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Characteristics and dynamics of arbuscular mycorrhizal fungal communities along a chronosequence of teak (*Tectona grandis*) plantations in Mt. Jianfengling, Hainan Island, China

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Abstract

Teak (*Tectona grandis* L.f.) is one of the most promising timber species in the tropical and subtropical areas in south China. Arbuscular mycorrhizal (AM) fungi play a crucial role in promoting plant growth, enhancing plant stress resistance and sustaining healthy ecosystem. However, little is known about mycorrhizal status in teak plantations. This study aims to characterize the dynamics of AM fungal communities in the rhizosphere of teak plantations at different ages. Fine roots and rhizosphere soils in teak plantations at varying ages (22, 35, 45 and 55 years old), and the adjacent native grassland without teak plantation (CK) were assessed for soil properties, and AM fungal communities using amplicon sequencing technology. With the increase of stand ages, catalase and ammonium nitrogen in the rhizosphere soil were also increased; soil organic carbon, total phosphorous (P), acid phosphatase, available potassium (AK) and available phosphorus (AP) were first increased and then declined at 55-year-old stand. In total, 12 and 9 AM fungal genera were detected in the rhizosphere soil and in teak root samples, respectively. The OTUs data revealed that AM fungi presented in the rhizosphere soil and roots were mostly belonged to *Glomus*. In the rhizosphere soil, the relative abundance of Glomus was first increased and then declined, while *Gigaspora* and *Scutellospora* were declined, although the diversity and richness of AM fungi showed no significant variation with stand ages. In roots, the composition of AM fungal community and its diversity did not change with stand ages, whereas the richness was increased with the stand age. The monte carlo permutation test indicated that AK, nitrate nitrogen and C/P ratio largely explained the shift in the composition of AM fungal community in the rhizosphere soil. The results demonstrated that AM fungal communities in the rhizosphere soil and teak roots shifted across plantation ages. These changes were largely attributed to the age-induced variation in soil properties.

Keywords: Tectona grandis; arbuscular mycorrhizal fungi; stand age; soil properties; plantations

Introduction

Arbuscular mycorrhizal (AM) fungi are a kind of widely distributed soil microbes that can form mutual association with over 80% of terrestrial plants, and act as a bridge connecting the belowground with the aboveground ecosystems (Wardle et al., 2004; van der Heijden et al., 2015). It is generally accepted that AM fungi can exert major effect on plant growth via promoting the nutrient uptake, especially phosphorus (P) (Smith and Read, 2008), protecting plants against abiotic stresses, such as drought, salinity, heavy metals, low temperature (Zou et al., 2015). Whereas, these physiological and ecological functions of AM fungi are highly dependent on their species diversity and community composition (Jiang et al., 2018). Therefore, a growing number of studies are

focusing on species diversity and mechanisms of community assembly of AM fungi in the ecosystems and exploring factors shaping AM fungal community composition.

Afforestation is an effective restoration technique via increasing vegetation and biodiversity for restoration of the degraded forest ecosystems (Wang et al., 2021). It can change composition and diversity of vegetation flora, soil physicochemical parameters, microclimate under the forest as well as accumulation and decomposition of litter through ecological succession (Zhao et al., 2018; Xu et al., 2020a). Thus, these shifts mentioned above may influence the AM community. The recipient symbiosis between plants and AM fungi is based on carbon cycle, specifically fatty acids (Jiang et al., 2017), suggesting that AM fungi may be influenced by variation of plant community composition and their growth and development (Lerat et al., 2003). For example, the developmental stage of host plant and plant communities also effect the composition and diversity of AM fungi (Chen et al., 2000; Hart et al., 2014).

Teak (*Tectona grandis* L.f.) is widely cultivated in the world due to its excellent timber qualities, high market demand, good economic and social value (Zhou et al., 2017; Huang et al., 2019). As a tropical hardwood tree species, teak is naturally distributed in India, Myanmar, Laos and Thailand (Yang et al., 2020). However, scarce information is available on diversity of AM fungi and changes in community structure over a period of time after afforestation. Considering the vital physiological and ecological functions of AM fungi in promoting plant growth and nutrient absorption, it is necessary to investigate AM fungal association with teak, screen out the potential AM fungi isolates and provide insights into the development of biological fertilizer for teak.

Using the high-throughput sequencing technique, this study investigated AM fungal communities in four teak plantations with varying ages and the adjacent native grassland in the Mt. Jianfengling, Hainan Province, China. The objectives of this study were (i) to analyze the AM fungal community characteristics and dynamics over increasing stand ages of teak plantation, and (ii) to explore key edaphic factors driving variation of AM fungal community.

Methodology

1- Study location and climate

The study was conducted at Mt. Jianfengling (18°20′—18°57′ N, 108°41′—109°12′ E) of Hainan, China. This region situated at an altitude of 60-750 m, and characterized by a tropical monsoon climate with an annual mean temperature of 24.5°C.

2- Experimental design and sampling

Teak plantations chronosequence included four age groups of 22, 35, 45 and 55 years. In addition, nearby native grassland, with no history of teak plantation was sampled as control and marked as CK (**Table 1**). Three standard sites with 20 m × 20 m were set up for each age group. The rhizosphere soil and fine roots were collected. Five dominant teak trees in each standard site were selected as the research objects. Top soil of 5 cm depth was removed in each selected site to get exposed the roots. The rhizosphere soil was obtained by gently brushing the root surface with a sterile brush. All samples of fine roots or the rhizosphere soil from each site were mixed to form a composite sample for roots or soil. The rhizosphere soil was divided into two parts, one for soil properties analysis; the other part and fine roots were stored in a deep freezer at -80℃ for DNA extraction.

Table 1： Characteristics of different stands of the teak plantations

Sites	Mean DBH (cm)	Mean height (m)	Canopy closure (%)	Altitude (m)	Slope degree (°)	Dominant species in understory
CK	–	–	–	118	11	*H. contortus*
22 Y	16.9	21.1	0.70	142	10	*E. odoratum*, *B. cristata*, *C. microphylla*
35 Y	20.3	32.9	0.76	104	17	*A. dioica*, *L. leucocephala*, *T. planicaule*
45 Y	23.4	35.5	0.67	96	13	*L. leucocephala*, *A. pavonlna*
55 Y	24.5	39.7	0.73	135	8	*E. odoratum*, *L. coromandelica*, *G. lobbianum*, *C. microphylla*

DBH, diameter at breast height; *H. contortus, Heteropogon contortus; E. odoratum*, *Eupatorium odoratum; B. cristata, Barleria cristata; C. microphylla, Carmona microphylla; A. dioica, Aporusa dioica; L. leucocephala, Leucaena leucocephala; T. planicaule, Tetrastigma planicaule; A. pavonlna, Adenanthera pavonlna; L. coromandelica, Lannea coromandelica; G. lobbianum, Gonocaryum lobbianum*. 22Y: 22-year stand; 35Y: 35-year stand; 45Y: 45-year stand; 55Y: 55-year stand; CK: control.

3-Soil properties and analysis

The pH of soil suspension (water ratio of 1: 2.5, w/v) was assessed using a glass electrode pH meter, and the total organic carbon (TOC) content by the high-temperature external heat dichromate oxidation-capacity method (Schinner et al., 1996). Total nitrogen (N) was measured by Kjeldahl method; the soil total phosphorous (P), total potassium (K) and available potassium (AK) content were measured by the methods reported by Ma *et al*. (Ma et al., 2020), and soil available phosphorous (AP) was extracted by HCl-NH_4F solution and determined by molybdenum-antimony resistance colorimetric method. The soil ammonium-N and nitrate-N were extracted with KCl solution and measured with the indophenol blue colorimetry and two-wavelength ultraviolet spectrometry method (Liu et al., 2020). The urease, acid phosphatase and catalase activity were measured as described by Zhen *et al.*(Zhen et al., 2019).

4-DNA extraction and PCR amplification

The DNA in the soil and root samples were extracted by the E.Z.N.A.® soil DNA Kit (Omega Bio-tek, Norcross, GA, U.S.) based on the protocols of the manufacturer. The partial small subunit (SSU) region of the 18S rRNA gene was amplified by nested PCR, using AML1/AML2 (5′-ATCAACTTTCGATGGTAGGATAGA-3′; 5′-GAACCCAAACACTTTGGTTTCC-3′) as the first primer with a thermocycle PCR system (GeneAmp 9700, ABI, USA) (Lumini et al., 2010). AMV4.5NF/AMDGR (5'-AAGCTCGTAGTTGAATTTCG-3'; 5'-CCCAACTATCCCTATTAATCAT-3') was used as the second primer, in the second round of PCR with the same procedure described above(Ji et al., 2020), except for the number of cycles, which was 30. Each sample was repeated three times.

5- Illumina Miseq sequencing

According to the protocols of the manufacturer, 2% agarose gel was used for extracting the PCR amplification product, and then purified by an AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA). The products were quantified using Quantus™ Fluorometer (Promega, USA). Thereafter, Purified amplicons were pooled in equimolar and paired-end sequenced on an Illumina MiSeq PE300 platform (Illumina, San Diego, USA).

6-Analysis of sequencing data

Trimmomatic was used to demultiplex the raw fastq files and for quality filtering, and the reads were merge by FLASH version 1.2.7. The chimeric sequences were identified and removed, the cleaned sequences were

clustered into Operational taxonomic units (OTUs) based on 97% similarity using the UPARSE (version 7.1)[1]. The most abundant sequence in an OUT was considered as the representative. The taxonomy of each OUT representative sequence was an analyzed by RDP Classifier (version 2.2)[2] against the PR2 (version 4.5)[3] and MaarjAM (version 081)[4] using confidence threshold of 0.7.

7-Statistical analysis

Significances in soil properties in the rhizosphere soil, AM fungal community diversity and composition among the different sampling sites were tested using one-way analysis of variance (ANOVA) followed by Duncan's multiple range test (DMRT) at $P \leq 0.05$. The Venn diagram were drawn using R (vision 3.6.1) to count the number of common and unique OTUs in soils and roots, the column and pie chart of AM fungal community composition were drawn using Origin 2021 (Origin Lab Corp, Northampton, MA, USA) based on the OTUs abundance table. The relationship between AM fungal diversity and richness, AM fungal spore density and colonization rate and soil properties were analyzed using Pearson correlation; the relationship between composition of AM fungal communities in soil and root, and soil parameters were analyzed using Spearman correlation. The non-metrical multidimensional scaling (NMDS) was performed to test whether the AM fungal composition was clearly separated across age groups, and the analysis of similarities (ANOSIM) and permutation multivariate analysis of variance (PERMANOVA) were conducted to test the statistically significant differences among AM fungal communities with the Bray-Curtis distances and 999 permutations. Redundancy analysis (RDA) was used to study the influence of soil properties on AM fungal community composition of rhizosphere soil and mycorrhizal associations in Canoco 5.0 software (Microcomputer Power, Inc, Ithaca, NY, USA).

Results

1. Soil characteristics

Soil parameters showed a tendency of increasing and then decreasing trend such as TOC, P, AP, AK, C/N ratio and urease enzyme activity. The rhizosphere soil was slightly acidic with a pH ranged from 6.53 to 6.77. Compared to the CK, N and NH_4^+-H contents was increased with the increase of stand ages, while K was decreased. The activities of the catalase and soil acid phosphatase enzymes in soils were also significantly affected by stand ages ($P<0.05$), which were larger than CK (**Table 2**).

2. Sequence summary and AM fungal diversity indices in soil and root

Overall, a total of 351843 and 250065 high-quality sequences of *Glomeromycota* were obtained from soil and root samples, respectively (**Table 3**). The results from the rarefaction curves showed the curve gradually flattened as the number of sampling reads increased, which indicated the sequencing depth was enough to reflect the AM fungal diversity, and it also highlighted higher AM fungal diversity in soil than in the roots (**Fig. 1**). There was no significant changes of the richness and diversity in soil with stand age. However, stand age significantly affected the Sobs index in roots ($P<0.05$). (**Table 4**). Further, there were significant differences in richness (Sobs and Chao 1) and diversity (Shannon) index between the soil and root samples (all $P<0.001$).

[1]http: // drive 5.com / uparse /

[2]http://sourceforge.net /projects/rdp-classifier/

[3]https://figshare.com/aticles/PR2_rRNA_gene_data

[4]https://www. maarjam.botany.ut.ee/

Table 2： Soil properties in the rhizosphere of teak plantations at 22, 35, 45 and 55 years old and adjacent grassland (CK) in the Mt. Jianfengling, Hainan Island, China

Soil properties	CK	22 Years	35 Years	45 Years	55 Years
pH	6.53±0.038b	6.40±0.052c	6.56±0.012b	6.56±0.009b	6.77±0.007a
TOC(g/kg)	26.07±1.123b	28.59±1.774b	54.44±5.082a	44.85±3.951a	32.43±1.213b
N(g/kg)	1.45±0.039c	1.69±0.059c	3.03±0.189ab	1.81±0.071bc	3.15±0.869a
P(g/kg)	0.28±0.005b	0.25±0.023b	0.43±0.056a	0.32±0.006b	0.31±0.012b
C/P	92.03±2.375a	117.14±9.793a	133.34±30.17a	140.11±11.66a	104.76±4.002a
C/N	18.01±0.485b	16.87±0.59b	17.9±0.76b	24.75±1.775a	11.57±2.377c
K(g/kg)	38.6±0.174a	21.38±0.327d	19.98±0.701d	34.85±1.413b	31.94±0.928c
AP(mg/kg)	2.35±0.116ab	1.42±0.094b	3.31±0.503a	1.68±0.345b	1.42±0.396b
AK(mg/kg)	78.8±7.833c	135.69±19.132c	146.77±5.332c	411.35±84.651a	287.15±7.666b
NO_3^--H(mg/kg)	13.98±1.582a	15.02±0.517a	14.67±1.049a	18.47±2.696a	17.26±1.247a
NH_4^+-H(mg/kg)	9.15±0.623c	10.87±0.297bc	11.22±0.455bc	15.19±2.505ab	19.16±2.69a
CATmL/g	9.78±1.576d	14.68±0.341bc	14.21±0.655c	17.8±1.328b	23.34±0.777a
S-AP(mg/g)	0.43±0.023d	0.51±0.013d	1.09±0.027a	0.9±0.049b	0.67±0.014c
Ure(mg/g)	5.78±0.296b	1.52±0.012c	10.2±0.7a	2.14±0.249c	1.13±0.159c

Values are expressed as mean ± stand error (n=3); in each column data followed with different letters indicate significant difference (P<0.05); C/P=TOC/P, C/N=TOC/N; NH_4^+-H: ammonium nitrogen; NO_3^--H: nitrate nitrogen; TOC: total organic carbon; N: soil total nitrogen; P: soil total phosphorous; K: soil total potassium; AP: available phosphorus; AK: available potassium; CAT: catalase; S-AP: soil acid phosphatase; Ure: urease.

Table 3： Number of sequences and mean length of sequences of AM fungi extracted from soil and root samples

Samples	Soil		Root	
	Sequence	Mean length	Sequence	Mean length
CK-1	23038	215.97	-	-
CK-2	23114	214.64	-	-
CK-3	24126	215.37	-	-
22-1	22891	216.62	20301	216.05
22-2	24386	216.13	17412	216.00
22-3	24035	215.84	23884	218.16
35-1	24451	215.58	18638	215.83
35-2	22788	215.46	18714	215.39
35-3	21634	216.42	21931	215.61
45-1	20492	215.61	19700	215.16
45-2	24598	216.12	20091	215.28
45-3	24792	216.22	23188	215.47
55-1	24074	215.77	22073	214.86
55-2	24403	216.37	23390	215.35
55-3	23021	216.20	20743	216.25

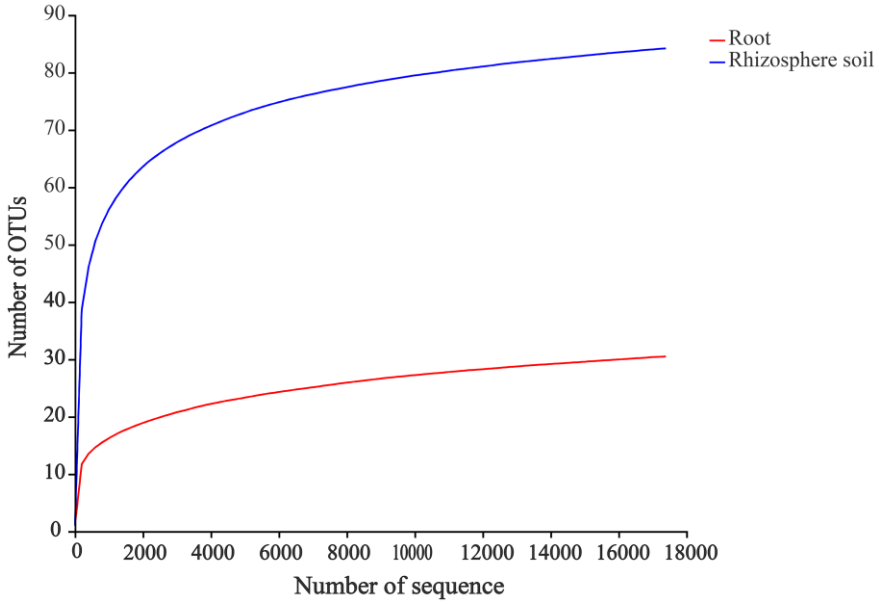

Fig. 1: Rarefaction curves plotted by teak root and rhizosphere soils along the chronosequences of teak plantations.

Table 4: AM fungal community diversity in soil and roots along stand age

Stand age (years)		Sobs	Shannon	Chao 1	Coverage
Soil	CK	80.0±16.3a	3.37±0.17a	94.97±9.62a	99.96%
	22 Years	91.3±10.4a	3.49±0.13a	96.02±9.27a	99.97%
	35 Years	66.7±15.2a	2.98±0.29a	84.83±28.98a	99.94%
	45 Years	109.0±7.1a	3.56±0.07a	119.12±9.31a	99.95%
	55 Years	82.0±27.3a	3.36±0.37a	91±31.11a	99.97%
Root	22 Years	14.0±5.3b	1.51±0.39a	14.50±5.22b	100%
	35 Years	21.3±7.7b	1.62±0.36a	25.67±10.17b	99.98%
	45 Years	56.3±7.7a	2.39±0.26a	70.36±7.88a	99.93%
	55 Years	35.0±9.0ab	1.46±0.38a	43.07±12.30ab	99.96%

Values are mean ± standard error except coverage which is expressed as mean percentage (n=3). The lowercase letters indicate a difference (p<0.05) between different stand ages. All the diversity indices (at 97% sequence similarity) are delineated at the OUT level.

3. AM fungal community composition and structure in soil and root

Majority of OTUs detected in soil and roots belonged to *Glomus* (**Fig. 2B and 2A**). The unclassified OTUs at the genera taxonomic level were mainly from the class Glomeromycetes. Compared with the soil samples, the root samples were comparatively rich in *Rhizophagus*, *Gigaspora* and *Scutellospora* mainly distributed in the soil of CK, but not detected in samples from the stand at age of 35, while *Acaulospora* and *Gigaspora* were mainly found in the root at age of 55 (**Fig. 2C** and **2D**).

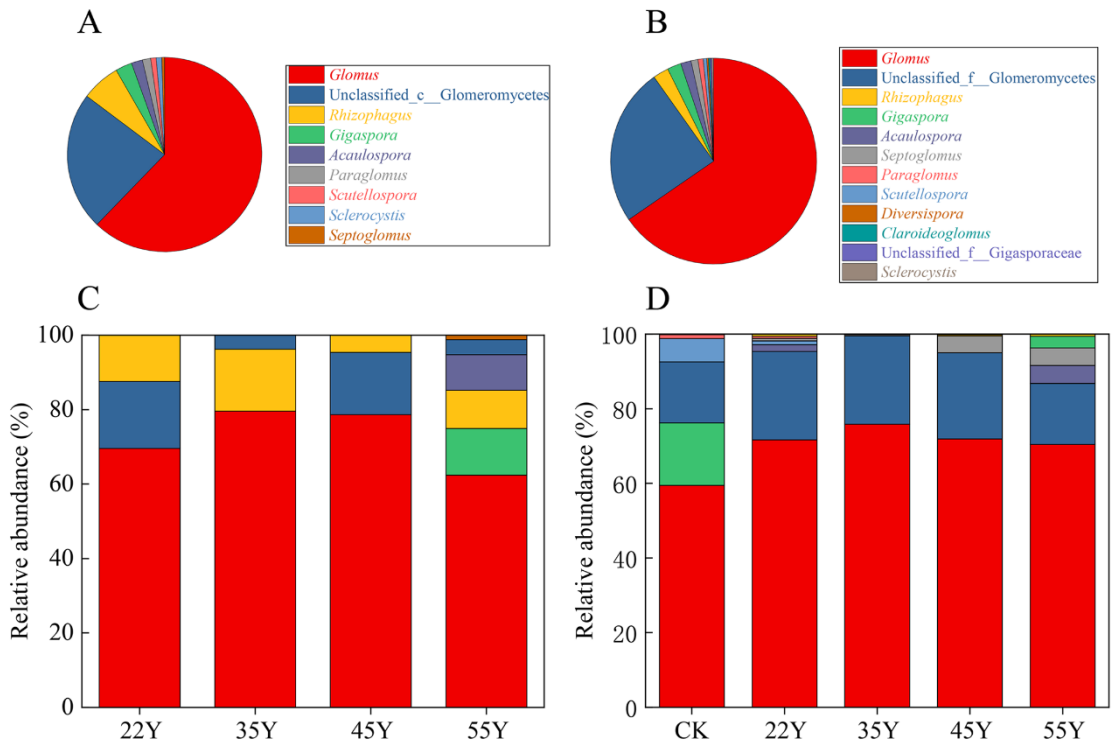

Fig. 2： The operational taxonomic units (OTUs) observed and the relative abundance of main AM fungal community composition at the genera levels in the rhizosphere soil (**B** and **D**) and in the root (**A** and **C**) among plantations with different stand ages. Values are mean ± standard error (n=12 and 15 in **A** and **B**; n=3 in **C** and **D**). 22Y: 22-year stand; 35Y: 35-year stand; 45Y: 45-year stand; 55Y: 55-year stand; CK: control.

The NMDS ordination exhibited that the sample sites of each age group were clearly separated along the stand age gradient, and the sample sites in CK were far apart from other samples (**Fig. 3B and 3A**). The analysis of ANOSIM and PERMANOVA demonstrated that AM fungal community structure significantly varied among samples from different stand ages in soil (P=0.001; P=0.001) and root (P=0.022; P=0.025) (**Table 5**), which supported the results of NMDS ordination analysis (**Fig. 4**).

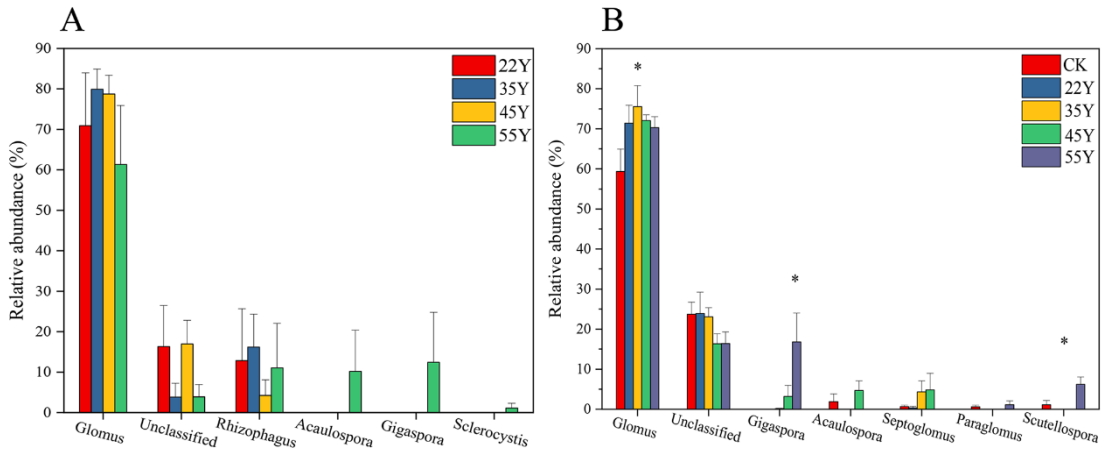

Fig. 3： The relative abundance (more than 1%) of AM fungal genus in the root (**A**) and the rhizosphere soil (**B**) samples. Unclassified: unclassified_c_Glomeromycetes. Bars are mean ± standard error, and asterisks (*) indicate significant difference between different age groups (P<0.05). 22Y: 22-year stand; 35Y: 35-year stand; 45Y: 45-year stand; 55Y: 55-year stand; CK: control.

Table 5: The statistical test of analysis of similarities (ANOSIM) and PERMANOVA (permutation multivariate analysis of variance) based on the Bray-curtis distances were conducted to test differences of age groups in root-associated and rhizosphere soil AM fungal community compositions between different ages.

Type	df	ANOSIM		PERMANOVA	
		R	*P*	*F*	*P*
Soil	4	0.74	0.001	1.997	0.001
Root	3	0.37	0.022	1.338	0.025

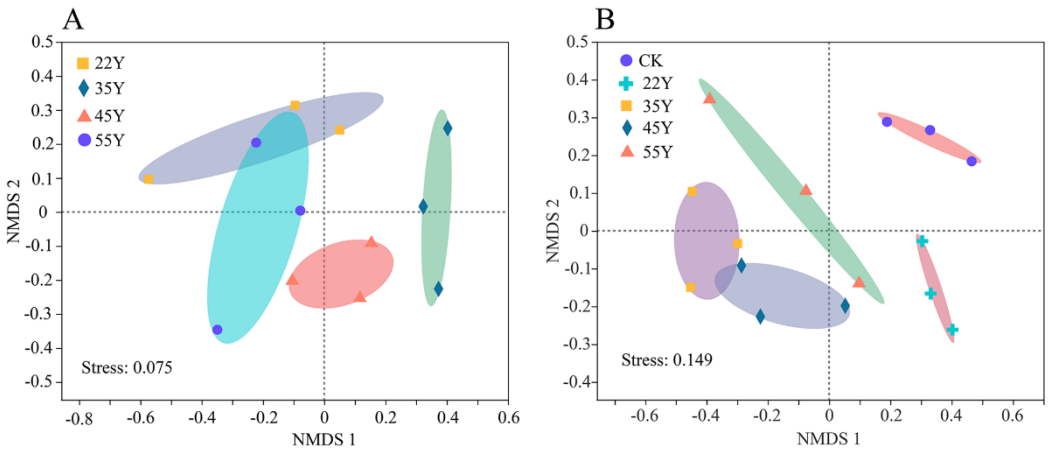

Fig. 4： Non-metric multidimensional scaling (NMDS) ordinations of the AM fungal community compositions (Bray-curtis) in the roots (**A**) and the rhizosphere soil (**B**) based on the abundance of OTUs among different stand age. 22Y: 22-year stand; 35Y: 35-year stand; 45Y: 45-year stand; 55Y: 55-year stand; CK: control.

4. Relationships between soil properties and AM fungal communities

The first and second axis of RDA analysis explained 54.56% and 19.13% of the variance of AM fungal communities in soil (**Fig. 5B**). Meanwhile, the correlation heat map of the soil physicochemical properties showed significant correlation with AM fungi community composition (**Fig. 6**). Moreover, the results of RDA analysis showed that the first two axes accounted for 30.98% and 17.28% of the variance of root-associated AM fungal community,(**Fig. 5A**).

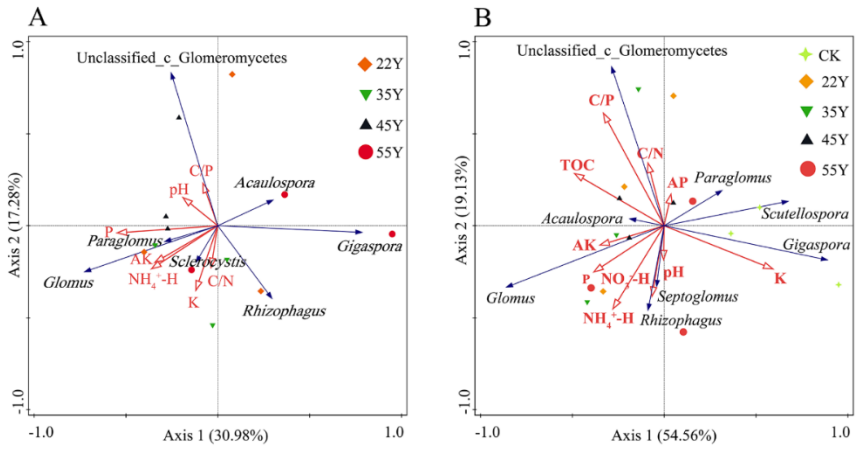

Fig. 5： Ordination plots of the redundancy analysis (RDA) showing the relationships between soil physicochemical properties (red arrows) and the AM fungal genera (blue arrows) with relative abundance greater than 1% in roots (**A**) and in the rhizosphere soils (**B**).NH_4^+-H: ammonium nitrogen; NO_3^--H: nitrate nitrogen; TOC: total organic carbon.22Y: 22-year stand; 35Y: 35-year stand; 45Y: 45-year stand; 55Y: 55-year stand; CK: control.

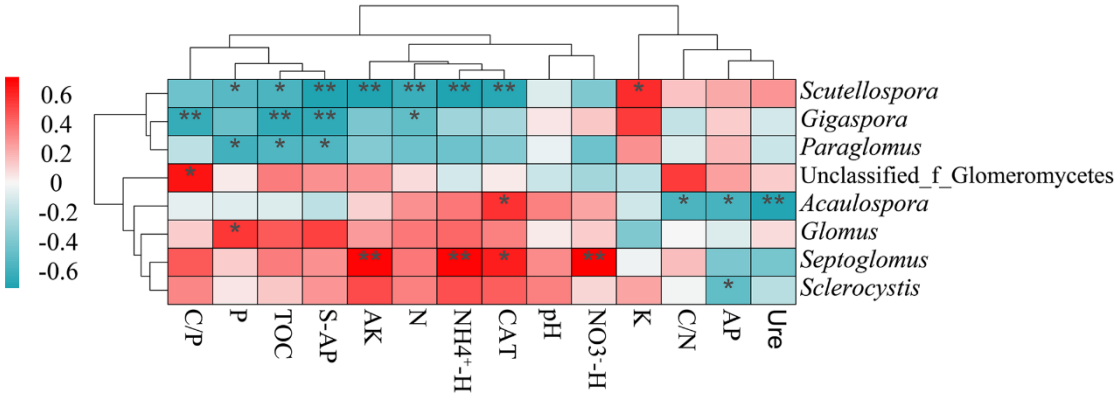

Fig. 6: Spearman correlation heat map of more than 1% average abundance of AM fungi genus in the rhizosphere soil and soil properties. NH_4^+-H: ammonium nitrogen; NO_3^--H: nitrate nitrogen; CAT: catalase; S-AP: soil acid phosphatase; Ure: urease. Significant differences by *p<0.05; **p<0.01.

A monte carlo permutation test showed that all soil chemical property parameters explanted the eigenvalues of 85.7%. K was the first factor that accounted for 20.6% of total variance (**Table 6**). Additionally, the monte carlo permutation test indicated that 7 variables could jointly explain 50.1% of the variations of the teak root-associated AM community composition, and P was the first factor that contributed to 10.3% of the total variation (**Table 6**).

Table 6: Conditional effects of soil physicochemical properties on AM fungal community composition in rhizosphere soil and root sample, which obtained from the summary of forward selection in the redundancy analysis (RDA).

Variables	Variables	Explains (%)	*F*-value	*P*-value
Soils	K	20.6	3.4	0.03*
	AK	18.4	3.6	0.022*
	NO_3^--H	14.1	3.3	0.022*
	C/P	11	3.1	0.028*
	NH_4^+-H	4.8	1.4	0.246
	C/N	6.4	2.1	0.122
	pH	2.1	0.7	0.57
	TOC	5.9	2.1	0.13
	P	1.6	0.5	0.684
	AP	0.8	0.2	0.868
Roots	P	10.3	1.1	0.304
	K	6.8	0.7	0.59
	AK	6.3	0.7	0.614
	NH_4^+-H	8.8	0.9	0.406
	pH	8.6	0.8	0.41
	C/P	4.1	0.4	0.658
	C/N	5.2	0.4	0.684

Explains (%): the explanation rate of single variable to total variable in rhizosphere soil root. NH_4^+-H: ammonium nitrogen; NO_3^--H: nitrate nitrogen; K: total potassium; AK: available K; TOC: total organic carbon; P: total phosphorus; AP: available P; TOC: total organic carbon. *p<0.05.

Discussion

1. AM fungal diversity and community composition

The data indicated the fungal richness (Sobs and Shannon) and diversity were higher in soil than in root (**Table 4, Fig. 1**), as observed in the study of *Achnatherum inebrians* (Zhong et al., 2021). This result may be explained as the location and density of AM fungal propagules (spores, extra radical mycelium and infected root fragments) in soil and root was different (Varela-Cervero et al., 2016).

Our result revealed that the AM fungal abundance and diversity in the rhizosphere soil of teak plantations varied along with the chronosequence, though these variations were not significant (**Table 4**). This phenomenon could attribute to that the high throughput-sequencing causes large variation in sequence abundance obtained between samples. Thus, it is needed to standardize the samples via rarefaction to a common sequencing depth per sample (Hart et al., 2015). Hence, the data obtained from the high-throughput sequence may not represent the original community (McMurdie and Holmes, 2014). Mounting studies had reported that high levels of P fertilizer application showed a lower richness (Camenzind et al., 2014; Chen et al., 2014) and diversity **(**Higo et al., 2018**)** of AM fungi because the benefit of the symbiosis was reduced. Conversely, the symbiosis relationship could be enhanced with limited supply of P (Dueñas et al., 2020). Our study observed that the content of P and AP significantly declined in the later succession. The host teak may seek to establish a symbiotic relationship with more AM fungal taxa to obtain P nutrient. Therefore, we found more AM fungal richness along with a chronosequence of teak plantations, and the AM fungal richness and diversity were positively correlated to the AP and P, respectively.

The present study showed that *Glomus* was the most abundant genus within all samples, varying between 59.34% to 70.32% in the rhizosphere soil, and 61.3% to 70.8% in the roots (**Table 7**).AM fungal taxa in root and soil samples. In fact, many studies had revealed that *Glomus* was dominant in forest lands in south China, such as *Eucalyptus* plantations (Chen et al., 2007), most forest ecosystems (Lu et al., 2019; Jing et al., 2020; Ji et al., 2021), reclamation land (Ezeokoli et al., 2020), desert vegetative sites (Vasar et al., 2021), agro-ecosystems (Wang et al., 2020), saline ecosystems (Min et al., 2019) and disturbed soil (Qin et al., 2017) indicating its wide adaptation in diverse ecosystems. They belonged to 12 and 9 AM fungal taxa, all could be found in rhizosphere soil.

Table 7： Relative abundance of main genera of AM fungi identified in the rhizosphere soil and fine roots of teak along the chronosequence of teak plantations. Values in the bar plot are mean (n=3).

Stand age (year)		CK	22 Years	35 Years	45 Years	55 Years
Soils	*Glomus*	59.34±5.64	71.42±4.45	75.53±5.27	72.06±1.44	70.32±2.75
	Rhizophagus	0.03 ±0.03	0.54±0.54	0	0.28 ±0.28	0.57 ±0.57
	Gigaspora	16.78±7.27	0.05 ±0.04	0	0.14 ±0.14	3.21 ±2.77
	Acaulospora	0	1.90 ±1.89	0	0	4.67 ±2.38
	Septoglomus	0	0.65 ±0.39	0.35 ±0.35	4.34 ±2.71	4.82 ±4.13
	Paraglomus	1.11±0.99	0.59 ±0.43	0	0	0.02 ±0.02
	Scutellospora	6.20±1.83	1.10 ±1.10	0	0	0
	Unclassified	16.41±2.91	23.74±2.98	23.92±5.28	23.08±2.29	16.35±2.48
Roots	*Glomus*	-	61.3±14.52	78.74±4.64	79.94±4.98	70.8±13.09
	Rhizophagus	-	11.02 ±11	4.23±3.85	16.17±8.18	12.8±12.83
	Acaulospora	-	10.1±10.18	0	0	0
	Gigaspora	-	12.4±12.39	0.05 ±0.03	0	0
	Sclerocystis	-	1.14±1.14	0	0	0
	Unclassified	-	3.87 ±3.06	16.96±5.84	3.82±3.39	16.2±10.19

Unclassified: unclassified_f_Glomeromycetes

Therefore, the taxa in the roots was a subset of the rhizosphere soil consistent with earlier reports (Miras-Avalos et al., 2011). The reasons according to the two-step theory, teak may exert selective force on the rhizosphere AM fungal taxa causing differentiation of community composition between the soil and root samples (Bulgarelli et al., 2013).

The relative abundance of *Glomus* in soil increased first and then declined with the chronosequence, and there was no significant change in root (**Fig. 3**). The results were contrary to earlier study that found the relative abundance of *Glomus* in soil decreased linearly over the age, and had no significant shift in root found by Sheng *et al* (Sheng et al., 2017). This may be due to the difference in response of AM fungal taxa in host plants (Lu et al., 2019). The possible reasons that the *Glomus* dominated all of stand age were due to the host-specificity preference, functional relevance, adaptation, and ease of propagation in the soil ecosystem (Cui et al., 2016; Ezeokoli et al., 2020). Moreover, *Gigaspora* and *Scutellospora* also changed significantly with stand ages (P<0.05).

2. Factors driving AM fungal communities

It is generally believed that the community assembly of AM fungi is scale-dependent (Jiang et al., 2018). Soil factors and host plants were the main driving factors for determining the spatial distribution of AM fungi on a local scale (van der Gast et al., 2011). Thus, the community assembly of AM fungi in this study may be determined by soil factors and host plants or both. The monte carlo permutation test showed that K, AK, NO_3^--H and C/P were significantly correlated with AM fungal community composition in the rhizosphere (**Table 6**). Evidence in the literature reported that pH played a central role in the assembly of soil AM fungal community (Qin et al., 2015; Wang et al., 2020). The underlying mechanisms that pH influenced the microbial communities may be due to nutrient availability (Vasar et al., 2021), pH value had a major impact of the mobility of multiple, hence, many connected biological processes in the soil also was affected (Neina and Science, 2019). However, the correlation and RDA analysis indicated that pH was not significantly correlated to the AM fungal community composition, richness and diversity. The phenomenon can attribute to quite small soil pH changes (from 6.40 to 6.77). Xiao et al. (Xiao et al., 2019) observed that soil NH_4^+-H was significantly related to AM fungal community composition, and another study found that AM fungal hyphae could predominantly assimilate NH_4^+-H, then transported to plants, due to priority synthesis of amino acids (Govindarajulu et al., 2005). Variation of NH_4^+-H in this study did not contribute significantly to the composition of AM fungal community, but NO_3^--H did (**Table 6**) indicating that not all N in the soil contributed to the shift of the AM fungal community.

The NMDS analysis had also proved that AM fungal community structure of root-associated was clearly affected by stand age (**Fig. 4A**). However, no soil parameters were found significantly influenced the composition of AM fungi (**Table 6**). Existing studies reported that the composition of neighboring plant species had a significant impact on the community structure of AM fungi in plant roots (Chaiyasen et al., 2014; Krüger et al., 2017). Therefore, the neighboring plant community composition might have an effect on the composition of root-associated AM fungal community.

Conclusions

Soil nutrient and enzymes increased in the early stages, but decreased significantly in later stages in terms of K, AK, P, N, acid phosphatase etc. K, AK, NO_3^-H and C/P in the rhizosphere soil were important driving factors that shift in the structure of the AM fungal community along the chronosequence. Whereas no significant changes affecting the structure of root-associated AM fungal community were observed. This study provided a general picture of the composition of the root-associated and rhizosphere soil of AM fungal community at different growth stages. Further research is needed to include newly established and young stands and to screen efficient AM fungi as biofertilizer inocula to improve the survival rate and productivity of afforested plantations.

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