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## **Influence of soil chemical properties on relative abundance of arbuscular mycorrhiza in forested soils in Malaysia**

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## Influence of soil chemical properties on relative abundance of arbuscular mycorrhiza in forested soils in Malaysia

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**Abstract:** The effect of soil chemical properties on the diversity and colonization of arbuscular mycorrhiza (AM) varies among ecosystems. This study was conducted to assess and compare the abundance of AM in a rehabilitated forest and a logged-over forest soil using the most probable number and spore number methods. *Glomus* (71.7%-82.1%) and *Acaulospora* (17.4%-19.5%) were found to be abundant in both sites, while *Gigaspora* was found only in the logged-over forest. The abundance of AM in the rehabilitated forest based on the spore count was significantly higher than in the logged-over forest by a 6-fold difference. Furthermore, root colonization in the rehabilitated forest was found to be almost 9-fold higher than in the logged-over forest. Such differences are linked to the soil chemical properties. The addition of organic matter during forest rehabilitation activity had created favorable conditions for AM sporulation. Soil P in both forests was positively related to the spore count ( $r > 0.68$ ,  $P < 0.001$ ) while the most probable number (MPN) was negatively influenced by soil K ( $r = -0.632$ ,  $P < 0.01$ ). In conclusion, this study showed that soil chemical properties have a direct effect on the abundance of AM.

**Key words:** Arbuscular mycorrhiza, logged-over forest, rehabilitated forest, soil nutrient

### Introduction

Deforestation is widespread in the tropics. It was estimated that in the 1980s Malaysia lost approximately 250,000 ha of forest annually (McMorrow and Talip 2001). Between 1990 and 2000, the deforestation rate in Malaysia was 86% and subsequently it was estimated that 140,200 ha of forest was lost annually from 2000 until 2005 (FAO 2005). Logging was reported to be a major cause of deforestation and forest degradation in Malaysia (Dauvergne 2001).

More recent estimates suggested that approximately 12.01 million ha or 72% of the total naturally forested areas in Malaysia consist of secondary forests (FAO

2007). Expansion of the secondary forest area is expected to continue, causing rapid reduction of primary forests. Therefore, secondary forests will become increasingly important, particularly for preservation of specific habitats and conservation of biodiversity (FAO 2007).

Improper logging activities caused soil compaction and also soil erosion (ITTO 2002). Such activities also create adverse conditions for regeneration of vegetation. Rehabilitation of such lands will rejuvenate forest areas by restoring vegetation cover and improve productive growth of trees. Rehabilitation of degraded forest is crucial for ecosystem enhancement (Kobayashi et al. 2001).

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Most soils in the tropics are low in fertility (Lal 1997). Mycorrhizas, which are associations between plant roots and soil fungi, help plants improve their ability to absorb nutrients in a low soil fertility condition (Berendse and Elberse 1990; Alexander et al. 1992). Smith and Read (1997) estimated that about 80% of plant species form associations with arbuscular mycorrhizal (AM) fungi. In tropical soils, AM fungi are predominant and vital for tree survival and growth, especially during the seedling stages, and their distribution and roles are well recognized (Johnson et al. 1997; Muthukumar et al. 2003). Mycorrhiza associations with roots of host plants are essential for recovery and stabilization of plant communities (Pankow et al. 1991). Failures of seedling establishment in disturbed forest areas are attributed to failed or slow root colonization by mycorrhiza and competition from other pioneer plant species (Janos 1992).

Sources of AM inoculum may comprise spores, hyphae, and fragments of colonized roots (Janos 1992). Losses of AM propagules as a result of soil disturbances have been documented (Janos 1988; McGee 1989). Such losses resulted in low infectivity rate and hence limit the establishment of vegetation in an area (Sylvia 1990).

Enhancements of P and Zn uptake, particularly in marginal soils, due to AM colonization have also been well documented (Smith and Read 1997). Colonization of host plant roots by AM fungi protects roots from infection by pathogens and nematodes (Azcon-Aguilar and Barea 1996; Johnson et al. 1997) and increase absorption of moisture (Graham 2001). Furthermore, AM mycelium assists in forming stable soil aggregates (Rillig 2004). Thus, AM are ecologically important as part of the soil biota, especially in maintaining interactions between plants and other soil communities.

This study was conducted with the aim of assessing and determining the distribution of AM within soils in 2 different types of forests, i.e. logged-over and rehabilitated forests. Relationships between most probable number (MPN), spore counts, and soil chemical properties were examined as well.

## **Materials and methods**

### **Study site and plot design**

The study was carried out in the forest reserve at Universiti Putra Malaysia (UPM) in Bintulu, Sarawak, Malaysia (3°12'N, 103°04'E). It is located at 60-110 m above sea level. The mean annual rainfall is 2327 mm. The soil is classified as Isohyperthermic Typic Dystropept (Nyalau series). Two adjacent forest areas, namely a logged-over and a rehabilitated forest, were chosen as study sites. Six plots of 20 × 20 m size were established in each forest type.

### **Logged-over forest**

This forest was selectively logged (low intensity harvesting activity) in 1971-1972. In 1994-1995, the area was illegally logged and a majority of the trees with a diameter at breast height (dbh) of 30 cm were felled. However, most of the logs were not extracted. More than 15 years later, it is still inhabited by a high diversity of flora with more than 70% canopy coverage. A total of 611 individual trees per ha (with a dbh of 10 cm and above), comprising 43 families and 159 species, has been reported and Dipterocarpaceae was recorded as the most dominant family (Yong 2010).

### **Rehabilitated forest**

The site was cleared in 1985 to establish a forest plantation but was abandoned, leaving the area covered with shrubs. A forest rehabilitation project was initiated in 1990 through a research partnership between UPM and the Yokohama University of Japan with financial support from the Mitsubishi Corporation of Japan. Miyawaki's ecological technique that utilizes indigenous tree species collected from nearby natural forests to create a native forest was adopted (Miyawaki and Box 1996). Selection of tree species was based on vegetation analyses of nearby areas and only native tree species were planted. Soil was plowed to a depth of 1 m and wood residue of multiple hardwood species from the nearby sawn timber factory was incorporated as organic matter during plowing. Approximately 1000 kg of wood was aided per hectare. The chemical properties of the wood residue mixed into the soil were not determined during this early stage of establishment. Wooden plains were installed along gradients to prevent erosion and landslides. Seedlings

were closely planted with 3 plants  $\text{m}^{-2}$  and covered with thick mulch after planting. After 17 years, a total of 22 tree species (45% Dipterocarpaceae) were established on site with *Shorea dasyphylla* as the dominant species. The latest inventory reported approximately 1125 trees  $\text{ha}^{-1}$  (dbh of 10 cm and above) still growing at the site (Su 2008).

### Soil sampling

Soil samplings for spore assessment were performed twice, in June 2008 and December 2009, which represent the lowest and highest precipitation periods of Bintulu (Figure). Ground litter and coarse roots were removed before sampling, and soil samples were collected to a depth of 20 cm using an auger. Soil samples were collected from all 6 plots of the 2 different forest types. To make a composite sample for each plot, 5 subsamples were collected from the 4 corners and the center of each plot and homogenized. There was a total of 12 composite samples, which were packed in polythene bags and stored at 4 °C until analysis.

### Soil chemical analysis

Prior to analysis, 4 subsamples were taken from each of the 12 composite samples, air-dried, and sieved through a 2 mm sieve. Soil pH was determined using a soil to water ratio of 1:2.5. Total organic carbon (TOC) was determined using the loss on ignition method as described by Dean (1974). The micro-Kjeldahl method was used to determine total N (Foster 1995). The double acid method was used for the extraction of P, K, Ca, and Mg, and quantification was done using an atomic absorption spectrophotometer (AAS). Phosphorus was determined by spectrophotometry.

### Most probable number (MPN)

Procedures for MPN determination followed methods described by Smith and Dickson (1997). Soil dilutions were made by mixing 200 g of forest soil with 1800 g of sterilized sand ( $10^{-1}$  dilution). Next, a  $10^{-2}$  dilution was prepared by taking 200 g of the  $10^{-1}$  dilution and mixing it thoroughly with 1800 g of sterilized sand. These steps were repeated to prepare soils of up to  $10^{-5}$  dilution. Undiluted soil served as the control. Four replications were prepared for each plot. *Setaria anceps* was selected and planted as the host plant in this experiment, as

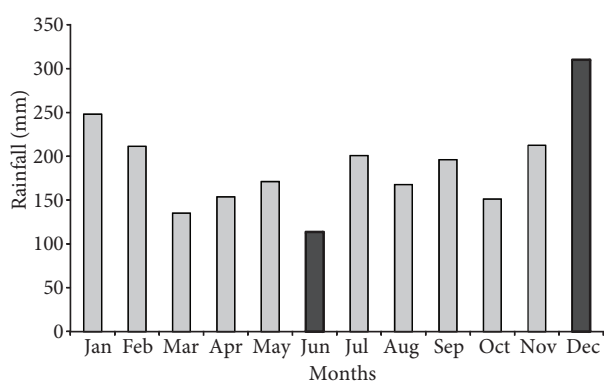


Figure. Monthly average rainfall from January 2005 to December 2009 taken from the nearest weather station, about 25 km from the study site (Source: Malaysian Meteorological Service).

a preliminary experiment found that this species was the best to promote sporulation (Lee, personal comm.). Moreover, *S. anceps* was commonly used as the host plant in propagating mycorrhiza in Malaysia (Alexander et al. 1992). The soil samples were put in pots (a total of 288 pots = 2 forests  $\times$  6 plots  $\times$  6 dilutions  $\times$  4 replications) and were kept moist to field capacity before a cutting of *S. anceps* was planted in each. The pots were placed on propagation beds in a nursery and the plants were water daily with care to avoid soil surface splashing between plots.

Roots and soil were sampled and assessed for AM colonization after 75 days. Root samples were thoroughly rinsed several times under running water to remove debris and soil particles, cleaned with KOH, and stained with acid fuchsin (Kormanik et al. 1980). Root segments were examined microscopically for the presence or absence of functional structures of AM (mycelium, vesicles, and arbuscules). The MPN was calculated based on the theory of probability of the presence or absence of AM colonization in successive dilutions (Smith and Dickson 1997).

### Spore count

AM spores were extracted using the wet sieving and centrifugation method (Brundrett et al. 1996). Air-dried soils of 20 g were decanted through 3 sieves (710, 150, and 63  $\mu\text{m}$ ). There were 4 replicates for each plot. Samples collected from the sieves were centrifuged at a speed of 3000 rpm ( $1409 \times g$ ) with water. The pellets that formed were resuspended in a 1.17 M sucrose solution and centrifuged. Spores in

the sucrose supernatant were then filtered through Whatman No. 1 filter paper and washed with water to remove excess sucrose. The extracted spores were transferred into petri dishes, viewed under dissecting microscopes (30×), and counted before being separated based on color and size. The spores were identified up to genus level following procedures described by Brundrett et al. (1996) and with reference to the International Culture Collection of (Vesicular) Arbuscular Mycorrhizal Fungi (<http://invam.caf.wvu.edu>).

The relative abundance of AM fungi was determined using the formula: (number of spores of a species or genus/total spores) × 100.

#### Data analysis

Data were analyzed using the t-test method to determine the effect of each site on chemical properties, MPN, and AM spore count. Pearson's correlation coefficients were used to determine the relationship between site chemical characteristics and AM spore density as well as MPN. SAS Version 9.1.3 (SAS Institute) was used in all the statistical analyses.

## Results

### Soil chemical characteristics

Soils of both sites were acidic with a pH value lower than 5 (Table 1). Both sites recorded TOC and N of 3.9%-4.4% and 0.16%-0.18% respectively (Table 1). There was a 3-fold difference in P concentration between both sites, with the rehabilitated forest recording a higher value of 10.16 ppm. Significant differences between sites were observed for pH, P, and Ca, with higher values recorded in the rehabilitated forest soil (Table 1). However, no significant difference was detected for TOC, N, K, and Mg (Table 1).

### Spore numbers, most probable number, and AM genus

The rehabilitated forest recorded a 6-fold higher number of spores ( $P < 0.05$ ) than the logged-over forest (Table 2) for both sampling times. A total of 287 and 234 spores per 20 g soil were isolated in the rehabilitated forest in June 2008 and December 2009 respectively. In contrast, only 46 and 41 spores per 20 g soil were isolated from the logged-over forest at the 2 sampling times. The difference in the 2 sampling intervals was not significant (Table 2). The estimated MPN value for the rehabilitated forest soil was almost 9 times higher ( $P < 0.05$ ) than the logged-over forest soil in both sampling intervals (Table 2). More than 900 propagules per 20 g soil were observed in the rehabilitated forest. On the other hand, the logged-over forest recorded less than 120 propagules per 20 g soil. The difference between sampling times for MPN was not significant (Table 2).

Three AM fungi genera were isolated in this study (Table 3), with spores from the genera *Glomus* and *Acaulospora* found in both forest soils during different sampling times. *Gigaspora* was found only in the logged-over forest. Nine species (*Glomus clarum* Nicol. & Schenck, *Glomus fasciculatum* (Thaxter) Gerd. & Trappe, *Glomus macrocarpum* Tulasne & Tulasne, *Glomus microcarpum* Tulasne & Tulasne, *Glomus multicaule* Gerd. & Bakshi, *Acaulospora laevis* Gerdemann & Trappe, *Acaulospora scrobiculata* Trappe, *Acaulospora spinosa* Walker & Trappe, and *Gigaspora margarita* Becker & Hall) of Glomales were isolated from sampling soils. Confirmation of a few of these identified species was also done using molecular characteristics (data not shown). Both sites recorded an equal number of species but were different in terms of species composition (Table 3). *Gigaspora margarita* was only found in the logged-over forest whilst *A. scrobiculata* was only recorded in the rehabilitated forest.

Table 1. Results of soil analysis

Forest type	pH	TOC (%)	Total N (%)	P (ppm)	K (mg/L)	Ca (mg/L)	Mg (mg/L)
Logged-over	4.52 <sup>b</sup> ± 0.05	4.36 <sup>a</sup> ± 0.73	0.18 <sup>a</sup> ± 0.05	3.57 <sup>b</sup> ± 0.35	4.38 <sup>a</sup> ± 0.78	0.99 <sup>b</sup> ± 0.62	2.43 <sup>a</sup> ± 0.69
Rehabilitated	4.66 <sup>a</sup> ± 0.15	3.93 <sup>a</sup> ± 1.27	0.16 <sup>a</sup> ± 0.05	10.16 <sup>a</sup> ± 2.73	4.31 <sup>a</sup> ± 1.47	2.40 <sup>a</sup> ± 1.38	3.00 <sup>a</sup> ± 1.25

Means ( $n = 24$ ) within columns with different letters differ significantly ( $P < 0.05$ ).



Table 2. Spore count (SC) and most probable number (MPN) estimates for the 2 forest sites.

Forest type	SC		MPN	
	June 2008	December 2009	June 2008	December 2009
Logged-over	46 <sup>ba</sup> (27-71)	41 <sup>ba</sup> (20-65)	112.6 <sup>ba</sup> (61.1-160.4)	104.2 <sup>ba</sup> (56.3-149.8)
Rehabilitated	287 <sup>aA</sup> (178-376)	234 <sup>aA</sup> (136-291)	956.3 <sup>aA</sup> (749.7-1356.3)	931.7 <sup>aA</sup> (689.1-1301.5)

Values in parentheses for SC are the ranges of spore numbers in 20 g of soil. Values in parentheses for MPN are confidence limits of median MPN propagules in 20 g of soil. Means ( $n = 24$ ) with different letters differ significantly ( $P < 0.05$ ). Small letters are comparing forest types while capital letters are comparing sampling times.

### Relationship between soil properties and AM

Spore count was positively correlated with P concentration of both logged-over ( $r = 0.842$ ,  $P < 0.001$ ) and rehabilitated ( $r = 0.688$ ,  $P < 0.001$ ) forest soils. MPN was negatively correlated with K ( $r = -0.632$ ,  $P < 0.01$ ) and Mg ( $r = -0.497$ ,  $P < 0.05$ ) in the rehabilitated forest, and with K ( $r = -0.816$ ,  $P < 0.001$ ) in the logged-over forest. Soil pH and P in the logged-over forest was positively correlated with MPN. No correlation between AM variables and TOC, N, or Ca was observed.

### Discussion

The current study assessed the influence of site and more specifically soil chemical properties on the diversity of mycorrhiza based on spore count and MPN. The rehabilitated forest was establishment after site preparation whilst the site of the secondary forest remains intact without any major disturbance. This might be the contributing factor that caused differences in a few of the soil chemical properties

between both forests. The extent of the wood residue influence on the rehabilitated forest's soil chemical properties was unable to be determined as there was no original chemical analysis done on the wood residue more than 17 years ago.

Soil pH in the rehabilitated forest was significantly higher than in the logged-over forest (Table 1). This was probably due to a higher volume of rain water on the forest floor in the logged-over forest (with a more open canopy - Yong 2010), which tends to leach out more base-forming cations such as Ca, leaving the exchange complex to be dominated by  $Al^{3+}$  and  $H^+$  ions, and thus resulting in lower soil pH. Although both types of forest are adjacent to each other, the rehabilitated forest recorded significantly higher P and Ca as compared to the logged-over forest (Table 1). This may have been the result of past silvicultural practices in the area where wood residue was added during site preparation. Decomposition of such materials can add to the availability of nutrients in the soil. In contrast, no silvicultural treatment was ever applied in the logged-over forest.

Table 3. AM genera and number of AM species identified (N), and their relative abundance (RA) in soils of the 2 study sites.

Forest type	Mycorrhiza genus	N	RA (%)	
			June 2008	December 2009
Logged-over	<i>Acaulospora</i>	2	17.4	19.5
	<i>Gigaspora</i>	1	10.9	4.9
	<i>Glomus</i>	5	71.7	75.6
Rehabilitated	<i>Acaulospora</i>	3	18.8	17.9
	<i>Glomus</i>	5	81.2	82.1

In this study, the number of spores recorded in the logged-over forest (Table 2) was in concordance with reports in other tropical forests (Zhao et al. 2001; Muthukumar et al. 2003; Shi et al. 2006). Similarly, Chubo et al. (2009) reported values ranging from 35 to 175 spores per 50 g soil at the Niah Forestry Research Station and in Niah National Park, Sarawak, Malaysia. A much lower spore count of 7 to 32 spores per 100 g soil was reported by Norani (1996) in disturbed to very disturbed sites at the Jengka Forest Reserve, Pahang, Malaysia. The number of spores found in the rehabilitated forest was significantly higher than in the secondary forest (Table 2). This value was within the range reported by Shi et al. (2006) (46 to 1499 spores per 100 g soil) for Meliaceae on Hainan Island, China. However, the value recorded for the current rehabilitation forest was higher than those reported by Norani (1996) and Chubo et al. (2009). An herbaceous layer was not found in either type of forest, thus the number of spores found in this study was related to the tree species found in the sampling plots.

Soil properties, microclimate, and land use can influence the occurrence of AM (Gai 2009). Host (dependency and age), AM species (sporulation potential, dormancy, and distribution pattern), and seasons could also affect the abundance of AM (Guadarrama and Alvarez-Sanchez 1999; Wubet et al. 2009). In the tropical wet forest of Mexico, Guadarrama and Alvarez-Sanchez (1999) reported that disturbance was a more dominant factor in influencing occurrence and richness of AM spores. A similar observation was also reported by Norani (1996). The lower spore number in the logged-over forest may be due to the availability of active growing roots all year round; thus less sporulation is required by the AM fungi present in the soil (Baylis 1969). Changes in soil physical and chemical properties (Table 1) could also cause the differences recorded. Several reasons may have contributed to the higher spore count in the rehabilitated forest as compared to the logged-over forest. Differences in tree species and density (a higher number of individual trees with a younger age), changes in pH and nutrient contents, and plowing and addition of wood residues could increase soil pore size and enhance AM hyphae growth and sporulation rate (Nadian et al. 1996; Norani 1996). Therefore, the higher total spore count in the rehabilitated forest was largely attributed to the

past silvicultural practices, particularly those done during site establishment.

In the current study, the difference between the MPN count and spore number reached an estimate of 2- to 3-fold. Higher MPN values from the experiment indicated that propagules (mycorrhizal spores, roots, and hyphae) rather than spores played a vital role as the inoculum. In the Jengka Forest Reserve, Pahang, Malaysia, Alexander et al. (1992) found that propagule density was 10 times greater than spore density in 3 different forest conditions. The lower MPN count in the logged-over forest as compared to the rehabilitated forest over time was likely due to the diminishing of mycorrhizal infected roots or a decline in infectivity rate or both (Alexander et al. 1992).

Brundrett et al. (1996) suggested that soil nutrient contents are a major factor in influencing site-mycorrhiza association. The results of this study were in contrast with findings by Muthukumar and Udaiyan (2002), who reported a negative correlation between spore count and soil P. They suggested that higher P content resulted in suppression in external hyphal growth or in an increase of P concentration in plant tissue. Suppression of growth and colonization rate of AM fungi due to high P content was also reported by Ahmed et al. (2000). Bolan (1991) suggested that mycorrhiza colonization would increase with increasing P levels in deficient soil before a reverse trend was observed. The positive relationship between spore count and soil available P in both forest types in the present study was probably due to the fact that the P concentration in the soil was low, thus allowing the enhancement of mycorrhizal sporulation.

Furlan and Bernier-Cardou (1989) reported a positive response between AM and soil K, while Ouimet et al. (1996) suggested that a minimum soil K was necessary for AM growth in certain plant species. In the present study, the correlation between K concentration and MPN was negative in both forest types. The high concentration of K in the soils could have caused a reduction instead of promoting propagule potential. The soil Mg and MPN in the rehabilitated forest was observed to be negatively correlated.

*Glomus* (71.7%-82.1%) was the most dominant genus found in both sites. The other genera, namely



*Acaulospora* and *Gigaspora*, gave values of 17.4%-19.5% and 4.9%-10.9%, respectively. *Glomus* has been reported to be a dominant species in other tropical forests (Zhao et al. 2001; Muthukumar et al. 2003; Shi et al. 2006; Chubo et al. 2009) regardless of the type and intensity of disturbance in the different ecosystems (Wubet et al. 2004; Muleta et al. 2008).

According to Gai (2009), *Glomus* has the ability to adjust its sporulation pattern in relation to environmental conditions, thus ensuring dominance in the soil rhizosphere. High adaptability will promote the ability of AM to colonize disturbed soils, while susceptible genera are generally eliminated (Muleta et al. 2008). Menéndez et al. (2001) found that *Glomus* was more resilient while *Entrophospora* was more sensitive to tillage. In the present study, *Gigaspora* was not detected in the rehabilitated forest. The absence of *Gigaspora* has also been reported in some tropical forest soils (Chubo et al. 2009). Meanwhile, the influence of plant species on the diversity of AM species is well known (Eriksson 2001). The higher number of tree species in the logged-over forest could have provided specific hosts for the survival of *Gigaspora* in that area.

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In conclusion, the results of this study indicated that *Glomus* was the most common genus in both forest types, thus confirming its dominance in the tropical forest soil. Spore and propagule densities were higher in the rehabilitated forest; however the values were within those reported earlier for tropical wet forests. The difference in spore counts and MPN between the 2 forests was most likely due to the influence of history, site preparation activity during the rehabilitation process, and host species availability. This study also found that AM abundance based on spore count and MPN was highly influenced by soil P and K, while soil pH and Mg had a slightly lower effect on MPN.

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