. Materials and Methods

2.1. Study Site

The study site was located in the Sanming Forest Ecosystem and Global Change National Observatory and Research Station, Fujian Province, China (26°11′ N, 117°228′ E). This region experiences a mean annual temperature of 19.5 °C and an average annual precipitation of 1630 mm. The soil at the site is classified as an oxisol [28]. In 2019, we selected a 16-year-old Chinese fir plantation on which to conduct a root and mycorrhizal hypha exclusion experiment. The plantation had an average tree density of 2858 trees per hm^2, with a mean tree height of 18.2 ± 2.0 m and an average diameter at breast height of 25.6 ± 1.5 cm.

2.2. Experimental Design

In July 2019, four 15 m × 20 m plots were established in a Chinese fir forest. Each plot contained fifteen subplots (0.5 m × 0.5 m) without woody plants. A minimum 40 cm deep ditch was dug using a shovel in each subplot, and a mesh with different pore sizes was wrapped around it to isolate roots or mycorrhizal hyphae before backfilling it with soil. The experimental setup, following a random block design, consisted of (1) five subplots with retained roots and mycorrhizal hyphae (CT), where no nylon mesh was installed to allow for the growth of roots and mycelia; (2) five subplots with root exclusion but retention of mycorrhizal hyphae (NR), where a 45 μm mesh was installed to exclude roots but allow mycorrhizal hyphae to grow in the soil; (3) five subplots with exclusion of both roots and mycorrhizal hyphae (NRH), where a 1 μm mesh was installed to exclude both roots and mycorrhizal hyphae from the soil. It is important to note that this mesh also excluded saprophytic fungal hyphae. A monthly removal of litterfall was implemented to minimize the influence of litterfall-derived C on microbial community and functions.

2.3. Soil Sampling

Soil samples were collected from the 0–10 cm depth using a 2.5 cm diameter stainless-steel sampler in July 2021. After removing stones, roots, and plant and animal residues, the samples were sieved through a 2 mm mesh. The sieved soil samples were divided into three subsamples. One subsample was stored at −80 °C for microbial community composition analysis. Another subsample was air-dried for soil pH, SOC, total nitrogen (TN), available phosphorus (AP), and total phosphorus (TP) analysis. The third subsample was stored at −4 °C for soil moisture (SWC), NH_4^+-N, NO_3^--N, DOC, and dissolved organic nitrogen (DON) analysis.

2.4. Soil Analyses

SOC and TN were quantified using a Carbon and Nitrogen Element Analyzer (Elementar Vario MAX, Hanau, Germany) based on combustion. Soil pH was determined using a pH meter (STARTER 300, OHAUS, Parsippany, NJ, USA) in a 1:2.5 soil–water suspension. SWC was measured gravimetrically. DOC and DON were extracted with deionized water in a soil-to-water ratio of 1:4, and their concentrations were measured using a total organic carbon analyzer (TOC-VCPH/CPN, Shimadzu, Kyoto, Japan). Soil NH_4^+-N and NO_3^--N were extracted with 2 M KCl and measured using a Continuous

Flow Analytic System (SAN++; Skalar, Breda, The Netherlands). Soil AP was extracted with a solution of 0.025 mol/L HCl and 0.03 mol/L NH_4F, and TP was digested using H_2SO_4-$HClO_4$. Phosphate concentrations in the extraction and digestion solutions were detected using a Continuous Flow Analytic System (SAN++; Skalar, The Netherlands).

2.5. Soil DNA Extraction and PCR Amplification

Soil DNA extraction was performed using the Fast DNA® SPIN Kit (MP Bio-medicals, Southern California, Santa Ana, CA, USA) following the manufacturer's instructions. The concentration and purification of the extracted DNA were determined using a NanoDrop 2000 ultraviolet visible spectrophotometer (Thermo Scientific, Wilmington, NC, USA). The quality of the DNA was checked using agarose gel electrophoresis.

High-throughput sequencing techniques were used to detect the soil bacterial and fungal community at the Illumina NovaSeq platform of the MAGIGENE Company in Guangdong, China. For bacterial community analysis, the V4 hypervariable region of the 16S rRNA gene was amplified using the primer pair 515F (5′-GTGCCAGCMGCCGCGGTAA-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′). For fungal community analysis, the ITS2 region was amplified using the primer pair ITS5-1737F (5′-AACTTTYRRCAAYGGATCwct-3′) and ITS2-2043R (5′-TCCCTCCGCTTATTGATATGC-3′). The PCR reactions were performed in triplicate in a 50 μL reaction system. The amplification protocol involved an initial denaturation step at 94 °C for 5 min, followed by 30 cycles of denaturation at 94 °C for 30 s, annealing at the specific annealing temperature for 30 s, and extension at 72 °C for 30 s. A final extension step was carried out at 72 °C for 10 min. The PCR reaction system contained 25 μL of 2× Premix Taq, 1 μL of each primer (10 μM), 50 ng of template DNA, and nuclease-free water.

2.6. Bioinformatic Analysis

The raw sequencing data were processed using cutadapt (v1.14) and usearch _fastq mergepairs (v10.0.240, http://www.drive5.com/usearch/, accessed on 20 January 2023). FastQC (version 0.11.5) was employed to assess the quality of the paired-end Illumina reads. After quality filtering, a total of 2,090,480 high-quality 16S rRNA gene sequences and 2,110,826 high-quality ITS sequences were obtained. The minimum sequence count per sample was 84,069 (for 16S rRNA genes) and 84,004 (for ITS regions), respectively. These high-quality sequences will serve as a reliable foundation for our subsequent analyses.

Based on the OTU abundance table, the indices of alpha diversity (Richness, Chao 1, Simpson, Shannon) were calculated using the usearch-alpha_div (V10) software (http://www.drive5.com/usearch/, accessed on 15 February 2023). To assess the beta diversity of the microbial community, non-metric multidimensional scaling (NMDS) analysis was performed based on Bray–Curtis distance matrices. The taxonomic annotation of species was obtained using the usearch-sintax tool, which aligned the representative sequences of each OTU against the SILVA (16S), RDP (16S), Greengenes (16S), SILVA (18S), and Unite (ITS) databases with a confidence cutoff of 80%.

2.7. Statistical Analysis

The effects of excluding roots and mycorrhizal hyphae on soil properties, microbial community diversity and composition, and the relative abundances of functional genes were examined using One-Way ANOVA with the "tidyverse" package in R. Differences between treatments were assessed using the Least Significant Difference (LSD) test implemented in the "agricolae" package. The Mantel test, which was conducted with the "vegan" package, was used to evaluate the correlations between soil microbial community, functional genes, and soil properties. The relationships between dominant bacterial and fungal species, microbial functional genes, and soil properties were established using the "psych" package. A correlation heatmap was generated using the "ggplot2" package. All analyses were performed in R version 4.0.4.

Microbial functions associated with C and N cycling were predicted using PICRUSt2 (version 2.3.0-b). To account for the influence of the copy number of the 16S marker gene on the species genome, the OTU abundance table was standardized by PICRUSt. Gene function annotation was performed by aligning the sequencing raw reads against the Kyoto Encyclopedia of Genes and Genomes (KEGG) Module database. The specific information regarding C degradation, C fixation, N fixation, and N degradation genes, including their KO numbers, can be found in Table S2.

3. Results

3.1. Soil Properties

The exclusion of roots and hyphae did not significantly affect pH, SWC, SOC, TN, C/N, TP, or AP ($p > 0.05$), but significantly decreased DOC, NH_4^+-N, NO_3^--N, mineral N, and DON in soil ($p < 0.05$, Table 1). In addition, no statistical differences in DOC, NH_4^+-N, NO_3^--N, mineral N, and DON were observed between NR and NRH treatments ($p > 0.05$, Table 1).

Table 1. Influences of excluding roots and mycorrhizal hyphae on soil properties in a subtropical Chinese fir plantation forest soil. CT, no exclusion of roots or mycorrhizal hyphae; NR, exclusion of roots but retention of mycorrhizal hyphae; NRH, exclusion of roots and mycorrhizal hyphae. SOC, soil organic carbon; DOC, dissolved organic carbon; DON, dissolved organic nitrogen; TN, total nitrogen; AP, available phosphorus; TP, total phosphorus. Values are mean ± SE ($n = 4$). Different letters indicate significant differences at $\alpha = 0.05$.

Soil Properties	Treatment		
	CT	NR	NRH
pH	4.34 ± 0.05 a	4.41 ± 0.05 a	4.4 ± 0.04 ba
Moisture	0.25 ± 0.02 a	0.23 ± 0.03 a	0.24 ± 0.02 a
SOC ($g \cdot kg^{-1}$)	21.34 ± 2.06 a	17.89 ± 2.62 a	17.05 ± 4.83 a
DOC ($mg \cdot kg^{-1}$)	39.43 ± 10.71 a	29.03 ± 7.78 ab	25.53 ± 4.26 b
NH_4^+-N ($mg \cdot kg^{-1}$)	12.68 ± 4.51 a	6.27 ± 1.93 b	5.1 ± 0.88 b
NO_3^--N ($mg \cdot kg^{-1}$)	5.32 ± 0.92 a	4.45 ± 0.8 ab	3.37 ± 0.63 b
Mineral N ($mg \cdot kg^{-1}$)	17.99 ± 4.57 a	11.3 ± 0.87 b	9.2 ± 1.9 b
DON ($mg \cdot kg^{-1}$)	0.92 ± 0.37 a	0.7 ± 0.36 ab	0.37 ± 0.08 b
TN ($g \cdot kg^{-1}$)	1.41 ± 0.13 a	1.24 ± 0.14 a	1.2 ± 0.19 a
C/N	15.17 ± 0.6 a	14.41 ± 0.99 a	13.98 ± 2.09 a
AP ($mg \cdot kg^{-1}$)	2.48 ± 0.54 a	2 ± 0.86 a	2.04 ± 0.78 a
TP ($g \cdot kg^{-1}$)	0.15 ± 0.01 a	0.15 ± 0.03 a	0.14 ± 0.01 a

3.2. Microbial Diversity and Community Composition

The richness and Chao1 indices of bacteria and fungi were significantly lower in the exclusion treatments compared to the CT treatment ($p < 0.05$, Table 2). However, there were no significant differences in these indices between the NR and NRH treatments ($p > 0.05$, Table 2). Regarding bacterial diversity, the Simpson and Shannon indices did not show significant differences between the root exclusion and CT treatments ($p > 0.05$), while the treatment excluding roots and hyphae resulted in an increased Simpson index ($p < 0.05$, Table 2). Nevertheless, the fungal Simpson and Shannon indices showed no significant differences among the three treatments ($p > 0.05$, Table 2). In addition, exclusion of roots or/and hyphae exerted a strong effect on bacterial β diversity ($p = 0.044$) but not fungi ($p > 0.05$, Figure 1).

Acidobacteria, *Proteobacteria*, *Chloroflexi*, *Verrucomicrobia*, *Planctomycetes*, and *Actinobacteria* were the most dominant bacterial phyla (Figure 2). Compared to the CT, exclusion of roots or/and hyphae significantly decreased the relative abundance of *Proteobacteria* and *Actinobacteria*, but increased that of *Acidobacteria* and *WPS2* ($p < 0.05$, Table S1). Moreover, the predominant fungal phyla were *Ascomycota*, *Mortierellomycota*, *Basidiomycota*, *Rozellomycota*, and *Mucoromycota* (Figure 2). The exclusion treatments increased the relative

abundances of *Rozellomycota* and *Glomeromycota*, but had no significance influence on other fungal phyla ($p < 0.05$, Table S1).

Table 2. Influences of excluding roots and mycorrhizal hyphae on microbial community diversity in a subtropical Chinese fir plantation forest soil. CT, no exclusion of roots or mycorrhizal hyphae; NR, exclusion of roots but retention of mycorrhizal hyphae; NRH, exclusion of roots and mycorrhizal hyphae. Values are mean ± SE (n = 4). Different letters indicate significant differences at α = 0.05.

Microbial Diversity		Treatment		
		CT	NR	NRH
Bacteria	richness	3607 ± 127 a	3298 ± 87 b	3158 ± 71 b
	chao1	3609 ± 127 a	3299 ± 87 b	3159 ± 71 b
	simpson	0.0056 ± 0.0005 b	0.0057 ± 0.0006 ab	0.0068 ± 0.0007 a
	shannon	3 ± 0.02 a	3 ± 0.03 a	3 ± 0.03 a
Fungi	richness	1380 ± 104 a	1147 ± 27 b	1059 ± 79 b
	chao1	1381 ± 104 a	1148 ± 27 b	1060 ± 79 b
	simpson	0.04 ± 0.032 a	0.027 ± 0.003 a	0.021 ± 0.014 a
	shannon	2 ± 0.15 a	2 ± 0.05 a	2 ± 0.11 a

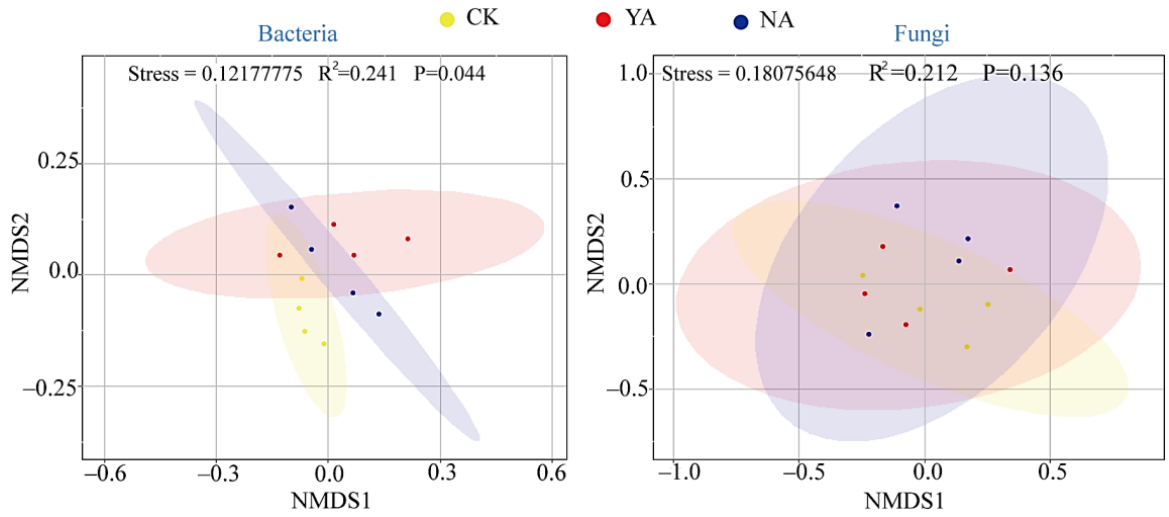

Figure 1. The unweighted nonmetric multidimensional scaling (NMDS) analysis of microbial community distances in a subtropical Chinese fir plantation forest soil. CT, no exclusion of roots or mycorrhizal hyphae; NR, exclusion of roots but retention of mycorrhizal hyphae; NRH, exclusion of roots and mycorrhizal hyphae.

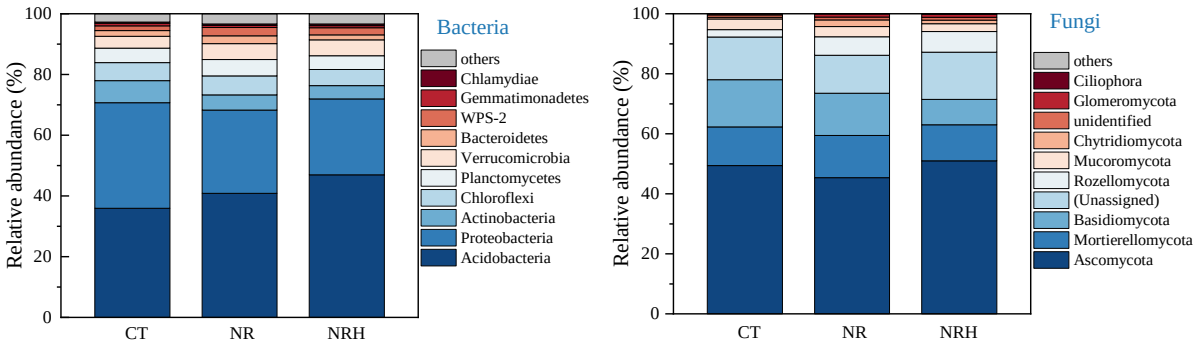

Figure 2. Effects of excluding roots and mycorrhizal hyphae on the relative abundances of dominant microbial species at the phylum level in a subtropical Chinese fir plantation forest soil. CT, no exclusion of roots or mycorrhizal hyphae; NR, exclusion of roots but retention of mycorrhizal hyphae; NRH, exclusion of roots and mycorrhizal hyphae.

3.3. Microbial Functional Prediction

Changes in soil microbial function for C or N degradation and fixation after excluding C sources from plant roots or/and mycorrhizal hyphae were observed (Figure 3). The results show that the exclusion of roots and hyphae significantly increased the relative abundances of six C degradation genes (i.e., *malQ*, *malZ*, *chi*, *rfbB*, *bglX*, *and ablA*) ($p < 0.05$) and four C fixation genes (i.e., *accA*, *icd*, *korA*, *and korB*) ($p < 0.05$), but decreased the abundance of four C fixation genes including *facA*, *coxL*, *coxM*, and *coxS* ($p < 0.05$, Figure 3). In addition, the exclusion of roots and hyphae induced an increase in the relative abundances of the N fixation gene (*nifS*) ($p < 0.05$), which resulted in a decrease in the N degradation genes of *nasA*, *nirB*, *ureC*, and *gdh2* ($p < 0.05$, Figure 3).

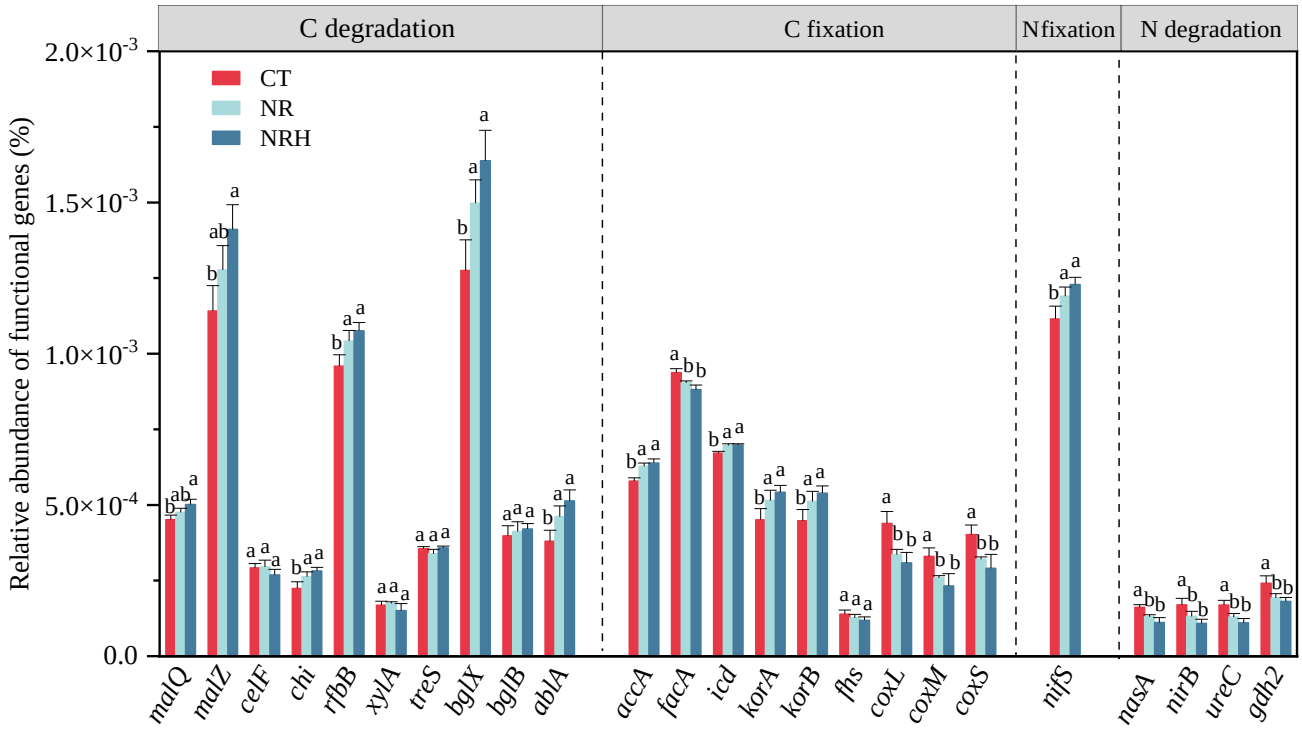

Figure 3. Effects of excluding roots and mycorrhizal hyphae on the relative abundances of microbial functional genes in a subtropical Chinese fir plantation forest soil. CT, no exclusion of roots or mycorrhizal hyphae; NR, exclusion of roots but retention of mycorrhizal hyphae; NRH, exclusion of roots and mycorrhizal hyphae. Different letters indicate significant differences at $\alpha = 0.05$.

3.4. Influencing Factors of Microbial Community and Function

The results of the Mantel test show soil available N (NH_4^+-N, NO_3^--N, and mineral N) as the dominant factor influencing bacterial and fungal diversity ($p < 0.01$, Figures 4 and S1). DOC and SWC act as the critical factors contributing to the changes in bacterial and fungal community composition ($p < 0.05$, Figure 4). Furthermore, we also found that DOC, NH_4^+-N, and mineral N were positively correlated with *Proteobacteria* and *Actinobacteria*, but negatively correlated with *Acidobacteria* and *Rozellomycota* ($p < 0.01$, Figure S2). In addition, the relative abundances of C and N degradation and fixation genes were significantly influenced by the changes in DOC, DON, NH_4^+-N, NO_3^--N, and mineral N ($p < 0.01$), rather than in pH, SOC, TN, C/N, TP, and AP ($p > 0.05$, Figure 5).

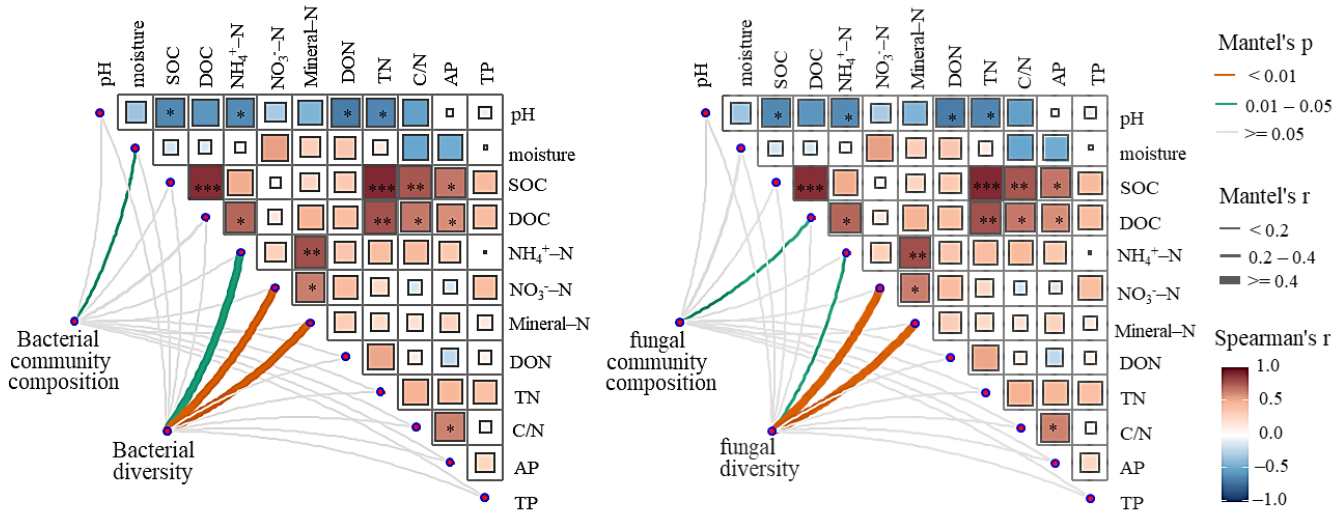

Figure 4. The dominant factors influencing microbial community and diversity based on the Mantel test. SOC, soil organic carbon; DOC, dissolved organic carbon; DON, dissolved organic nitrogen; TN, total nitrogen; AP, available phosphorus; TP, total phosphorus. *, **, and *** indicate significant levels at 0.05, 0.01, and 0.001, respectively. The yellow line, green line, and gray line represent significant levels at 0.01, 0.05, and >0.05, respectively.

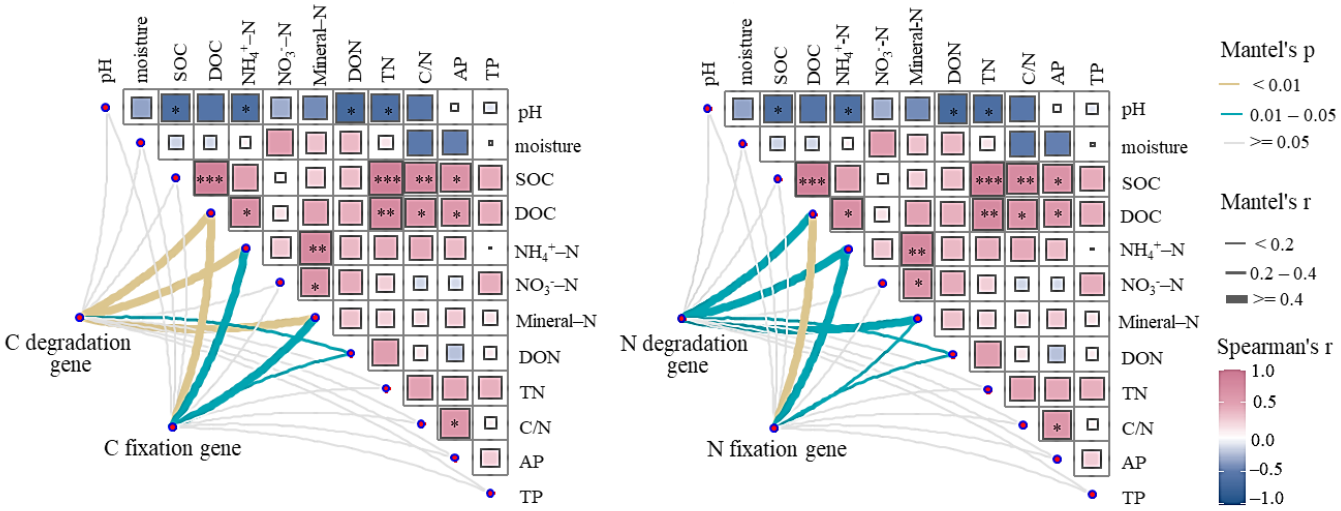

Figure 5. The dominant factors influencing the relative abundance of functional genes based on the Mantel test. SOC, soil organic carbon; DOC, dissolved organic carbon; DON, dissolved organic nitrogen; TN, total nitrogen; AP, available phosphorus; TP, total phosphorus. *, **, and *** indicate significant levels at 0.05, 0.01, and 0.001, respectively. The yellow line, green line, and gray line represent significant levels at 0.01, 0.05, and >0.05, respectively.

3.5. Relationships between Microbial Community Composition and Function

The majority of C and N degradation and fixation genes showed strong correlations with *Acidobacteria*, *Proteobacteria*, *Verrucomicrobia*, *Actinobacteria*, *Rozellomycota*, and *Glomeromycota* ($p < 0.05$, Figure 6). Specifically, the relative abundances of genes for C degradation (i.e., *malQ*, *malZ*, *celF*, and *chi*), C fixation (i.e., *facA*, *coxL*, *coxM*, and *coxS*), and N degradation (i.e., *nasA*, *nirB*, *ureC*, and *gdh2*) were positively correlated with *Proteobacteria* and *Actinobacteria* ($p < 0.01$), while showing negative relationships with *Acidobacteria*, *Verrucomicrobia*, *Rozellomycota*, and *Glomeromycota* ($p < 0.05$, Figure 6). Conversely, there were positive relationships of C (i.e., *accA*, *icd*, *korA*, and *korB*) and N fixation (*nifS*) genes and *Proteobacteria* and *Actinobacteria* ($p < 0.01$), but negative relationships between these C

and N fixation genes and *Acidobacteria*, *Verrucomicrobia*, *Rozellomycota*, and *Glomeromycota* ($p < 0.05$, Figure 6).

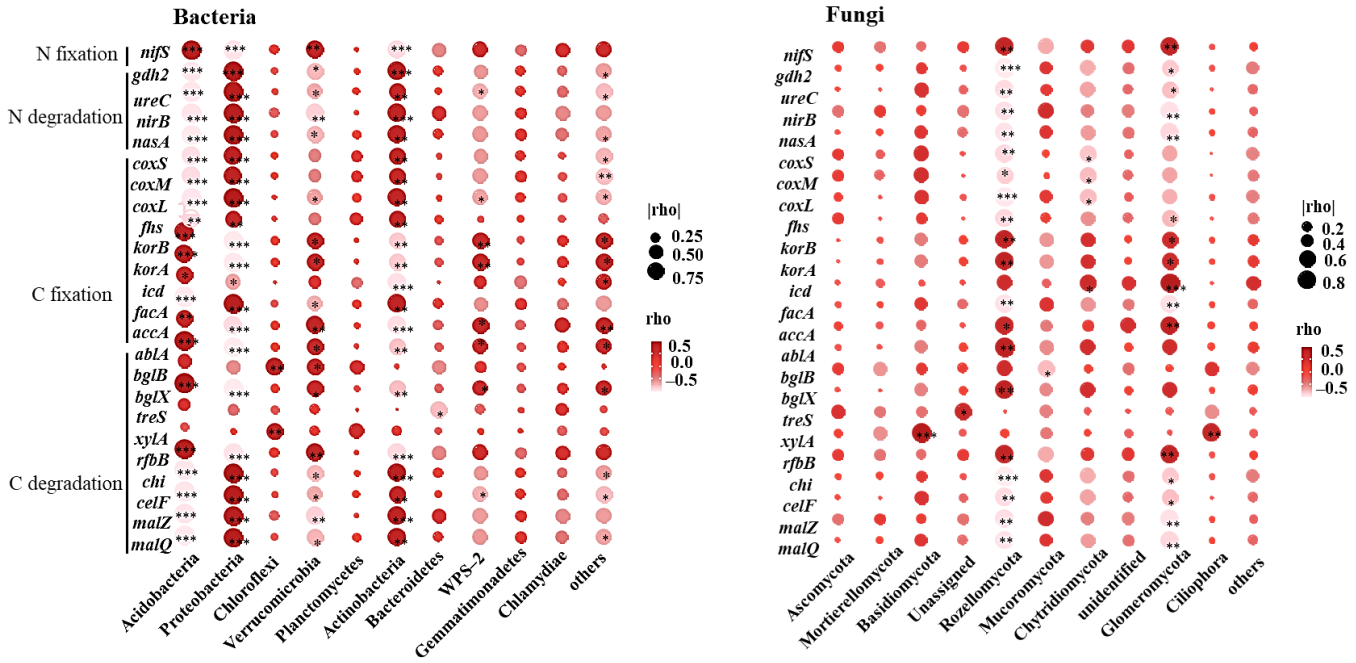

Figure 6. Correlations between the relative abundance of dominant microbial species and functional genes for carbon and nitrogen cycling. *, **, and *** indicate significant levels at 0.05, 0.01, and 0.001, respectively.

4. Discussions

4.1. Influence of Excluding Roots and Mycorrhizal Hyphae on Soil Properties

The roots and mycorrhizal hyphae are considered the primary pathway for plant photosynthetic C input into soil, and changes in their prevalence are often associated with variations in soil C content [17,29]. In this study, we observed a significant decrease in DOC in both exclusion treatments ($p < 0.05$, Table 1). This finding is consistent with the understanding that root exudates serve as the dominant source of labile C, such as DOC, in the soil [1,30]. Thus, when plant photosynthetic C is excluded, the DOC content is expected to decrease. However, excluding roots and mycorrhizal hyphae did not significantly alter SOC content ($p < 0.05$, Table 1). There are several explanations for this. Firstly, the duration of plant photosynthetic C exclusion in our study was relatively short (2 years), and significant changes in SOC may require a longer-term experiment to manifest. Secondly, excluding root exudates not only reduces the input of C into the soil, but also decreases the loss of SOC by decreasing the priming effect [6,31], thus contributing to the unchanged SOC observed in our study.

Similar to SOC, there were no significant changes in TN, TP, or C:N in the treatments excluding roots and hyphae ($p > 0.05$, Table 1). This suggests that excluding plant roots and mycorrhizal hyphae for a period of 2 years did not have an impact on the total C, N, and P in soil. However, it is worth noting that the available N, such as NH_4^+-N, NO_3^--N, and DON, exhibited a significant decrease in the NR and NRH treatments ($p < 0.05$, Table 1). This finding is intriguing because under normal circumstances, the exclusion of plant roots and mycorrhizal hyphae would be expected to increase soil available N, as the reduction in plant N uptake leads to a decrease in N output from the soil. One plausible explanation for the decrease in available N could be the reduction in N sources resulting from the decomposition of plant roots in the root and hypha exclusion treatments. Additionally, without plant roots, there may be an increased risk of N leaching, which could contribute to a decline in N availability in the surface soil. Taken together, excluding plant photosynthetic

C did not significantly alter total C, N, and P, but it did lead to a reduction in available C and N in the soil. This reduction may induce microbial C and N limitation, thereby impacting microbial community and function.

4.2. Influence of Excluding Roots and Mycorrhizal Hyphae on Microbial Diversity and Community Composition

Soil labile C serves as an energy source for microbes [1,32], and a reduction in C availability therefore exerts a negative effect on microbial diversity. We found that the exclusion of roots and hyphae decreased microbial diversity (e.g., richness and Chao1) ($p < 0.05$, Table 2). In general, the lower concentration of DOC resulting from the exclusion of plant roots and mycorrhizal hyphae could limit microbial biomass and lead to a decline in microbial diversity [16,17]. Additionally, we found that the availability of NH_4^+-N was the dominant factor influencing bacterial and fungal richness (Figure S1), suggesting that the decrease in N availability could be another reason for the decline in microbial diversity. Previous studies have also shown that rare species are more sensitive to environmental changes compared to common species [14,33]. Moreover, we observed different responses in bacterial and fungal β diversity to the exclusion of plant roots and mycorrhizal hyphae, with significant changes occurring in bacteria but not in fungi (Figure 1). This result suggests that bacteria are more sensitive to low C availability compared to fungi. This observation aligns with the consensus that bacterial growth relies more on labile C, such as DOC, while fungi have a greater ability to utilize recalcitrant C when labile C is scarce.

The changes in microbial composition in response to the exclusion of plant roots and mycorrhizal hyphae were characterized by decreased relative abundances of *Proteobacteria* and *Actinobacteria* phyla and increased abundances of *Acidobacteria*, *WPS2*, *Rozellomycota*, and *Glomeromycota* phyla ($p < 0.05$, Table S1). These changes in dominant phyla are likely associated with the ecological strategies of microbes under different environmental conditions, such as copiotrophs and oligotrophs [12,21,34]. Copiotrophic species are abundant in soils with high resource availability, such as available C or N, while oligotrophic species are more common in soils with limited resource availability [20,21]. Therefore, the decrease in the *Proteobacteria* phylum (copiotrophic species) and the increase in the oligotrophic *Acidobacteria* and *Rozellomycota* phyla could be attributed to the low availability of labile C and N (as indicated by decreased DOC, DON, and mineral N) in root and hypha exclusion treatments [12].This observation is further supported by previous studies showing higher abundance of *Proteobacteria* but lower abundance of the *Acidobacteria* phylum in rhizosphere soil (rich in C and N) compared with bulk soil (low C and N) [21]. However, considering the different functions of various microbial phyla in driving soil C or N turnover, the changes in microbial composition in the root and hypha exclusion treatments also imply altered microbial abilities to influence carbon and nitrogen availability in the soil. The specific impacts of these compositional changes on soil C and N dynamics would require further investigation.

4.3. Influence of Excluding Roots and Mycorrhizal Hyphae on Microbial Function in Meditating Soil C and N Availability

As anticipated, the exclusion of roots and mycorrhizal hyphae led to changes in microbial composition, which subsequently impacted the microbial-mediated availability of C and nitrogen N in the soil. Notably, the relative abundances of genes involved in C and N degradation and fixation (NR and NRH) significantly increased ($p < 0.05$, Figure 3), indicating that microbes enhanced their capacity to acquire C and N from the soil. This adaptation could be attributed to a microbial strategy for dealing with limitations in C or N availability [35,36], further supported by the robust correlations observed between these genes and the levels of accessible C (DOC) and N (NH_4^+-N, NO_3^--N, and DON) (Figure 5).

Among these C degradation genes, *malQ*, *malZ*, and *bglX* are responsible for starch and sucrose metabolism [23,37], and the *rfbB* gene plays a critical role in the metabolism of amino sugar and nucleotide sugar [38]. Excluding plant roots and mycorrhizal hyphae results in a shortage of labile C, leading to microbial C limitation. The upregulation of these

genes may accelerate the decomposition of soil organic C, such as starch and sucrose, as well as stable organic C, such as amino sugar and nucleotide sugar, thereby releasing labile C and alleviating microbial C limitation.

In contrast to the increased abundance of C degradation genes, the relative abundances of N degradation genes were observed to decrease in the exclusion treatments ($p < 0.05$, Figure 3). The decreased expression of *nirB* and *gdh2* genes, which encode oxidoreductases involved in decomposing organic matter and releasing available N [39,40], suggests a decline in microbial ability to mineralize organic N. This provides an explanation for the reduction in available N in root and hypha exclusion treatments. Typically, microbes activate genes related to N acquisition when soil N availability is low [25]. However, in this study, the N degradation genes exhibited a decrease. This could be attributed to the microbial C limitation induced by the exclusion of roots and mycorrhizal hyphae, which inhibits the expression of N mineralization genes due to insufficient energy. Conversely, the relative abundance of the N fixation gene, such as *nifS* [41], was increased ($p < 0.05$, Figure 3), indicating an enhanced microbial N fixation ability after excluding roots and mycorrhizal hyphae. Consequently, considering the decreased abundance of N degradation genes and increased abundance of the N fixation gene, one possible strategy employed by microbes to alleviate N deficiency is to enhance microbial N fixation rather than relying on the mineralization of organic N in the soil.

5. Conclusions

After two years of excluding roots and mycorrhizal hyphae, the total C and N content in the Chinese fir soil remained unchanged, but there was a significant decrease in available C and N. This reduction in C and N availability might reduce the energy and nutrient supply for microbes, leading to a decline in microbial diversity. This decline is evident from the decreased richness and Chao1 indices observed after the exclusion of roots and hyphae. Moreover, the exclusion of roots and hyphae also impacts the microbial composition. For example, the relative abundances of dominant copiotrophic phyla (e.g., *Proteobacteria*) decreased, while the relative abundances of oligotrophic phyla (e.g., *Acidobacteria* and *Rozellomycota*) increased in response to the decline in soil available C and N. In this scenario, soil microorganisms upregulated C degradation and fixation genes to enhance organic matter decomposition and C fixation in order to acquire C. Regarding microbial N acquisition, biological N fixation appeared to be more important than mineral N, as evidenced by the increased expression of N fixation genes rather than N degradation genes. This suggests that microbes employ different strategies to alleviate C or N limitation induced by the removal of roots and mycorrhizal hyphae. Overall, the observed responses of soil properties, microbial diversity, and microbial functions to changes in belowground C input have implications for predicting and modeling C and N cycling in forest ecosystems.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/f14091847/s1, Figure S1: The dominant factors that contribute to microbial community diversity after removal of root and mycorrhizal hypha in a subtropical Chinese fir plantation forest soil. SOC, soil organic carbon; DOC, dissolved organic carbon; DON, dissolved organic nitrogen; TN, total nitrogen; AP, available phosphorus; TP, total phosphorus. * and ** indicate significant level at 0.05 and 0.01, respectively; Figure S2: Correlations between the relative abundance of dominant microbial species and soil properties in a subtropical Chinese fir plantation forest soil. SOC, soil organic carbon; DOC, dissolved organic carbon; DON, dissolved organic nitrogen; TN, total nitrogen; AP, available phosphorus; TP, total phosphorus. *, **, *** indicate significant level at 0.05, 0.01, and 0.001, respectively; Table S1: Influences of excluding root and mycorrhizal hypha on the relative abundance of dominant species at phylum level in a subtropical Chinese fir plantation forest soil. CT, no exclusion of root or mycorrhizal hypha; NR, exclusion of root but remain mycorrhizal hypha; NRH, exclusion of root and mycorrhizal hypha. SOC, soil organic carbon; DOC, dissolved organic carbon; DON, dissolved organic nitrogen; TN, total nitrogen; AP, available phosphorus; TP, total phosphorus. Different letters indicate significant differences at $\alpha = 0.05$; Table S2: Information of microbial functional genes involved in carbon or nitrogen degradation and fixation in this study.

Author Contributions: P.L. wrote the paper. L.Y. provided the research data, planned the research, guided the research framework, and provided project funding support. K.Y. provided the English-language editing and provided project funding support. L.X. analyzed the satellite image data. All authors have read and agreed to the published version of the manuscript.

Funding: This research was supported by the National Natural Science Foundation of China (Nos. 32171587, 32192433, 41977090, and 32271633).

Data Availability Statement: Date will be made available on request.

Acknowledgments: We gratefully acknowledge Xia Yun for the assistance in experimental operations and data analysis. Additionally, we extend our appreciation to the reviewers and editors for their valuable suggestions, which significantly contributed to the quality of this study.

Conflicts of Interest: The authors declare no conflict of interest.

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