

## Article

# Influence of Intraspecific Competition Stress on Soil Fungal Diversity and Composition in Relation to Tree Growth and Soil Fertility in Sub-Tropical Soils under Chinese Fir Monoculture

Taimoor Hassan Farooq<sup>1,2,\*,†</sup> , Uttam Kumar<sup>3,†</sup> , Awais Shakoor<sup>4</sup> , Gadah Albasher<sup>5</sup>, Saad Alkahtani<sup>5</sup> , Humaira Rizwana<sup>6</sup>, Muhammad Tayyab<sup>7</sup> , Jalpa Dobaria<sup>8</sup>, Muhammad Iftikhar Hussain<sup>9</sup> and Pengfei Wu<sup>2,\*</sup>

- <sup>1</sup> Bangor College China, A Joint Unit of Bangor University and Central South University of Forestry and Technology, Changsha 410004, China
- <sup>2</sup> College of Forestry, Fujian Agriculture and Forestry University, Fuzhou 350002, China
- <sup>3</sup> Institute of Applied Ecology, College of Plant Protection, Fujian Agriculture and Forestry University, Fuzhou 350002, China; uttam5454@gmail.com
- <sup>4</sup> Department of Environment and Soil Sciences, University of Lleida, Avinguda Alcalde Rovira Roure 191, 25198 Lleida, Spain; awais.shakoor@udl.cat
- <sup>5</sup> Department of Zoology, College of Science, King Saud University, Riyadh 11451, Saudi Arabia; Galbeshr3@gmail.com (G.A.); salkahtani@Ksu.edu.sa (S.A.)
- <sup>6</sup> Department of Botany and Microbiology, College of Science, King Saud University, Riyadh 11451, Saudi Arabia; hrizwana@ksu.edu.sa
- <sup>7</sup> College of Crop Science, Fujian Agriculture and Forestry University, Fuzhou 350002, China; tyb.pk@hotmail.com
- <sup>8</sup> Department of Biochemistry, B.A. College of Agriculture, Anand Agricultural University, Anand 388110, Gujarat, India; Jalpadobaria@aau.in
- <sup>9</sup> Plant Biology & Soil Science Department, University of Vigo, 36310 Vigo, Spain; iftikhar@uvigo.es
- \* Correspondence: t.farooq@bangor.ac.uk (T.H.F.); ffwupengfei@fafu.edu.cn (P.W.)
- † These authors contributed equally to this work.



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**Abstract:** Soil microorganisms provide valuable ecosystem services, such as nutrient cycling, soil remediation, and biotic and abiotic stress resistance. There is increasing interest in exploring total belowground biodiversity across ecological scales to understand better how different ecological aspects, such as stand density, soil properties, soil depth, and plant growth parameters, influence belowground communities. In various environments, microbial components of belowground communities, such as soil fungi, respond differently to soil features; however, little is known about their response to standing density and vertical soil profiles in a Chinese fir monoculture plantation. This research examined the assemblage of soil fungal communities in different density stands (high, intermediate, and low) and soil depth profiles (0–20 cm and 20–40 cm). This research also looked into the relationship between soil fungi and tree canopy characteristics (mean tilt angle of the leaf (MTA), leaf area index (LAI), and canopy openness index (DIFN)), and general growth parameters, such as diameter, height, and biomass. The results showed that low-density stand soil had higher fungal alpha diversity than intermediate- and high-density stand soils. *Ascomycota*, *Basidiomycota*, *Mucromycota*, and *Mortierellomycota* were the most common phyla of the soil fungal communities, in that order. *Saitozyma*, *Penicillium*, *Umbelopsis*, and *Talaromyces* were the most abundant fungal genera. Stand density composition was the dominant factor in changing fungal community structure compared to soil properties and soil depth profiles. The most significant soil elements in soil fungal community alterations were macronutrients. In addition, the canopy openness index and fungal community structure have a positive association in the low-density stand. Soil biota is a nutrient cycling driver that can promote better plant growth in forest ecosystems by supporting nutrient cycling. Hence, this research will be critical in understanding soil fungal dynamics, improving stand growth and productivity, and improving soil quality in intensively managed Chinese fir plantations.

**Keywords:** soil fungal composition; vertical soil profiles; stand density; soil health; sub-tropical forest; Southern China

## 1. Introduction

Soil microorganisms are an essential component of forest soils, as they help with energy flow and nutrient conversion in forest ecosystems. In addition, by assisting in the degradation of soil organic matter (SOM), they also serve as indicators of soil health, soil toxicology, and tree planting conditions [1,2] because soil microbiota is most sensitive to any changes in the soil microenvironment [3]. Despite extensive research on soil microorganisms worldwide, the functions of the majority of microbes remain unknown, as only a small percentage (1–2%) of microbes can be isolated, cultured, and identified [4]. Fungi (eukaryotic microorganisms) play an important ecological fundamental role as mutualists, decomposers, and pathogens of animals and plants [5]. They mediate plant mineral nutritional status, promote carbon (C) cycling in soils, and alleviate C limitations in other soil microbiota [6].

Plantation productivity, stand growth, canopy structure, and soil fertility all are influenced by various forest management practices, such as stand density and spacing [7–9], stand structure [10], species genotype [11], use of cutting [12], seed germination methods [13,14], use of inorganic and organic amendments [15], and planting pattern [16]. Among them, plantation stand density is a crucial subject in silvicultural techniques. By altering root distribution and canopy density, stand density and pattern directly affect plant survival and growth and indirectly influence the composition of understory vegetation and soil ecosystem functioning. Variable stand density has been identified as a significant factor in plant-soil feedback in various studies [17,18]. In terms of productivity, Farooq et al. [7] mentioned that the general growth and survival rate of individual trees was usually better in the low-density stand; still, overall stand yield and biomass production were higher in intermediate and high-density stands.

Furthermore, the light intensity was abundant in the low-density stands, allowing for rapid decomposition of litter and, ultimately, the accumulation of acid matter in the soil [19]. In terms of soil fertility, Lei et al. [20] demonstrated that soil nutrients in less dense stands were more critical for improving soil health than soil nutrients in densely planted stands. According to Farooq et al. [8], the low-density stand had more soil total nitrogen (TN), soil available nitrogen (AN), and total phosphorus (TP), while the intermediate-density stand had higher soil available phosphorus (AP) and soil organic matter (SOM). Although these researchers made significant contributions to our knowledge of the impacts of planting density on stand growth and soil properties in forest ecosystems, they paid less attention to how planting density and spacing affect soil fungal communities. Soil fungal functioning under different forest management practices can directly impact tree survival, tree growth, and overall stand productivity, specifically because of root and mycorrhizal interaction. Moreover, fungi contribute to the decomposition of SOM and provide essential nutrients for plant growth. Thus, their role is vital in plant protection as biological agents, which directly influences soil health.

Plantations play an important role in Chinese forest ecosystems; in these plantations, the Chinese fir (*Cunninghamia lanceolata* (Lamb.) Hook.) is a common species. It is a species of evergreen coniferous tree known for its high yield, superior timber quality, and rapid growth [21]. Chinese fir plantations play a vital role in climate regulation as well as for soil and water conservation. It is widely distributed across 16 provinces of China, primarily in central and southern China [22]. Since the 1980s, due to numerous afforestation projects, the area under Chinese fir plantations has experienced significant growth [23]. It covers more than 11 million hectares, accounting for 24% of China's total forest plantations [8]. Plantations are usually fast-growing species with shorter rotation cycles compared to broadleaved natural forests and mixed-species forests. These plantations are artificial and managed differently than natural forest stands. Moreover, natural forests have typically stable vegetation with natural rotation cycles and less human interference than plantations. Soil fertility in forest ecosystems can be influenced by forest composition and structure, and different silvicultural methods can directly influence the soil microbiota.

Some studies about soil fungal diversity and composition in soils under Chinese fir monoculture have been conducted; however, more emphasis has been placed on different aged plantations, chronosequence, and provenance trails. There has been insufficient research on fungal soil dynamics concerning stand growth and soil properties of coniferous plantations established at various stand density levels. Therefore, the objectives of this are (i) to look at the impact of altering stand density on soil fungal diversity and community composition and (ii) to analyze the association between soil fungal diversity, tree canopy growth, component biomass production, and soil quality indicators in Chinese fir monoculture. We hypothesized that (i) varying stand density and planting spacing would significantly influence soil fungal diversity and composition, and (ii) the relationship of soil fungal dynamics with canopy growth, tree component biomass, and soil quality parameters would be strongly positive under varying stand densities.

## 2. Materials and Methods

### 2.1. Study Site and Plantation Establishment

The experiment was conducted in Chinese fir plantation stands at the Xinkou Research Forest Farm in Sanming City, Fujian Province, Southern China (26°10' N and 117°27' E). Silty Oxisol was a type of soil that was acidic. The research area has a humid subtropical monsoon climate with a mean annual temperature of 19 °C, average annual rainfall of 1612 mm, and average annual relative humidity of 80%. According to research objectives, three Chinese fir monoculture stands established at different stand densities, i.e., low density (1450 trees hm<sup>2</sup> with 2.36 × 2.36 m spacing), intermediate density (2460 trees hm<sup>2</sup> with 1.83 × 1.83 m spacing), and high density (1450 trees hm<sup>2</sup> with 1.44 × 1.44 m spacing) were selected. These stands were named PD1, PD2, and PD3, respectively. The selected stands were located at an elevation range of 205 to 500 (a.s.l.). These experimental stands were established in 2007–2008 from seedlings (1-year-old) after clear-cutting. For the first three years, weeding was done twice a year, followed by once a year after that. All the selected experimental stands are present in the same research station and share the same agronomic practices and climatic conditions. The understory vegetation layer was composed of *Callicarpa kochiana* Makino, *Woodwardia japonica* (L.f.) Sm., *Ilex pubescens* Hook. & Arn., *Selaginella moellendorffii* Hieron., *Alpinia japonica* Miq. and *Maesa japonica* Moritzi ex Zoll. Average DBH in PD1, PD2, and PD3 was 13.05 cm, 12.46 cm, and 11.04 cm, respectively, whereas average height was 12.41 m, 12.01 m, and 11.63 m, respectively. Moreover, the average mortality rate was 8.37%, 9.69%, and 11.99%, respectively. Details of growth parameters and biomass production are reported in our published paper [7].

### 2.2. Soil Sampling and Physicochemical Analysis

Three 20 × 20 m plots were established for each density stand to explore the fungal flora for nine plots. To avoid pseudoreplication, the minimum distance between the established plots was kept at 400 m. A soil auger (3.5 cm diameter) was used for soil sampling at two depths (i.e., 0–20 (U) and 20–40 cm (L)) within a 25-cm radius of the tree root. A five-point sampling method with an S-shaped pattern was used to minimize spatial heterogeneity-related inaccuracy to make one soil sample with three replicates. After sampling, roots were softly shaken to eradicate tenuously connected soil, and firmly connected soil was swept aside. Finally, three composite samples per density per soil depth were obtained, separated into two portions and instantly stored in an icebox. In the laboratory, one portion was kept at −80 °C to isolate DNA for fungal flora analysis, and the other portion was air-dried for soil physicochemical analysis. Methods for soil physicochemical analysis are mentioned in (Table 1).

**Table 1.** Methods used for soil physicochemical properties analysis [8].

Analysis	Method/Equipment
pH	Potentiometric method (1:2.5 soil:water)
Electrical conductivity (EC)	Conductivity meter
Bulk density (BD)	The core method of the Nanjing Institute of Soil Science (1978)
Total nitrogen (TN)	CN elemental analyzer
Total phosphorus (TP)	Molybdenum-antimony colourimetric method
Total potassium (TK)	Flame photometry
Total calcium (TCa)	CN elemental analyzer
Total magnesium (TMg)	CN elemental analyzer
C:N ratio	CN elemental analyzer
Soil moisture content (SMC)	Calculated based on a wet and dry weight

To minimize the direct influence of shrubs and herbs, we chose those trees for sampling where the presence of shrubs and herbs around the canopy projection area of trees was considerably less. Still, these are open (field) conditions; hence, the indirect influence might be possible. Moreover, we tried to select the plots with uniform topography to minimize the local terrain impact on trees/vegetation.

### 2.3. Soil Physiochemical Properties and Canopy Dynamics

Soil pH, SOM, and TMg were significantly lower in PD1, while between PD2 and PD3 there was no significant change observed. Soil BD, EC, SMC, TK, TCa and C:N showed no significant change with the stand density of Chinese fir. However, TN and TP showed a variable trend with the change in density stand of Chinese fir (Table 2). Moreover, LAI and MTA grew as the stand density increased, whereas the DIFN followed an opposite pattern (Table 3). This article briefly stated the soil properties and canopy dynamics; however, the soil fertility indices are detailed in [8] and canopy dynamics in [9].

**Table 2.** The physicochemical features of soil in selected Chinese fir stands of various densities. Different small letters indicate the significant difference among stand densities at  $p > 0.05$  [8].

(A)						
Stand Density	pH	<sup>a</sup> BD (g/m <sup>3</sup> )	EC	SOM (g/kg)	SMC (%)	Soil Type
Low (PD1)	4.21 b	1.22 ab	0.02 a	31.90 b	11.49 b	SO
Intermediate (PD2)	4.31 a	1.28 a	0.02 a	36.56 a	9.53 b	SO
High (PD3)	4.27 a	1.16 b	0.02 a	35.47 a	17.2 b	SO
(B)						
Stand Density	TN (g/kg)	TP (g/kg)	TK (g/kg)	TMg (g/kg)	TCa (g/kg)	C/N
Low (PD1)	0.99 a	0.59 a	22.5 a	7.02 b	2.23 a	15.21 a
Intermediate (PD2)	0.74 c	0.48 b	21.2 a	8.51 a	2.25 a	15.80 a
High (PD3)	0.92 b	0.59 a	21.8 a	7.79 ab	2.14 a	15.59 a

**Note:** Shown values of <sup>a</sup>BD, soil bulk density; EC, soil electrical conductivity; SOM, soil organic matter; SMC, soil moisture content; TN, soil total nitrogen; TP, soil total phosphors; TK, soil total potassium; TMg, soil total magnesium; TCa, soil total calcium; C/N, carbon to nitrogen ratio.

**Table 3.** Canopy growth properties of selected Chinese fir different density stands. Different small letters indicate the significant difference among stand densities at  $p > 0.05$  [9].

Stand Density	<sup>a</sup> LAI	LAI <sub>e</sub> (m <sup>2</sup> m <sup>-2</sup> )	MTA (°)	DIFN	ACF	FNC (g/kg)
Low (PD1)	3.97 c	2.48 c	34.8 b	0.15 a	0.990 b	16.92 a
Intermediate (PD2)	4.56 b	2.85 b	44.5 ab	0.11 b	0.993 ab	13.51 b
High (PD3)	5.07 a	3.17 a	48.7 a	0.09 b	0.995 a	15.93 a

**Note:** Shown values of <sup>a</sup>LAI, leaf area index; LAI<sub>e</sub>, leaf area index effective; MTA, mean tilt angle of leaf; DIFN, canopy openness index; ACF, apparent clustering factor; FNC, foliar nitrogen concentration.

#### 2.4. DNA Extraction, PCR Amplification, and Illumina Hiseq 2500 Sequencing

Extraction of DNA was done from soil samples according to the manufacturer's instructions using the EZNA stool D.N.A. Kit (Omega Bio-Tech, Norcross, GA, United States). By using primers ITS3\_KYO2F and ITS4R, PCR was used to amplify the ITS region of the eukaryotic ribosomal RNA gene (95 °C for 2 min, then 98 °C for 10 s, 62 °C for 30 s, and 68 °C for 27 cycles) 30 s, and then extended for 10 min at 68 °C). PCR reactions were carried out in triplicate with a 50-μL mixture containing 5 μL of 10× KOD. Buffer, 5 μL of 2.5 mM dNTPs, 1.5 μL of each primer (5 μM), 1 μL of KOD. Polymerase, and 100 ng of template DNA. The AxyPrep D.N.A. Gel Extraction Kit (Axygen Biosciences, Union City, CA, US) was used to extract amplicons from 2% agarose gels, purify them, and quantify them using QuantiFluor ST (Promega, Madison, WI, US). Purified amplicons were pooled in equimolar quantities and sequenced using standard procedures on an Illumina platform.

#### 2.5. Sequence Processing and Functional Assignment

The unprocessed ITS gene sequencing reads were demultiplexed, quality-filtered by Trimmomatic, then combined by FLASH with a baseline overlap of 10 bp and a mismatch error rate of 2%. (version 1.2.11). Using UPARSE (version 7.1), operational taxonomic units (OTUs) with a 97 percent similarity criterion were grouped, and chimeric sequences were discovered and deleted. The taxonomy of each OTU. representative sequence was examined using the RDP Classifier, and its ITS units were compared to the UNITE database. QIIME produced Chao1, Simpson, and all of the other alpha diversity indices using a confidence level of 0.7 in all of the comparisons (version 1.9.1). QIIME also plotted the OTU. rarefaction curve and rank abundance curves. The functional group (guild) of the O.T.U.s was determined using FUNGuild (v1.0) [24,25].

#### 2.6. Statistical Analysis

One-way analysis of variance (ANOVA) and LSD multiple comparisons were carried out using SPSS 19.0 software to perform a significance test between groups and evaluate the differences for total fungal abundance and diversity indices and taxa of different soils and densities. ACE, Chao1, Simpson and Shannon indices calculated the alpha diversity of fungal colonies in the soil. The beta-diversity for soil samples was estimated using weighted (WT) and un-weighted (UWT.) UniFrac distance matrices based on sensitivity to rare taxa. We used redundancy analysis (RDA) to correlate soil physiochemical properties with soil biotic communities and created a heat map using Pearson correlation between different fungal phyla and also between different alpha diversity indices to better know the fundamental drivers of diversity in soil fungal communities [25].

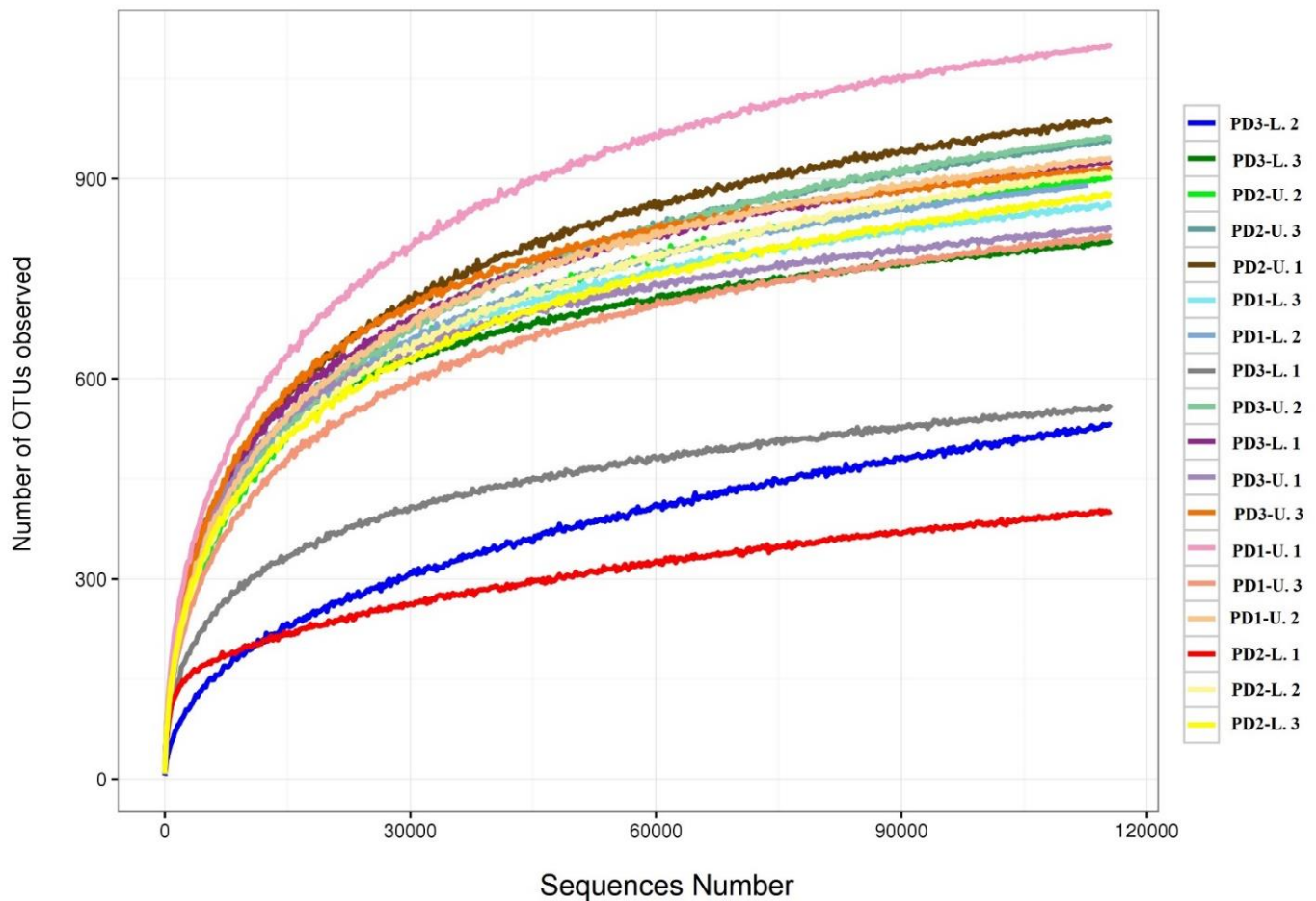
### 3. Results

#### 3.1. Distribution of Soil Fungal Communities

At all soil depths and stand densities, a total of 837,336 (average 139,556) reads were obtained with a maximum length of 449 and a minimum length of 201 from all the soil samples. Per sample, the number of ITS gene sequences ranged from 32,587 to 36,786. (Figure 1). The fungal OUTs ranged from 753 to 977, depending on soil samples and were categorized into OTUs (97 percent similarity according to rarefaction curve analysis,



Figure 1). These findings proved that the soil sampling depth was adequate to attain the richness and diversity of all soil samples. Subsequently, short, ambiguous, and low-quality pyrotags, singletons and replicates reads were eliminated. The relative abundances of the fungal taxa were examined at the phylum and genus levels to determine whether there were any significant alterations in the composition of the fungal communities in different density stands with two different vertical soil profiles.



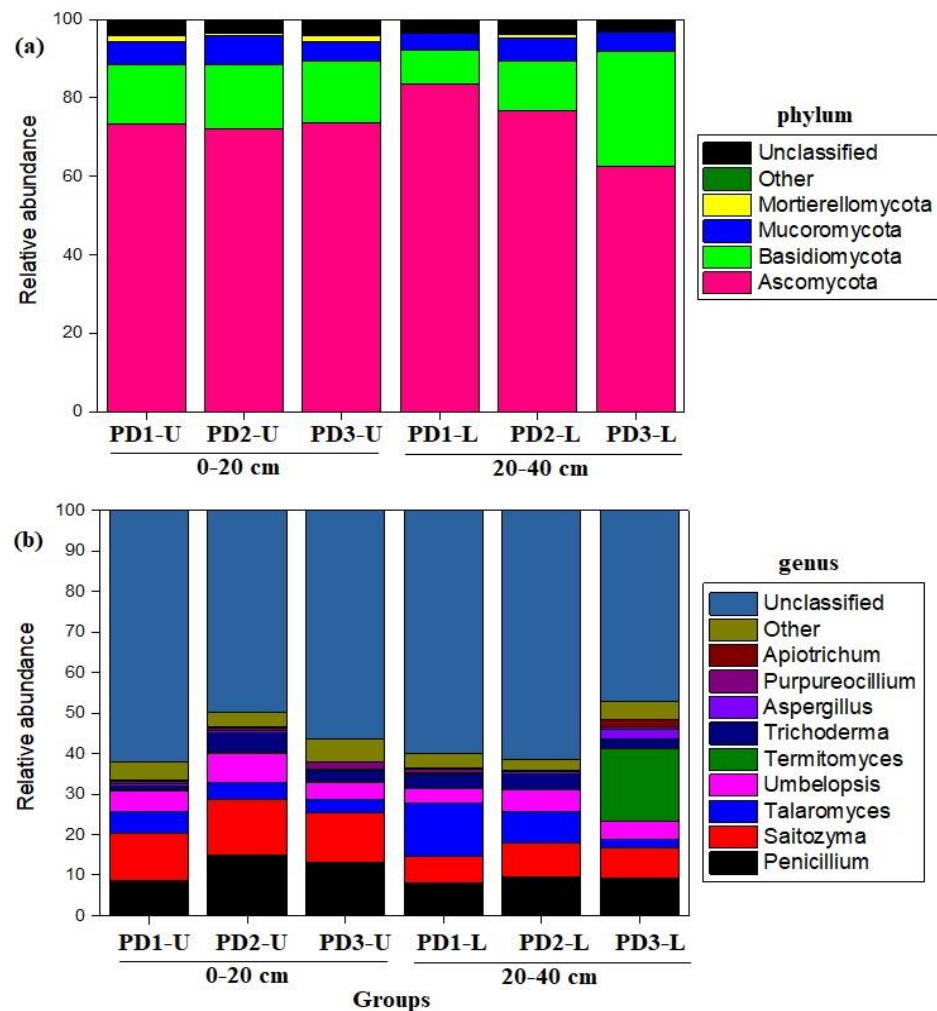
**Figure 1.** A rarefaction curve based on 97 percent similarity was created. PD1, PD2, and PD3 denote low-, intermediate-, and high-density Chinese fir stands, respectively, and “U” and “L” denote the upper (10–20 cm) and lower (20–40 cm) soil layers, respectively.

Many different phyla were detected in all soil samples in three different density stands. However, *Ascomycota*, *Basidiomycota*, *Mucromycota*, and *Mortierellomycota*, respectively, were most abundant in both vertical soil profiles and detected in all samples, representing 90–96% of fungal sequences (Figure 2a). Out of all total soil samples, *Ascomycota* was the most dominant phylum with 73.2%, followed by *Basidiomycota* and *Mucromycota* with 16.88% and 5.46%, respectively. *Saitozyma* was the most prevalent genus in the upper layer of all density stands, followed by *Penicillium*, *Umbelopsis*, and *Talaromyces* (Figure 2b). *Talaromyces* was the most prevalent genus in the lowest layer of low-density stands, followed by *Penicillium*. *Termitomyces* was the most prevalent genus in the lowest layer of high-density stands, followed by *Penicillium* (Figure 2b).

### 3.2. Soil Fungal Diversity and Communities Structure

ACE and Chao1 estimation demonstrated that the upper soil profile of each density stand showed a higher fungal community richness than the lower vertical soil profile (Figure 3a,b). However, In PD3-L soil samples, Simpson diversity and Shannon (SSD)

indices, were highest compared to all other soil samples. In comparison to PD3-L, there was also a significant decrease in PD1-L and PD2-L. SSD levels increased significantly in PD2-U and PD3-U, but there were no substantial differences in PD1-U across all samples (Figure 3c,d).

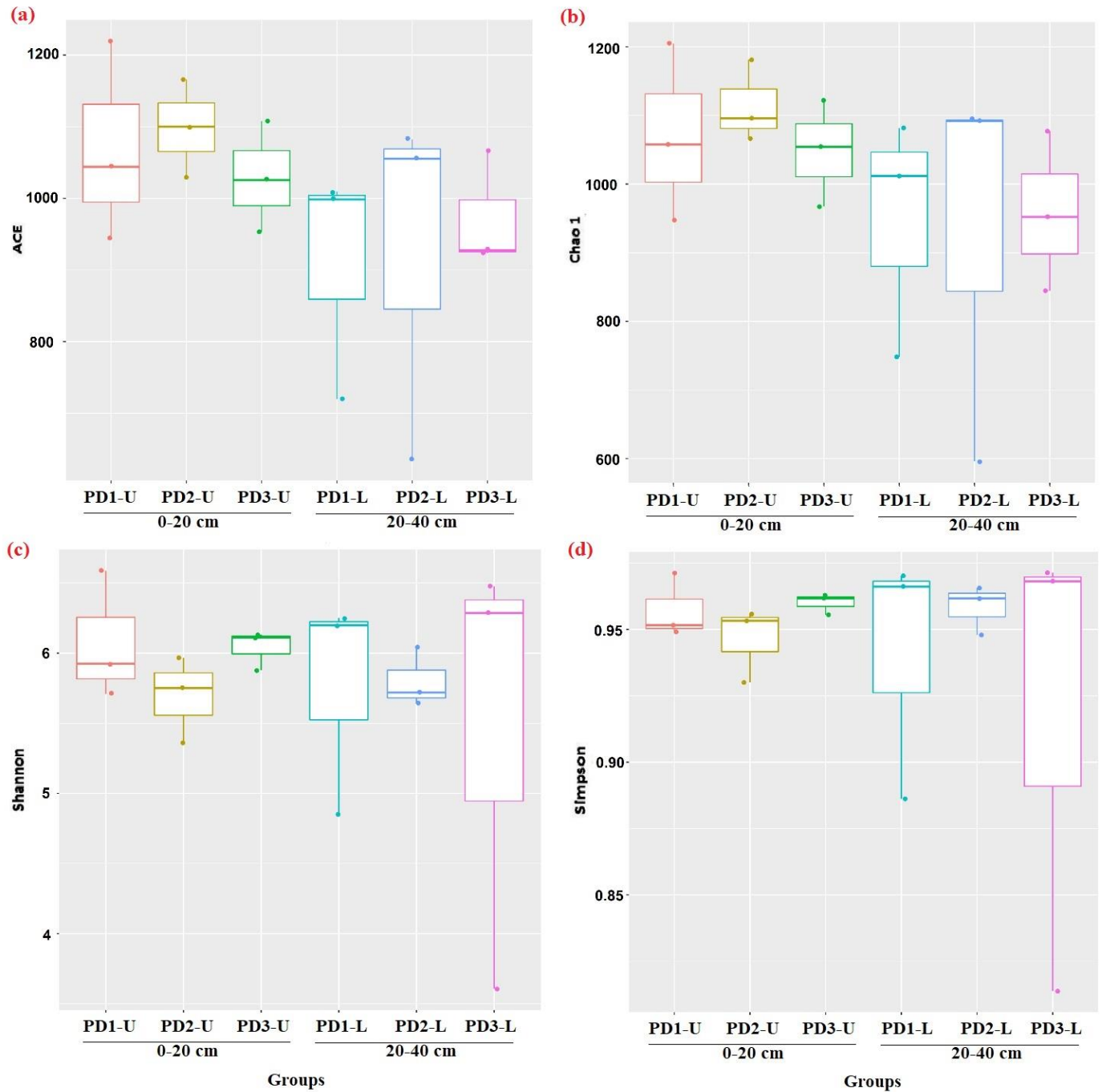


**Figure 2.** (a) Distribution of the most abundant fungal phyla and (b) fungal genera in PD1-U, PD2-U, PD3-U, PD1-L, PD2-L, and PD3-L soils under Chinese fir plantations. <sup>a</sup> U means upper layer (10–20 cm), and L means lower layer (20–40 cm). <sup>b</sup> PD1, low-density stand; PD2, intermediate-density stand; PD3, high-density stand.

According to the WT UniFrac non-metric multidimensional scaling (NMDS) analysis, soil samples from various soil depths and stand ages created distinct and intersecting clusters in ordination space (Figure 4a,b), indicating that fungal communities PD1-U and PD3-L were distinguished from those in other soils. The clustering of PD2-U, PD3-U, PD1-L and PD2-L was observed along axis-1, whereas PD1-U and PD3-L were clustered along axis-2. Furthermore, U.W.T. UniFrac NMDS analysis demonstrated that all of the soil samples' fungal populations clustered along axis-1 (Figure 4a,b). The differences in the organization of fungal communities among distinct samples were demonstrated using the UWT pair group approach with arithmetic mean clustering analysis (UPGMA). *Ascomycota* had the maximum relative abundance according to UPGMA analysis, and followed by *Basidiomycota*, *Mucromycota*, and *Mortierellomycota*, respectively (Figure 5). Moreover, all groups fungal taxonomy of different stand densities and soil depths is shown in (Figure 6).

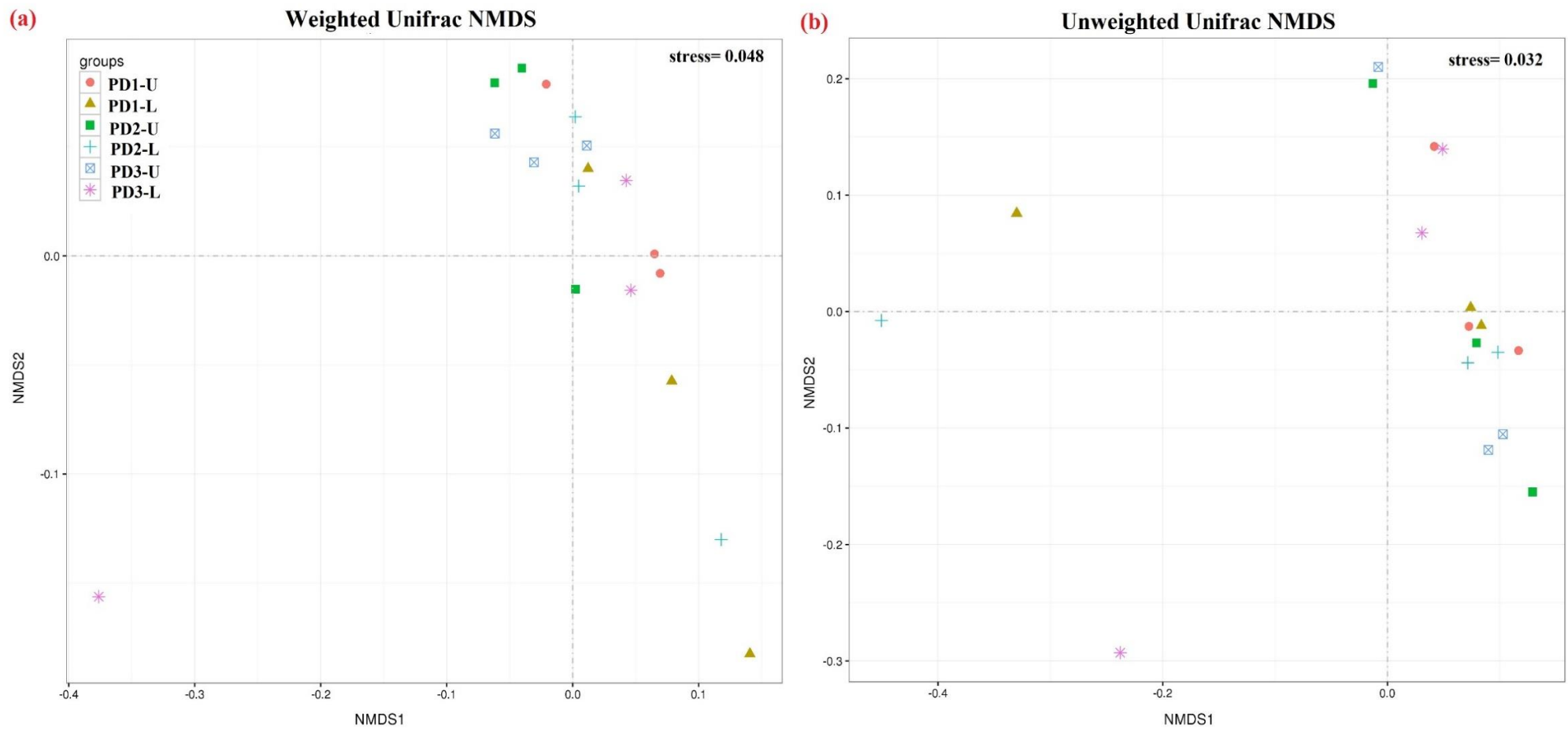
FUNGuild provided more information on the trophic mechanisms of fungal colonies seen in all soil samples (Figure 7). Animal pathogen fungi were the most prevalent in all of

the soil samples, followed by fungal parasites and soil saprotrophs. Fungal functions were split among the OTUs acquired from all soil samples, while “Unassigned” was applied to all taxa that did not match any taxa in the database (Figure 7).

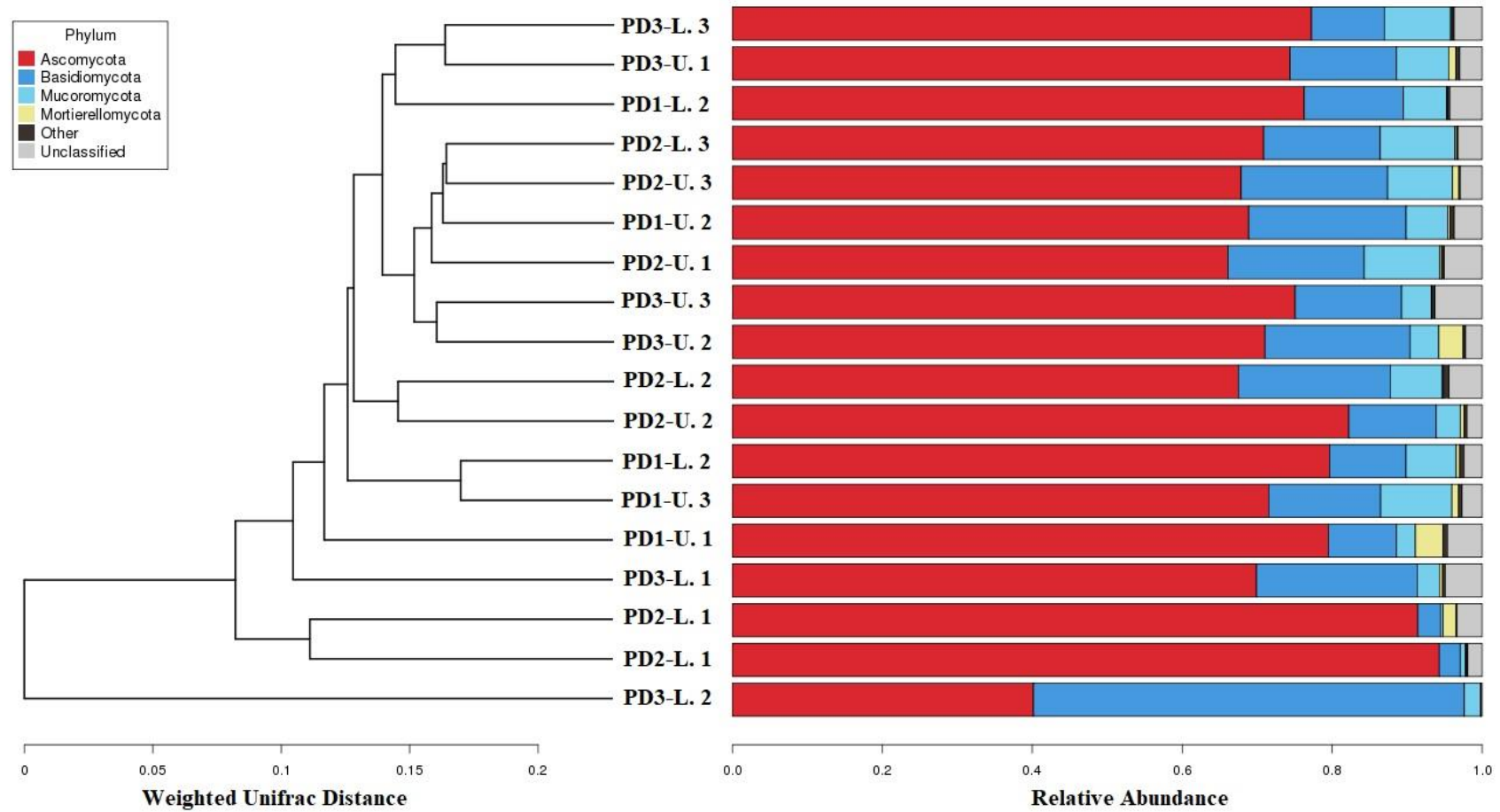


**Figure 3.**  $\alpha$ -Diversity indices containing calculations of (a) abundance-based coverage estimators (ACE), (b) Chao1, (c) Shannon, and (d) Simpson in low-density stand (PD1), intermediate-density stand (PD2) and high-density Chinese fir stand (PD3) at 0–20 cm (U) and 20–40 cm (L) soil layers.





**Figure 4.** Biplot ordination of non-metric multidimensional scaling (NMDS) of (a) weighted UniFrac and (b) unweighted UniFrac distances in two soil layers of different density stands of Chinese fir plantations. <sup>a</sup> Soil layers, (10–20 (U) and 20–40 cm (L)). <sup>b</sup> PD1, low-density stands; PD2, intermediate-density stands; PD3, high-density stand.



**Figure 5.** UPGMA/hierarchical clustering analysis based on weighted UniFrac distances showing the relative abundance of the most abundant fungal phylum in two soil layers of different density stands of Chinese fir plantations (1, 2, and 3 are the replications). <sup>a</sup> U means upper layer (10–20 cm), and L means lower layer (20–40 cm). <sup>b</sup> PD1, low-density stand; PD2, intermediate-density stand; PD3, high-density stand.

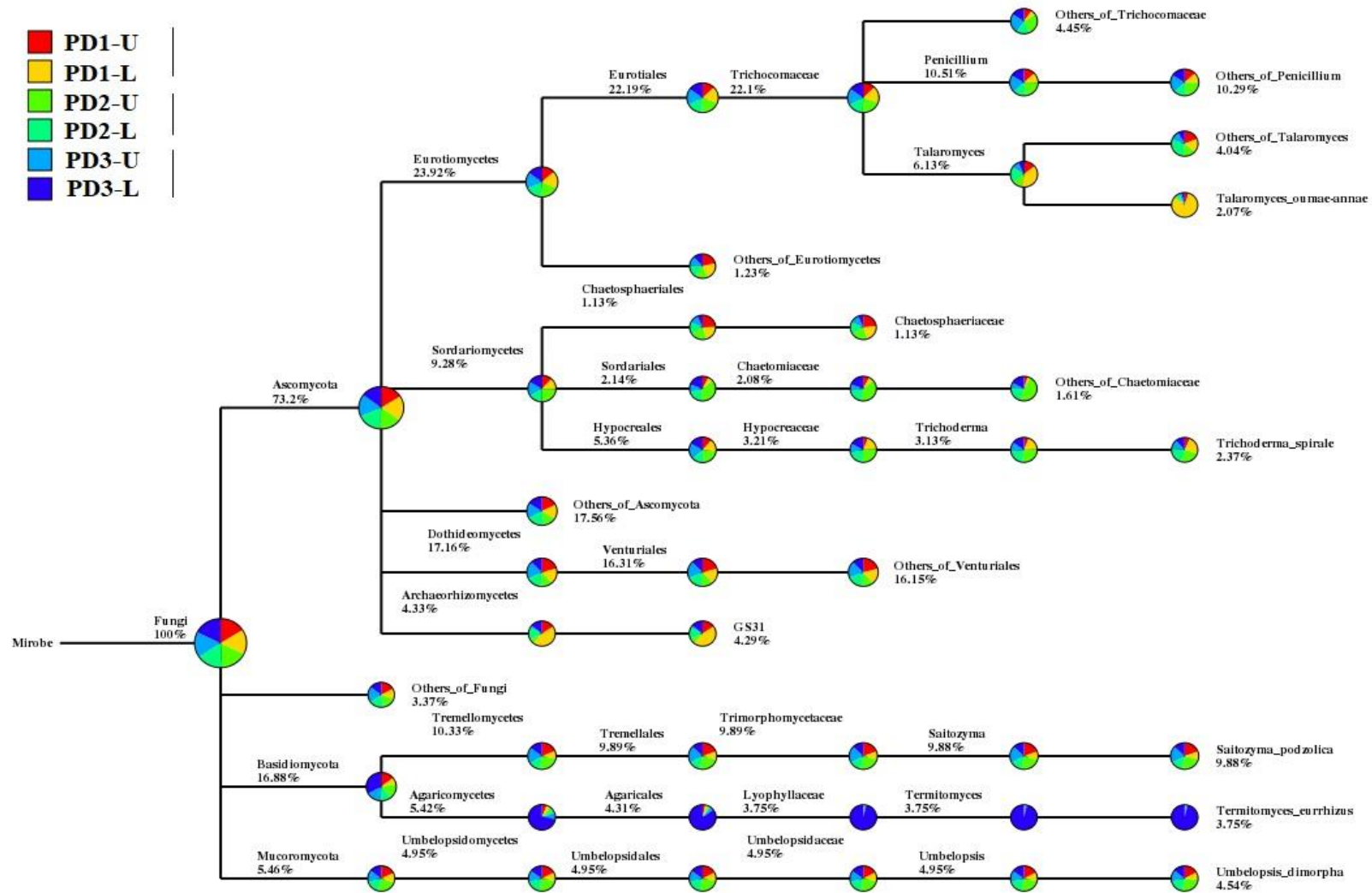
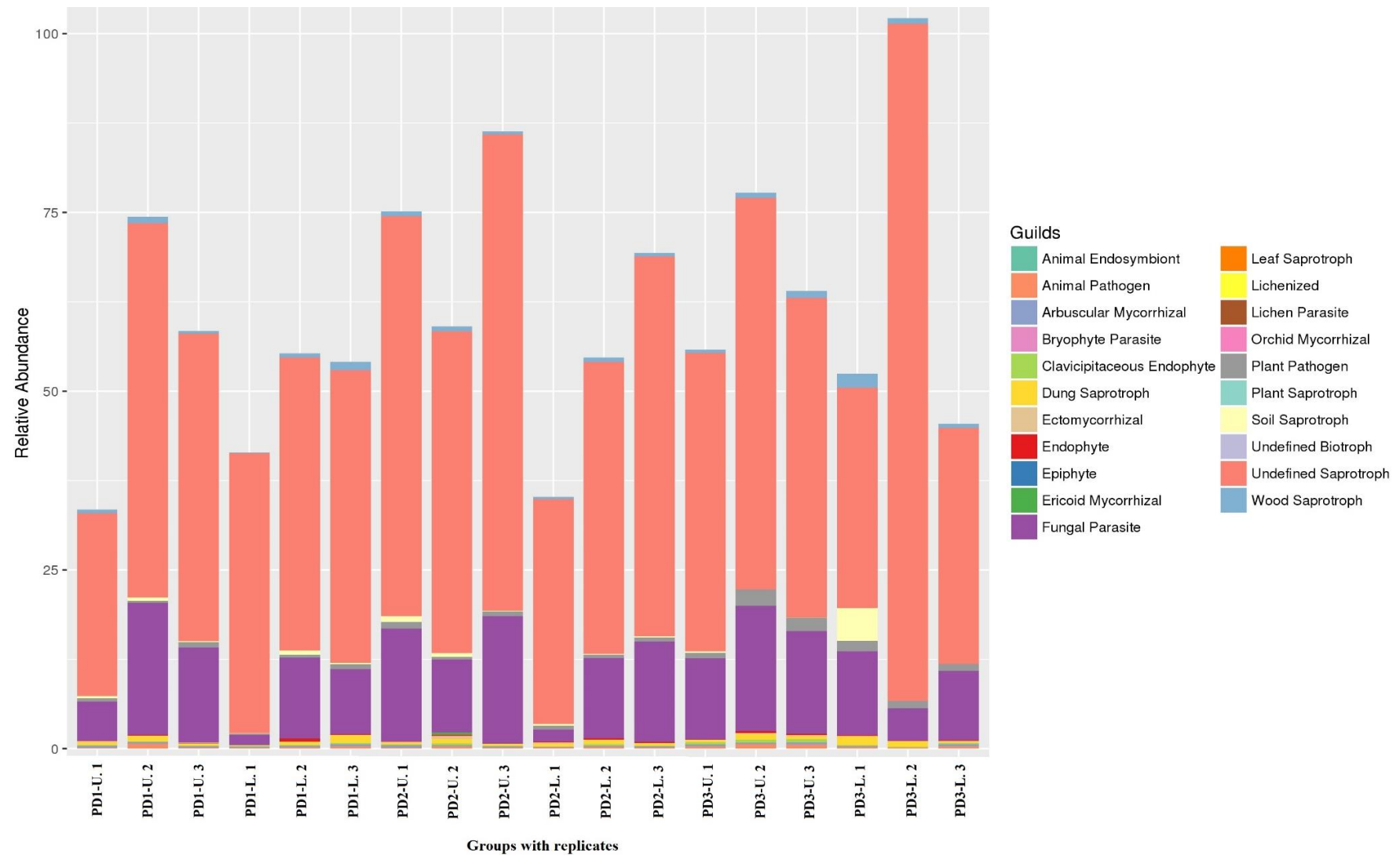


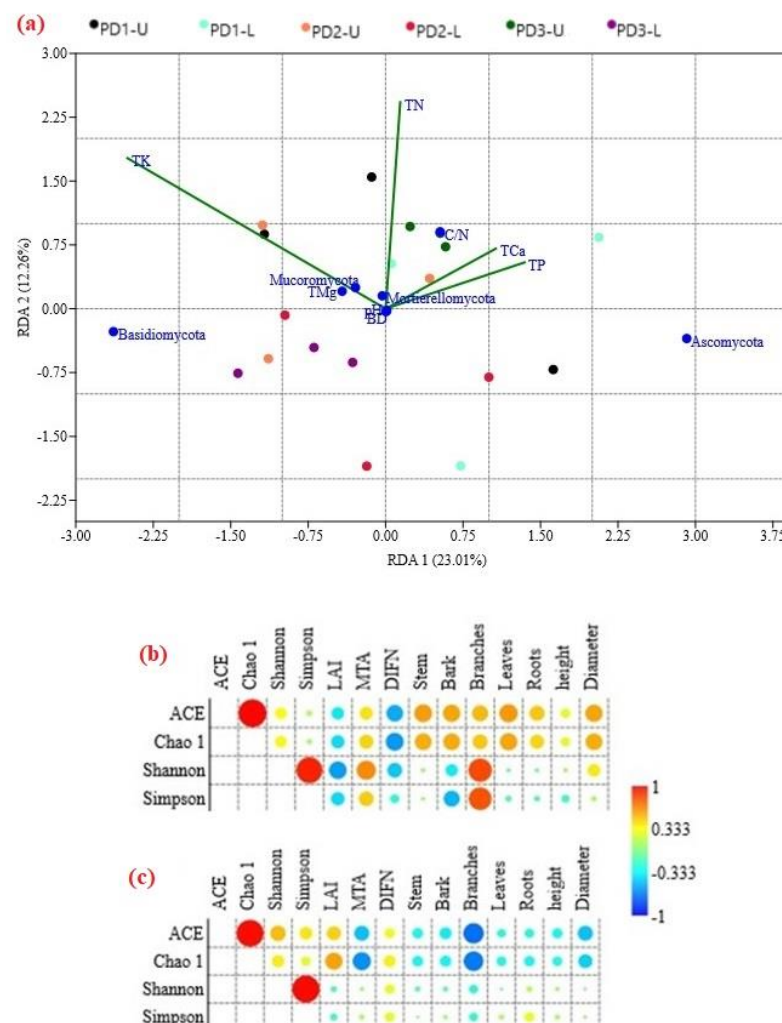
Figure 6. All groups fungal taxonomy in low-density stand (PD1), intermediate-density stands (PD2), and high-density Chinese fir stand (PD3) at 0–20 cm (U) and 20–40 cm (L) soil layers.



**Figure 7.** Variation in fungal function and the structure of the fungal functional groups (guilds) observed in low-density stands (PD1), intermediate-density stands (PD2), and high-density Chinese fir stands (PD3) at 0–20 cm (U) and 20–40 cm (L) soil layers.

### 3.3. Potential Drivers of Soil Fungal Diversity

The abundance of distinct kinds of soil fungal communities was variable and correlated differentially with soil physicochemical parameters and alpha diversity indicators. RDA results showed that soil physicochemical properties accounted for 35.27% of the total migration of the soil fungal communities. RDA indicated that TK and TN were the most dominating physicochemical properties for the soil fungal diversity, followed by TP, TCa, C:N, BD, and pH. *Mucoromycota* showed a substantial positive connection with TK and TMg. Similarly, RDA also indicated a positive correlation among *Mortierellomycota* with TP and TCa (Figure 8a). In the upper layer of the soil profile, the correlation between different alpha diversity analyses revealed a negative correlation between the DIFN with ACE and Chao1. Similarly, ACE and Chao1 had a substantial positive link with diameter and leaves, bark, and stem biomass, while SSD had a significant negative correlation with branch biomass. Aside from that, MTA was found to have a good relationship with SSD. Branch biomass had a negative connection with ACE and Chao1 in the lowest layer of the soil profile. While MTA showed a negative correlation with Chao1 (Figure 8b,c).



**Figure 8.** (a) Distance-based redundancy analysis (RDA) of the correlation between the most abundant phylum of fungi and soil physicochemical properties, such as total nitrogen (TN), total phosphorus (TP), total potassium (TK), total calcium (TCa), total magnesium (TMg), C/N ratio, soil pH, and soil bulk density (BD) in two soil layers of different density stands of Chinese fir plantations. (b) Correlation of the  $\alpha$ -diversity indices at 10–20 cm and (c) 20–40 cm soil layer with LAI, MTA, DIFN, stem biomass, bark biomass, branches biomass, leaves biomass, roots biomass, average height, and mean diameter of different density stands of Chinese fir plantations in Xinkou forest plantations, Sanming, Fujian, eastern China. <sup>a</sup> Soil layers, 10–20 (U) and 20–40 cm (L). <sup>b</sup> PD1, low-density stands; PD2, intermediate-density stands; PD3, high-density stands.



#### 4. Discussion

Soil pH is commonly recognized as a critical factor for plant growth and development. In study plots, the soil pH ranged from 4.2 to 4.3, indicating an acidic nature of the soil; this is somewhat lower than the ideal soil pH range for regular growth, which is 4.5 to 6.5 and metabolism of the Chinese fir plant [20,26]. Previous research depicted that soil pH is an important factor in determining the composition of fungal communities [27,28]. This is an important factor because soil pH can affect fungal diversity and population dynamics by affecting nutrient availability or imposing physiological constraints on fungal growth. These phenomena are also supported by some previous research indicating that soil pH significantly impacts fungal populations [29–32].

The fungal phylum of *Ascomycota* and *Basidiomycota* was significantly higher in terms of relative abundance in all the soils. *Ascomycota* is the most numerous and diverse phylum of eukaryotes in agricultural soils and the decomposers of organic substrates (e.g., leaf litter, wood, and manure) [25,33]. Moreover, as a key decomposer, *Basidiomycota* produces enzymes (peroxide) that degrade plant components such as cellulose and lignin, hence increasing the soil's overall carbon pool [34]. The fungal genus of *Saitozyma* was most abundant in all densities; similar results were also seen in pine forest areas by [35]. The species belonging to this genus are typically soil-borne yeasts and often also found in environmental sequencing studies [36]. In the soil and litter of forests, species from this genus are also most abundant and involved in the decomposition dead plant biomass [37]. Overall, this genus is involved in enhancing the total carbon pool of forest soils. The abundance of *Saitozyma* in forest soils was in accordance with the [38]. The fungal genus of *Penicillium* was second most abundant in all the soil samples, followed by *Talaromyces*. These species are involved in nutrient cycling, especially P cycling and decomposition of organic materials [39].

In both vertical soil profiles, fungal alpha diversity fell dramatically from low-density to intermediate-density, followed by an increase in the high-density stand, which directly links with the variations in soil pH. An important influence of soil pH on fungal alpha-diversity has been reported in various soils [40–42], proving that soil pH is a determinant of fungal diversity. A global meta-analysis has corroborated this finding that soil pH is one of the most important indicator for any modification in soil fungal diversity [43]. NMDS analysis revealed that there are considerable changes in the fungal community of the soil among different stand density soils, which were possibly due to direct influence by plant root exudates [44], suggested that the fungal community assembly is seriously affected by plants because it has an essential role in structuring fungal communities. Combined with RDA results, we can assume that the fungal community structure has undergone significant changes under different stand density compositions, which can be attributed to differences in soil chemical properties [45].

Changes in soil nutrient status significantly impact the organization and makeup of the soil microbial population [25,46]. In our present study, soil TP, TN and TK were the main driving force in changing fungal community compositions in both soil vertical profiles [47]. Other than this, soil TCa and TMg were also associated with the abundance of few fungal classes. In our study, P was positively correlated with phylum *Mortierellomycota*, which is in accordance with Zhang et al. [48] and negatively associated with unclassified communities of phylum *Basidiomycota*, this is in line with the findings of prior studies by Lauber et al. [46] and Gao et al. [49], they stated that members of phylum *Ascomycota* were abundant and *Basidiomycota* were fewer in the soils with a higher concentration of P and lower concentration of P respectively. These results also suggest that the fungal communities play an important role in the plants' utilization and absorption of soil P. Similarly, Ca is also an important element in regulating fungal cells, especially in the phylum *Basidiomycota* [50]. Soil K had a positive correlation with members of phylum *Mucoromycota*, which is in accordance with the findings of [51]. The findings of FUNGuild revealed that soil samples from sites with higher levels of organic materials effectively reduced the enormous number of endophytes and plant pathogens while increasing the

abundance of animal pathogens [25,51–53]. The Pearson correlation analysis between different alpha diversity indices and different plant morphological indices showed a significant correlation. For example, the correlation of ACE and Chao1 indices with DIFN was significantly negative in the upper layer of vertical soil profile. The possible reason behind this was that an open canopy provides greater daytime heating of the soil to many fungal communities [54,55]. These associations are likely to have substantial consequences for fungal and other belowground communities [1]. Stand density solely does not influence the microbial biota and soil health directly. But, with a combination and an interplay of other biotic and abiotic factors such as species autecology [10], organic amendments [56,57], plant growth regulator [58], nutrient distribution [59,60], different stress conditions [61–63], greenhouse gas emissions [64–67], soil pollutants different [68], planting materials and forest composition [69], and atmospheric deposition levels.

## 5. Conclusions

We find substantiation of the complexity of belowground ecology by displaying a divergence of patterns of relative abundance and richness between soil fungi at different stand density levels using various factors such as vertical soil profiles, stand density, and soil physicochemical properties to study the composition and diversity of soil fungi across Chinese fir plantation systems. The results showed that low stand density soil had more alpha diversity of soil fungi than the high-density stand soils. The most abundant soil fungal phylum were *Ascomycota*, *Basidiomycota*, *Mucromycota* and *Mortierellomycota*, respectively. Although soil physicochemical properties and vertical soil profiles were contributing factors for the fungal beta diversity, compared to different stand density compositions, these were less influencing the structure of fungal communities. Out of all stand densities, low-density stand and DIFN were the significant factors influencing the fungal beta diversity. The major influencers for modifications in soil fungal communities are TN, TP, and TK. This research will aid in a better understanding of the diversity and organization of key soil fungal communities in response to varying stand density levels, plant morphology, soil parameters. It will provide a conceptual framework for more sustainable forest management in the Chinese fir ecosystem and other plantation crops.

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