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Mycorrhizal Fungal Diversity and Its Relationship with Soil Properties in *Camellia oleifera*

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Abstract: Arbuscular mycorrhizal fungi (AMF) are well known for their important physiological implications on diversified host plants, while the information on AMF diversity and its relationship with soil properties of *Camellia oleifera* is yet not fully understood. In the proposed study, high-throughput sequencing of small subunit ribosomal RNA was performed to analyze the AMF diversity of the rhizosphere and endosphere of 20-year-old *C. oleifera* Xianglin in the field at Wuhan (China) and their relationship with soil physico-chemical properties. As high as 30.73–41.68% of the roots of *C. oleifera* were colonized by indigenous AMF with a spore density of 66–111 spores/10 g soil. The surface soil (0–20 cm) showed significantly higher root fungal colonization, spore density, soil hyphal length, and easily extractable glomalin-related soil protein content than the sub-surface soil (20–40 cm). Soil pH value, available K, and NO_3^--N content affected the root and soil mycorrhizal development, whilst soil pH proved to be the most influential soil property governing their variability. A total of 467 OTUs associated with AMF were detected from the endosphere and rhizosphere, representing 10 genera and 138 species, of which 295 OTUs and 9 genera were jointly observed. The genus *Glomus* displayed maximum relative abundance (>86%) in both endosphere and rhizosphere. *Scutellospora* was detected in the endosphere, but absent in the rhizosphere. The endosphere recorded a relatively higher number of OTUs and alpha diversity indices (Shannon, Simpson, and PD index) of AMF than rhizosphere. Our study, hence, revealed that *C. oleifera* in fields was mainly colonized by *Glomus*, coupled with comparatively greater AMF diversity in the endosphere than in the rhizosphere, governed predominantly by soil pH, NO_3^--N content, and available K content.

Keywords: *Glomus*; high-throughput sequencing; mycorrhiza; OTU; oil plant

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1. Introduction

Camellia oleifera is an evergreen woody edible oil plant, widely distributed in the subtropical areas of southern China, and less frequently in northern Southeast Asia [1]. Seeds of *C. oleifera* are extracted for oils (tea oil), as well known for fragrance, nutritive value, and long storage. Tea oil is a high-quality edible oil that protects the liver from oxidative damage caused by CCl_4 [2]. *C. oleifera* is also used in the industry as a pesticide as well as the fertilizer, with the ability to improve water storage capacity of farmland and pests' suppressive ability in rice fields [3,4]. Pharmacological studies showed that seeds of *C. oleifera* contained various bioactive substances and are traditionally used as folk medicine against bacterial infections, stomach pains, and burns [5]. The demand for *C. oleifera* has increased greatly in recent years, encouraging the planting area increasing by leaps and bounds. However, the seed yield of *C. oleifera* is not high, reported as 21–25 t/hm^2 [6].

Arbuscular mycorrhizal fungi (AMF), a kind of soil beneficial fungi, are well known for their association with roots of terrestrial plants to form arbuscular mycorrhizas [7]. AMF have been extensively reported to improve plant stress tolerance, fruit quality, plant growth response, nutrient acquisition capacity, soil environment, and soil microbial diversity [8–12], suggesting their diversified roles. Previous studies have demonstrated the good population diversity of AMF within rhizosphere of tea trees [13], but the information regarding AMF diversity in the rhizosphere and endosphere of *C. oleifera* is scarce. Li and Chen [14] firstly observed the extraradical mycorrhizal hyphae, vesicles, arbuscules, intraradical hyphae, and subtanding hyphae in roots of *C. oleifera*, indicating the mycorrhizal dependence. Wang et al. [15] studied the inoculation responses of *C. oleifera* seedlings with six *Glomus* spp., which revealed an increase in plant height, diameter, shoot biomass, root biomass, soluble sugar content, and leaf relative water content, dependent upon AMF species, whilst *G. versiforme* exhibited a higher magnitude of response. In addition to growth promotion, AMF (e.g., *Funneliformis mosseae*) significantly improved root morphology, photosynthetic rate, and leaf and root P concentration of *C. oleifera* [16]. These results demonstrated the positive benefits of AMF on multiple physiological functions of *C. oleifera*.

The physiological and ecological functions of AMF are closely associated with their community, and different AM fungal communities usually have distinctive functional traits [17]. The objective of the proposed study was to investigate the root AMF colonization and their soil influencing factors of *C. oleifera* in fields in relation to the diversity of AMF in endosphere and rhizosphere.

2. Materials and Methods

2.1. Experimental Setup

The experimental site was located at the garden (30°18′34″ N, 114°15′37″ E) of *C. oleifera* in the Demonstration Park of Wuhan Forestry and Fruit Ecological Science and Technology, Jiangxia District, Wuhan, China. The site was characterized by a humid monsoon climate with an annual mean temperature of 16.8 °C, annual mean precipitation of 1347.7 mm, and annual sunshine duration of 1450–2050 h. The 20-year-old *C. oleifera* 'Xianglin no. 11' grafted on *C. oleifera* 'Xianglin no. 15' planted with a 2 × 3 m spacing was selected as the tested plant material, without any additional fertilizer during the 2018–2020 period.

2.2. Sampling

Rhizosphere soil and fine root samples within the tree canopy were isolated in 16 August 2020, whilst the rhizosphere soil was collected by shaking off the soil attached to the root surface [18]. Each plot had three trees with uniform growth vigor, and the samples collected from three trees were used as a replicate, resulting in a total of three replicates defined as CR1, CR2, and CR3 in endosphere (roots) and CS1, CS2, and CS3 in rhizosphere (soils), respectively. At the time of sampling, we divided the soil into two parts, surface soil (0–20 cm) and sub-surface soil (20–40 cm), and about 500 g rhizosphere soil/tree and 20 g roots/tree were collected.

2.3. Determinations of Root Mycorrhizal Colonization

Root mycorrhizal colonization was determined using the protocol suggested by Zhang et al. [19] after clearing with 10% KOH solution, bleaching with 30% H_2O_2 solution, acidifying with 0.1 mM HCl solution, and staining with 0.05% trypan blue solution in lactoglycerol. The root mycorrhiza was observed under a binocular biomicroscope (NE610, Ningbo Yongxin Optics Co., Ltd., Ningbo, China). Root mycorrhizal colonization degree was expressed as the percentage of mycorrhiza-colonized root lengths against total observed root lengths.

2.4. Determinations of Soil Spore Density and Soil Hyphal Length

Mycorrhizal spore density in the soil (20 g) was determined following the wet sieving–sucrose centrifugation method as described by Daniels and Skipper [20]. Mycorrhizal hyphal length in the soil was measured as per the method of Bethlenfalvay and Ames [21]. A 0.5-g soil sample was incubated with 6 mL 0.1 mol/L phosphate buffer followed through mixing. The 0.8 mL of the above solution was mixed with 0.4 mL of 0.05% trypan blue solution at 70 °C for 20 min, cooled to room temperature, and finally examined under a stereo microscope (SMZ-161-BLED, Motic China Group Co., Ltd., Xiamen, China).

2.5. Determinations of Soil Properties

Soil pH value was determined by a pH meter (pH828+, Smart Sensor, Dongguan, China) using a 10-g air-dried soil mixed with 25 mL of distilled water. Soil easily-extractable glomalin-related soil protein (EE-GRSP) levels were measured using the protocol suggested by He et al. [8] based on the extraction of 8 mL of 20-mM sodium citrate buffers (pH 7.0) at 0.11 MPa for 30 min. Soil NH_4^+-N content, NO_3^--N content, Olsen-P content, and available K content were determined using the Soil Nutrient Detector (HM-TYD, Shandong Hengmei Electronic Technology Co., Ltd., Weifang, China) based on the user manual.

2.6. Analysis of AMF Diversity of Rhizosphere and Endosphere

AMF diversity of rhizosphere and endosphere was assayed in accordance with the protocol of Wu et al. [13]. The procedure was followed through genomic DNA extraction using the DNA purification ELISA kit, followed by the detection of DNA concentrations by electrophoresis. The specific AMF primers with Barcode (AML1 (F): 5′-ATCAACTTTCGATGGTAGGATAGA-3′; AML2 (R): 5′-GAACCCAAACACTTTGGTTTCC-3′; AMV4.5NF (F): 5′-AAGCTCGTAGTTGAATTTCG-3′; AMDGR (R): 5′-CCCAACTATCCC TATTAA TCAT-3′) were used to amplify the specified region of the sample with 18S genes. The PCR mixture (25 μL) consisted of 1 × PCR buffer, 1.5 mM of $MgCl_2$, 0.4 μM of deoxynucleoside triphosphate, 1.0 μM of primers, 0.5 U of KOD-Plus-Neo (TOYOBO), and 10 ng of template DNA. The PCR amplification was described by Wu et al. [13] in detail. The analysis was performed through three replicates for each sample, and each PCR reaction was terminated in the linear amplification period. The PCR products were recovered by cutting the gel with the gel recovery kit, and the target DNA fragments were recovered by the elution with TE buffer. The PCR products were quantified by Qubit 2.0 with reference to preliminary electrophoresis results, and then mixed in an appropriate proportion according to the sequencing volume requirement of each sample.

The library was constructed using the Illumina TruSeq DNA PCR-Free Sample Prep Kit, and sequenced using PE300 based on the Illumina MiSeq Reagent Kit v3 (600 cycles). The raw image data files from high-throughput sequencing were converted into raw sequenced sequences, called Raw Reads, after base identification by CASAVA software. The double-ended sequences were spliced into one sequence (Raw Tag) using FLASH7 according to the overlap relationship between PE reads [22]. To obtain high quality sequences, the sequences were quality controlled and the sequence obtained after quality control was called Clean Tag. The sequences were then clustered to generate the classification units at operation, viz., OTU (Operational Taxonomic Units), and OTU clustering analysis was performed at 97% similarity levels. Representative sequences of OTUs were annotated taxonomically utilizing the UCLUST classification [23]. Sequences of the subphylum Coccidioides were selected, and community composition analysis [24] and alpha diversity analysis [25] were performed on AMF by means of OTU data.

2.7. Data Analysis

The data of endosphere and rhizosphere variables were analyzed through SAS software (SAS Institute, Inc., Cary, NC, USA). The Duncan's multiple range test was utilized to compare the significant ($p < 0.05$) difference between treatments. Alpha diversity index

of AMF and correlation between species derived from high-throughput sequencing were analyzed using the R (4.0.5) software (www.r-project.org, released on 31 March 2021).

3. Results

3.1. Changes in Soil Chemical Traits

The soil of the sample site growing *C. oleifera* was observed weakly acidic (Table 1). The pH value of soil sample S1 (surface layer of 0–20 cm) was significantly lower than that of soil sample S2 (sub-surface layer of 20–40 cm). Soil NH_4^+-N content, NO_3^--N content, and available K content of sample S1 were significantly higher than that of sample S2, whereas Olsen-P content showed no significant difference between the S1 and S2.

Table 1. Physico-chemical characteristics of different soils at two contrasting depths of *Camellia oleifera*.

Soil Samples	pH	NH_4^+-N Content (mg/kg)	NO_3^--N Content (mg/kg)	Available K Content (mg/kg)	Olsen-P Content (mg/kg)
S1	6.13 ± 0.10 [b]	78.88 ± 9.31 [a]	130.43 ± 31.55 [a]	215.68 ± 35.15 [a]	104.96 ± 36.03 [a]
S2	6.51 ± 0.09 [a]	61.68 ± 7.47 [b]	74.62 ± 21.64 [b]	143.85 ± 38.51 [b]	102.50 ± 13.95 [a]

Data in the same column followed by different letters indicate significant ($p < 0.05$) differences. S1 and S2 denote the soil of the 0–20 cm layer and 20–40 cm layer, respectively.

3.2. Changes in Mycorrhizal Status of Rhizosphere and Endosphere

The root of *Camellia oleifera* was actively colonized by native AMF (Figure 1a), up to 30.73–41.68%, and indigenous spores of AMF (66–111 spores/10 g soil) were observed in the rhizosphere (Figure 1b; Table 2). Soil hyphal length ranged from 3.99 to 8.94 cm/g soil, and soil EE-GRSP levels varied from 0.53 to 0.64 mg/g soil (Table 2). The surface soil of 0–20 cm (S1) recorded significantly higher root AMF colonization degree, spore density, hyphal length, and EE-GRSP levels than the sub-surface soil of 20–40 cm (S2).

Table 2. Root and soil mycorrhizal status of *Camellia oleifera* in two soil depths.

Samples	Root AMF Colonization (%)	Soil Spore Density (#/10 g soil)	Soil Hyphal Length (cm/g)	Soil EE-GRSP Level (mg/g)
S1	41.68 ± 7.01 [a]	111.25 ± 12.56 [a]	8.94 ± 0.89 [a]	0.64 ± 0.02 [a]
S2	30.73 ± 4.27 [b]	66.33 ± 8.14 [b]	3.99 ± 0.34 [b]	0.53 ± 0.06 [b]

Data in the same column followed by different letters indicate significant ($p < 0.05$) differences. S1 and S2 denote the soil of the 0–20 cm layer and 20–40 cm layer, respectively.

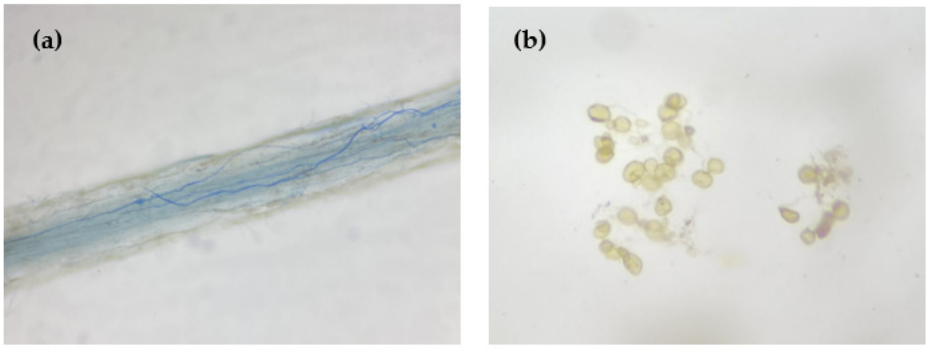

Figure 1. Root colonization (**a**) and soil indigenous spores (**b**) of *Camellia oleifera* by native AMF.

3.3. Correlation between Soil Properties and Mycorrhizal Traits

Root AMF colonization was significantly and positively correlated with soil spore density and available K content (Table 3). Soil spore density was significantly and positively correlated with soil hyphal length and NO_3^--N content, and soil hyphal length was significantly and positively correlated with EE-GRSP and NO_3^--N content. Soil spore

density, hyphal length, EE-GRSP levels, and root AMF colonization were all significantly and negatively correlated with soil pH value.

Table 3. Correlation of soil physico-chemical characteristics and mycorrhizal traits.

	Root Colonization	Soil Spore Density	Soil Hyphal Length	EE-GRSP	pH	Soil NH_4^+-N	Soil NO_3^--N	Soil Available K	Soil Olsen-P
Root colonization	1.00	0.80 *	0.68	0.433	−0.77 *	0.30	0.56	0.74 *	−0.08
Soil spore density		1.00	0.95 **	0.682	−0.92 **	0.59	0.89 **	0.65	−0.08
Soil hyphal length			1.00	0.74 *	−0.93 **	0.68	0.84 **	0.61	−0.05
EE-GRSP				1.00	−0.84 **	0.68	0.71 *	0.73 *	−0.11

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

3.4. Sequencing Quality Analysis

As many as 42,021 to 47,469 original sequences were detected in endosphere and rhizosphere samples of *C. oleifera*, of which 35,431 to 43,030 valid sequences (88.5% to 92.5%) were obtained after filtering and splicing, having average length of valid sequences ranging from 261 to 263 (Table 4). Figure 2 showed the trend of species richness with sequencing depth. With an increase of the number of reads, the curve gradually leveled off, indicating that the sequencing depth basically covered all the species in the samples. Therefore, our measured data reflected the real situation of AMF communities in the test samples.

Table 4. Sequence data of root and soil samples collected from *Camellia oleifera*.

Samples	Sample Number	Raw PE	Clean Tags	Effective Tags	Effective Rates (%)	Average Length (nt)
Root	CR1	47,469	44,188	43,030	90.65	263
	CR2	45,960	41,329	40,105	87.26	262
	CR3	44,237	39,958	35,431	86.88	261
Soil	CS1	43,739	40,814	39,320	89.90	262
	CS2	42,021	39,852	38,437	91.47	261
	CS3	47,477	43,361	42,042	88.55	262

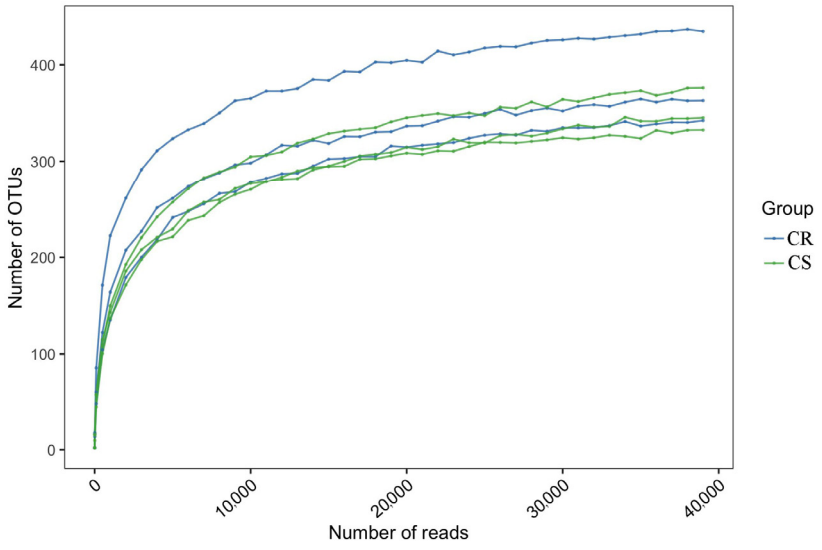

Figure 2. Sample richness dilution curve of OTUs in endosphere (CR) and rhizosphere (CS) samples from *Camellia oleifera*.

3.5. Changes in OTUs in Endosphere and Rhizosphere

A total of 467 OTUs belonging to AMF were obtained after clustering and classifying 97% of OTUs. Using the Wayne diagram, we visualized the common and unique OTUs of

AMF communities in endosphere (CR) samples and rhizosphere (CS) samples (Figure 3). The number of OTUs common to both CR and CS samples was 295, accounting for 63.17% of the total number of OTU sequences; the number of OTUs unique to the CR sample was 116, accounting for 24.84% of the total number of OTU sequences; the number of OTUs unique to the CS sample was 56, accounting for 11.99% of the total number of OTU sequences. The number of OTUs in endosphere, therefore, was higher than that in rhizosphere.

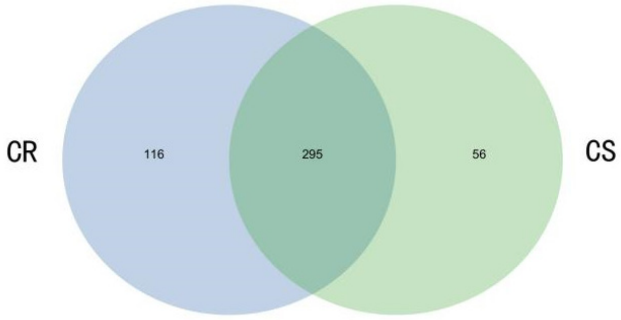

Figure 3. Venn diagram of OTUs of AMF of endosphere (CR) and rhizosphere (CS) of *Camellia oleifera*.

3.6. Changes in Relative Abundance of AMF in the Genus Level

As many as 467 OTUs of AMF (after sequence alignment of OTUs) were grouped into 10 genera and 138 species, of which a total of 10 genera (*Ambispora*, *Acaulospora*, *Archaeospora*, *Claroideoglomus*, *Diversispora*, *Gigaspora*, *Glomus*, *Paraglomus*, *Redeckera*, and *Scutellospora*) and 133 species were found in the CR sample (Figure 4). As many as 9 genera (*Ambispora, Acaulospora, Archaeospora, Claroideoglomus, Diversispora, Gigaspora, Glomus, Paraglomus*, and *Redeckera*) and 114 species were observed in the CS sample (Figure 4). Compared with the CR sample, the CS sample failed to detect *Scutellospora*. The AMF genus *Glomus* was observed most dominantly in the CR sample with maximum relative abundance of 86.77%, followed by *Paraglomus* (7.48%), *Claroideoglomus* (3.00%), *Diversispora* (1.61%), *Ambispora* (0.62%), and *Acaulospora* (0.26%), respectively, in decreasing order. The remaining 4 genera viz., *Archaeospora*, *Gigaspora, Redeckera*, and *Scutellospora*, had a low relative abundance of 0.25%. In the CS sample, the relative abundance of *Glomus* was 88.59%, followed by *Claroideoglomus* (7.73%), *Diversispora* (2.83%), and *Paraglomus* (0.84%), respectively. The rest of the other genera such as *Ambispora*, *Acaulospora*, *Archaeospora*, *Gigaspora*, and *Redeckera* made very low contributions (up to 0.01%). The genus *Glomus* was the dominant genus in both CR and CS samples. The relative abundance of *Paraglomus* was relatively higher in the CR sample than in the CS sample, and the relative abundance of *Claroideoglomus* and *Diversispora* in the CR sample was substantially lower than that in the CS sample.

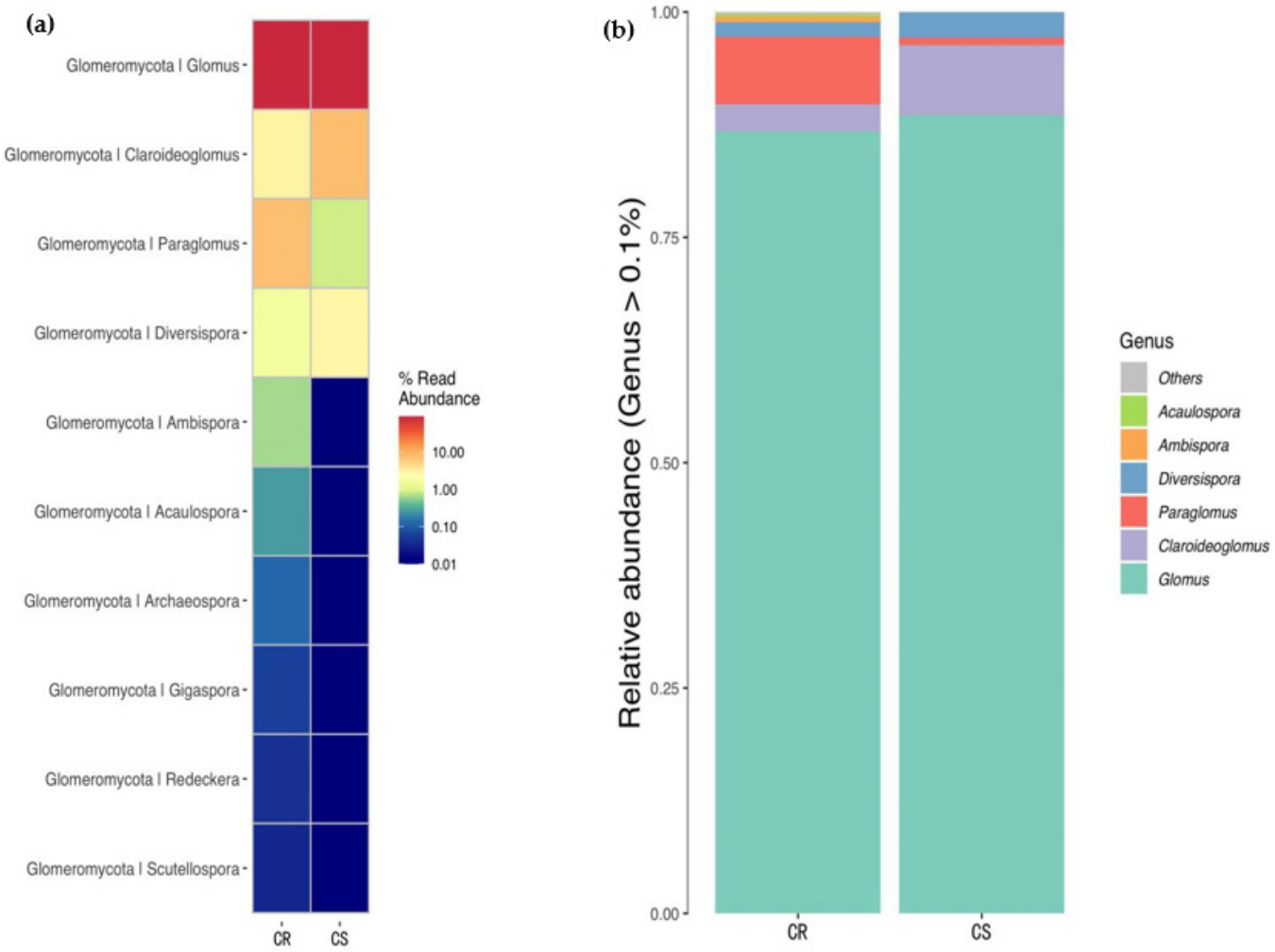

Figure 4. Read abundance (**a**) and genus level richness (**b**) of AMF in endosphere (CR) and rhizosphere (CS) of *Camellia oleifera*.

3.7. Changes in Alpha Diversity Index of AMF

The OTU coverage of *C. oleifera* in the CR and CS samples was observed beyond 0.998, indicating the data represented the diversity between the two contrasting sources of samples. There were no significant differences in the three indices of AMF alpha diversity including the observed, Chao1, and ACE indices between the CR and CS samples (Table 5), indicating that the total amount of OTUs in the two samples was not significantly different. However, another three indices of AMF alpha diversity such as Shannon, Simpson, and phylogenetic Diversity (PD) showed significantly higher in endosphere than in rhizosphere, further implying that endosphere displayed a greater AMF alpha diversity than rhizosphere alone.

Table 5. Comparison of AMF alpha diversity indexes.

	Observed	Chao1	ACE Estimator	Shannon	Simpson	Phylogenetic Diversity
CR	257.67 ± 14.57 ^{a}	286.81 ± 10.99 ^{a}	292.10 ± 5.16 ^{a}	3.39 ± 0.27 ^{a}	0.93 ± 0.03 ^{a}	12.70 ± 1.14 ^{a}
CS	234.67 ± 12.50 ^{a}	286.73 ± 9.65 ^{a}	279.92 ± 8.87 ^{a}	2.85 ± 0.13 ^{b}	0.83 ± 0.05 ^{b}	10.56 ± 0.53 ^{b}

Data in the same column followed by different letters indicate significant ($p < 0.05$) differences. CR and CS mean the samples from the endosphere and rhizosphere of *Camellia oleifera*, respectively.

4. Discussion

In our study, AMF spores, root AMF colonization, and soil mycorrhizal hyphae were found in *C. oleifera*, indicating that *C. oleifera* is a mycorrhiza-dependent plant, in agreement with the earlier findings of Li and Chen [14]. In this study, root mycorrhizal colonization degree, spore density, soil hyphal length, and EE-GRSP content were significantly higher in the 0–20 cm soil than in the 20–40 cm soil. Similar results were earlier obtained by Wu et al. [26] in the rhizosphere of *Citrus unshiu*. Such differences are accountable to the fact that AMF are aerobic fungi and the surface soil possesses higher capacity of air exchange than sub-surface soil [27,28], thus, registering higher AMF diversity of surface soil versus sub-surface soil. Correlation analysis further showed that soil pH value was the primary factor and negatively influenced the mycorrhizal status of rhizosphere and endosphere. Guo and Gong [29] also observed soil pH as the principal factor in shaping the AMF composition in a salt-stressed ecosystem, because soil pH affected spore germination and hyphal growth [30]. The other soil properties such as NO_3^--N content and available K content also contributed towards mycorrhizal development in endosphere and rhizosphere of *C. oleifera*, similar to the observation earlier made by Liu et al. [31] in *Robinia pseudoacacia* plantation in the hilly region of the Loess Plateau, China.

In this study, as many 10 genera representing 138 AMF species from 8 families were detected, where the relative abundance of *Glomus* was observed the maximum. Singh et al. [32] reported *Glomus* as the dominant genus in tea. In fact, *Glomus* is the most common and largest genus within the phylum Glomeromycota [33]. According to the latest classification system, AMF are classified into 259 species from 25 genera in 11 families [34]. The AMF species identified in our study accounted for 53.28% of the currently known AMF species. We further observed 411 and 351 OTUs from endosphere and rhizosphere, respectively, with 295 OTUs common to both, and significantly higher OTUs in the endosphere than in the rhizosphere, consistent with the earlier results of Wu et al. [35] in citrus.

A total of 9 genera and 10 genera of AMF were identified respectively in the rhizosphere and the endosphere, indicating almost similar AMF diversity within two contrasting sources of samples in the genus structure [13]. We obtained little difference in the Chao1 index between the rhizosphere and endosphere, but the other indices such as Shannon index and Simpson index of the endosphere were significantly greater than those of the rhizosphere, indicating former having comparatively higher functional diversity of AMF than latter [35].

It is documented that the population of AMF was affected by various factors, such as sampling time, climatic environment, soil physic-chemical properties, etc. [29,30,32]. However, the present study conducted only a sampling, not repeated from subsequent years. Therefore, future studies still need to take multiple and successive samples to analyze the temporal and spatial diversity of AMF in *C. oleifera*.

5. Conclusions

AMF diversity in *C. oleifera* was abundant, along with higher diversity of AMF in endosphere versus rhizosphere. Moreover, AMF growth could be affected by soil traits, especially pH, NO_3^-N, and available K, also dependent upon soil depth. Such results provide a scientific basis for onward studies on the role of AMF in field management of *C. oleifera* in order to raise the current low productivity.

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References

1. Zhang, S.; Li, X.-Z. Inhibition of α-glucosidase by polysaccharides from the fruit hull of *Camellia oleifera* Abel. *Carbohydr. Polym.* **2015**, *115*, 38–43. [CrossRef]
2. Lee, C.-P.; Shih, P.-H.; Hsu, C.-L.; Yen, G.-C. Hepatoprotection of tea seed oil (*Camellia oleifera* Abel.) against CCl_4-induced oxidative damage in rats. *Food Chem. Toxicol.* **2007**, *45*, 888–895. [CrossRef]
3. Lin, P.; Wang, K.; Zhou, C.; Xie, Y.; Yao, X.; Yin, H. Seed transcriptomics analysis in *Camellia oleifera* uncovers genes associated with oil content and fatty acid composition. *Int. J. Mol. Sci.* **2018**, *19*, 118. [CrossRef]
4. Li, T.; Zhang, H.; Wu, C.-E. Screening of antioxidant and antitumor activities of major ingredients from defatted *Camellia oleifera* seeds. *Food Sci. Biotechnol.* **2014**, *23*, 873–880. [CrossRef]
5. Zhang, Z.; Meng, J.; Pan, D.; Yang, C.; Li, Y. Mating system and progeny genetic diversity of *Camellia oleifera* 'Ruan Zhi'. *J. For. Res.* **2018**, *30*, 1805–1810. [CrossRef]
6. Peng, J.J.; Zhong, Z.X.; Li, T.T.; Wang, B.M. The effect of bio-fertilizer on the fruit and yield of *Camellia oleifera*. *Anhui Agric. Sci. Bull.* **2020**, *26*, 90–94.
7. Zhang, F.; Zou, Y.N.; Wu, Q.S.; Kuča, K. Arbuscular mycorrhizas modulate root polyamine metabolism to enhance drought tolerance of trifoliate orange. *Environ. Exp. Bot.* **2020**, *171*, 103962. [CrossRef]
8. He, J.-D.; Chi, G.-G.; Zou, Y.-N.; Shu, B.; Wu, Q.-S.; Srivastava, A.; Kuča, K. Contribution of glomalin-related soil proteins to soil organic carbon in trifoliate orange. *Appl. Soil Ecol.* **2020**, *154*, 103592. [CrossRef]
9. Wu, Q.S.; Gao, W.Q.; Srivastava, A.K.; Zhang, F.; Zou, Y.N. Nutrient acquisition and fruit quality of Ponkan mandarin in response to AMF inoculation. *Indian J. Agric. Sci.* **2020**, *90*, 1563–1567.
10. Cheng, H.-Q.; Giri, B.; Wu, Q.-S.; Zou, Y.-N.; Kuča, K. Arbuscular mycorrhizal fungi mitigate drought stress in citrus by modulating root microenvironment. *Arch. Agron. Soil Sci.* **2021**. [CrossRef]
11. Yang, L.; Zou, Y.-N.; Tian, Z.-H.; Wu, Q.-S.; Kuča, K. Effects of beneficial endophytic fungal inoculants on plant growth and nutrient absorption of trifoliate orange seedlings. *Sci. Hortic.* **2021**, *277*, 109815. [CrossRef]
12. Zou, Y.-N.; Zhang, F.; Srivastava, A.K.; Wu, Q.-S.; Kuča, K. Arbuscular mycorrhizal fungi regulate polyamine homeostasis in roots of trifoliate orange for improved adaptation to soil moisture deficit stress. *Front. Plant Sci.* **2021**, *11*, 600792. [CrossRef]
13. Wu, Q.S.; Shao, Y.D.; Gao, X.B.; Xia, T.J.; Kuča, K. Characterization of AMF-diversity of endosphere versus rhizosphere of tea (*Camellia sinensis*) crops. *Indian J. Agric. Sci.* **2019**, *89*, 348–352.
14. Li, X.Q.; Chen, Y.C. Relationship between endophytic mycorrhizal distribution of *Camellia oleifera* and environmental condi-tion. *For. Sci. Technol.* **1982**, *11*, 20–21.
15. Wang, D.X.; Zhang, N.Y.; Chen, G.C. Effects of AM fungi on the growth and drought-resistance of *Camellia oleifera*. *Guangxi For. Sci.* **2011**, *40*, 259–261, 273.
16. Lin, Y.L.; Li, Z.Y.; Zhang, L.P.; Wu, F.; Yang, Y.; Tan, M.X.; Hu, D.N. Effects of organic phosphorus and AM fungi on growth, root morphology and photosynthetic characteristics of *Camellia oleifera*. *Non-Wood For. Res.* **2021**, *39*, 121–128.
17. Jiang, S.; Liu, Y.; Luo, J.; Qin, M.; Johnson, N.C.; Öpik, M.; Vasar, M.; Chai, Y.; Zhou, X.; Mao, L.; et al. Dynamics of arbuscular mycorrhizal fungal community structure and functioning along a nitrogen enrichment gradient in an alpine meadow ecosystem. *New Phytol.* **2018**, *220*, 1222–1235. [CrossRef]
18. Riley, D.; Barber, S.A. Bicarbonate accumulation and ph changes at the soybean (*Glycine max* (L.) Merr.) root-soil interface. *Soil Sci. Soc. Am. J.* **1969**, *33*, 905–908. [CrossRef]
19. Zhang, F.; Wang, P.; Zou, Y.-N.; Wu, Q.-S.; Kuča, K. Effects of mycorrhizal fungi on root-hair growth and hormone levels of taproot and lateral roots in trifoliate orange under drought stress. *Arch. Agron. Soil Sci.* **2019**, *65*, 1316–1330. [CrossRef]
20. Daniels, B.A.; Skipper, H.D. Methods for the recovery and quantitative estimation of propagules from soil. In *Methods and Principles of Mycorrhizal Research*; Schenck, N.C., Ed.; American Phytopathological Society: St. Paul, MN, USA, 1982; pp. 29–35.
21. Bethlenfalvay, G.J.; Ames, R.N. Comparison of two methods for quantifying extraradical mycelium of vesicular-arbuscular mycorrhizal fungi. *Soil Sci. Soc. Am. J.* **1987**, *51*, 834–837. [CrossRef]
22. Mago, T.; Salzberg, S.L. FLASH: Fast length adjustment of short reads to improve genome assemblies. *Bioinformatics* **2011**, *27*, 2957–2963. [CrossRef] [PubMed]
23. Edgar, R.C. Search and clustering orders of magnitude faster than BLAST. *Bioinformatics* **2010**, *26*, 2460–2461. [CrossRef] [PubMed]

24. Oberauner, L.; Zachow, C.; Lackner, S.; Högenauer, C.; Smolle, K.H.; Berg, G. The ignored diversity: Complex bacterial com-munities in intensive care units revealed by 16S pyrosequencing. *Sci. Rep.* **2013**, *3*, 1413. [CrossRef] [PubMed]
25. Tuomisto, H. A diversity of beta diversities: Straightening up a concept gone awry. Part 1. Defining beta diversity as a func-tion of alpha and gamma diversity. *Ecography* **2010**, *33*, 2–22. [CrossRef]
26. Wu, Q.S.; He, X.H.; Zou, Y.N.; He, K.P.; Sun, Y.H.; Cao, M.Q. Spatial distribution of glomalin-related soil protein and its re-lationships with root mycorrhization, soil aggregates, carbohydrates, activity of protease and β-glucosidase in the rhizosphere of Citrus unshiu. *Soil Biol. Biochem.* **2012**, *45*, 181–183. [CrossRef]
27. Brady, N.C.; Wei, R.R. Organisms and ecology of the soil. *Nat. Prop. Soil* **1996**, *11*, 328–360.
28. Wu, Q.S.; Wang, S.; Cao, M.Q.; Zou, Y.N.; Yao, Y.X. Tempo-spatial distribution and related functionings of root glomalin and glomalin-related soil protein in a citrus rhizosphere. *J. Anim. Plant Sci.* **2014**, *24*, 245–251.
29. Guo, X.; Gong, J. Differential effects of abiotic factors and host plant traits on diversity and community composition of root-colonizing arbuscular mycorrhizal fungi in a salt-stressed ecosystem. *Mycorrhiza* **2014**, *24*, 79–94. [CrossRef]
30. Gai, J.P.; Feng, G.; Li, X.L. Diversity of arbuscular mycorrhizal fungi in field soils from North China. *Biodivers. Sci.* **2004**, *12*, 435–440.
31. Liu, Z.K.; Tian, S.; Tang, M. Spatial distribution of arbuscular mycorrhizal fungi and its relationship with soil factors in the rhizosphere of Robinia pseudoacacia at different ages. *Sci. Silvae Sin.* **2013**, *49*, 89–95.
32. Singh, S.; Pandey, A.; Chaurasia, B.; Palni, L.M.S. Diversity of arbuscular mycorrhizal fungi associated with the rhizosphere of tea growing in 'natural' and 'cultivated' ecosites. *Biol. Fertil. Soils* **2008**, *44*, 491–500. [CrossRef]
33. Rodrigues, K.M.; Rodrigues, B.F. Glomus. In *Beneficial Microbes in Agro-Ecology: Bateria and Fungi*; Amaresan, N., Kumar, S.M., Annapurna, K., Kumar, K., Sankaranarayanan, A., Eds.; Academic Press: London, UK, 2020; pp. 561–569.
34. Redecker, D.; Schüβler, A.; Stockinger, H.; Stürmer, S.L.; Morton, J.B.; Walker, C. An evidence-based consensus for the classi-fication of arbuscular mycorrhizal fungi (Glomeromycota). *Mycorrhiza* **2013**, *23*, 515–531. [CrossRef] [PubMed]
35. Wu, Q.S.; Sun, P.; Srivastava, A.K. AMF diversity in citrus rhizosphere. *Indian J. Agric. Sci.* **2017**, *87*, 653–659.