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Arbuscular Mycorrhizal Fungi Associated with Tree Species in a Planted Forest of Eastern China

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Received: 17 April 2019; Accepted: 15 May 2019; Published: 16 May 2019

Abstract: Arbuscular mycorrhizal fungi (AMF) play an important role in the establishment and maintenance of plant communities in forest ecosystems. Most previous studies about AMF have been conducted in natural forests, and little attention has been paid to trees in planted forests. This study investigated AMF associated with tree species and the relationships between edaphic factors and AMF communities in a planted forest of eastern China. We found high total AMF colonization rates in the roots of *Carya illinoensis* (Wangenh.) K. Koch, *Zelkova serrata* (Thunb.) Makinoz, *Taxodium 'zhongshansha'*, *Eucommia ulmoides* Oliv., and *Elaeagnus pungens* Thunb., ranging from 62.07% to 100%, indicating that AMF can establish effective symbiotic relationships with these tree species. The AMF colonization rate was significantly and negatively correlated with soil phosphorus, while AMF colonization intensity was significantly and negatively correlated with soil moisture content, total carbon, and organic matter content. Spore density was in the range of 4.38 to 76.38 spores per g soil. In total, 35 AMF species from 10 genera were identified. *Glomus* and *Acaulospora* were the dominant genera. *Acaulospora foveata* and *Septoglomus constrictum* were the dominant species. AMF communities differed among the tree species and were closely related to edaphic factors, and AMF diversity was significantly related to soil carbon and pH. Our results revealed the colonization, community, and diversity of AMF associated with tree species, as well as their relationships with edaphic factors, in planted forests. Our findings can be used to provide insight on the utilization and management of AMF to maintain sustainable management of planted forests.

Keywords: colonization; AMF composition; diversity; edaphic factors; tree species

1. Introduction

Planted forests play an increasingly important role in meeting the demand for wood and environmental conservation, and 20% of forests in China are categorized as planted forest land, covering an area of about one-third of the world's planted forest land. Decreasing soil quality is the principal threat to sustainable forest management, particularly in planted forests. Harnessing soil microorganisms provides an eco-friendly and cost-effective method to solve that problem. Soil microorganisms play important roles in soil formation, nutrient cycling, nutrient uptake, and reclamation of ecosystems [1–3]. In particular, arbuscular mycorrhizal fungi (AMF) form symbiotic associations with the roots of

more than 80% of plant species [4], and they play a vital role in the successful establishment and maintenance of plant communities [5]. AMF hyphae can take up phosphorous (P), which cannot be absorbed by root hairs [6], and the AMF soil mycelial network provides many benefits to host plants [7], including plant growth promotion [8,9] and improvement of plant resistance to stress and disease [10,11]. In addition, AMF can be beneficial to soil aggregation as a result of the actions of hyphae and glomalin secretion [1,12]. Hence, the occurrence and colonization of AMF would be beneficial to the survival of tree seedlings and the sustainable management of forests. Investigations on the mycorrhizal colonization statuses of tree species were mainly made in natural forest ecosystems, and have rarely been made in planted forests.

Moreover, the AMF species associated with plant species have different functions to hosts and important influences on the distribution, diversity, and regeneration of plant communities [13]. The biological and functional diversity of AMF is vitally important to forest ecosystems, and can be decisive for plant community structures and productivity [14,15]. Previous studies on AMF diversity in forests have been conducted mainly in Brazil [16,17], Mexico [18], USA [19], India [20,21], Bangladesh [22], Ethiopia [23], and China [24]. In China, AMF diversity was examined in grassland ecosystems [25,26], an agroecosystem [27], a wetland ecosystem [28], and a natural forest ecosystem [29], mostly concentrating on medicinal plants [30,31] and herbaceous plants [32]. However, knowledge about the diversity of AMF associated with tree species in planted forests is scanty. It is well known that AMF are widespread in various ecosystems, and their colonization and community are also influenced by soil physicochemical properties [33]. It is important to consider the influence of abiotic factors on root colonization and fungal community composition when investigating the diversity of AMF.

In the present study, *Carya illinoensis* (Wangenh.) K. Koch, *Populus lasiocarpa* Oliv., *Metasequoia glyptostroboides* Hu et Cheng, *Zelkova serrata* (Thunb.) Makinoz, *Taxodium 'zhongshansha'*, *Eucommia ulmoides* Oliv., *Ginkgo biloba* Linn., and *Elaeagnus pungens* Thunb. in a planted forest of eastern China were selected as studied tree species because of their important ecological and economic values. Although some of the tree species, such as *C. illinoensis*, have been described as forming mycorrhizas with ectomycorrhizal fungi Tuber [34], they might also form mycorrhizas with AMF. Here, we focused on the AMF associated with trees. The AMF colonization statuses and spore community compositions in the rhizospheres of the eight tree species were estimated. The relationship between edaphic factors and AMF was been studied. The results of this study would provide insight on the utilization and management of AMF to maintain sustainable management of planted forests.

2. Material and Methods

2.1. Study Sites and Sampling

The Jiangsu coastal area is located in eastern China. The beach area is 6520 million square meters, accounting for more than one quarter of the total beach area in China, which is expanding by 26–33 million square meters per year. Although this beach area is a valuable reserve land resource, poor soil fertility, high salt content, a shallow water table, frequent inclement weather, and a vulnerable ecosystem make it very difficult to construct a protective forest in this area. The Chinese government has been trying to develop forestry in the Jiangsu coastal area. The Dafeng forest farm (33°03′ N, 120°43′ E) of Jiangsu Province was established to develop plantations in the 1950s, and different tree species were transplanted to the farm at different times. Eight ecological and economic tree species, *C. illinoensis*, *P. lasiocarpa*, *M. glyptostroboides*, *Z. serrata*, *T. 'zhongshansha'*, *E. ulmoides*, *G. biloba*, and *E. pungens*, were imported from local tree nurseries in 1990–1998. The tree species were randomly planted in different plots (one tree species per plot), and each tree species was planted in more than three plots. The plots were more than 6000 m^2, and the distance between plots was more than 1000 m. These plots were of light saline land, and the growths of trees were not seriously affected by salt in the soil.

Samples were collected from the planted forest in the Dafeng forest farm in October 2017. The region is located in the subtropical and warm–wet transition zone, with a mean annual temperature of 14 °C and mean annual rainfall of approximately 1058.4 mm [35]. The eight ecological and economic tree species mentioned above were examined in this study, with three sampling plots employed for each tree species. Each plot included triplicate samples, and the distances between the replicates were greater than 10 m. The fine roots and rhizosphere soils (soils adhering to roots) (about 1500 g each) were collected at depths of 5–30 cm from triplicate samples and mixed together as one sample. A total of 24 samples were collected. The soil samples were divided into two parts, with one part used for soil physicochemical analysis and the other used for spore isolation studies. The soil samples were air-dried for about 2 weeks, and the physical and chemical properties of rhizosphere soils adhered to roots of tree species were measured. The roots were washed carefully with tap water, and both the soil and root samples were stored in a refrigerator at 4 °C until used.

2.2. Soil Physicochemical Analysis

The air-dried soil samples were sieved through a 2 mm grid. Soil pH was measured in a soil:water suspension (1:5 (w/v)) with a digital pH meter (PHS-3D, Shanghai Leici Instrument Limited Company, Shanghai, China). To determine the soil moisture content (SM), the soil samples were dried at 105 °C for 48 h, and SM was calculated as water (%) by mass = [(wet mass − dry mass)/dry mass]. Soil electric conductivity (EC) was measured in a soil:water suspension (1:5 (w/v)) using a conductivity meter (DDS-11C, Shanghai Hong Yi Instrument Company). Nitrate nitrogen content (NN) was determined using the phenol disulfonic acid method [36]. Available phosphorus (AP) and total phosphorus (TP) were extracted by sodium bicarbonate, and their concentrations were estimated using molybdenum blue colorimetry [37]. Available potassium (AK) and total potassium (TK) were extracted by ammonium acetate, and their contents were determined using a flame photometer (Sherwood, Model 425 Flame Photometer, Sherwood, Chicago, IL, USA). Organic matter content (OM) was measured by wet oxidation with potassium dichromate [38]. Soil total nitrogen (TN), total carbon (C), and total sulfur (S) contents were determined using an elemental analyzer (Vario MACRO cube, Elementar Trading Shanghai, Shanghai, China).

2.3. Assessment of AMF Colonization

The clearly washed fine roots were soaked in 10% (w/v) KOH, and then stained with 0.05% trypan blue solution in lactic acid–glycerol as described by Phillips and Hayman [39]. The percentage of root colonization including total AMF colonization (RLC, %), vesicle colonization (RLV, %), arbuscules colonization (RLA, %), hyphae colonization (RLH, %), and the colonization intensity were estimated based on a previously described method [40].

2.4. AMF Spore Quantification and Identification

The AMF spores were extracted from a sample of 100 g air-dried soil by wet sieving and sucrose density centrifugation [41]. For determining the spore density (SD), the isolated intact healthy spores were collected on a filter paper and separated by morphotype, including spore size, color, ornamentations, wall layers, and hyphal attachments, and then counted under a dissecting microscope at 45 × magnification. The spores were mounted in polyvinyl lactoglycerol (PVLG) and PVLG + Melzer's reagent (1:1, v/v) [42]. The spores were identified according to the identification manuals provided by [43], recent advances in Glomeromycota taxonomy [44–46], and the reference culture descriptions (http://invam.wvu.edu/the-fungi/classification and http://www.zor.zut.edu.p1/Glomeromycota).

2.5. Diversity Studies and Statistical Analyses

The frequency of occurrence (FO), relative abundance (RA), importance value (IV), spore density (SD), species richness (SR), Shannon–Wiener index (H), and evenness (E) were used to estimate the structure of the AMF community. These parameters can be calculated by the following formulas:

$$FO = (\text{number of samples in which the species or genus was observed/total samples}) \times 100\%$$

where species were put into the following categories, based on percent occurrence: Dominant (50%), most common (31%–50%), common (10%–30%), and rare (<10%), according to Zhang et al. [47].

$RA = (\text{spore number of species or genus/total spore number}) \times 100\%$

$IV = (FO + RA)/2$

SD = spore number/100 g air-dried soil

SR = species number/soil sample

$H = -\sum_{i=1}^{k}(P_i \ln P_i)$; $P_i = n_i/N$, where n_i is the spore number of a species and N is the total number of identified spore samples [48].

$E = H/H_{max}$; $H_{max} = \ln S$, where S is the total number of identified species.

Correlation analysis was conducted using SPSS 17.0 to test for a possible correlation between the colonization rate, diversity of AMF, and soil properties. Redundancy analysis (RDA) and canonical correspondence analysis (CCA) were performed with Canoco for Windows (version 5.0, Microcomputer Power, Ithaca, NY, USA) to reveal the influence of edaphic properties on the composition of AMF communities. Rare species were removed and AMF spore number data were transformed to log $(x + 1)$ for the analysis.

3. Results

3.1. AMF Colonization and SD

The SD and AMF colonization statuses for the eight ecological and economic tree species studied are shown in Table 1. The results revealed that all the tree species examined could be colonized by AMF. Among the eight tree species studied, the RLA, RLH, and SD of *E. ulmoides* were the highest, corresponding to 83.91%, 87.36%, and 12,281 per 100 g air-dried soil, respectively. *C. illinoensis* and *T. 'zhongshansha'* presented the highest RLV (59.00%) and RLC (90.30%). The RLC values of *Z. serrata* and *E. pungens* were more than 70.00%, and their spores were 3871 and 1880 per 100 g air-dried soil, respectively. Although the RLC of *Z. serrata* was not low, the colonization intensity was inferior. Furthermore, the variations in RLC of *M. glyptostroboides* and *P. lasiocarpa* were high, ranging from 12.90% to 86.65%, whereas that of *P. lasiocarpa* ranged from 25.81% to 90.00%. The RLC, RLV, RLA, RLH, and SD of *G. biloba* were lower than those of the other tree species, with values corresponding to 12.26%, 8.93%, 6.70%, 11.15%, and 9.18% per 100 g air-dried soil, respectively. These results indicated that *C. illinoensis*, *P. lasiocarpa*, *M. glyptostroboides*, *T. 'zhongshansha'*, *E. ulmoides*, and *E. pungens* could form a good symbiotic relationship with AMF, whereas *Z. serrata* and *G. biloba* did not rely on AMF.

Table 1. Arbuscular mycorrhizal fungi (AMF) colonization statuses of roots and spore density in the rhizosphere soils of trees.

Tree Species	AMF Colonization/%				Colonization Intensity	SD (No. Per 100 g Air-Dried Soil)
	RLC	RLV	RLA	RLH		
CI	90.3 ± 11.6 a	45.6 ± 40.9 ab	71.8 ± 14.3 ab	81.8 ± 8.6 ab	Medium-strong	934.7 ± 608.2 b
PL	67.2 ± 35.7 a	38.5 ± 38.8 ab	54.7 ± 33.9 ab	67.2 ± 35.9 ab	Inferior-strong	2075.7 ± 403.6 ab
MG	52.1 ± 34.3 a	32.2 ± 28.0 ab	37.7 ± 29.9 bc	47.6 ± 30.1 b	Inferior-medium	1876.0 ± 734.0 ab
ZS	73.1 ± 15.3 a	39.1 ± 14.5 ab	68.4 ± 25.0 ab	73.1 ± 15.3 ab	Inferior	3971.0 ± 2684.9 a
TZ	88.7 ± 9.8 a	59.0 ± 8.7 a	74.5 ± 14.6 ab	86.5 ± 12.1 a	Medium-strong	1478.7 ± 1125.3 ab
EU	87.4 ± 19.0 a	31.5 ± 25.6 ab	83.9 ± 24.9 a	87.4 ± 19.0 a	Strong	4207.0 ± 3069.4 a
GB	12.3 ± 15.4 b	8.9 ± 9.6 b	6.7 ± 5.7 c	11.2 ± 13.4 c	Inferior-medium	918.7 ± 247.4 b
EP	86.7 ± 6.7 a	56.7 ± 13.5 ab	64.6 ± 16.6 ab	86.7 ± 6.7 a	Medium-strong	1880.0 ± 284.0 ab

Tree species: CI, *Carya illinoensis*; PL, *Populus lasiocarpa*; MG, *Metasequoia glyptostroboides*; ZS, *Zelkova serrata*; TZ, *Taxodium 'zhongshansha'*; GB, *Ginkgo biloba*; EP, *Elaeagnus pungens*. RLC, RLV, RLA, and RLH are the percentages of root length with total, vesicle, arbuscules, and hyphae colonization, respectively. SD is the spore density. Different letters indicate significant differences at $p < 0.05$.

3.2. Identification of Spores and AMF Community Composition and Diversity

A total of 35 AMF species were identified in the rhizosphere soils of the eight tree species examined (Figure 1; Table 2). These AMF belonged to 10 genera and six families (Glomeraceae, Claroideoglomeraceae, Gigasporaceae, Acaulosporaceae, Ambisporaceae, and Archaeosporaceae). A total of 79.51% of AMF spores were from the Glomeraceae family, and 49.28% were from the genus *Rhizophagus*. *Acaulospora foveata* and *Septoglomus constrictum* were the dominant species, with FO values of more than 50%. *Acaulospora foveata* was found in the rhizosphere soils of all tree species studied, while *Rhizophagus clarus* (28.99%) was the most abundant AMF species.

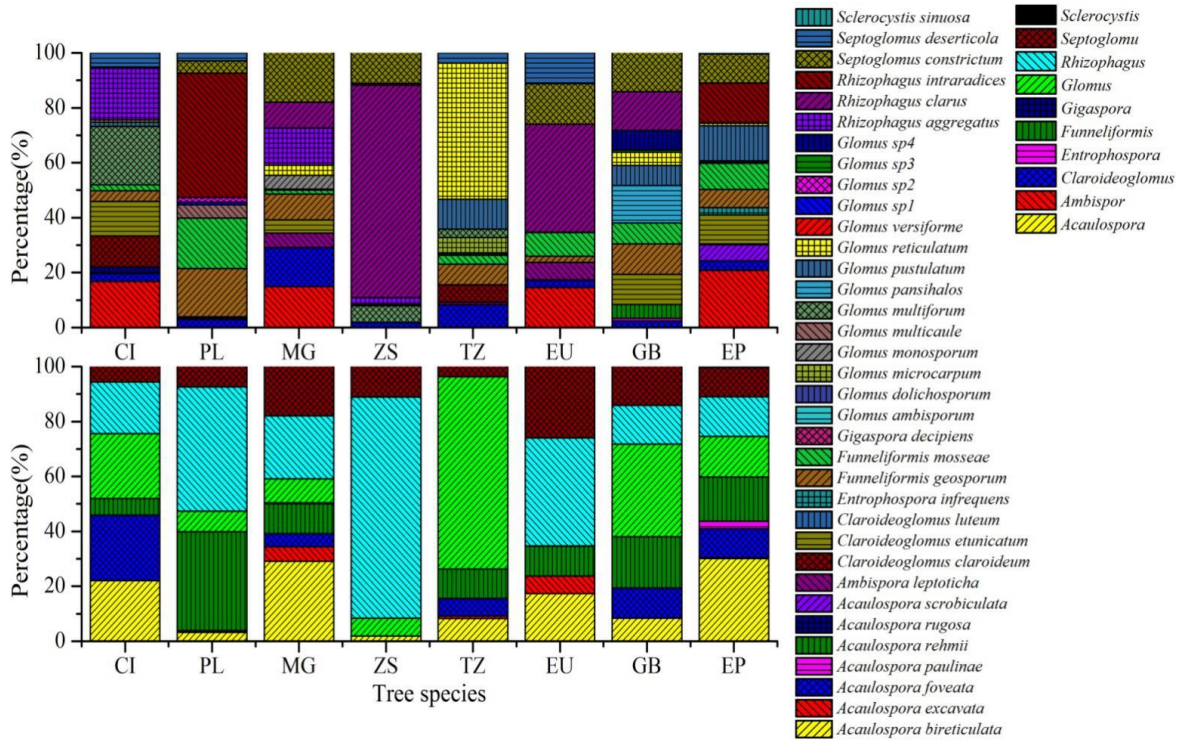

Figure 1. AMF community composition in the rhizosphere soils of different tree species. Tree species: CI, *Carya illinoensis*; PL, *Populus lasiocarpa*; MG, *Metasequoia glyptostroboides*; ZS, *Zelkova serrata*; TZ, *Taxodium 'zhongshansha'*; GB, *Ginkgo biloba*; EP, *Elaeagnus pungens*.

Table 2. Occurrence frequency, relative abundance, and important values of arbuscular mycorrhizal (AM) fungi species for trees.

Species No.	AM Fungi	Tree Species								FO (%)	RA (%)	IV (%)
		CI	PL	MG	ZS	TZ	EU	GB	EP			
1	*Acaulospora bireticulata*	−	−	−	−	−	−	+	−	4.17	0.008	2.09
2	*Acaulospora excavata*	+	−	+	−	−	+	−	+	25	8.317	16.66
3	*Acaulospora foveata*	+	+	+	+	+	+	+	+	50	4.352	27.18
4	*Acaulospora paulinae*	−	−	−	−	−	−	+	−	4.17	0.047	2.11
5	*Acaulospora rehmii*	−	+	−	−	−	+	+	−	12.5	0.318	6.41
6	*Acaulospora rugosa*	+	−	−	−	−	−	−	−	4.17	0.124	2.15
7	*Acaulospora scrobiculata*	−	−	−	−	−	−	−	+	4.17	0.628	2.40
8	*Ambispora leptoticha*	−	−	+	−	+	+	−	+	26.67	2.219	14.44
9	*Claroideoglomus claroideum*	+	−	−	−	+	−	−	−	12.5	1.156	6.83
10	*Claroideoglomus etunicatum*	+	+	+	−	−	−	+	+	33.33	2.964	18.15
11	*Claroideoglomus luteum*	−	+	−	−	−	−	−	−	4.17	0.031	2.10
12	*Entrophospora infrequens*	−	−	−	−	−	−	−	+	4.17	0.287	2.23
13	*Funneliformis geosporum*	+	+	+	−	+	+	+	+	37.5	5.842	21.67
14	*Funneliformis mosseae*	+	+	+	−	+	+	+	+	37.5	6.307	21.90
15	*Gigaspora decipiens*	−	−	+	−	−	−	−	−	4.17	0.039	2.10
16	*Glomus ambisporum*	−	−	−	−	−	+	−	−	4.17	0.008	2.09
17	*Glomus dolichosporum*	−	−	−	−	+	−	−	−	4.17	0.062	2.12
18	*Glomus microcarpum*	−	−	−	−	+	−	−	−	4.17	0.489	2.33
19	*Glomus monosporum*	−	−	+	−	−	−	−	−	8.33	0.551	4.44
20	*Glomus multicaule*	−	+	−	−	−	−	−	+	8.33	0.628	4.48
21	*Glomus multiforum*	+	−	−	+	+	−	−	+	16.67	2.832	9.75
22	*Glomus pansihalos*	−	−	−	−	−	−	+	−	4.17	0.721	2.45
23	*Glomus pustulatum*	+	−	−	−	+	−	+	+	29.17	2.785	15.98
24	*Glomus reticulatum*	+	−	+	+	+	−	+	+	29.17	5.112	17.14
25	*Glomus versiforme*	−	−	−	−	−	−	−	+	4.17	0.031	2.10
26	*Glomus sp1*	−	+	−	−	−	−	−	−	4.17	0.116	2.14
27	*Glomus sp2*	−	+	−	−	−	−	−	−	4.17	0.209	2.19
28	*Glomus sp3*	−	−	−	−	−	−	+	−	4.17	0.039	2.10
29	*Glomus sp4*	−	−	−	−	−	−	+	−	4.17	0.380	2.28
30	*Rhizophagus aggregatus*	+	−	+	+	−	−	−	−	16.67	3.049	9.86
31	*Rhizophagus clarus*	−	−	+	+	−	+	+	−	29.17	28.991	29.08
32	*Rhizophagus intraradices*	−	+	−	+	−	−	−	+	20.83	7.145	13.99
33	*Septoglomus constrictum*	+	+	+	+	−	+	+	+	54.17	10.512	32.34
34	*Septoglomus deserticola*	+	+	−	−	+	+	−	−	16.17	3.662	9.92
35	*Sclerocystis sinuosa*	−	−	−	−	−	−	−	+	4.17	0.039	2.10
Species richness		13	12	12	7	11	10	14	16			

Tree species: CI, *Carya illinoensis*; PL, *Populus lasiocarpa*; MG, *Metasequoia glyptostroboides*; ZS, *Zelkova serrata*; TZ, *Taxodium 'zhongshansha'*; GB, *Ginkgo biloba*; EP, *Elaeagnus pungens*. FO, RA, and IV are the frequency of occurrence, relative abundance, and importance value, respectively.

Among the eight tree species investigated, the SR of AMF was the highest for *E. pungens* and lowest for *Z. serrata* (Table 3). Interestingly, the most abundant AMF species and genera differed with the tree species. *R. clarus* was the most abundant AMF species for *Z. serrata* and *E. ulmoides*, *Glomus reticulatum* was the most abundant AMF species for *T. 'zhongshansha'*, and *Rhizophagus intraradices* was the most abundant AMF species for *P. lasiocarpa*. Furthermore, *Rhizophagus* was the most abundant AMF genus for *P. lasiocarpa*, *Z. serrata*, and *E. ulmoides*; *Glomus* was the most abundant AMF genus for *T. 'zhongshansha'* and *G. biloba*; and *Acaulospora* was the most abundant genus for *M. glyptostroboides* and *E. pungens*. The SR for *E. ulmoides* was significantly lower than those for *E. pungens* and *C. illinoensis*. The H values for *E. ulmoides* and *Z. serrata* were significantly lower than those for *C. illinoensis*, *M. glyptostroboides*, and *G. biloba*, and the E value for *M. glyptostroboides* was significantly higher than that for *Z. serrata* (Table 3).

Table 3. Diversity indices of AMF communities in the rhizosphere soils of trees.

	CI	PL	MG	ZS	TZ	EU	GB	EP
SR	6.33 ± 2.08 a	5.67 ± 2.31 ab	5.33 ± 0.58 ab	4.00 ± 1.00 ab	500 ± 1.73 ab	3.33 ± 0.58 b	5.67 ± 1.15 ab	6.67 ± 1.53 a
H	1.37 ± 0.36 a	1.29 ± 0.24 ab	1.52 ± 0.11 a	0.81 ± 0.40 b	1.11 ± 0.34 ab	0.82 ± 0.10 b	1.54 ± 0.17 a	1.30 ± 0.31 ab
E	0.76 ± 0.06 ab	0.80 ± 0.11 ab	0.91 ± 0.05 a	0.58 ± 0.22 b	0.74 ± 0.27 ab	0.71 ± 0.17 ab	0.89 ± 0.03 ab	0.70 ± 0.19 ab

Tree species: CI, *Carya illinoensis*; PL, *Populus lasiocarpa*; MG, *Metasequoia glyptostroboides*; ZS, *Zelkova serrata*; TZ, *Taxodium 'zhongshansha'*; GB, *Ginkgo biloba*; EP, *Elaeagnus pungens*. SR, H, and E are the species richness, Shannon–Wiener index, and evenness of the AMF community, respectively. Different letters indicate significant differences at $p < 0.05$.

3.3. Chemical and Physical Soil Parameters

The soil properties of the rhizospheres of the eight ecological and economic tree species examined are presented in Table 4. The SM of the rhizosphere of *Z. serrata* was significantly higher than those of the rhizospheres of *E. ulmoides* and *G. biloba*. The soil TN in the rhizosphere of *E. pungens* was significantly higher than that in the rhizosphere of *E. ulmoides*. Furthermore, the soil TP in the rhizosphere of *G. biloba* was the highest, and was significantly higher than those in the rhizospheres of the other tree species, while the soil AP ranged from 2.31 to 17.11 mg/kg, with the highest soil AP being found in the rhizosphere of *M. glyptostroboides*. The soil OM, C, and S in the rhizospheres of *Z. serrata* and *M. glyptostroboides* were higher than those in the rhizospheres of the other tree species. However, no differences in soil pH, EC, NN, AK, and TK were found among all the soil samples from the rhizospheres of the examined tree species.

Table 4. Chemical properties of soil in the rhizospheres of eight tree species.

Species	pH	SM (%)	EC (μS/cm)	NN (mg/kg)	TN (%)	AP (mg/kg)	TP (mg/kg)	AK (mg/kg)	TK (g/kg)	OM (g/kg)	C (%)	S (‰)
CI	8.05 a	28.13 a	298.3 a	37.99 a	0.400 ab	12.50 ab	854.7 b	156.4 a	14.35 a	4.23 c	1.60 c	0.285 abc
PL	7.95 a	21.22 ab	259.0 a	35.47 a	0.300 ab	1.75 b	737.0 b	134.2 a	15.54 a	4.91 c	1.67 bc	0.252 abcd
MG	7.78 a	22.71 ab	200.6 a	52.43 a	0.253 ab	17.11 a	906.5 b	175.9 a	14.40 a	11.32 ab	2.00 ab	0.299 ab
ZS	7.77 a	29.26 a	248.3 a	33.72 a	0.297 ab	2.31 b	829.5 b	184.6 a	13.46 a	12.87 a	2.20 a	0.307 a
TZ	7.97 a	20.04 ab	191.1 a	26.63 a	0.327 ab	3.00 b	788.9 b	110.9 a	15.60 a	5.26 bc	1.72 bc	0.225 cd
EU	7.88 a	14.78 b	178.4 a	69.14 a	0.187 b	4.93 b	811.8 b	167.1 a	13.96 a	7.09 abc	1.84 abc	0.232 bcd
GB	8.13 a	19.89 b	145.6 a	38.24 a	0.417 ab	7.77 ab	1272.8 a	134.4 a	14.06 a	2.98 c	1.58 c	0.192 d
EP	8.11 a	25.75 ab	180.4 a	13.48 a	0.727 a	6.07 b	931.9 b	120.2 a	14.02 a	4.35 c	1.73 bc	0.215 cd

Tree species: CI, *Carya illinoensis*; PL, *Populus lasiocarpa*; MG, *Metasequoia glyptostroboides*; ZS, *Zelkova serrata*; TZ, *Taxodium 'zhongshansha'*; GB, *Ginkgo biloba*; EP, *Elaeagnus pungens*. SM, organic matter; EC, soil electric conductivity; NN, nitrate nitrogen content; TN, soil total nitrogen; AP, available phosphorus; TP, total phosphorus; AK, available potassium; TK, total potassium; OM, organic matter; C, total carbon; S, total sulfur. Different letters indicate significant differences at $p < 0.05$.

3.4. Relationship between Soil Factors and AMF

AMF colonization and community diversity were significantly related to soil properties (Table 5). While RLC, RLA, and RLH were negatively and significantly correlated with TP ($p < 0.01$), a significant positive correlation was observed between RLV and TK ($r = 0.483$, $p < 0.05$). Colonization intensity was noted to be negatively and significantly correlated with SM ($r = -0.417$, $p < 0.05$), OM ($r = -0.467$, $p < 0.05$), C ($r = 0.496$, $p < 0.05$), and S ($r = 0.453$, $p < 0.05$). Moreover, while there was no significant correlation between SD and soil properties, a significant positive correlation was found between SR and pH ($r = 0.478$, $p < 0.05$). Besides, C negatively and significantly affected SR ($r = -0.415$, $p < 0.05$) and H ($r = -0.408$, $p < 0.05$).

Table 5. Pearson's correlation coefficients between AMF community parameters and edaphic factors.

	pH	SM	EC	NN	TN	AP	TP	AK	TK	OM	C	S
RLC	−0.092	−0.044	0.067	−0.246	0.086	−0.112	−0.635 **	−0.094	0.284	−0.051	−0.006	0.170
RLV	−0.220	−0.061	−0.045	−0.266	0.235	−0.161	−0.378	−0.190	0.483 *	−0.030	−0.026	0.067
RLA	−0.151	−0.151	0.030	−0.223	0.033	−0.184	−0.568 **	−0.076	0.192	0.029	0.028	0.163
RLH	−0.105	−0.060	0.078	−0.233	0.081	−0.173	−0.627 **	−0.114	0.298	−0.020	0.019	0.160
COI	0.331	−0.417 *	−0.257	0.020	−0.018	−0.064	−0.269	−0.101	0.400	−0.467 *	−0.496 *	−0.453 *
SD	−0.112	−0.340	−0.074	−0.020	−0.117	0.012	−0.056	−0.074	−0.115	0.100	0.034	−0.038
SR	0.478 *	0.006	−0.337	−0.291	0.279	−0.005	0.095	−0.129	−0.074	−0.300	−0.415 *	−0.373
H	0.169	0.002	−0.332	−0.102	0.274	0.318	0.374	−0.215	−0.130	−0.316	−0.408 *	−0.305
E	−0.146	−0.004	−0.131	0.115	0.121	0.365	0.346	−0.212	−0.028	−0.228	−0.218	−0.099
Acaulospora	0.169	−0.108	−0.232	−0.031	−0.072	−0.009	−0.248	0.179	−0.110	0.216	0.198	−0.041
Ambispora	−0.108	−0.445 *	−0.126	0.051	−0.190	0.272	−0.037	−0.026	−0.069	−0.002	−0.033	−0.076
Claroideoglomus	0.600 **	0.232	0.274	−0.116	0.104	0.066	0.000	−0.055	0.070	−0.280	−0.329	0.011
Funneliformis	−0.211	−0.080	0.092	0.368	0.012	−0.056	−0.062	0.009	−0.033	−0.326	−0.086	−0.148
Glomus	0.047	−0.099	−0.087	−0.169	0.050	−0.231	0.048	−0.168	0.483 *	−0.019	0.050	0.003
Rhizophagus	−0.360	−0.026	0.148	−0.108	−0.039	−0.099	−0.073	0.110	−0.215	0.347	0.334	0.295
Septoglomus	−0.243	−0.415 *	−0.151	−0.063	−0.080	0.073	−0.103	0.055	−0.263	0.274	0.215	0.027
Glomeraceae	−0.394	−0.175	0.078	−0.074	−0.036	−0.142	−0.087	0.059	−0.116	0.282	0.328	0.220

SM, organic matter; EC, soil electric conductivity; NN, nitrate nitrogen content; TN, soil total nitrogen; AP, available phosphorus; TP, total phosphorus; AK, available potassium; TK, total potassium; OM, organic matter; C, total carbon; S, total sulfur. RLC, RLV, RLA, and RLH are percentages of root length with total, vesicle, arbuscules, and hyphae colonization, respectively. COI, colonization intensity; SD, spore density; SR, H, and E are species richness, Shannon–Wiener index, and evenness of the AMF community, respectively. * $p < 0.05$, ** $p < 0.01$.

Based on the RDA results related to AMF community composition (Figure 2), it was noted that pH significantly related to *Claroideoglomus* ($p < 0.01$), SM significantly related to *Ambispora* and *Septoglomus* ($p < 0.05$), and TK significantly related to *Glomus* ($p < 0.05$). However, none of the soil properties had significant relationships with *Acaulospora*, *Rhizophagus*, *Funneliformis*, and Glomeraceae.

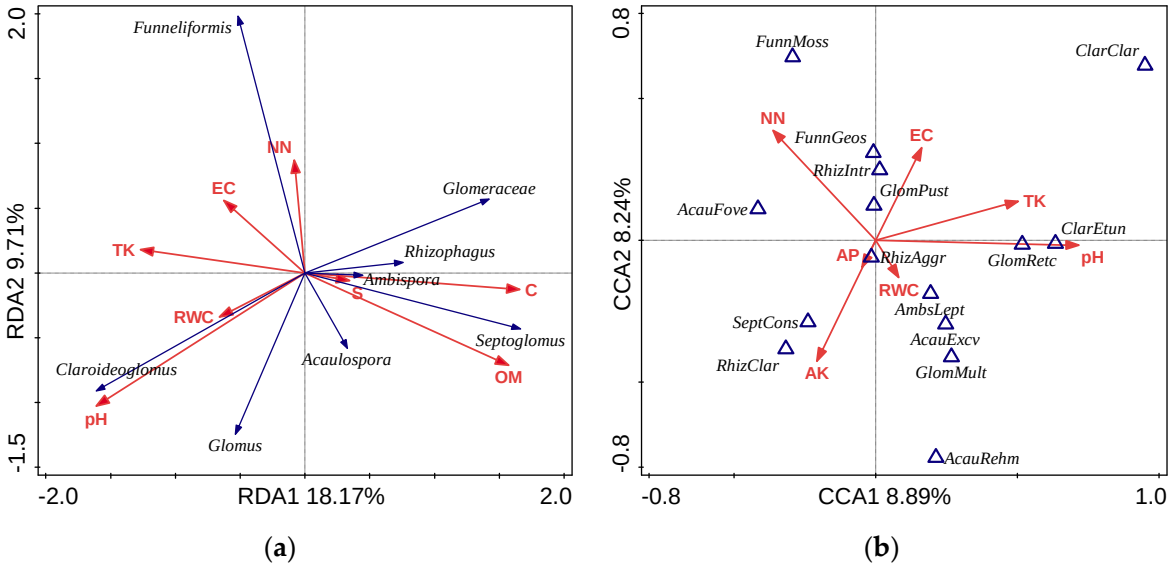

Figure 2. The redundancy analysis (RDA) (**a**) and canonical correspondence analysis (CCA) (**b**) of AMF genera and species with edaphic factors. AcauExcv, *Acaulospora excavate*; AcauFove, *Acaulospora foveata*; AcauRehm, *Acaulospora rehmii*; AmbsLept, *Ambispora leptoticha*; ClarClar, *Claroideoglomus claroideum*; ClarEtum, *Claroideoglomus etunicatum*; FunnGeos, *Funneliformis geosporum*; FunnMoss, *Funneliformis mosseae*; GlomMult, *Glomus multiforum*; GlomPust, *Glomus pustulatum*; GlomRetc, *Glomus reticulatum*; RhizAggr, *Rhizophagus aggregatus*; RhizClar, *Rhizophagus clarus*; Rhizintr, *Rhizophagus intraradices*; SeptCons, *Septoglomus constrictum*.

CCA revealed that edaphic factors could explain only 17.17% of the variations in AMF species composition and diversity (Figure 2). According to the CCA results, soil pH and TK were positively correlated with the occurrence of *Acaulospora excavate, Claroideoglomus etunicatum*, and *G. reticulatum*; soil EC and NN were positively correlated with the occurrence of *Acaulospora*

foveata, *Claroideoglomus claroideum*, *Funneliformis geosporum*, *Funneliformis mosseae*, *Glomus pustulatum*, and *Rhizophagus intraradices*; and SM, AP, and AK were positively correlated with the occurrence of *Acaulospora rehmii*, *Ambispora leptoticha*, *Glomus multiforum*, *Rhizophagus aggregates*, *Rhizophagus clarus*, and *Septoglomus constrictum*.

4. Discussion

The results of the present study showed that the eight tree species examined in the planted forest were colonized by AMF. However, the AMF colonization status varied significantly depending on sampling point and the planted tree species, consistent with the results of a previous study on AMF of tree species in different forests [22]. Similar variations in AMF colonization have also been found in other types of forests, such as tropical rainforests [24,49] and peat swamp forests [50]. These variances in the colonization of AMF may be caused by mycorrhizal dependencies of different host plants [51], by colonization abilities of various AMF species, and by climatic and edaphic factors [52]. In the present study, when compared with other tree species, the total AMF colonization rates for *C. illinoensis*, *Z. serrata*, *T. 'zhongshansha'*, *E. ulmoides*, and *E. pungens* were high, ranging from 62.07% to 100%. A previous study demonstrated that *C. illinoensis* formed mycorrhizas with ectomycorrhizal fungi Tuber [34]. Our study indicated that AMF can establish better symbiotic relationships with these five tree species above, including *C. illinoensis*, compared with other tree species.

It is well known that soil properties have an important influence on the colonization of AMF. High soil fertility can affect the sporulation and colonization ability of AMF. Among all the nutrient elements, P is most closely associated with mycorrhizal formation, playing an important role in regulating the colonization rate of AMF [51]. A fertilization experiment conducted by Shi et al. showed that the colonization rate of AMF was reduced after the addition of a phosphatic fertilizer to the soil [53]. In the present study, the differences of rhizosphere soil physical and chemical properties might be due to the effects of tree species, but in turn could affect AMF colonization and community composition. Correlation analysis showed that RLC, RLA, and RLH were negatively and significantly correlated with TP ($p < 0.01$). It has been speculated that soil containing a high P content might have a negative impact on the transduction of phosphatase secreted by AMF, restraining the growth of AMF [54]. A significant and positive correlation was observed between RLC and TK; however, its underlying reason needs further research. The OM, which serves as a nutrient sink for plants, could also regulate the intensity of AMF colonization [55], and soil humidity levels have been found to have an important effect on AMF colonization and mycorrhizal efficiency [56]. In the present study, the colonization intensity of AMF was significantly and negatively correlated with SM, C, and OM.

Evaluation of the percent population of AMF spores in the rhizosphere soils of the eight tree species revealed values of SD in the range of 4.38–76.38 per g soil, which are much higher than those reported in the Hazarikhil forest in Bangladesh (0.35–4.32 spores per g soil) [22] and Amazonian terra firme forest in Brazil (1.5–9.4 spores per g soil) [16], comparable with those found in the tropical rainforest of Xishuangbana in China (0.6–19.1 spores per g soil) [57] and the subtropical forest of Huangshan in China (0.45–32.50 spores per g soil) [29], and much lower than those noted in a primary forest in the French Guiana (50–154 spores per g soil) [58]. Besides, no significant relationship was found between SD and edaphic factors, whereas a significant and positive association was detected between SD and AMF colonization rates. These results are in accordance with the research reported by Louis and Lim [59], but contrast to the results of Chaiyasen et al. [60]. These varying findings could be due to plant root traits, spore germination, AMF colonization capacities, and environmental factors.

Glomerospores have key characteristics for identification at the species level, and many studies on AMF have been successfully conducted based on morphological identification [61,62]. In the present study, a total of 35 AMF species were detected via spore morphology in the planted forest, compared with 27 AMF species found in a tropical rainforest [57], 58 AMF species observed in the Brazilian Atlantic forest [63], and 25 AMF species noted in a subtropical forest [29]. Our results demonstrated a high AMF diversity associated with the ecological and economic trees in planted forests. Besides,

in the present study, Glomeraceae was the most abundant family, *Glomus* and *Acaulospora* were the dominant genera, and *Acaulospora foveata* and *Septoglomus constrictum* were the dominant species observed in the rhizosphere of the examined planted forest. Previous studies have also reported *Glomus* and *Acaulospora* to be the dominant genera in other forests [33,57], and they might form a functional complementarity with the host. The dominance of *Glomus* and *Acaulospora* in planted forests may be related to their smaller spore size, which allows them to easily produce more spores in a short time period. Furthermore, the high isolation frequencies of *Acaulospora foveata* and *Septoglomus constrictum* noted in the present study demonstrated the ability of these fungi to adapt well to the local environment. Nevertheless, although *Acaulospora foveata* and *Septoglomus constrictum* were widely distributed in soil, their relative abundances were not high, which may be due to their moderate sporulation ability, but strong mycelial network, which can extend over a large area, leading to a wide distribution in soil [64].

The AMF SR and communities in the rhizosphere soils of different tree species varied, which is in accordance with those reported in previous studies [65,66]. These differences in the AMF SR and communities may be owing to the preferences of host plants to AMF [67]. In addition, correlation analysis revealed that AMF SR and H were significantly related to soil C, which could possibly be owing to the abilities of host plants to deliver different levels of C to the soil and indirectly affect the sporulation, growth, and survival of different AMF [14,68]. It must be noted that the differential microenvironments that host plants offer can influence AMF sporulation, community abundance, and diversity. Accordingly, in the present study, *Rhizophagus clarus* spores were abundant in the rhizosphere soil of *Z. serrata*, whereas *Glomus reticulatum* spores were high in the rhizosphere soil of *T. 'zhongshansha'*. Thus, these findings could be potentially valuable for selecting high-efficiency AMF for the afforestation of different tree species.

Edaphic factors had a strong impact on the composition of AMF communities [27]. Soil pH is considered to be a key environmental factor that shapes the AMF community structure [69] by influencing the availability of some ions and nutrients from the soil to plants [70]. A previous study showed that soil P was the most significant factor affecting the AMF communities [52], because high P levels in soil could reduce the content and change the composition of root exudates that carry materials and energy necessary for AMF [71]. In addition, soil texture [72], OM [73], and EC [27] have also been reported to affect AMF communities. In the present study, RDA and CCA suggested that soil C and pH were the significant drivers of AMF composition and diversity, followed by soil SM, TP, and TK. Furthermore, in accordance with a previous study suggesting that glomoid species thrive in high pH (7.8) [74], the high abundance and richness of *Funneliformis*, *Glomus*, *Rhizophagus*, and *Septoglomus* observed in the present study, all of which belong to Glomeraceae, might be attributed to the high soil pH (7.62–8.56). Besides, high soil pH might also be responsible for the relatively low abundance of *Acaulospora*, which is more widely distributed in acidic soils. Unlike soil pH, the relationship between soil C and AMF communities has rarely been examined. Moreover, the relative abundance of *Ambispora* and *Septoglomus* was significantly and positively related to SM, indicating that they may be not adapted to dry conditions. In addition, climatic conditions have been indicated to have an important effect on AMF communities [52], which requires further research.

5. Conclusions

In the studied planted forest, the roots of most trees were colonized by AMF. Among them, the roots of *C. illinoensis*, *Z. serrata*, *T. 'zhongshansha'*, *E. ulmoides*, and *E. pungens* were readily colonized by AMF, which indicates that the application of AMF in afforestation of these tree species has great potential. The AMF colonization rate was significantly and negatively correlated with soil P, which suggests that the application of AMF in afforestation is more suitable in low P soil. In the rhizosphere soils of all the examined tree species, abundant spore numbers and high diversities of AMF species were found. *Glomus* and *Acaulospora* were the dominant genera, meaning that they are the most promising genera for application in afforestation. The AMF community composition and diversity were highly related to the host trees and edaphic factors, which indicates that local edaphic factors should be

considered when applying AMF in afforestation. In total, the results of this study could be beneficial for the sustainable management of planted forests.

Author Contributions: J.Z., G.G.W., and J.W. conceived the experiments. J.W., Z.Y., Z.F., L.Z., and S.M. conducted the experiments. J.W. and Y.Y. interpreted the data. J.W. wrote the manuscript. B.Z. revised the manuscript. All authors read and approved the final manuscript.

Funding: This work was financially supported by the Agricultural Science and Technology Independent Innovation Fund of Jiangsu province of China (Grant No. CX (17) 004), the National Special Fund for Forestry Scientific Research in the Public Interest (Grant No. 201504406), the Major Fund for Natural Science of Jiangsu Higher Education Institutions (Grant No. 15KJA220004), the Priority Academic Program Development of Jiangsu Higher Education Institutions (PAPD), and the Doctorate Fellowship Foundation of Nanjing Forestry University (2169125).

Acknowledgments: B.Z. was supported by the Greater Everglades Priority Ecosystem Science program and UC Davis Chancellors' postdoc fellowship. The authors thank Yang of Nanjing Forestry University for his suggestions during the writing of this manuscript and the staff at the Dafeng forest farm for their assistance in the field work. We also thank Donald L. De Angelis of the University of Miami and International Science Editing (http://www.internationalscienceediting.com) for editing this manuscript.

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

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