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A comparison between the ectomycorrhizal fungal communities associated with the natural and plantation populations of *Dipterocarpus alatus*

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Abstract. *Kaewgrajang T, Yamato M, Polamart T, Sangwanit U. 2023. A comparison between the ectomycorrhizal fungal communities associated with the natural and plantation populations of* Dipterocarpus alatus*. Biodiversitas 24: 2088-2098. Dipterocarpus alatus* Roxb. is a native tree species of Southeast Asian countries and one of the most economically important dipterocarp species. We investigated the ectomycorrhizal (ECM) fungi associated with *D. alatus* using molecular identifications on putative fruiting bodies and ECM roots collected from three natural forests and 10 *D. alatus* plantations. In this investigation, we aimed to report the ECM fungal communities of *D. alatus* in natural forests and plantations. Combining the results of fruiting bodies and ECM roots, 82 taxa belonging to 12 families were identified as ECM fungi. The ECM fungal community comprised many rare species and a few frequently found species. The *Sebacina* genus was found the most frequently in natural forests and plantations. The ECM fungal diversity was higher in plantations compared to natural forests. However, the frequently occurring ECM fungal taxa were not different between the natural forests and plantations. The result suggests that ECM fungal communities could be maintained in *D. alatus* plantations even if a reduction occurs in the natural population of dipterocarp forest.

Keywords: Aboveground fungi, belowground fungi, dipterocarps, ectomycorrhiza, Sebacinaceae

INTRODUCTION

Dipterocarps are distributed throughout the world's tropical regions, including Asia, Africa, and South America. In particular, they are a valuable component of the tropical rainforests in Southeast Asian countries, i.e., Burma, Cambodia, Laos, Vietnam, Malaysia, Indonesia, and Thailand (Ghazoul 2016). They are important in ecosystem services and have economic value as well. Currently, the timber obtained from dipterocarps dominates the international topical timber market in many Southeast Asian countries, especially of the species from the genera *Dipterocarpus*, *Hopea*, *Shorea*, and *Vatica* (Chan 2017). However, the dipterocarps species are threatened by continuing global climate change, leading to habitat loss and forest fragmentation in many countries such as Vietnam (Tran et al. 2017), the Philippines (Pang et al. 2021), and Malaysia (Barstow and Bartholomew 2022). Deb et al. (2017) reported that populations of *Shorea robusta* Gaertn. and *Dipterocarpus turbinatus* C.F.Gaertn. in South Asia and the continental parts of Southeast Asia will decline by 20-30% by the year 2070 due to climate change. In October 2021, all 162 endemic dipterocarp species were listed in the Red List of Bornean Endemic Dipterocarps, with 99 species (or 66%) being threatened with extinction (Bartholomew et al. 2021).

Ectomycorrhizae are symbiotic associations between plants and fungi. Ectomycorrhizal (ECM) symbiosis is essential for tree growth in infertile soils in natural forests (Tedersoo and Brundrett 2017). An estimated 6,000-7,000 species host ECM, which includes only 2% of the vascular plants (Tedersoo and Brundrett 2017; Brundrett and Tedersoo 2018). Nevertheless, many ecologically and economically important plant families, such as Pinaceae, Fagaceae, Fabaceae, and Dipterocarpaceae, have ECM associations (Tedersoo and Brundrett 2017). Around 700 known species in 17 genera in Dipterocapaceae (Pooma et al. 2017), whereas over 500 species have been reported to form ECM associations (Brearley 2012; Tedersoo and Brundrett 2017). Furthermore, the ECM association was reported as one of the factors facilitating the dominance of Dipterocarpaceae in the tropical rain forests of Southeast Asia (Brearley 2012; Steidinger et al. 2019; Tedersoo et al. 2020). Furthermore, four Endogonamycetes lineages, 33-34 Pezizomycetes lineages, and 45-48 Agaricomycetes lineages have been classified as ECM fungi (Tedersoo and Smith 2017). In the case of dipterocarps, ECM fungi of the fungal families, i.e., Thelephoraceae, Russulaceae, Sclerodermataceae, Cortinariaceae, Agaricaceae, and Boletaceae, were identified as the mycobionts (Phosri et al. 2012; Smith et al. 2013; Tedersoo and Nara 2010). In particular, *Tomentella*, *Thelephora*, *Russula*, and *Lactarius*

were reported to be abundant as ECM fungal genera in the dipterocarps (Brearley 2012; Phosri et al. 2012; Kaewgrajang et al. 2014; Essene et al. 2017). However, fewer studies have been conducted on the structure of ECM fungal communities in tropical forests, especially dipterocarp forests, compared to temperate forests.

In this study, we focused on *Dipterocarpus alatus* Roxb. ex G. Don, which is a native tree species of the Southeast Asian countries, i.e., Bangladesh, lower Myanmar, Thailand, Laos PDR, Cambodia, South Vietnam, Philippines, and northern Malaysia (Pooma et al. 2017). Generally, suitable habitats of *D. alatus* are distributed in evergreen and deciduous forests. The optimum conditions for this species are as follows: humidity between 75-85%, precipitation between 1,500-2,200 mm, mean annual temperature between 25-27°C, and a dry season duration of 4-6 months (Pooma et al. 2017). *D. alatus* is one of the most economically important dipterocarp species having both direct and indirect benefits, such as the production of good quality wood, oleoresin, solid resin, medicinal bioactive compounds, and several ecosystem services (Dyrmose et al. 2017; Yongram et al. 2019). Nevertheless, the distribution and numbers of this species have been decreasing at an alarming rate, and it is now listed as a threatened species (Tam et al. 2014). Furthermore, the habitat of *D. alatus* is severely affected by deforestation, forest fragmentation, and unsustainable forest management. Therefore, it is now reduced to grow in scattered patches of populations. Recently, Chokthaweepanich et al. (2022) found that some *D. alatus* populations are at risk of imminent extinction because of losing their genetic diversity in Thailand.

Several attempts have been made to establish dipterocarp plantations in Southeast Asian countries. Previously, dipterocarp plantations in Thailand were mainly established for conservation by various Thai government sectors, i.e., the Royal Forest Department, the Department of National Parks, Wildlife and Plant Conservation, and the Forest Industry Organization. Nowadays, the concern in such plantations has been driven by the private sector with the help of local communities. The plantation can have multiple benefits, such as wood, resins, and edible ECM mushroom production. Furthermore, because fruiting bodies of ECM fungi are often found in *D. alatus* plantations, such plantations can be important for conserving diverse ECM fungi, especially after a reduction in the natural dipterocarp population. Therefore, we propose that the remaining dipterocarp forests and the plantations could be considered as a genetic resource for ECM fungi associated with the dipterocarp. In the future, some fungi may be utilized effectively in dipterocarp plantations. In this study, we identified the ECM fungal communities associated with *D. alatus* using fruiting bodies and ECM roots through morphological and molecular analyses in natural forests and plantations to evaluate the ECM fungal community in such environments.

MATERIALS AND METHODS

Sampling sites

Three natural forests with *D. alatus* trees and 10 *D. alatus* plantations were selected as sampling sites in seven Thailand provinces—Chumphon, Surat Thani, Kamphaeng Phet, Chachoengsao, Phichit, Kalasin, and Nakhon Ratchasrima (Figure 1). Three natural forests are located in Chumphon, Phichit, and Nakhon Ratchasrima, with the D. alatus being the dominant canopy tree species. The natural forest in Chumphon was observed as an evergreen hill forest spread over 192 ha. The dipterocarp trees observed in this study are *D. alatus*, *Shorea talura* Roxb., and *Hopea* spp. While the understory vegetation are *Croton* spp., various bamboo species (Poaceae), and palms (Arecaceae). The natural forest in Phichit was reported as a moist evergreen forest within 164 ha. The dominant species is *D. alatus* trees, with an average diameter at breast height (DBH) of more than 80 cm. Next, other tree species, such as *Butea monosperma* (Lam.) Kuntze, *Cassia fistula* L., *Bauhinia malabarica* Roxb., *Samanea saman* (Jacq.) Merr. (Fabaceae), and *Lagerstroemia calyculata* Kurz (Lythraceae). The natural forest in Nakhon Ratchasrima was a dry evergreen forest with various tree species, such as *D. alatus*, *Hopea ferrea* Laness., *Hopea odorata* Roxb., *Shorea roxburghii* G.Don (Dipterocarpaceae), *Afzelia xylocarpa* (Kurz) Craib, and *Senna siamea* (Lam.) H.S.Irwin & Barneby (Fabaceae), within a total area of 716 ha.

Most plantations were pure stands of *D. alatus*, established on degraded natural forests with only a few dipterocarp trees. Therefore, the Royal Forest Department and the Forest Industry Organization initiated the plantation of dipterocarps. The ten trial plantations were located in five provinces—Kamphaeng Phet, Nakhon Ratchasrima, Surat Thani, Chachoengsao, and Kalasin. The location of the three natural forests and ten trial plantations are listed along with the tree spacing, age, average DBH, and climate information shown in Table 1. In addition, the mean annual temperature and annual precipitation were measured at the nearest meteorological station and ranged between 26.2-28.3°C and 1,019-1,809 mm, respectively (Table 1).

Soil analysis

At each sampling site, five soil cores (100 g; at depths between 5-10 cm) were randomly collected, ranging at 10 m from each soil core, and mixed together to make one soil sample. For the soil samples, soil texture, soil organic matter, pH (soil: water, 1:1 v/v), and available phosphorous (Bray II method) were measured. In addition, the total carbon and nitrogen were also analyzed using the dry combustion method.

The sampling of ECM fungal fruiting bodies

Four plots of 100 m^2 (10 m×10 m) were randomly set at each sampling site, and the fruiting bodies of putative ECM fungi were collected during the rainy season. In addition, the putative ECM fungal fruiting bodies from outside the plot were occasionally collected, as a few were isolated

inside the plots. The fresh fruiting bodies were gently brushed to remove soil debris, photographed, and characterized according to their macroscopic and microscopic features. Finally, the collected fruiting bodies were oven dried at 48°C for 48-72 h and deposited as voucher specimens in the herbarium at the Department of Forest Biology, Faculty of Forestry, Kasetsart University, Bangkok, Thailand.

The sampling of ECM roots

Ten *D. alatus* trees, distributed within a 0.5 ha area in each sampling site, were randomly selected to examine the ECM roots. The distance between each selected *D. alatus* tree was about 10-20 m. The average DBH of the selected trees at each sampling site ranged from 77.8-97.8 cm for the natural forests and 8.7-50.9 cm for the plantations (Table 1). One soil core (100 g; 5 cm in depth) was taken at a distance of 1.5 m from the base of each sample tree. Hence, 130 soil cores (10 per site) were collected and kept under cool conditions until they were further processed (within 2-4 days). Root samples were gently washed on a sieve using tap water. The ECM morphotypes were distinguished based on the root color, mantle texture, emanating hyphae, rhizomorphs, and ECM branching pattern. In addition, the total number of ECM tips in each soil core was counted using a dissecting microscope.

Molecular analysis

A part of the dried fruiting bodies of each fungus (25-75 mg) was ground to a powder form and placed in liquid nitrogen. Then the DNA was extracted using the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. On the ECM roots molecular identification, three root tips of each morphotype from each soil core were individually placed in a 1.5 mL tube. Next, those tubes containing 20 µL of PrepMan Ultra Reagent (Applied Biosystems, Foster City, CA, USA) and the DNA was extracted from the ECM roots according to the manufacturer's instruction.

Figure 1. The sampling sites for the study on the ectomycorrhizal fungal community in *Dipterocarpus alatus*

Table 1. Sampling sites with environmental conditions for the study on the ectomycorrhizal fungal community in *Dipterocarpus alatus*

Sampling site	Forest/ plantation	Spacing (m)	Tree age (years)	Average DBH (cm)	Area (ha)	Mean annual temperature (°C)	Annual precipitation (mm)
Phichit	Forest		—	98	164.0	28.0	1,343
Wang Nam Khiew	Forest		—	91	761.0	26.2	1,030
Chumphon	Forest		—	78	192.0	28.2	1,809
Tri Trung	Plantation	5 x 5	50	51	8.0	28.4	1,252
Kamphaengphet-1	Plantation	4 x 4	21	19	1.6	28.3	1,353
Kamphaengphet-2	Plantation	4 x 4	15	14	6.2	28.3	1,353
Kamphaengphet-3	Plantation	4 x 4	9	8.7	1.0	28.3	1,353
Somdet	Plantation	4 x 4	32	28	1.0	27.7	1,232
Sakaerat-1	Plantation	4 x 4	23	21	1.5	27.2	1,019
Sakaerat-2	Plantation	4 x 4	16	16	1.5	27.2	1,019
Ladkrating	Plantation	4 x 4	40	40	1.0	27.3	1,154
Surat Thani	Plantation	4 x 4	13	10	32.0	27.3	1,700
Garnjanadit	Plantation	3 x 8	37	23	20.8	27.5	1,734

Notes: * DBH: diameter at breast height

From the extracted DNA, the internal transcribed spacer region of the fungal nuclear ribosomal DNA (ITS rDNA) was amplified by using the polymerase chain reaction (PCR) using TaKaRa Ex Taq Hot Start Version (Takara Bio, Kusatsu, Japan) with a fungal-specific primer ITS1F and a universal primer ITS4. On the PCR reaction, the mixture contained: 1 μL of the extracted DNA solution, 0.375 units of Taq polymerases, 0.125 μM of each primer, 100 μM of each deoxynucleotide triphosphate, and 1.5 μl of the supplied PCR buffer in a total volume of 15 μl. The PCR program on a PC-818S Program Temp Control System (Astec, Fukuoka, Japan), with an initial denaturation step at 94°C for 2 min, followed by a step of 35 cycles at 94°C for 20 sec, 55°C for 30 sec, 72°C for 1 min, and a final extension step at 72°C for 5 min. Next, the amplified PCR products were purified using a HiYield™ Gel/PCR DNA-Fragments Extraction Kit (RBC Bioscience, Taipei, Taiwan). The purified PCR products were cloned into pGEM-T Easy Vector System I (Promega, Madison, USA), and three colonies with inserted DNA were randomly chosen for sequencing. The inserted DNA was sequenced using a BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Tokyo, Japan) with T7 and SP6 promoter primers. A consensus sequence was made from two or three identical sequences for the three sequences obtained from the one root tip. The obtained consensus sequences were divided based on the 97.0% sequence similarity, and the putative taxa were inferred using the basic local alignment search tool (BLAST). One representative sequence of each putative ECM fungal taxon was deposited in the DNA Data Bank of Japan (DDBJ).

Data analysis

Species accumulation curve (Sobs) and incidence-based species richness estimates using the Chao 2 and Jackknife 2 procedures were computed on the Estimate S ver. 8.2.0 software. These analyses used soil cores as sampling units, and the ECM fungal taxa were randomly sampled without replacement. In addition, a heat map was made based on the number of soil core samples with ECM fungal detection.

RESULTS AND DISCUSSION

Soil chemical properties

Most of the soils collected were sandy loams in texture. The soil pH ranged from 4.63 to 6.16, and the percentage of soil organic matter was between 0.48 to 8.51% (Table 2). Other soil chemical properties, such as the total nitrogen were between 0.03 and 0.37%; the total carbon was between 0.76 and 4.79 g kg^{-1}, and the available phosphorus was between 0.98 and 49.90 mg kg^{-1} (Table 2). Among the sampling sites, the Phichit site had the highest soil fertility, probably because of the dung deposited by the open-billed stork (*Anastomus oscitans* Boddaert 1783), which nests in the crowns of *D. alatus* trees at the site.

Detection of ectomycorrhizal (ECM) fungi

The ECM fungi of *D. alatus* detected in this study were divided into 82 taxa and 12 families (Table 3). Representative sequences of the fungal taxa were submitted to the DDBJ under the accession numbers AB854645-AB854726 (Table 3). Among these, 50 taxa in 13 genera and 9 families were obtained from the fruiting bodies. The taxon number (Figure 2.A) was the highest in Russulaceae (24 taxa), followed by Amanitaceae (8 taxa) and Inocybaceae (6 taxa), and they accounted for 47%, 13%, and 11%, respectively (Figure 2.B).

For the belowground ECM fungi, 76 out of the 130 soil cores (58.5%) contained ECM roots, and the number of ECM root tips in one soil core ranged between 49.3 to 283. In these soil samples, the ECM roots were distinguished into 12 morphotypes (Figure 3). Then, 270 root tips (at least 3 of each morphotype from each soil core) were randomly collected for molecular identification. Therefore, 73% of the ITS region's root tips (196 out of 270) DNA fragments were successfully amplified and sequenced. Accordingly, 336 sequences of ITS regions, ranging from 533 to 777 base pairs, were obtained. These sequences were divided into 3 taxa in the Ascomycota and 33 taxa in the Basidiomycota belonging to 7 families, i.e., the Sebacinaceae (13 taxa), Ceratobasidiaceae (1 taxon), Russulaceae (5 taxa), Thelephoraceae (9 taxa), Inocybaceae (1 taxon), Clavulinaceae (3 taxa), and Cortinariaceae (1 taxon) (Table 3, Figure 4a). Among them, the Sebacinaceae formed the majority in both natural forests and plantations and accounted for 77% of the obtained sequences (Figure 4b). The belowground ECM fungal communities differed greatly from those of the fruiting bodies. Only three sequences of belowground fungi (Ino1, Rus1, and Rus2) matched the sequences of the fruiting bodies with more than 99% similarity.

Comparison of ECM fungi in the natural forests and plantations

Combining the fungal taxa obtained from the ECM fungal fruiting bodies and the belowground ECM roots resulted in 27 taxa in 11 families in the natural forests and 73 taxa in 12 families in the plantations. It should be mentioned that the number of soil samples from the natural forests (30 soil cores) was less than that obtained from the plantations (100 soil cores). The Chao 2 and Jackknife 2 procedures suggested that the minimum taxon richness in the plantations (50 and 52, respectively) was greater than that of the natural forests (29 and 28, respectively) (Figure 5). Interestingly, there was no difference in the most frequently occurring fungal taxa between sampled natural forests and plantations (Figure 6). The top six most frequent taxa—Seb13, Seb12, Seb7, Seb5, Cer1, and Seb10—(Figure 6) made up 79.2% of all the obtained sequences from the natural forests and 76.1% from the plantations.

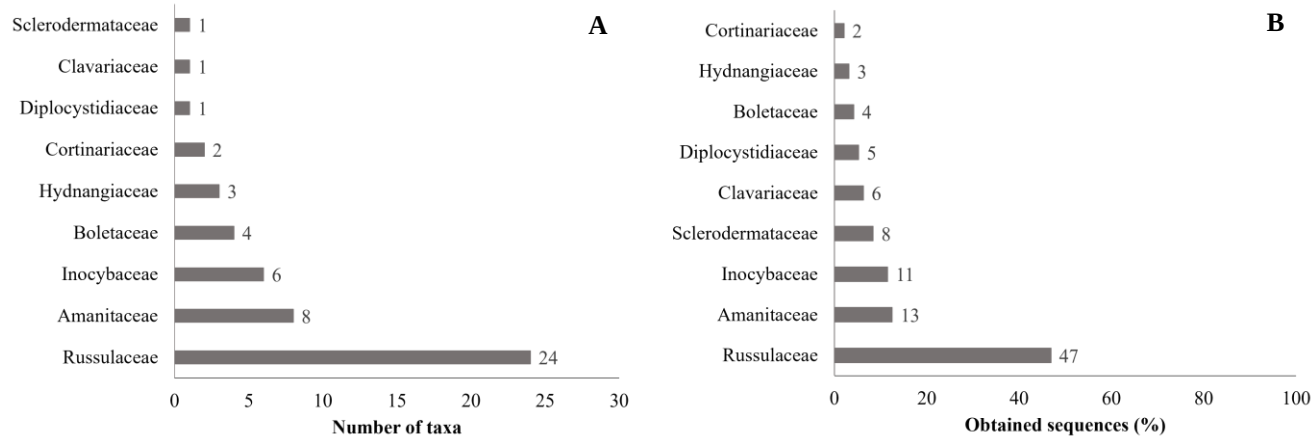

Figure 2. Number of taxa (A) and the obtained sequences (%) (B) from fruit body specimens

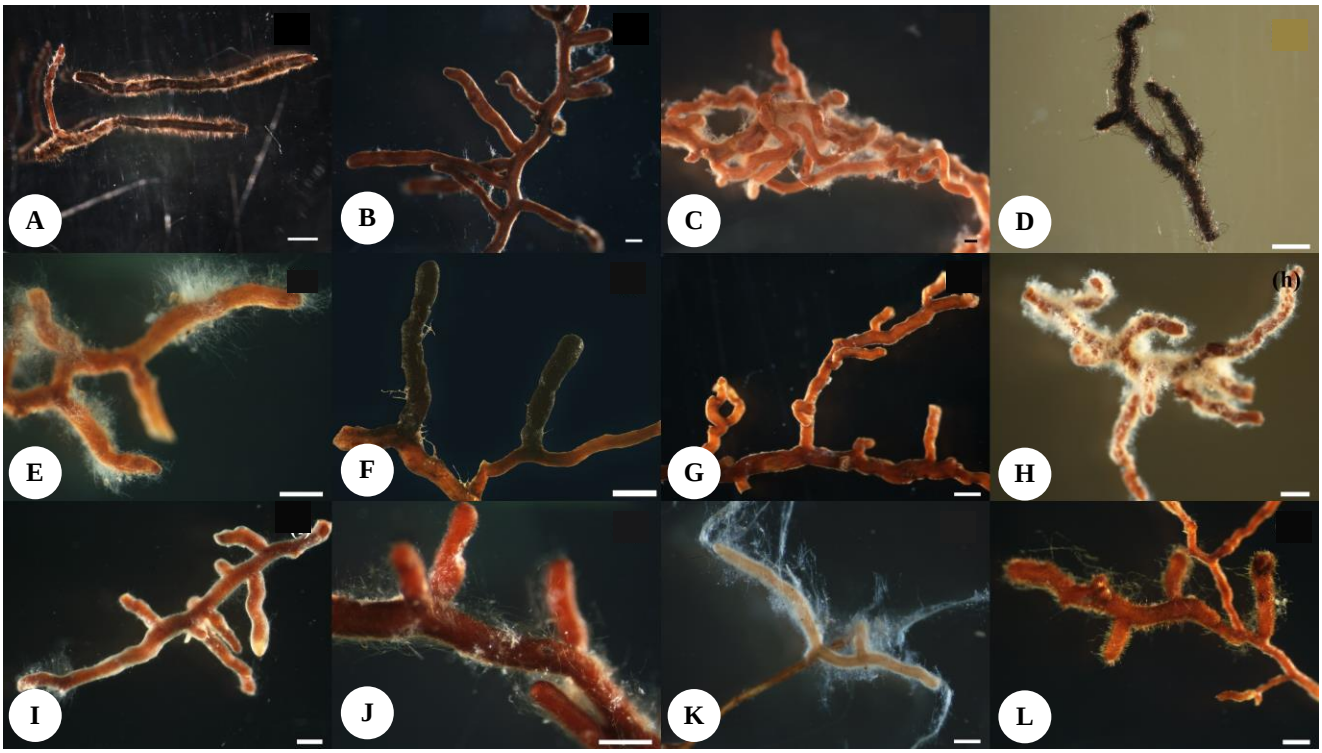

Figure 3. Twelve ectomycorrhizal morphotypes obtained from *Dipterocarpus alatus* roots in natural forests and plantations (A) blackish brown to black, velvety, and unbranched without rhizomorphs (B) dark brown, smooth, and irregular branches without rhizomorphs (C) pale brown, cottony, and irregular pinnate without rhizomorphs (D) black, spiny, and few branches with black rhizomorphs (E) pale brown, smooth to cottony, and monopodial pinnate without rhizomorphs (F) black, smooth to grainy, and unbranched with black rhizomorphs (G) brown, smooth, and compound pinnate without rhizomorphs (H) brown, smooth, and compound pinnate without rhizomorphs (I) brown, cottony, and irregular pinnate without rhizomorphs (J) reddish brown, cottony, and irregular pinnate without rhizomorphs (K) white to cream, cottony, and few branched without rhizomorphs (L) brown, spiny, and irregular pinnate (scale bar = 400 µm)

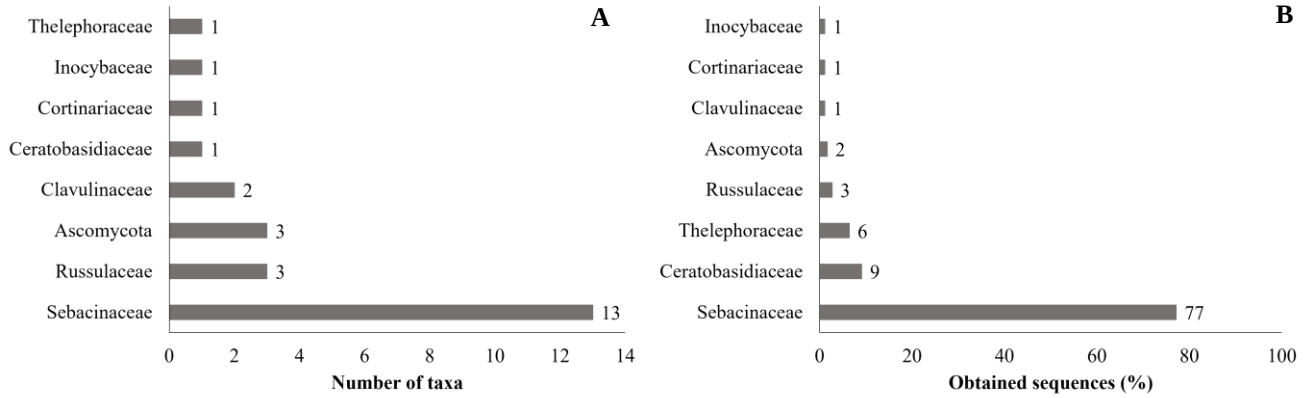

Figure 4. Number of taxa (A) and the obtained sequences (%) (B) from ectomycorrhizal root tips

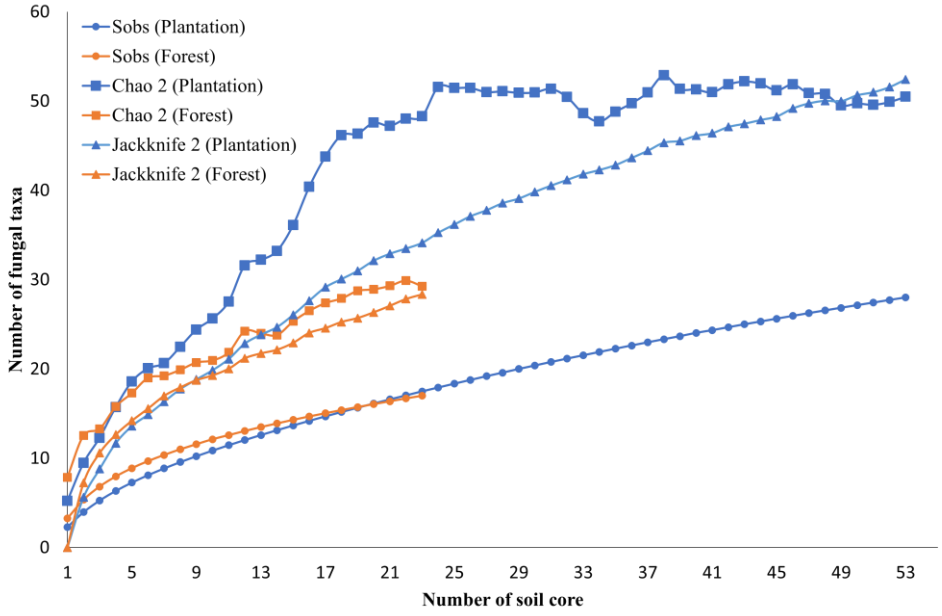

Figure 5. Species accumulation curve (Sobs), Chao2, and Jackknife2 species richness estimate for whole fungal communities of *Dipterocarpus alatus* in natural forests and plantations. Plots were randomly sampled without replacement using 1000 permutations for each sampling size

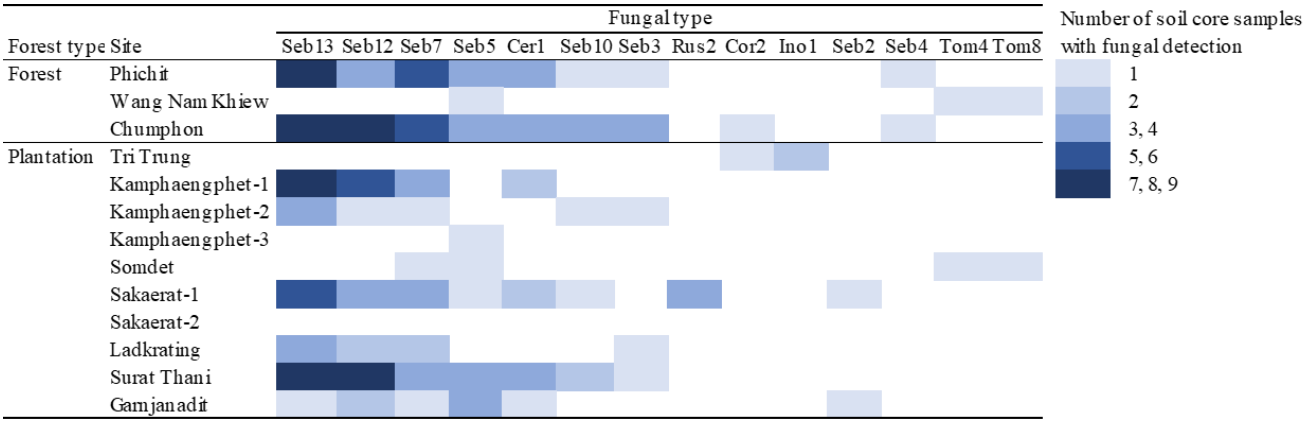

Figure 6. A heat map to show the number of soil core samples with ECM fungal detection collected from three sites of forest and 10 sites of plantations. The ECM fungal type with detection in at least two sites were selected and shown

Table 3. Fungal taxa and the representative ITS rDNA sequences obtained from ectomycorrhizal (ECM) fruiting bodies and ECM roots of *Dipterocarpus alatus* and their similarities to DNA sequences in GenBank database and their occurrences in forests or plantations in this study

Family/ Phylum	Fungal taxa	Sequences obtained part (F*/R*)	Accession number	Base pairs	Similarity to the DNA sequences in GenBank			Number of occurrences in the sampling sites	
					GenBank closest species match (Accession number)	E-value	Identity (%)	Forest	Plantation
Amanitaceae	Ama1	F	AB854645	561	*Amanita hemibapha* subsp. *javanica* (AB451969)	0	100	1	2
	Ama2	F	AB854646	575	*Amanita* sp. H909 (GQ925403)	0	92	0	3
	Ama3	F	AB854647	695	uncultured *Amanita* (JQ347157)	0	93	0	1
	Ama4	F	AB854648	598	*Amanita alboflavescens* (FJ441037)	2.00E-137	100	0	1
	Ama5	F	AB854649	597	*Amanita vaginata* (AB015691)	3.00E-125	83	0	1
	Ama6	F	AB854650	686	*Amanita longipes* (FJ596833)	2.00E-156	88	0	1
	Ama7	F	AB854651	674	*Amanita griseofolia* (FJ441043)	0	94	1	0
	Ama8	F	AB854652	604	*Amanita alboflavescens* (FJ441037)	8.00E-137	84	0	1
Diplocystidiaceae	Ast1	F	AB854653	656	*Astraeus odoratus* (AJ629879)	0	99	1	3
Boletaceae	Bol1	F	AB854654	699	*Boletus sp.* MHM075 (EU569236)	1.00E-117	100	1	0
	Bol2	F	AB854655	747	uncultured Basidiomycota (GU328546)	4.00E-103	100	0	1
	Xer1	F	AB854656	809	*Boletus bicolor* (GQ166877)	5.00E-110	98	0	1
	Xer2	F	AB854657	777	uncultured ectomycorrhizal fungus (FR731312)	1.00E-99	90	0	1
Ceratobasidiaceae	Cer1	R	AB854658	723	uncultured Ceratobasidiaceae (HQ667794)	0	99	2	5
Clavulinaceae	Cla1	F	AB854659	690	*Clavulina cf. cristata* O 65398 (EU862205)	0	99	1	5
	Cla2	R	AB854660	694	uncultured Clavulinaceae (GQ268599)	0	90	0	1
	Cla3	R	AB854661	669	uncultured Clavulinaceae (FJ454922)	0	94	0	1
	Cla4	R	AB854662	718	uncultured Clavulinaceae (FJ454901)	1.00E-88	98	0	1
Cortinariaceae	Cor1	F	AB854663	578	*Cortinarius keralensis* (AY083188)	0	98	0	1
	Cor2	R	AB854664	606	uncultured *Cortinarius* (EU668226)	0	93	1	1
	Ana1	F	AB854665	693	*Anamika angustilamellata* (AY575917)	0	96	0	1
Hydnangiaceae	Lac1	F	AB854666	689	*Laccaria* sp. GMM6784 (JX504135)	0	94	0	1
	Lac2	F	AB854667	686	*Laccaria* sp. GMM6784 (JX504135)	0	96	0	1
	Lac3	F	AB854668	693	*Laccaria vinaceoavellanea* (AB453023)	0	94	0	1
Inocybaceae	Ino1	F, R	AB854669	696	*Inocybe umbrinella* (FJ904166)	2.00E-115	88	2	3
	Ino2	F	AB854670	687	*Inocybe* sp. ZT10102 (GQ893019)	0	96	0	1
	Ino3	F	AB854671	657	*Inocybe rimosa* (EU523562)	3.00E-158	85	0	1
	Ino4	F	AB854672	706	uncultured *Inocybe* (JN544499)	1.00E-110	92	0	2
	Ino5	F	AB854673	558	uncultured *Inocybe* (HE687064)	6.00E-86	98	0	1
	Ino6	F	AB854674	631	*Inocybe* sp. ZT10097 (GQ893008)	0	100	0	2
Russulaceae	Lact1	F	AB854675	682	*Lactarius* cf. *piperatus* SY-2008 (AB459515)	0	99	0	2
	Lact2	F	AB854676	678	uncultured fungus (JN969388)	0	99	0	3
	Lact3	F	AB854677	722	mitochondrion *Lactarius imperceptus* (JQ272401)	0	92	0	4
	Lact4	F	AB854678	705	uncultured *Lactarius* (JF519140)	0	94	0	1
	Rus1	F, R	AB854679	676	*Russula* cf. *rosacea* SY-2008 (AB459514)	0	98	1	1
	Rus2	F, R	AB854680	681	*Russula siamensis* (AB206535)	0	99	2	2
	Rus3	R	AB854681	702	*Russula cf. viscida SY-2008* (AB458684)	0	99	0	1
	Rus4	F	AB854682	694	*Russula virescens* (AB453021)	0	99	0	1

	Rus5	F	AB854683	702	*Russula* sp. RW-3 (AF345248)	0	99	0	2
	Rus6	F	AB854684	684	*Russula* cf. *foetens* UE18.07.2003-7 (DQ422023)	0	95	1	1
	Rus7	F	AB854685	720	uncultured fungus (JN969374)	0	93	0	3
	Rus8	F	AB854686	733	uncultured fungus (JN969374)	0	99	0	3
	Rus9	F	AB854687	696	*Gymnomyces fallax* (AY239349)	0	92	0	3
	Rus10	F	AB854688	665	*Russula* sp. TH9568 (KC155397)	0	89	0	1
	Rus11	F	AB854689	703	*Russula brevipes* (FJ845429)	0	92	0	1
	Rus12	F	AB854690	639	uncultured *Russula* (GQ268645)	0	93	0	2
	Rus13	F	AB854691	686	*Russula brevipes* (FJ845429)	0	92	0	1
	Rus14	F	AB854692	684	*Russula cf. foetens* UE18.07.2003-7 (DQ422023)	0	96	0	1
	Rus15	F	AB854693	722	uncultured fungus (JN969386)	0	99	0	2
	Rus16	F	AB854694	733	uncultured fungus (JN969374)	0	93	0	1
	Rus17	F	AB854695	676	*Russula* sp. R42 (AF350065)	0	99	0	2
	Rus18	F	AB854696	701	*Gymnomyces parksii* (AY239335)	0	93	0	1
	Rus19	F	AB854697	745	uncultured Russulaceae (GQ268648)	0	92	0	1
	Rus20	F	AB854698	691	*Russula densifolia* (AB291755)	0	96	0	1
	Rus21	F	AB854699	731	uncultured Russulaceae (GQ268648)	0	90	0	1
Sclerodermataceae	Scl1	F	AB854700	672	*Scleroderma columnare* (AB459512)	0	99	2	6
Sebacinaceae	Seb1	R	AB854701	621	uncultured *Sebacina* (JQ420940)	0	95	0	1
	Seb2	R	AB854702	597	*Sebacina epigaea* (AF490397)	0	98	0	2
	Seb3	R	AB854703	658	uncultured Sebacinales (EU625988)	0	87	2	3
	Seb4	R	AB854704	594	uncultured *Sebacina* (EU909223)	0	99	2	0
	Seb5	R	AB854705	609	uncultured *Sebacina* (GQ219896)	0	99	3	6
	Seb6	R	AB854706	631	mitochondrion Sebacinales sp. 1 RB-2011 (JQ272332)	0	96	0	1
	Seb7	R	AB854707	651	uncultured Sebacinales (EU625988)	0	95	2	7
	Seb8	R	AB854708	647	uncultured Sebacinales (HM230841)	0	93	0	1
	Seb9	R	AB854709	638	uncultured *Sebacina* (HQ211493)	0	89	1	0
	Seb10	R	AB854710	651	uncultured *Sebacina* (HQ211527)	0	98	2	3
	Seb11	R	AB854711	613	uncultured *Sebacina* (JQ420940)	0	99	1	0
	Seb12	R	AB854712	652	uncultured *Sebacina* (JQ420983)	0	96	2	6
	Seb13	R	AB854713	652	uncultured *Sebacina* (HQ211818)	0	97	2	6
Thelephoraceae	The1	R	AB854714	669	*Thelephora* sp. Nan-CU5 (AB453032)	0	93	0	1
	Tom1	R	AB854715	662	uncultured *Tomentella* (EU625861)	0	93	0	1
	Tom2	R	AB854716	667	uncultured *Tomentella* (EU625851)	0	91	1	0
	Tom3	R	AB854717	670	uncultured *Tomentella* (FJ378811)	0	91	0	1
	Tom4	R	AB854718	667	*Tomentella brunneocystidia* (DQ848613)	0	93	1	1
	Tom5	R	AB854719	652	uncultured *Tomentella* (HM488595)	0	88	0	1
	Tom6	R	AB854720	664	uncultured *Tomentella* (EU625888)	0	91	0	1
	Tom7	R	AB854721	671	*Tomentella* sp. TU103595 (AM412297)	0	99	1	0
	Tom8	R	AB854722	687	uncultured *Tomentella* (EF218826)	0	90	1	1
	Tom9	R	AB854723	669	uncultured *Tomentella* (EU625838)	0	88	1	0
Ascomycota	Asc1	R	AB854724	595	*Rhizoscyphus ericae* (JQ711893)	0	99	0	1
	Asc2	R	AB854725	533	*Cenococcum geophilum* (AY394913)	0	95	0	1
	Asc3	R	AB854726	559	*Cenococcum geophilum* (AY394913)	0	97	1	0

Notes: *F: fruit bodies, *R: ectomycorrhizal roots

Table 2. Soil chemical properties of sampling sites

Sampling Site	Soil Texture	pH	Organic matter (%)	Total N (%)	Available P (mg kg^{-1})	Total C (g kg^{-1})
Phichit	Clay loam	5.60	8.51	0.37	49.90	4.79
Wang Nam Khiew	Sandy loam	6.16	2.65	0.08	2.88	0.96
Chumphon	Loamy sand	5.86	2.02	0.12	1.96	1.57
Tri Trung	Sandy loam	5.06	1.80	0.08	11.61	1.25
Kamphaengphet-1	Loamy sand	5.49	0.48	0.04	2.22	0.91
Kamphaengphet-2	Sandy loam	5.59	2.45	0.07	3.58	1.26
Kamphaengphet-3	Loamy sand	5.29	0.62	0.03	20.32	0.93
Somdet	Sandy loam	5.40	1.95	0.07	3.19	0.97
Sakaerat-1	Clay loam	4.79	3.15	0.19	1.31	2.24
Sakaerat-2	Sandy clay loam	4.64	1.11	0.09	2.17	1.06
Ladkrating	Sandy loam	5.14	2.09	0.08	2.23	1.11
Surat Thani	Sandy loam	5.11	1.30	0.08	2.58	0.76
Garnjanadit	Sandy loam	5.52	1.28	0.09	0.98	1.07
Average		5.36	2.26	0.11	8.07	1.45

Discussion

Diversity and community of ECM fungi in Dipterocarpus alatus

The ECM fungi associated with *D. alatus* comprised 82 fungal taxa in this study, obtained from the putative ECM fruiting bodies and ECM roots in three natural forests and 10 plantations. Among the aboveground fungi, the most fungal taxa were recorded from the Russulaceae, especially members of the genus *Russula* (17 taxa). Tedersoo and Nara (2010) also revealed that *Russula-Lactarius* lineages were relatively more diverse in tropical than temperate forests. Diverse Russulaceae fungi have also been obtained in tropical forests, in Peninsular Malaysia (Lee et al. 2002), in India (Verma et al. 2018), in West Africa (Bâ et al. 2012), in Thailand (Phosri et al. 2012), and in Malaysia (Essene et al. 2017; Peay et al. 2010). However, 15 taxa of *Russula* could not be identified at the species level in this study because their morphological features differed from the described species. Watling and Lee (2007) suggested that there are probably around 60 undescribed species of *Russula* and *Lactarius* in Thailand and Malaysia. Therefore, several *Russula* taxa in this study are supposed to be novel species. Recently, it was reported that six Thai *Russula* species (Wisitrassameewong et al. 2022) and 17 Thai *Lactarius* species, which constitute 87% of this genus collected from the northern part of Thailand (Hyde et al. 2018), were newly identified species.

The belowground fungi were identified as Ascomycota and Basidiomycota fungi. The Ascomycota fungi have sequence similarities to *Rhizoscyphus ericae* (JQ711893) and *Cenococcum geophilum* (AY394913), which were detected from ECM roots in previous studies (Table 3). The Basidiomycota fungi were divided into seven families, the Sebacinaceae, Ceratobasidiaceae, Russulaceae, Thelephoraceae, Inocybaceae, Clavulinaceae, and Cortinariaceae. These fungal families have also been reported earlier as ECM fungi associated with dipterocarps (e.g., Peay et al. 2010; Phosri et al. 2012; Essene et al. 2017). Sebacinaceae was the most taxon-rich fungal family in this study. This family has a wide host range (Weiß et al. 2011) and is an important component in other dipterocarp species (Phosri et al. 2012). Peay et al. (2010) also detected Sebacinales associated with dipterocarp species in the rain forest; however, the ratio on ECM root tips was not high than other fungal orders such as Russulales, Boletales, Agaricales, Cantharellales and Thelephorales. In addition, previous studies revealed that fungi from the Thelephoraceae were common on the roots of dipterocarp trees (Phosri et al. 2012; Nuytinck et al. 2023). These results suggest that the dominant fungal families may differ among dipterocarp forests.

The ECM fungal community usually comprised a few frequently occurring taxa and many rare taxa (e.g., Tedersoo et al. 2008). However, no fruiting bodies were found in this study for more than 90% of the belowground ECM fungal taxa. Similarly, the communities of belowground ECM fungi rarely match those of the aboveground ECM fungi in most ecosystems (Tedersoo et al. 2008). Therefore, neither fruiting bodies nor ECM roots could sufficiently explain the diversity of ECM communities.

In the current study, the rarefied species accumulation curve and minimum taxon richness estimated by the Chao 2 and Jackknife 2 procedures did not level off in either natural forests nor plantations, indicating that the sample was insufficient to characterize the ECM fungal taxon composition fully. Therefore, more ECM fungal taxa could be detected with an increased sampling effort.

Comparison of ECM fungi in natural forests and plantations

Based on the observed ECM fungal fruiting bodies and ECM roots, the number of ECM fungal taxa obtained in the plantations was greater than that in natural forests. Moreover, the minimal species richness levels determined using the Chao 2 and Jackknife 2 procedures for the plantations were higher than those in the forests. The ages of *D. alatus* trees distributed in the natural forests in the current study were estimated to be more than 100 years old because their mean DBH was twice as large as that of the 50-year-old *D. alatus* trees at the Tri Trung plantation. Therefore, the stand age could have resulted in the lower

ECM diversity observed in the natural forests. Our finding differs from those of Martín-Pinto et al. (2022), who reported that the species richness of ECM fungi was higher in the old age stands than in the younger ones. Kranabetter et al. (2005) also found that ECM fungal richness was high in the older age class of pine forests. In our study, the low root density of mature stands in the natural forests might have resulted in lower ECM fungal richness. Hence, increasing the number of soil samples in future studies would help explain the ECM fungal diversity in the natural forests dominated by *D. alatus*. However, many factors can influence the community structure of ECM fungi in the dipterocarp forests, such as soil types (Essene et al. 2017) and soil nutrient contents (Peay et al. 2010).

This study also revealed no difference in the frequently occurring ECM fungal taxa between the natural forests and the plantations. In addition, most of the abundant species were from the Sebacinaceae, Sclerodermataceae, and Ceratobasidiaceae families. This suggests that the diversity of ECM fungal communities could be maintained in *D. alatus* plantations to compensate for decreasing the natural dipterocarp forests. Furthermore, ECM fungi can effectively produce dipterocarp seedlings to succeed in the plantation of dipterocarp (Kaewgrajang et al. 2013; 2019). Therefore, we expect such fungal communities to be a genetic resource in future dipterocarp plantations.

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