DIVERSITY OF ARBUSCULAR MYCORRHIZAL FUNGI IN RELATION TO SOIL PROPERTIES AND DISTURBANCE

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ABSTRACT

Arbuscular mycorrhizal (AM) fungi play an important role in terrestrial symbiosis, but little is known about how soil AM fungal community composition varies in relation to soil properties and disturbance. Diversity of AM fungi was investigated in soil under trees in disturbed rainforest of Jeli District, Kelantan, Malaysia. Sixty rhizosphere soil samples were collected from this rainforest and 234 AM fungal spores (or sporocarp) samples were obtained using the wet-sieve method. Twenty-six species of AM fungi were identified from the collections. The species of AM fungi were of the genera *Acaulospora* (6 species), *Gigaspora* (1 species), *Glomus* (14 species), *Sclerocystis* (1 species) and *Scutellospora* (4 species). *Glomus* was dominant at the study site. The AM fungal spore density ranged from 65 to 1582 per 100 g dry soil (average 839), and the species richness of AM fungi ranged from 1-7 (average = 3.93). The diversity indexes in this study showed that low AM fungi diversity was found in the study site and its tend to be relatively influenced by soil properties and disturbance.

Key words: Arbuscular mycorrhizal (AM) fungi, soil properties, disturbance, rainforest

INTRODUCTION

The tropical rainforest in Malaysia is considered a major reserve of biodiversity, mainly of plant species which may contribute to a greater diversity of microorganisms inhabiting the soil [1]. Agriculture is the most dominant land use in Malaysia where a number of Malaysian rainforest is cut down for agricultural

activities to be developed. Land-use change, particularly the conversion of forests to pasture or cultivated fields, resulted in loss of biodiversity. This disturbance affects the biological processes that important for maintaining productivity and sustainability of ecosystems [2]. There is great diversity of living organisms that live in the soil, but unfortunately biodiversity loss is known to influence the species that live in that habitat, as well as organic material produced in the soil [3]. Forest management is known to give significant impact on the structure of microbial communities in soil [4], however the effects of disturbance on soil forest populations is poorly understood which may have serious implications for both the reestablishment of natural forests and the viability of agroecosystem.

Arbuscular mycorrhizal (AM) fungi (phylum Glomeromycota) colonize the roots of most terrestrial plants, play an essential role in capture nutrient and develop symbiotic or pathogenic associations with plants and animals besides interacting with other microorganisms [5], [6]. It is one of the important components of the soil microbial community which act as primary decomposers of organic residues in soil. The roles of AM fungi in the soil are complex and critical to maintain the functionality of the biomass. Fungal taxa can influence plant growth and performance [7], disease resistance, water relations and improve soil quality [8]. AM fungi originating from different ecosystems contributed directly to the survival of plant species, development of the earth's biodiversity and consequently to the equilibrium of ecosystems [9], [10], [11], [12].

It was shown that AM fungal communities exhibit differing compositions in broadly defined habitat such as forest, grassland and arable fields [13], [14]. Nowadays, most attention has been focussed on the effect of land-use conversion in tropical rainforests on AM fungal communities and specifically those found in agricultural ecosystems. Agricultural management practices have been reported to have correlation with a decrease in AM fungi species richness and diversity, depending on the intensity of crop management [15]. Previous study conducted by [16] shows that soil disturbance that results from land-use conversion can have significant and long-term effects on soil carbon and nutrient contents, soil texture, and pH. It is well known that fertilization also reduces composition of AM fungi and soil nutrient [17]. Therefore, to assess the impact of such disturbance on AM fungi, the present

study investigated the types and diversity of AM fungi in disturbed rainforest of Jeli district, Kelantan, Malaysia which already changed to agricultural regions.

MATERIALS AND METHODS

Sixty soil samples were collected from different plant rhizospheres to a depth of 5 – 30cm from a disturbed forest in Jeli district, Kelantan (6.1644° N, 102.2825° E). The samples were air dried at room temperature for two weeks and stored in sealed plastic bags at 4ºC before used for the test.

Soil analysis

Soil sample was air-dried, finely ground, sieved to pass a 2-mm screen. The soil textures were determined by using textural triangle [18]. The soil pH was determined using a glass and reference electrode with a pH meter (HI 3220, HANNA Instruments, Inc., 584 Park East Drive, Woonsocket, RI 02895) on a 1:1 suspension (5 g scoop of soil to 5 ml water) [19]. Soil CEC was determined by ammonium acetate method at pH 7.00 [20]. The determination of soil organic carbon was based on the Walkley-Black chromic acid wet oxidation method [21]. The total bacterial counts were determined by standard spread-plate dilution method described by [22] and all the data were

expressed as colony forming units (CFU) per gram of dry soil. Meanwhile, chemical analysis for the entire element was determined using Inductively Coupled Plasma (ICP) and elemental analysis.

AMF spore isolation and identification

Wet sieving and decanting method were used to extract AM fungi spores. Twenty grams of soils was suspended in 250 ml of distilled water and stirred with a magnetic stirrer for 10 minutes. Suspension was decanted through a series of 250, 180, 125 and 63 µm sieves. Spores and debris were collected on 125 and 63 µm sieves, washed into a beaker with water, and filtered through filter paper. Then, it was placed in a 9 cm Petri dish for examination under a dissecting microscope 40x magnification for spore number counted. Sporocarps and spore clusters were considered as one unit. Each type of AM fungi spore was mounted in water, lactophenol, PVA and Melzer's reagent, respectively for identification. The identification was based on morphological characteristic using identification keys from an internet-published reference culture database established by Morton (http://invam.caf.wvu. edu/Myc Info/Taxonomy/species.htm), AM fungi phylogeny (www.amf-phylogeny.com), [23] and [24].

Statistical analysis

AM fungal composition in field samples were evaluated based on occurrence frequency, species distribution, spore density (SD), species richness (SR), Shannon Wiener index of diversity (H'), Simpson diversity index (D) and Sorenson's similarity coefficients (Cs).

RESULTS AND DISCUSSION

Soil physicochemical and microbial analyses

The results for soil physicochemical and microbial analyses of disturbed rainforest in Jeli, Kelantan were summarized in Table 1. It is noted that the soil sample was categorized as loam soil (20.5% clay, 40.1% silt, and 39.3% sand). The values of organic matter and CEC are 4.23 and 1.07, respectively. In addition, the soil pH of this soil series was acidic (4.69). Besides, the total bacterial counts in this soil sample were 2.02 x105 CFUg[-1]. Meanwhile, chemical analysis for the entire element present in the soil samples were summarized in Table 2. Previous studies have demonstrated that soil properties including soil texture [25], soil pH [26],

[27] and soil nitrogen availability [28] is a major determinant of microbial community composition. Further evidence reported that AM fungi exhibit host-specific and different structure depending upon habitat soil characteristics [29], [30], [31]. It is interesting to note that the P concentration (1.098 mg/kg) and soil nitrogen availability were very low (0.25%) compared to other elements (Table 2) suggesting that extractable soil P and C: N ratios may be an important regulator of the biogeographical patterns exhibited by fungal communities in this rainforest. This finding is in line with a study conducted by [32] who reported that the changes in fungal communities were more closely associated with P concentrations than land-use type. Moreover, the disturbance soil (cultivated and pasture) tended to have lower soil C: N ratios. In connection to other studies, [28] and [33] found the shifts in substrate quality (high vs low C: N) and availability (nitrogen fertilization) affect the fungal abundance.

Table 1: Soil physico-chemicals and microbial analysis

Parameters	
Soil texture	loam
Clay, %	20.54
Silt, %	40.13
Sand, %	39.34
Organic matter, %	4.2287
Cation exchange capacity, meq 100g[-1]	1.0746
pH	4.69
Total bacterial count, CFU g[-1]	2.02×10^5

Abbreviations: meq, milliequivalents; CFU, colony forming units

Table 2: Soil chemical analysis

Property	(mg/kg)
Available Mg	27.865
Available K	67.564
Available P	1.098
Available Ca	51.287
Available Cu	1.9746
Available Zn	20.651
Available Fe	9737.9
Available N (%)	0.252

AM fungi species and their occurrence frequencies

Two-hundred and thirty-four AM fungal spore (or sporocarp) samples were wet-sieved from the 60 soil samples, from which 26 species of AM fungi were identified. The species diversity of AM fungi was not as high as we had previously thought, as 26 species of AM fungi were far less than the 60 host plants examined. It was found that the Shannon-Wiener index of diversity (H'), Simpson's diversity index (D), and Sorenson's similarity coefficients (Cs) of AM fungal community composition were low which means less communities have in common (Table 3). This low diversity of AM fungi species in disturbed forest has also been reported elsewhere [34], [35]. Previous studies have shown a significant impact of soil disturbance on AM fungal community composition [36], [37], [38]. A number of researchers have reported that a variety of agricultural practices such as tillage, crop rotation, crop residue retention, and fertilizer application have positive influence on soil microbiota including AM fungal species [39], [40], [41].

The identified species of AM fungi belonged to the genera of *Acaulospora* (6 species), *Gigaspora* (1 species), *Glomus* (14 species), *Sclerocystis* (4 species) and *Scutellospora* (6 species). The occurrence frequency of the five genera was 38.03%, 0.43%, 57.70%, 1.28% and 2.56%, respectively (Table 4). The results indicated that *Glomus* was the dominant genera, where *G. monosporum* and *G. claroideum* were the dominant species in the tropical rainforest of Jeli, Kelantan (Table 4). The fact that *Glomus* is dominant genera in this district must be related to their sporogenous characteristics and its soil habitat. It has been found that *Glomus* species usually produce more spores than *Gigaspora* and *Scutellospora* species in the same environment [42].

Table 3: Diversity indices of AM fungi

Diversity Index	
Shanon-Weiner (H')	1.0407
Simpson,s diversity (D)	0.0903
Sorenson's Coefficients (Cs)	0.3850

Table 4: Identified AM fungi and their occurrence frequencies

No.	AM fungi	Occurrence times	Occurrence frequency (%)
	Acaulospora	89	38.03
1	*A. spinosa* **Walker & Trappe**	24	10.26
2	*A. denticulata* **Sieverding & Toro**	13	5.56
3	*A. scrobiculata Trappe*	4	1.71
4	*Acaulospora* sp.1	14	5.98
5	*Acaulospora* sp.2	24	10.26
6	*Acaulospora* sp.3	10	4.27
	Glomus	135	57.70
7	*G. clarum* **Nicol. & Schenck**	6	2.56
8	*G. etunicatum* **Becker & Gerd.**	2	0.85
9	*G. mossae* **(Nicol & Gerd.) Walker**	18	7.69
10	*G. monosporum* **Gerd & Trappe**	37	15.81
11	*G. claroideum* **Schenck & Smith**	31	13.25
12	*G. constrictum* **Trappe**	13	5.56
13	*G. aggregatum* **Schenck & Smith**	1	0.43
14	*Glomus* sp.1	2	0.85
15	*Glomus* sp.2	12	5.13
16	*Glomus* sp.3	2	0.85
17	*Glomus* sp.4	8	3.42
18	*Glomus* sp.5	1	0.43
19	*Glomus* sp.6	1	0.43
20	*Glomus* sp.7	1	0.43
	Scutellospora	6	2.56
21	*S. heterogama* **Walker & Sanders**	3	1.28
22	*Scutellospora* sp.1	1	0.43
23	*Scutellospora* sp.2	1	0.43
24	*Scutellospora* s.p3	1	0.43
	Sclerocytis	3	1.28
25	*Sclerocytis* sp.1	3	1.28
	Gigaspora	1	0.43
26	*Gigaspora* sp.1	1	0.43
	Total : AM fungi=26 species	234	100

Spore density and species richness of AM fungi

The distribution of the 26 identified species of AM fungi in the 60 soil samples, the spore density (spores/100g soil) and the species richness per soil sample is given in Table 5. Fungal spore density ranged from 65 to 1582 per 100 g dry soil ($\overline{x}$ = 839) and species richness ranged from 1-7 ($\overline{x}$ = 3.93). The spore density was usually positively related to the species richness. The distribution of AM fungal species was relatively even, with 90% samples in the range of 2-6 (3.93 ± 1.5). Conversely, it was found that the spore density was uneven, with 62% samples in the range of 463-1171 (839 ± 368). The unevenness of spore density must be due to differences in the ability of AM fungi species to sporulate [42]. The average number of spores recorded from soil rhizosphere in this study is higher to that found by [43] in agroforestry systems (141 spores/100 g of soil), but likely similar with a study

conducted by [6] for tropical rainforest of Xishuagbanna, Southwest China (675 spores/100 g of soil).

Past study conducted by [44] demonstrated that soil forest factors could affect the development and distribution pattern of AM fungi spores in the rhizosphere. The rhizosphere is a site of complex interactions between AM fungi with other plants or microorganisms, where environmental factors such as soil physico-chemical parameters as well as fertilizers or cultivation practices may have large effect on microbial communities. Later on, [45] showed that the spore density, isolation frequency, and species richness varied greatly with different soil characteristics. According to [46], soil forest disturbance also affects the abundance and richness of mycorrhizal spores. These results from previous studies strongly suggest that the complex below ground structure of tropical rainforests is major factors that affect the spore density.

Table 5: AM fungi species distribution, spore density (SD) and species richness (SR)

Soil Sample	AM fungi	SD	SR	Soil Sample	AM fungi	SD	SR
1	1*2 11 12 25	676	5	31	1 9 10	775	3
2	1 2 5 11	929	4	32	2 4 15 17	415	4
3	1 6 12 15	463	4	33	2 10 15	726	3
4	1 2 5 10 25	1020	5	34	10 11	899	2
5	1 5 10	897	3	35	4 5 7 9 10 11	1455	6
6	1 4 11 17	735	4	36	7 10 11 12 15 26	1171	6
7	1 9	282	2	37	1 4 9 10 11 15	1422	6
8	5 9	392	2	38	7 9 11 12	698	4
9	1 9	282	2	39	9 11	541	2
10	1 4 6 10	849	4	40	1 5 7 10 11 14 15	1467	7
11	5 10 12 24	879	4	41	1 3 10 11 12 16 17	1258	7
12	1 4 10 12	887	4	42	1 10 11 17	1101	5
13	11	406	1	43	6 10 12	695	3
14	6 10 11	981	3	44	4 5 10	877	3
15	2 11 13 14 22 25	546	6	45	4 6 10 11	1108	4
16	1 5	404	2	46	1 2 3 5 10 11	1451	6
17	1 5 10	897	3	47	5 7 9 10 11 12	1042	6
18	1 5 9 10 11 15	1582	6	48	1 5 6 10 11 12	1505	7
19	16 17 23	65	3	49	4 15	241	2
20	1 5 10	897	3	50	10 11 15	1013	3
21	1 5 10	897	3	51	10 11 15 17	1068	4
22	11 20	409	2	52	2 4 9 10	874	4
23	3 5 17 18	345	4	53	4 6 11	615	3
24	10 11 12 15	1133	4	54	4 6 9 10	837	4
25	1 5 9 10 11 17 19	1501	7	55	5 10	750	2
26	3 4 8 9 12 21	420	6	56	5 10 12	870	3
27	1 5 15 21	522	4	57	2 8 10 11	1023	4
28	9 11	541	2	58	2 5 7 9 10	1041	5
29	6 9	217	2	59	2 5 7 10 11	1312	5
30	2 6 9 11	742	4	60	2 5 10 11 21	1279	5
Total: soil samples = 60; Average spore density = 839 ± 368; Species richness = 3.93 ± 1.5							

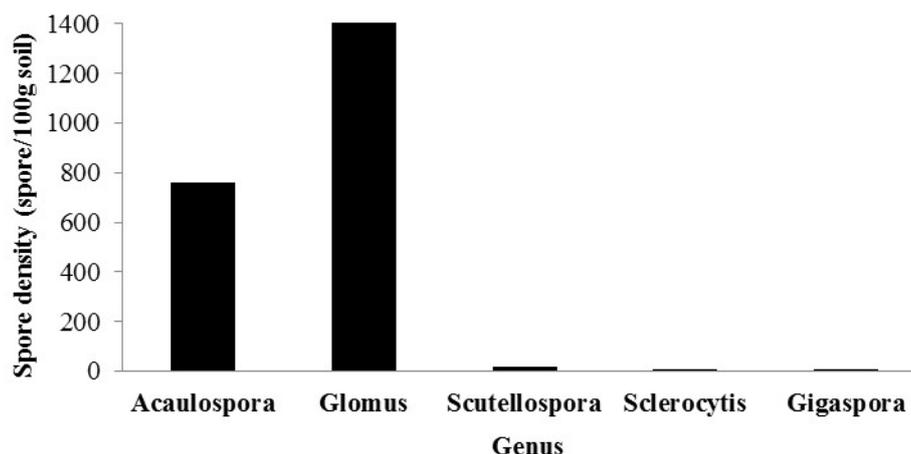*Numbers in this column refers to the codes of AM fungi species in Table 4.

Figure 1: Spore density of five AM fungi genera isolated from study site

Spore density of five AM fungus genera isolated from disturbed forest is given in Figure 1. The dominant genus *Glomus* had the highest spore density (1403±10.8 spores per 100g soil) and followed by second dominant genus *Acaulospora*, which had 761±14.4 spores per 100g soil. The results of the present investigation suggested that disturbance affect the abundance of AM fungi spores. Reduction in spore density due to agricultural practices has been described by many studies. It has been reported that land use change can reduce AM fungi spores and density in tropical [47] and subtropical [48] (Guadarrama *et al*. 2014) ecosystems, and also affect its composition [49]. [50] reported that the mean spore density of AM fungi was significantly decreased in overgrazed as compared to non-grazed plots in the Inner Mongolia steppe. Another work conducted

by [35] demonstrate that genera *Glomus* and *Acaulospora* had high spore density in undisturbed forest of Karbi Assam Hill in India. [51] reported that highest spore density occurred in uncultivated field, slightly lower in old field and lowest in cultivated field in hot and arid ecosystem of Southwest China. According to [52], reduced spore density can be explained by the fact that disturbance disrupts the soil fungi by removing above ground biomass on which these obligate symbionts depend for their carbon source and by breaking the hyphal network leading to a reduction in mycorrhizal colonization

CONCLUSION

In conclusion, the forests of this district contained a low AM fungal diversity. It was shown that soil physico-chemical properties and across disturbance by agricultural activity can have a

significant effect on fungal population and diversity. The study conducted however, have some limitation where the soil sampling was confined only to selected disturbed area. There is need for a wider study area so as a complete representation of the AM fungi diversity in disturbed and non-disturbed rainforest could be discover. This study also can catalogue the AM fungi species so that they can be used in future for restoration and regeneration of degraded forests and maintenance of sustainable forestry.

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