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Responses of Fungal Community Structure and Functional Composition to Short-Term Fertilization and Dry Season Irrigation in *Eucalyptus urophylla* × *Eucalyptus grandis* Plantation Soils

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Abstract: Plantation forests productivity is severely limited by the seasonal drought and fertilization practices in South China. Soil nutrient and water availability influence soil fungal community, functional group diversity and the variation of plant productivity; however, the effects of irrigation and fertilization on fungal responses have rarely been studied. Here, we investigate the responses of fungal community structure and functional groups in *Eucalyptus* plantation soils to short-term fertilization (F), dry-season irrigation (W), short-term fertilization combined with dry-season irrigation (FW), and control (CK) treatments for ten months. A higher proportion of Basidiomycota was observed in the irrigation and/or fertilization treatments; conversely, lower proportions of Ascomycota and Mucoromycotina were observed in the only irrigation and fertilization treatments. Higher soil carbon contents and symbiotroph fungi (mainly Ectomycorrhizas) proportion were detected in the FW treatment, while low proportions of saprophytic and pathogenic fungi were observed in the FW treatment when compared with those in other treatments. These results may indicate that *Eucalyptus* tree growth under irrigation and fertilization condition was better than under fertilization only, irrigation only, or neither management. The results highlight that short-term fertilization and dry-season irrigation can shift fungal community structure and functional groups by regulating available soil moisture and nutrients. They also provide a theoretical basis for the development of more appropriate management approaches in the early stages of forest plantation.

Keywords: soil fungi; fungal diversity; seasonal drought; irrigation; fertilization; *Eucalyptus*; ectomycorrhizal fungi

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1. Introduction

Soil fungi are extensively distributed in forest ecosystems, with crucial roles in energy flow regulation and nutrient transformation in forest soils [1–3]. Fungal communities also participate in biogeochemical cycling, including nutrient and soil carbon (C) cycling, generally by decomposing organic matter, or producing extracellular enzymes to decompose cellulose and lignin [4–6], which enhance forest ecosystem sustainability and productivity [4]. Some studies have investigated the effects of fertilization or water treatment on soil fungal biomass [5,7], soil fungal functional composition [4,8], and arbuscular mycorrhizal (AM) fungal community structure [9] in terrestrial ecosystems. Recently, microorganisms

have also been integrated into ecosystem process models to improve the predictive powers of the models to underground C and nitrogen (N) cycle dynamics [10].

Fungi are considered sensitive indicators of soil health and quality and are strongly influenced by agricultural and forestry management practices [2,6,11]. Therefore, a comprehensive understanding of the responses of fungal community structure and function in soils could facilitate the development of more appropriate management practices in agroforestry ecosystems. However, unraveling the complex interactions between fungi and their environments is challenging. Notably, the functional compositions of the fungal communities rather than their taxonomy compositions in natural environments are closely linked to environmental factors [2,6,11]; in other words, fungal community functions are similar in similar environments, although the compositions of the functional species could vary greatly across distinct ecological environments [12,13].

Most previous studies have focused on the responses of fungal community composition or soil extracellular enzymes to environmental change [14,15]; on the contrary, relatively few studies have explored the responses of fungal functional groups to change in biotic or abiotic factors [16]. Therefore, it is essential to investigate the functional characteristics of soil fungal communities [2,3]. A relatively recently developed fungi annotation tool, FUNGuild, offers methods of analyzing trophic dynamics of fungi, which facilitates the determination of fungal functions, as well as interactions among fungi, and associating them with ecological processes and functions [16]. Such tools have facilitated the comprehensive study of fungal dynamics in different ecosystems.

Soil available nutrients are one of key factors influencing wood productivity in forest plantations. Fertilization in forest ecosystems can influence soil ecological processes by directly improving nutrient availability [17,18]. However, previous studies have shown that the inorganic fertilizer amendment has negative, neutral [19,20], or positive impacts on fungi [21], so that the effects of fertilization on fungal communities and function remain controversial. Such inconsistencies could be related to fertilizer type, local nutrient status and vegetation type [6,22,23]. In addition, the soil moisture content is a key factor influencing soil nutrient availability and as well as soil fungal community structure and function [24,25]. Therefore, shifts in soil fungal community structure and functional groups could be influenced by soil nutrients and water availability.

Eucalyptus sp. is an extremely fast-growing tree species that is cultivated globally [26,27]. To achieve high wood productivity, *Eucalyptus urophylla* × *Eucalyptus grandis* and *E. grandis* are planted under intensive management practices during their short-rotation plantations [26]. Due to their high capacity to assimilate nutrients and water from soils, silvicultural practices (such as fertilizer application, irrigation and weed control) positively influence the wood productivity and quality in *Eucalyptus* plantations [26–28]. Today, fertilization is an essential practice for the early growth of *Eucalyptus* plantations [27]. Forest plantation productivity is severely limited by seasonal drought in South China due to drought stress [27,29]. Furthermore, poor management practices and environmental change could affect microbial community structure and function, with potential risks to forest soil health and productivity [30].

Only a few studies have attempted to assess the responses of fungal communities to soil water supplementation in forest plantations. The productivity of plantation forests in South China is severely limited by the seasonal drought [28]. Furthermore, in tropical and subtropical forests with high amounts of rainfall, experimental studies with a combination of irrigation and fertilization treatments have hardly been carried out to investigate their impacts on soil fungal community structure and activity [17]. It is necessary to investigate the responses of fungi to dry-season irrigation and short-term fertilization in plantation soils.

In the present study, we investigated the influence of dry-season irrigation and short-term fertilization on soil fungal community structure and functional groups. We hypothesized that (1) short-term fertilization and dry-season irrigation management practices have distinct impacts on *Eucalyptus* growth, soil environmental factors, and fungal community composition; (2) different fungal functional groups respond differently to fertilization and

irrigation; (3) and soil environmental characteristics and fungi composition are correlated with *Eucalyptus* growth characteristics.

To test the hypotheses above, we carried out field experiments based on short-term irrigation and fertilization treatments, or their combination, in tropical forests in South China, to investigate potential shifts in fungal community structure and functional groups in *E. urophylla* × *E. grandis* plantation soils, using the 18S rRNA amplicon sequencing and FUNGuild.

2. Materials and Methods

2.1. Experimental Sites and Soil Sampling

The present study was conducted at a Teaching and Research station (23°14′48 N, 113°38′20 E) affiliated with the South China Agricultural University, in Zengcheng District, Guangzhou City, Guangdong Province, southern China [27]. The average annual temperature in the area is 21.91 °C and the average annual precipitation is approximately 2004.5 mm. The area experiences a rainy season (April to September) and a dry season (October to next March), which account for approximately 82.69% and 17.31% of the whole-year rainfall, respectively. The soil in the area is a typical red soil, and the main soil properties (0–20 cm depth) are as follows: pH = 4.92; soil organic matter = 7.19 g kg^{-1}; TN = 0.37 g kg^{-1}; TP = 0.16 g kg^{-1}; AK = 0.29 mg kg^{-1}, and total K (TK) = 8.83 g kg^{-1} [27].

Three-month-old clones of *E. urophylla* × *E. grandis* (DH32-29) seedlings were used in the present study. They were cultured in vitro by Guangdong Gaoyao Forestry Development Co., Ltd., China. Seedlings were planted with 3 × 2-m spacing (1650 plants/ha planting density) in April 2017. An orthogonal experimental design, as described by Yu et al. [27], was adopted for the water and fertilizer treatment. The total study area was 0.536 hectares with five terraces using the adoption of the horizontal terraced land preparation method. Each terrace had four plots with different treatments as described in Hua et al. [29]. The four treatments were: control (CK) (without irrigation or fertilization treatment), fertilization only (F) (anhydrous fertilization treatment), dry-season irrigation only (W), and short-term fertilization combined with dry-season irrigation (FW) treatments. In the irrigation treatments, the relative WC of the soil from a depth of 40 cm and 40 cm away from the trees was maintained at 90% for three days in the dry season [27]. Fertilization (F and FW treatment) was applied at the beginning of planting with top dressing fertilizer (40 kg N ha^{-1} $year^{-1}$; 24 g N, 72 g P_2O_5, and 24 g K_2O) and localized placement (75 kg N ha^{-1} $year^{-1}$; 45 g N, 21 g P_2O_5, 24 g K_2O) was applied in July 2017. Water supplementation in the dry season (W and FW treatment) was carried out only in the dry season, with irrigation carried out twice a week. Irrigation was conducted for 4 h each time, with 4 L of water applied per hour for each plant, yielding 32 L of water for 8 h each week for each plant. The irrigation period was from 1 October 2017 to January 2018, for a total of four months. Each plant was supplied with 512 L of water in total.

All 20 plots were sampled on 7 January 2018. In each plot, 10 soil cores (0–20-cm depth and 4-cm diameter) were randomly obtained using a cylindrical soil borer along with each plant at 5 m from each other. The litter layer was discarded, and the samples were homogenized thoroughly. Each soil sample was sieved through a 2-mm mesh to remove plant roots and stones and then divided into three parts. One part was immediately frozen in liquid N and then stored at −80 °C for total DNA isolation, another part was air-dried and stored at room temperature for soil physicochemical property analyses, and the other part was stored at 4 °C for use in soil microbial biomass and available N and P analyses.

2.2. Soil Physicochemical Property Analyses

A small aluminum box of soil samples was taken from three random points in each experimental plot. All the boxes were weighed and placed in an oven at 105 °C until the weights were constant. Dry weights were recorded and the soil WC (%) = (fresh weight − dry weight)/(dry weight − aluminum box weight) × 100% was calculated. Soil pH was determined using a pH-meter (PHS-3E, INESA Scientific Instrument Co., Ltd., Shanghai,

China) in a 1:2.5 soil/water suspension. Soil total nitrogen (TN) and soil organic carbon (SOC) were evaluated as described previously by Finzi et al. [31,32]. Total phosphorus (TP) and total potassium (TK) were determined using the NaOH molten–molybdenum antimony colorimetric method [33]. Soil inorganic N was extracted with 2 mol L^{-1} KCl, and the nitrate-nitrogen (NO_3^--N) and ammonia-nitrogen (NH_4^+-N) levels were calorimetrically using a continuous flow analyzer (Lachat Instruments, Mequon, WI, USA) [33]. AP was extracted using the ascorbic acid reductant method, and AK was assessed using an atomic absorption spectrophotometer [33]. Five soil samples were analyzed in each treatment.

2.3. Soil Microbial Biomass

Microbial biomass carbon (MBC), dissolved organic C (DOC), and microbial biomass nitrogen (MBN) were determined according to methods in previous studies [32,34].

2.4. Soil Total DNA Extraction

Soil total DNA was extracted from approximately 0.5 g of wet soil per sample using a PowerSoil DNA Isolation Kit (MoBio Laboratories, Carlsbad, CA, USA) according to the manufacturer's instructions. The DNA concentrations and quality were measured using a Nanodrop 2000 Spectrophotometer (Nanodrop Technologies, Wilmington, DE, USA). DNA integrity and purity were measured on 0.8% agarose gels. DNA was diluted with sterile water to a final concentration of 1 ng μL^{-1}.

2.5. PCR Amplification and Illumina Sequencing

The V5–V7 region of fungal was amplified by an ABI GeneAmp® 9700 PCR thermocycler (Applied Biosystems, Carlsbad, CA, USA) as described by Zhao et al. [35]. Polymerase chain reaction (PCR) was performed as follows: 4 µL 5 × TransStart FastPfu buffer, 2 µL 2.5 mM dNTPs, 0.8 µL forward primer (5 µM), 0.8 µL reverse primer (5 µM), 0.4 µL TransStart FastPfu DNA Polymerase, 10 ng template DNA, and finally up to 20 µL ddH_2O. The following thermocycling conditions were applied: initial denaturation at 95 °C for 3 min, followed by 30 cycles of 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 45 s, with a single extension at 72 °C for 10 min, and termination at 4 °C. PCR experiments were performed with five biological replicates according to a previous report [36]. The PCR products were extracted from 2% agarose gel and purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) according to the manufacturer's instructions, and quantified using Quantus™ Fluorometer (Promega Corporation, Madison, WI, USA). An equimolar mix of all three amplicon libraries was used for sequencing on an Illumina MiSeq platform (Illumina, San Diego, CA, USA) at Shanghai Majorbio Bio-Pharm Technology Co. Ltd. (Shanghai, China) The sequencing raw data with the FASTQ files were quality filtered using Trimmomatic and merged using FLASH v1.2.11 (https://ccb.jhu.edu/software/FLASH/, accessed on 1 December 2019) based on recommended criteria, and the raw data were deposited in the NCBI Sequence Read Archive under accession no: PRJNA637913 [37].

2.6. Bioinformatics Analyses

All data were processed on the Majorbio Cloud Platform (http://www.majorbio.com, accessed on 3 May 2020). Sequences were processed and analyzed according to the procedure described by Yu [38]. Sequences that had the following three criteria were maintained: (1) precise primers and bar-codes; (2) quality score > 20; and (3) length > 200 bp. The operational taxonomic units (OTUs) with 97% similarity were clustered using UPARSE v7.1 (http://drive5.com/uparse/, accessed on 3 May 2020), and chimeric sequences were identified and removed [39]. The taxonomy of each OTU representative sequence was analyzed using RDP Classifier (http://rdp.cme.msu.edu/, accessed on 3 May 2020) against the Silva SSU132 database (https://www.arb-silva.de/, accessed on 3 May 2020) using a confidence threshold of 0.7. Identified OTUs were assigned to functional groups (Trophic modes and guilds) using the FUNGuild annotation tool [16]

according to Nie et al. 2018 [6]. Fungal alpha diversity was calculated based on OTUs and fungal community diversity using the Shannon index, Simpson index, and Chao1 index using MOTHUR v.1.30.1 [40]. The ANOSIM (999 permutations, non-parametric) and non-metric multidimensional scaling (NMDS) with the Bray-Curtis were used to examine the dissimilarities of the fungal community compositions among treatments [41,42].

2.7. Statistical Analysis

Potential soil environmental factors influencing soil fungal community structure and functional groups were selected as environmental variables, including WC, soil pH, N parameters (TN, NO_3^--N and NH_4^+-N), TP, AP, AK, SOC, DOC, MBC and MBN. Detrended correspondence analysis (DCA) was performed using species data (97% similarity sample OTU table), and the first axis of lengths of the gradient in the analysis was less than 3.5. Variations in the fungal community structure, fungal functional groups, and environmental factors were evaluated using redundancy analysis RDA by the "vegan" package in R v3.6.3, the environmental variables and soil microbial biomass from different treatments were subjected to a one-way analysis of variance (Duncan test, $p < 0.05$) using the IBM SPSS Statistics 22.0 (IBM Corp., Armonk, NY, USA). Significant differences between mean values were indicated using different letters. Correlations between tree growth and physicochemical properties and fungal community structure were calculated using the linear regression model method. GraphPad Prism 6.0 (GraphPad Software, San Diego, CA, USA) was used to draw figures.

3. Results

3.1. Effects of Fertilization and Irrigation on Eucalyptus Growth

The heights, crown diameter, ground diameters (GD), and diameter at breast height (DBH) of *E. urophylla* × *E. grandis* were significantly higher in the fertilization and/or irrigation treatments than in the CK treatment and were in the order of FW > F > W > CK (Table 1). The heights and DBHs of *E. urophylla* × *E. grandis* in the fertilization treatments were more than 2.52-fold those in the CK treatment, respectively. However, the clear bole height (CBH) of *E. urophylla* × *E. grandis* increased significantly in the fertilization treatment when compared with that in the control, whereas plant CBH decreased in the fertilization treatment when compared with that in the control, independent of the water supply condition (Table 1). Overall, according to the results above, dry season irrigation and short-term fertilization promoted *Eucalyptus* growth significantly.

Table 1. *Eucalyptus* plantation growth under the irrigation and fertilization in January 2018.

Treatment	Number	Height/cm	CBH/cm	Crown Diameter/m	GD/mm	DBH/mm
CK	168	163.02 ± 84.03 d	24.35 ± 8.32 b	1.20 ± 0.46 d	25.85 ± 14.68 c	17.23 ± 8.04 c
W	194	191.39 ± 85.02 c	29.61 ± 9.67 a	1.30 ± 0.40 c	29.54 ± 14.56 b	20.57 ± 8.24 b
F	195	451.55 ± 106.67 b	22.18 ± 8.85 c	2.18 ± 0.29 b	63.41 ± 14.59 a	43.39 ± 13.56 a
FW	186	488.94 ± 91.98 a	25.86 ± 9.36 b	2.27 ± 0.20 a	66.14 ± 12.80 a	45.99 ± 11.85 a [1]

[1] CK: control; W: irrigation only; F: fertilization only; FW: fertilization and irrigation; CBH: Clear Bole Height; GD: Ground Diameter; DBH: Diameter at Breast Height. Different letters indicate significant differences in individual parameters among the four treatments detected using the Duncan test ($p < 0.05$). Mean ± standard error of the mean (SEM) ($n = 5$).

3.2. Effects of Fertilization and Irrigation on Soil Physicochemical Properties

The effects of the fertilization and dry season irrigation treatments on soil physicochemical properties in the *Eucalyptus* plantation topsoil (0–20 cm depth) are presented in Table 2. When compared with the CK treatment, the only irrigation treatment increased soil water content (WC) remarkably, and significantly increased soil nutrients (such as ammonium-N (NH_4^+-N), total phosphorus (P; TP), and available potassium (K; AK)). In contrast, WC decreased in the F treatment, but soil nutrient contents (such as total N(TN)), nitrate-N (NO_3^--N), NH_4^+-N, TP, and AK) increased significantly (Table 2), when com-

pared to the levels in the CK. In addition, in the fertilization combined with irrigation treatment, not only WC but also soil nutrients (such as soil TN, NO_3^--N, NH_4^+-N, TP, AP, and AK) increased significantly, when compared to the levels in the CK. Conversely, in the only fertilization or irrigation treatment, soil pH decreased significantly, and increased slightly in the fertilization combined with irrigation treatment, when compared to the levels in the CK treatment. Soil organic C (SOC) content in the fertilization combined with irrigation treatment was significantly higher than in the CK treatment (Table 2). However, there were no differences in SOC contents between the CK and F treatments, and SOC contents were significantly lower in the W treatment. Compared to the CK treatment, the DOC contents were significantly higher in fertilization and/or irrigation treatments, and microbial biomass C (MBC) contents were higher in the fertilization treatments; furthermore, there were no differences in MBC contents among the fertilization and/or irrigation treatments. The microbial biomass nitrogen (MBN) was higher in the fertilization treatment than in the CK treatment. The results suggest that dry season irrigation and/or short-term fertilization alter soil physiochemical properties, while only dry season irrigation treatment did not influence MBC and MBN contents.

Table 2. Physicochemical properties of the *Eucalyptus* plantation top-soil (0–20-cm depth) under dry season irrigation and short-term fertilization.

Treatment	CK	W	F	FW
Moisture (%)	10.09 ± 0.45 c	15.40 ± 0.29 a	9.36 ± 0.30 d	11.68 ± 0.36 b
Soil pH	4.94 ± 0.18 a	4.76 ± 0.03 b	4.64 ± 0.04 b	5.03 ± 0.04 a
TN (g kg^{-1})	0.18 ± 0.00 b	0.19 ± 0.01 b	0.22 ± 0.02 a	0.21 ± 0.00 a
NO_3^--N (mg kg^{-1})	0.93 ± 0.17 b	0.96 ± 0.13 b	1.85 ± 0.83 a	2.17 ± 0.24 a
NH_4^+-N (mg kg^{-1})	4.80 ± 0.63 b	6.63 ± 0.40 a	6.66 ± 0.57 a	7.04 ± 0.95 a
TP (g kg^{-1})	0.13 ± 0.00 c	0.14 ± 0.01 b	0.15 ± 0.00 a	0.16 ± 0.00 a
AP (mg kg^{-1})	0.20 ± 0.00 b	0.23 ± 0.02 b	0.42 ± 0.03 b	6.02 ± 0.78 a
AK (mg kg^{-1})	9.49 ± 0.25 c	10.52 ± 0.23 b	11.75 ± 0.58 a	11.41 ± 0.41 a
SOC (g kg^{-1})	7.53 ± 0.19 b	6.64 ± 0.55 c	7.81 ± 0.50 b	8.40 ± 0.33 a
DOC (mg kg^{-1})	342.325 ± 17.48 b	376.44 ± 33.02 a	407.88 ± 22.29 a	408.67 ± 4.90 a
MBC (mg kg^{-1})	49.22 ± 13.40 b	52.83 ± 3.63 b	75.61 ± 9.13 a	81.93 ± 8.73 a
MBN (mg kg^{-1})	22.60 ± 4.81 b	29.00 ± 3.44 ab	31.90 ± 13.72 a	32.63 ± 8.08 a [1]

[1] CK: control; W: irrigation only; F: fertilization only; FW: irrigation and fertilization. TN: total nitrogen; NO_3^--N: nitrate nitrogen; NH_4^+-N: ammonium nitrogen; TP: total phosphorus; AP: available phosphorus; AK: available potassium; MBN: microbial biomass nitrogen; SOC: Soil organic carbon; DOC: dissolved organic carbon; MBC: microbial biomass carbon. Different letters indicate significant differences in individual parameters among the four treatments detected by Duncan test ($p < 0.05$). Mean ± standard error of the mean (SEM) ($n = 5$).

3.3. Effects of Fertilization and Irrigation on Soil Fungal Community Structure

After quality filtering, the remaining 1,150,303 high-quality sequences were clustered into 219 operational taxonomic units (OTUs). The majority of the sequences across all 20 samples examined belonged to the phyla Basidiomycota (59.96–74.43%) and Ascomycota (23.34–44.50%), and other taxa were present at low proportions (Figure 1a). At the phylum, the proportion of Basidiomycota was significantly increased, while the proportion of Ascomycota was decreased in the only irrigation or fertilization treatments than in the CK treatment, respectively (Figures 1 and A1). However, the difference in proportions of Basidiomycota and Ascomycota between the fertilization combined with irrigation treatment and CK treatment was not significant. The proportions of Glomeromycota and Mucoromycotina declined considerably in the fertilization (F and FW) treatments when compared with those samples in the CK or only irrigation treatment, whereas the proportion of Glomeromycota was higher in the only irrigation treatment than in the CK treatment. At the class level, the dominant taxa were Agaricomycetes, Eurotiomycetes, and Sordariomycetes in the top-soil of the *Eucalyptus* plantation (Figure 1b). The proportion of Agaricomycetes showed F > WF > W > CK, conversely, the Archaeorhizaomycetes was F/WF < W < CK. Compared with the CK treatment, the proportion of Eurotiomycetes was significantly decreased in the only irrigation or fertilization treatment, which was significantly increased in the fertilization combined with irrigation treatment. The proportion of Sordariomycetes presented as WF < F < W < CK (Figure 1b).

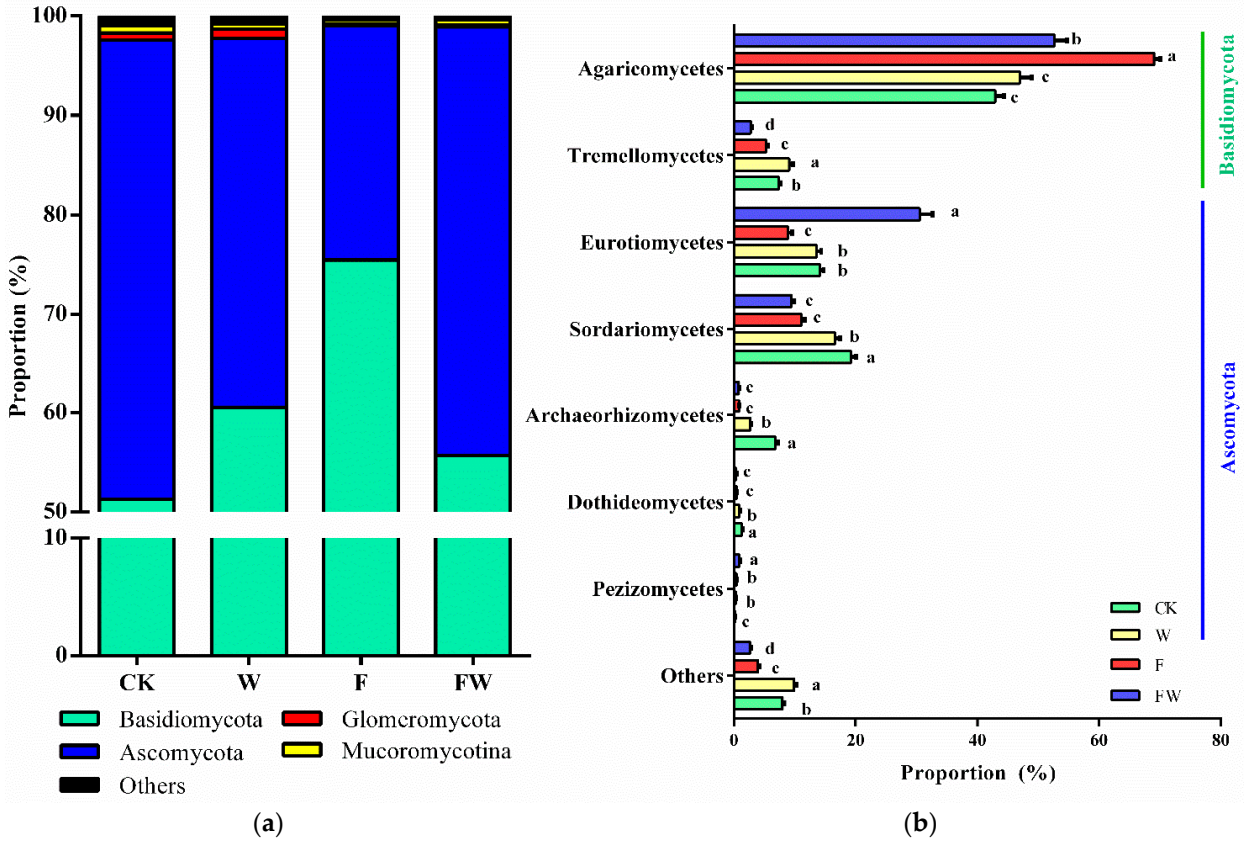

Figure 1. The dominant (Proportion more than 1%) soil fungal communities in the different treatments at (**a**) phylum and (**b**) class levels. Different letters indicate significant difference between treatments detected using the Duncan test ($p < 0.05$). CK: control; W: irrigation only; F: fertilization only; FW: irrigation combined with fertilization.

The α-diversity (including Shannon, Simpson, and Chao1) indices of the fungal community in the fertilized soils (F and FW treatments) were significantly lower than those in the non-fertilized soils (W and CK treatments) (Table 3). Moreover, nonmetric multidimensional scaling (NMDS) analysis was used to compare β-diversity among different treatments (Figure 2). According to the NMDS results, each treatment formed a group that was well separated from the other treatments (Analysis of Similarities (ANOSIM) = 0.926, $p = 0.001$) (Figures 2 and A2). Overall, the results indicated that irrigation and fertilization influenced soil fungal community structure considerably.

Table 3. Diversity parameters from high throughput sequencing of fungal 18S rRNA operational taxonomic units (OTUs) amplified from *Eucalyptus* plantation soils under irrigation and fertilization.

Treatment	Shannon Index	Simpson Index	Chao1 Index
CK	4.46 ± 0.08 a	0.91 ± 0.01 a	423.24 ± 19.84 a
W	4.41 ± 0.09 a	0.91 ± 0.01 a	424.82 ± 22.67 a
F	3.44 ± 0.12 b	0.81 ± 0.02 b	353.36 ± 28.10 b
FW	3.33 ± 0.25 b	0.81 ± 0.04 b	342.83 ± 16.36 b [1]

[1] CK: control; W: irrigation only; F: fertilization only; FW: irrigation combined with fertilization. Different letters indicate significant differences in individual parameters among the four treatments detected using the Duncan test ($p < 0.01$). Mean ± SEM ($n = 5$).

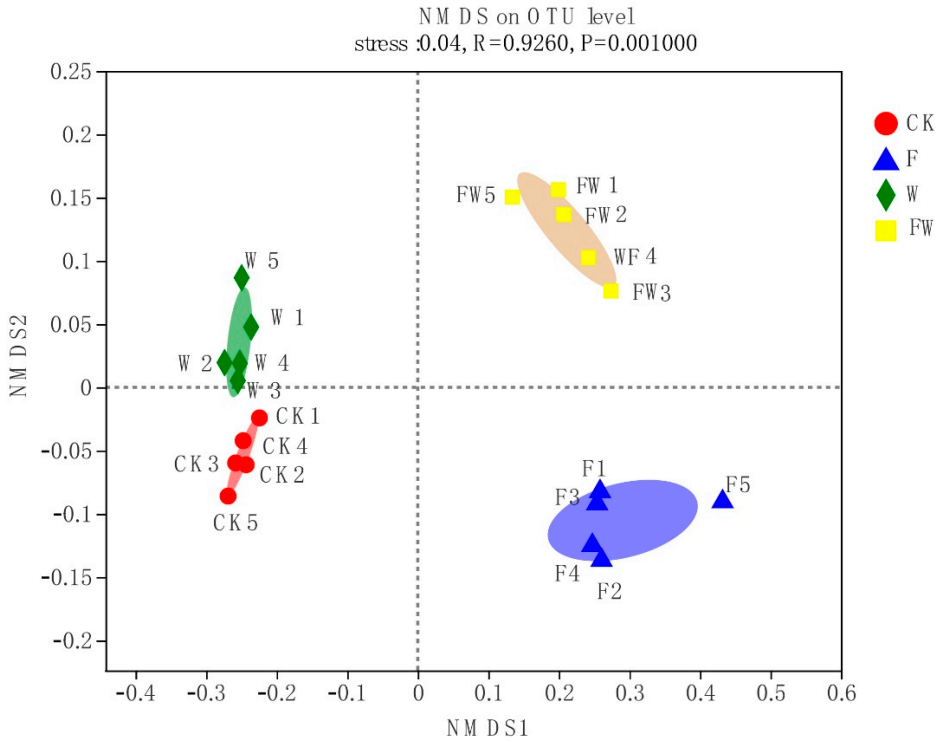

Figure 2. Non-metric dimensional scaling (NMDS) visualization of the β-diversity and fungal community structure in the soils under four treatments. CK: control; W: irrigation only; F: fertilization only; FW: irrigation combined with fertilization.

3.4. Fungi Trophic Groups and Guild Assignment

FUNGuild analysis was performed to predict the distributions of trophic modes with strict definition and reference. OTUs with 97% similarity were assigned into specific trophic modes, and then subdivided further into specific ecological communities (16). The three dominantly trophic modes (symbiotroph, saprotroph, and pathotroph) were identified, and the rest were considered unidentified fungi (Figure 3a). Symbiotrophs accounted for 9.65%, 8.66%, 72.13%, and 43.63% of the fungi across the CK, W, F, and FW treatments, respectively. In addition, there were no significant differences in the proportion of symbiotroph fungi between the W and CK treatments. The proportions of saprotroph fungi in the F (12.71%) and W (38.98%) treatments were significantly lower than those in the FW (43.60%) and CK (46.23%) treatments. Moreover, the proportion of pathotrophs was significantly higher in the CK treatment (22.63%) than in the W (18.31%), F (6.91%), and FW (6.65%) treatments (Figure 3a).

To obtain further detailed information on trophic modes across all the soil samples, we investigated the proportion of 11 trophic functional groups (including AM, Ectomycorrhizas, Endophyte, Soil saprotroph, Wood saprotroph, Dung saprotroph, Undefined Saprotroph, Stem saprotroph-wood saprotroph, Endophyte-fungal parasite, Animal pathogen, and Plant pathogen) using FUNGuild (Figures A1, 3b and 4a–c). Symbiotrophs were dominated by ectomycorrhizas, AM, and endophyte guilds, and the fungal taxa with the greatest proportions of ectomycorrhizal fungi, AM fungi, and endophyte guilds were *Pisolithus* (Basidiomycota), *Glomeromycota* (Glomeromycota), and *Phialophora verrucose* (Ascomycota), respectively (Figure 4d–f). The proportion of *Pisolithus* in the F (71.77%) and FW (43.19%) treatments increased significantly when compared with those samples in the CK treatment (7.31%); conversely, the proportion of AM fungi (*Glomeromycota*) and endophytes (*P. verrucose*) decreased significantly in the fertilization treatments when compared with those in the CK treatment. However, the only irrigation treatment did not significantly influence the proportion of *Pisolithus* (Figure 4d–f).

The dominant soil saprotroph, wood saprotroph, and undefined saprotroph taxa in all treatments were *Archaeorhizomyces*, *Sarcosomataceae*, and *Eurotiales*, respectively (Figure 4g). Specifically, the proportions of *Archaeorhizomyces* and *Sarcosomataceae* were decreased significantly in the fertilization (F and FW) treatments; the proportion of *Eurotiales* increased significantly in the fertilization combined with irrigation treatment, whereas the proportion of *Eurotiales* decreased significantly in the only irrigation treatment when compared with the proportions in the CK treatment. In addition, *Tremellales*, *Hypocrea*, and *Metarhizium* were the dominant taxa among the undefined fungi, endophyte-fungal parasite, and animal pathogen functional groups, respectively. Furthermore, the proportions of *Hypocrea* and *Metarhizium* decreased significantly in the fertilization and/or irrigation treatments when compared with the proportions in the CK treatment, whereas the proportion of *Tremellales* in the FW treatment was lower than that in the only irrigation or fertilization sample (Figure 4h). Overall, these results indicate that dry season irrigation and/or short-term fertilization had obvious effects on soil fungi functional groups.

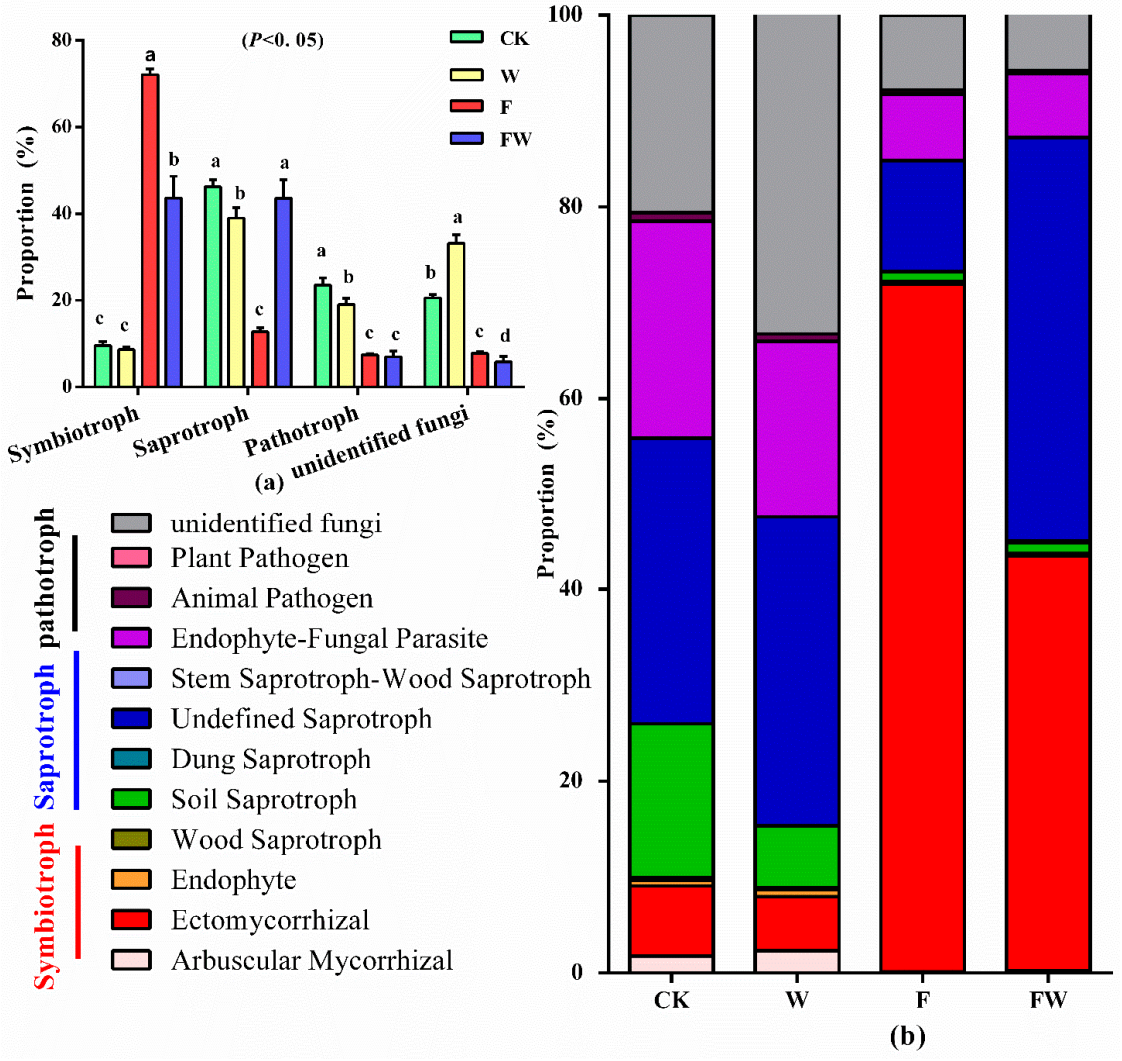

Figure 3. Variations in fungal function (**a**) and (**b**) compositions of fungal functional groups (guilds) inferred using FUNGuild. CK: control; W: irrigation only; F: fertilization only; FW: irrigation combined with fertilization. Different letters indicate significant differences in individual parameters among four treatments detected using the Duncan test ($p < 0.05$). Mean ± SEM ($n = 5$).

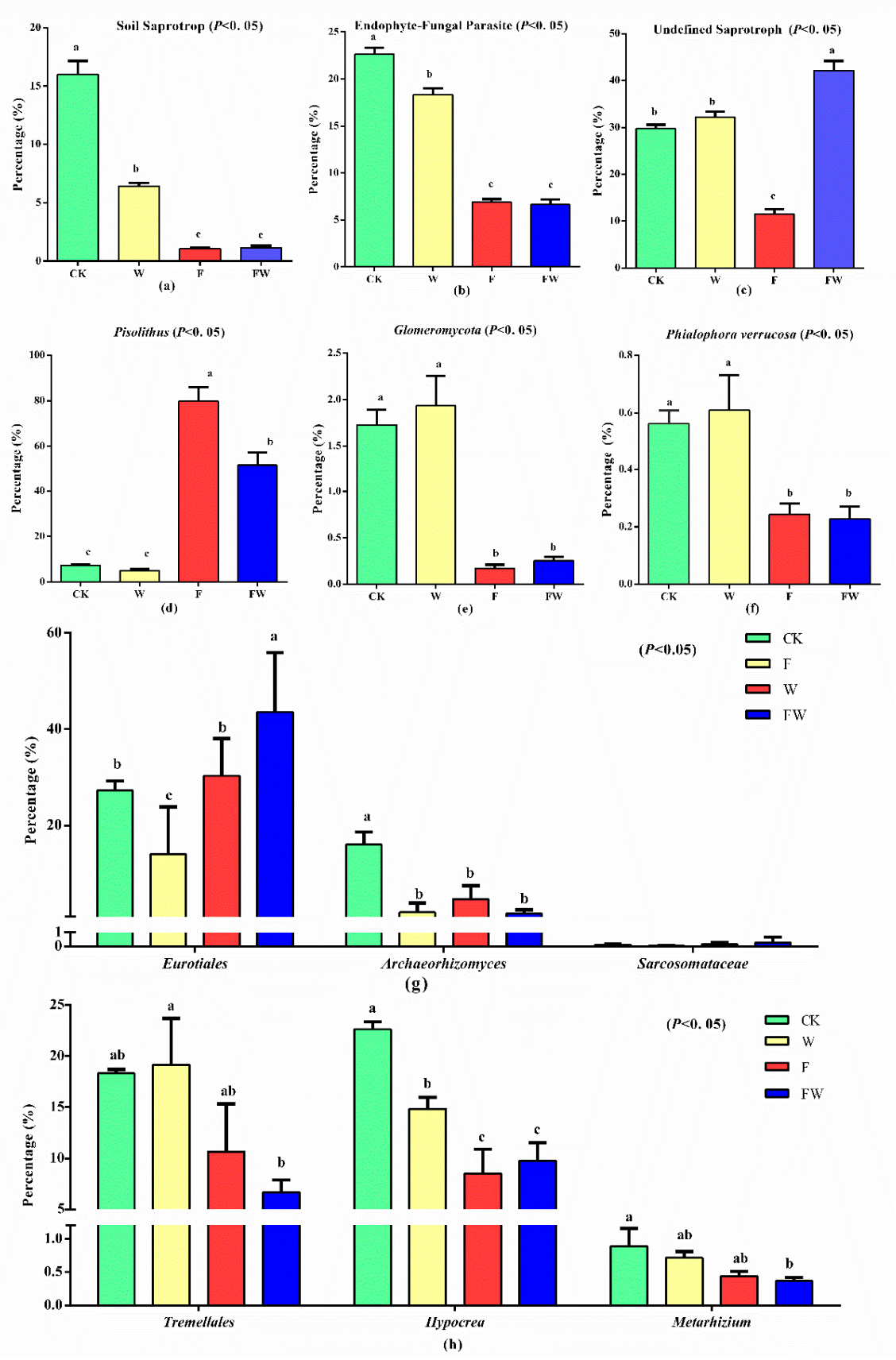

Figure 4. Percentage of dominant taxa including (**a**) arbuscular mycorrhizal, (**b**) ectomycorrhizal, (**c**) endophytes, (**d**) soil saprotroph, (**e**) endophyte-fungal parasite, (**f**) undefined saprotroph, (**g**) und-

efined fungi and (**h**) pathotroph in total identified GUIDs, respectively. CK: control; W: irrigation only; F: fertilization only; FW: irrigation combined with fertilization. Different letters indicate significant differences in individual parameters among the four treatments detected using the Duncan test ($p < 0.05$). Mean ± SEM ($n = 5$).

3.5. Effects of Environmental Factors on Fungal Community Structure and Functional Groups

To determine the relationships among fungal communities, functional groups, and environmental factors, redundancy analysis (RDA) was carried out. According to the results, soil physicochemical properties significantly explained shifts in fungal community structure (Figure 5). The first and second axes accounted for 74.56% and 15.3% of the shifts in fungal community structure, respectively (Figure 5a); similarly, Mantel test results showed that soil environmental factors (including WC, pH, NO_3^--N, NH_4^+-N, TN, MBC, microbial biomass N (MBN), TP, AP, AK, and dissolved organic C(DOC)) were the optimal predictors of fungal community structure ($p < 0.05$).

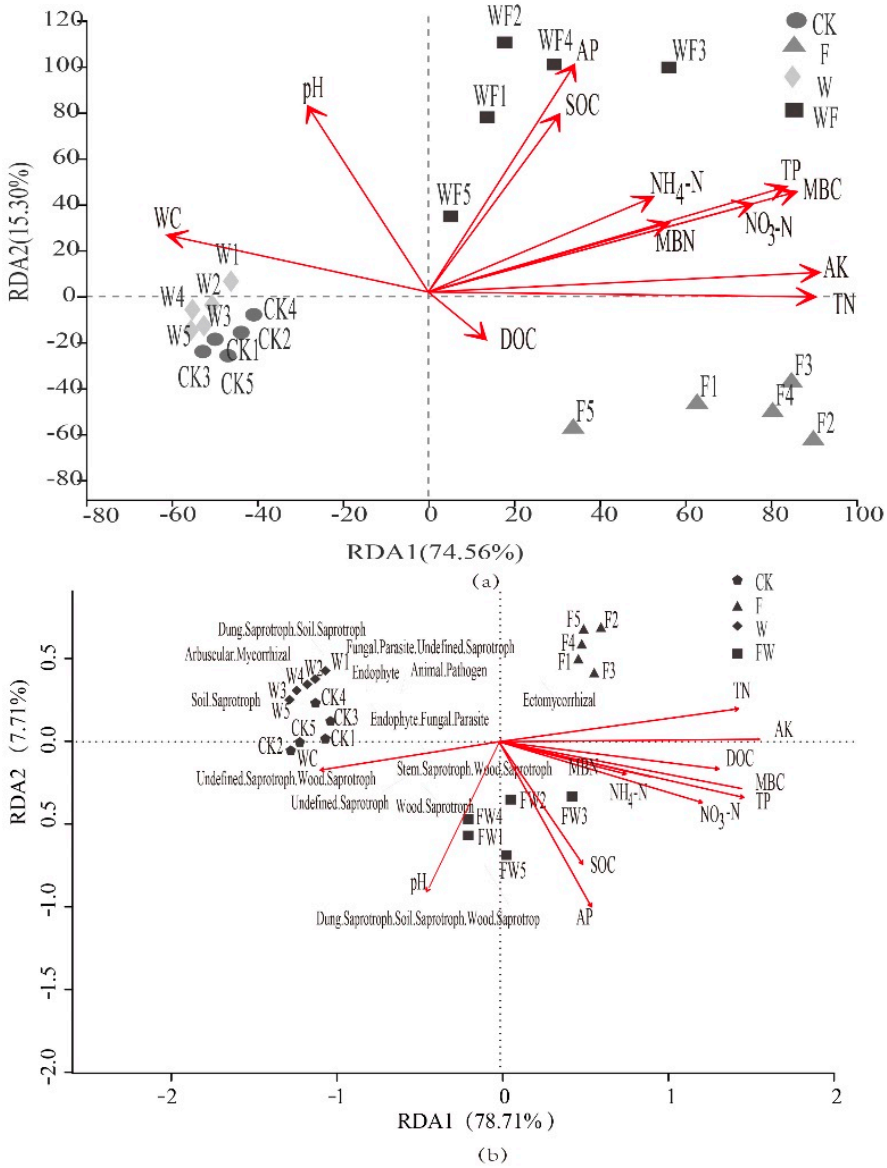

Figure 5. Redundancy analysis (RDA) of community structure for (**a**) the fungal order-level taxonomy and (**b**) function category for fungal function in different treatment soils ($n = 5$). ● CK (control); ▲ F (fertilization only); ◇ W (irrigation only); ■ FW (irrigation combined with fertilization); Arrows represent the environmental variables.

Furthermore, the relationships between fungal functional groups and environmental factors were analyzed (Figure 5b); the first and second axes accounted for 78.71% and 7.71% of the variation in fungal trophic functional guilds, respectively. In addition, according to the Mantel test results, environmental factors (including WC, pH, NO_3^--N, NH_4^+-N, TP) were the optimal predictors of variation in fungal trophic functional guilds. The results demonstrated that soil nutrient status and WC influence soil fungal communities and functional groups.

3.6. Correlation between Tree Growth and Partial Fungal Taxon or Physicochemical Properties in the Eucalyptus Plantation Soils

Eucalyptus growth characteristics (Height, Crown diameter, GD and DBH) were positively associated with the proportion of the symbiotroph fungal group, and significantly negatively associated with the proportion of the pathotroph fungal group (Table A1). Specifically, *Eucalyptus* height, Crown diameter, GD and DBH were positively correlated with the proportion of Ectomycorrhizal fungi (the most dominant fungi, $r > 0.889$), which increased significantly in the fertilization treatments when compared to the proportion in the only irrigation or CK treatment (Figure 3). The *Eucalyptus* height, Crown diameter, GD and DBH were positively correlated with the proportion of *Pisolithus* ($r > 0.91$), but negatively correlated with *Glomeromycota* ($r < -0.93$) and *P. verrucose* ($r < -0.979$, $p < 0.05$) proportion. In addition, *Eucalyptus* height, Crown diameter, GD and DBH were significantly negatively ($r < -0.951$) correlated with the AM and Endophyte fungi (Table A1) proportion. Furthermore, *Eucalyptus* Height, Crown diameter, GD, and DBH were significantly positively correlated with SOC, MBC, and NO_3^--N contents, and positively and negatively associated with the soil nutrient contents and WC, respectively, although non-significantly (Table A2).

The results suggest that the proportion of symbiotroph fungi (mainly *Ectomycorrhizas*) and nutrient contents (especially SOC, MBC, and NO_3^--N) were strongly positively associated with *Eucalyptus* growth in field under the fertilization (F and FW) treatments.

4. Discussion

Microbial biodiversity and physicochemical properties could influence soil degradation or amelioration processes [6]. Furthermore, water availability and fertilization could directly affect soil fungal diversity, fungal community composition, and fungal functional groups; however, the effects of fertilization (N, P) could be altered by water availability levels [4,7,9]. Similar to previous reports, in the present study, major soil nutrients (N, P, K, and C contents) increased significantly under the only fertilization treatment, increased slightly in the only irrigation treatment, and were the highest in the fertilization combined with irrigation treatment, when compared with the levels in the CK treatment in the *Eucalyptus* plantation soils (Table 2 in [4,6]). Among soil physicochemical properties, pH and WC were the factors controlling soil fungal biodiversity in forest ecosystems [2,4,9]. In the present study, the only fertilization treatment led to a decrease in pH in the *Eucalyptus* plantation soil, and pH was the main driver of soil fungal community structure (Table 2, Figure 5). The result is consistent with the finding of a previous study carried out in an agricultural ecosystem [43]. In addition, the dry season irrigation management decreased pH and WC were the key factors influencing soil fungal community structure and functional groups. The soil moisture could have increased the mobility of solutes and enzymes, which would facilitate substrate supply to microorganisms [44]. In addition, increased soil water availability could enhance the metabolic activity of most microorganisms and soil organic matter degradation, which would increase soil nutrient availability (Table 2 and Figure 1 in [45]). The fertilization combined with irrigation treatment had the highest N, P, K and C contents, and the greatest tree growth, which suggests that irrigation and fertiliz ation synergistically increased soil fertility. Therefore, dry season irrigation and fertilization significantly enhanced soil fertility, which could directly promote *E. urophylla* × *E. grandis* growth.

High microbial biodiversity indicates good soil quality, better substrate-use efficiency and high nutrient availability [3,6]. According to our results, the alpha-diversity of soil fungi

was lower in the only fertilization treatment than in the CK treatment, which may be due to the lower soil pH values and nutrient contents under the fertilization treatments [36,46]. In addition, beta-diversity varied across treatments. However, similar fungal diversity between the only irrigation and CK treatments or between the fertilization treatments does not imply similar fungal community function [8]. Soil fungal diversity decreased in the forest and desert but increased in cropland under N fertilization [47]; however, the effects of N fertilization could be altered by water availability conditions [8,38]. Furthermore, soil fungal diversity could be influenced by soil type, forestry management practices, the type of fertilization approach (single type or multiple applications, nutrient amounts, and water supplementation, and treatment time and periods (season, long-term or short-term) [4,5,47,48].

The impacts of management practices on soil fungal diversity could be difficult to base on single-factor experiments. In different ecosystems, the responses of microbial community composition and functional groups to soil WC and soil nutrients vary [6,7,22,47]. In natural ecosystems, increased N inputs decrease microbial biomass and microorganisms in soils in upland plants [6,49]. However, in the present study, fertilization significantly increased soil C and microbial biomass accumulation, which are key indicators of soil quality and productivity [6,49]. Soil moisture content is a key factor influencing C and N cycles in soil, as well as plant growth and soil microbial community structure and function in ecosystems [50]. In the present study, dry season irrigation and fertilization significantly influenced soil water availability, fungal community composition, and fungal functional groups in *Eucalyptus* soil, which is consistent with the findings of a previous study [51]. In addition, in the present study, fungal communities responded more strongly to fertilization than to water supplementation management in the dry season, and fertilization treatment decreased soil WC and fungal diversity, as reported in previous studies [52,53]. A previous meta-analysis also showed that fertilization increased the total SOC and improved soil quality [54].

In the fertilization (F and FW) treatments, the proportion of symbiotic fungi was increased significantly, whereas the proportions of saprotrophs and pathotrophs were reduced, when compared with the CK or only irrigation treatment. Moreover, tree growth was positively associated with symbiotrophs, which were significantly negatively associated with pathotrophs. Symbiotrophs (symbionts) receive nutrients from host-cell assimilation products and supply mineral nutrients to host cells in exchange; conversely, pathotrophs (pathogens) receive nutrients by harming host cells [6,16]. Symbiotic fungi increase the surface areas of plant roots, enhancing plant capacity to obtain nutrients and water in return for C substrates from photosynthesis [53]. According to these results, symbiotrophs enhanced the growth and adaptation of *Eucalyptus* plantations under fertilization (F and FW) treatments. In addition, the proportion of AM fungi (especially on *Glomeromycota*) decreased significantly, while the proportion of ectomycorrhizal fungi (especially on *Pisolithus*) increased significantly. Moreover, the proportion of AM was significantly negatively correlated with plant growth (tree height, crown diameter, GD, and DBH). A previous study reported that *Glomeromycota* is an indicator of changes in soil moisture content and that the AM (*Glomeromycota*) fungi proportion decreased under combined N and P additions in grassland ecosystems [55]. *Glomeromycota* relative abundance could decrease in cases where they are less valuable to their hosts and less plant C is available under additional N and P fertilization treatment [55].

Ectomycorrhizal fungi can mobilize more stable soil organic N and promote N cycling in ecosystems [56]. An increase in ectomycorrhizal fungi (such as *Pisolithus*) belonging to Basidiomycota in the present study could facilitate plant growth and SOC accumulation. Notably, AP contents in the fertilization combined with irrigation treatment were more than 14-fold those in the other treatments, although there was no significant correlation between AP and tree growth. This may be because the promotion of *Eucalyptus* growth by ectomycorrhizal fungi is highly correlated with seedling P content, external hyphae biomass, and shoot weight (Table A1 and Table S2 [57]). Another potential reason is the

typical acid red soil in south China, which is very deficient in P in the subtropical forest ecosystem [29]. Fertilization treatment effectively increased ectomycorrhizal fungi and P availability, which was the primary factor limiting *Eucalyptus* growth in a plantation in a dry season in South China [29]. Therefore, these findings suggest that short-term fertilization and dry season irrigation management shift the fungal community composition and alter the proportion of ectomycorrhizal fungi, which was positively correlated with plant growth under fertilization management.

Fertilization is an essential management approach in forest plantations for directly increasing soil fertility. Soil pH and NO_3^--N are edaphic properties with considerable influence on fungal community structure [58]. In the present study, RDA analysis results showed that the fertilization treatments resulted in soil fungal communities distinct from those in the only irrigation and CK treatments. In the present study, soil pH and edaphic properties (NO_3^--N, NH_4^+-N, and TP) were the major factors influencing fungal functional groups. Fertilization directly increased soil fertility, and increased the DOC, MBC, and MBN contents in soils significantly. Furthermore, tree growth was significantly positively correlated with NO_3^--N, SOC, and MBC, which indicated that the fertilization treatment enhanced *Eucalyptus* growth and soil quality by increasing soil nutrient availability. The healthy *Eucalyptus* plantations could have in turn increased SOC via plant litter decomposition and root exudates [8].

In the present study, fertilization improved soil nutrient contents and led to a significant shift in fungal community composition. Compared to the CK treatment, the proportion of the symbiotic fungi increased, whereas saprophytic and pathogenic fungi populations decreased in the fertilized soils. This result suggested that the soil nutrient contents were the major factor influencing fungal community structure. In wet tropical forests at La Selva, Costa Rica, reduced precipitation (with a combination of shelters and trenching) in the field decreases the abundance of soil fungal communities significantly, which in turn alters the fungal/bacterial ratio leading to shifts in the dynamics of soil microbial communities [51]. Our results also showed that dry season irrigation significantly altered soil fungal community structure and functional groups and alleviated the negative impacts of short-term fertilization on soil fungi biodiversity, leading to the enhancement of the growth of *Eucalyptus* plantations in the dry season in South China.

In summary, according to the results of the present study, dry season irrigation and short-term fertilization practices significantly influence fungal community structure and functional composition in *Eucalyptus* plantation soils. The symbiotrophic fungi population in plantation soils under short-term fertilization application was significantly higher than that in the control soils, and the fungi populations were dominated by ectomycorrhizal fungi, specifically the genera *Pisolithus*. Such an increase in symbiotic fungi could enhance nutrient cycling in plant rhizospheres. Nevertheless, the proportion of the pathotrophic fungi was significantly lower under the combined irrigation and fertilization treatment soils than in the control soils. Such shifts in fungal community structure and low pathogenic fungi proportions enhance soil quality and increase the fungal community diversity in *Eucalyptus* plantation soils. Soil fungal community structure and functional composition, which were closely correlated with soil nutrient availability, soil pH, and WC, were significantly altered under short-term fertilization and dry season irrigation. Future studies should evaluate the effects of long-term irrigation and fertilization practices on forestry ecosystems, which could have a significant impact on soil biogeochemistry and plantation productivity and on fungal community biodiversity in *Eucalyptus* plantation soils.

Author Contributions: Conceptualization, S.G., Q.H., J.L. and Z.C.; Data curation, Q.H., J.L. and Z.C.; Formal analysis, D.H. and Z.W.; Funding acquisition, X.X., Q.Q., J.L. and Z.C.; Investigation, S.G. and Z.C.; Methodology, S.G. and Z.C.; Project administration, Q.H., Y.S., J.L. and Z.C.; Resources, S.G., Q.H., Y.S., Q.Q., J.L. and Z.C.; Software, S.G., D.H. and Z.C.; Supervision, J.L. and Z.C.; Visualization, D.H. and Z.W.; Writing—Original draft, S.G., Z.C.; Writing—Review & editing, X.X., D.H., J.M., J.L. and Z.C. All authors have read and agreed to the published version of the manuscript.

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Informed Consent Statement: Not applicable.

Data Availability Statement: The raw sequence files for each sample can be accessed at the NCBI SRA BioProject database (Accession Number: PRJNA637913).

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Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

Appendix A

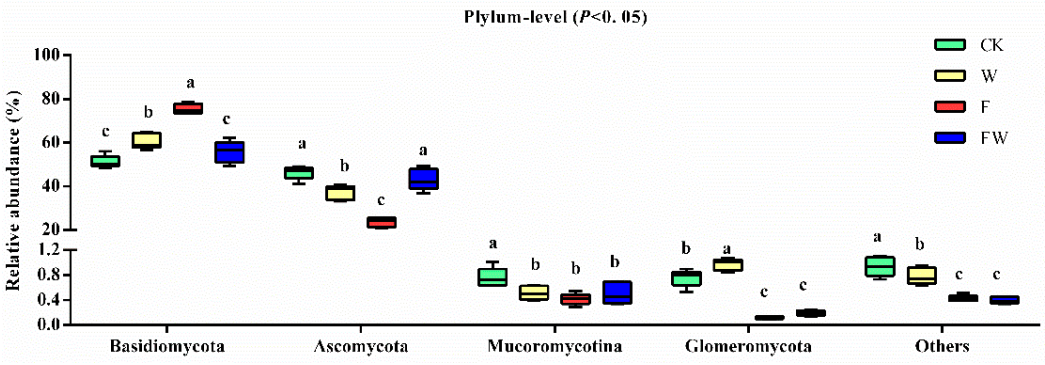

Figure A1. Dry season irrigation and short-term fertilization treatments influenced *Eucalyptus* soil fungal community structure at the phylum-level. CK: control; W: irrigation only; F: fertilization only; FW: irrigation combined with fertilization. Different letters indicate significant differences in individual parameters among the four treatments detected based on the Duncan test ($p < 0.05$). Mean ± SEM ($n = 5$).

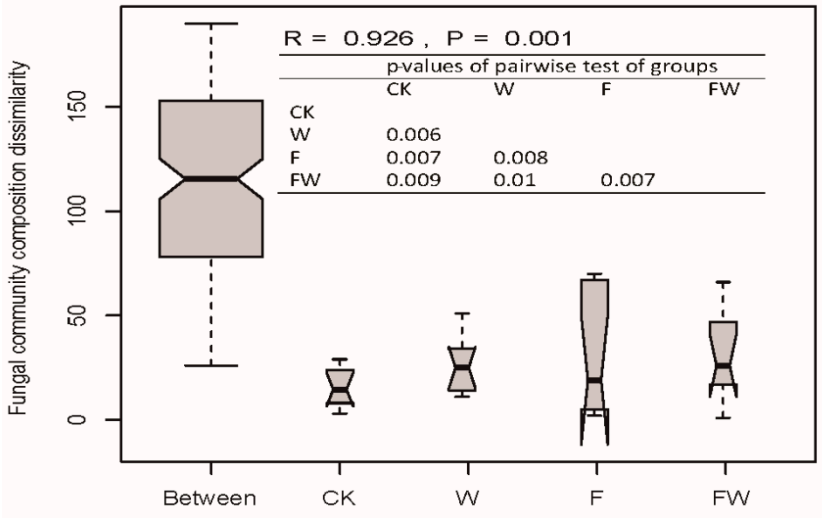

Figure A2. Fungal community composition dissimilarity based on Analysis of similarities. CK: control; W: irrigation only; F: fertilization only; FW: irrigation combined with fertilization.

Table A1. Correlation coefficients between tree growth and partial fungal taxon in the *Eucalyptus* plantation soils based on the linear regression model method.

Fungi	Height/cm	CBH/cm	Crown Diameter /m	GD/mm	DBH/mm
Symbiotroph	0.883	−0.697	0.895	0.9	0.89
Saprotroph	−0.478	0.555	−0.501	−0.511	−0.498
Pathotroph	−0.986 *	0.376	−0.988 *	−0.988 *	−0.990 **
unidentified fungi	−0.884	0.746	−0.883	−0.883	−0.873
Arbuscular Mycorrhizal	−0.956 *	0.691	−0.958 *	−0.959 *	−0.951 *
Ectomycorrhizal	0.889	−0.699	0.901	0.905	0.895
Endophyte	−0.962 *	0.656	−0.963 *	−0.963 *	−0.957 *
Pisolithus	0.91	−0.689	0.921	0.925	0.916
Glomeromycota	−0.93	0.564	−0.939	−0.943	−0.938
Phialophora verrucosa	−0.979 *	0.615	−0.983 *	−0.984 *	−0.979 *
Scleroderma	0.422	−0.21	0.443	0.451	0.448
Ambispora	−0.73	0.936	−0.738	−0.742	−0.723

* represents $p < 0.05$, ** represents $p < 0.01$.

Table A2. Correlation coefficients between tree growth and physicochemical properties in the *Eucalyptus* plantation soils based on the linear regression model method.

Physicochemical Property	Height/cm	CBH/cm	Crown Diameter/m	GD/mm	DBH/mm
SOC (g/Kg)	0.986 *	−0.322	0.981 *	0.979 *	0.984 *
MBC (mg/Kg)	0.997 **	−0.4	0.995 **	0.994 **	0.996 **
MBN (mg/Kg)	0.815	0.038	0.816	0.816	0.828
Moisture (%)	−0.388	0.983 *	−0.397	−0.401	−0.376
Soil pH	0.004	0.172	−0.021	−0.031	−0.023
TN (g kg^{-1})	0.938	−0.472	0.946	0.949	0.947
NO_3^--N (mg kg^{-1})	0.992 **	−0.422	0.988 *	0.986 *	0.988 *
NH_4^+-N (mg kg^{-1})	0.712	0.228	0.71	0.709	0.726
TP (g kg^{-1})	0.939	−0.12	0.932	0.93	0.94
AP (mg kg^{-1})	0.672	0.053	0.652	0.644	0.657
AK (mg kg^{-1})	0.909	−0.271	0.915	0.918	0.921
MBN (mg kg^{-1})	0.857	−0.022	0.858	0.858	0.869
SOC (g/Kg)	0.986 *	−0.322	0.981 *	0.979 *	0.984 *
MBC (mg/Kg)	0.997 **	−0.4	0.995 **	0.994 **	0.996 **

* represents $p < 0.05$, ** represents $p < 0.01$.

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