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Species richness and composition of arbuscular mycorrhizal fungi occurring on eucalypt trees (*Eucalyptus camaldulensis* Dehnh.) in rainy and dry season

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Abstract

River red gum (*Eucalyptus camaldulensis* Dehnh.), the most commonly planted eucalypt species globally, has several advantages and is widely used for many purposes, which makes the tree important. Mycorrhizal establishment in eucalyptus has been known for many years, and the benefits of this symbiosis have been commercially explored. The main goal of this research was to assess the diversity and distribution of arbuscular mycorrhizal fungi (AMF) on eucalyptus planted in agricultural fields in the rainy and dry season. Fields were chosen in ten different sites located in four provinces in the northeast of Thailand. Rhizosphere soil and root samples were collected and the number of AMF spores and AMF root colonization were assessed. The number of AMF spores was higher in the rainy season than in the dry season, while AMF root colonization was higher in the dry season than in the rainy season. On the basis of morphological identification of AMF, a total of 35 AMF fungal taxa in eight genera were identified, ten belonging to *Acaulospora*, one to *Dentiscutata*, one to *Entrophospora*, 16 to *Glomus*, three to *Gigaspora*, one to *Racocetra*, two to *Scutellospora*, and one to *Septoglomus*. *Glomus* was the dominant genus followed by *Acaulospora*. Relative abundance, and frequency of occurrence were higher in the rainy season than in the dry season. *Racocetra fulgida* was the most common species with a frequency of occurrence of 90% in rainy season, and 80% in dry season. Species richness, Simpson's index of dominance and Shannon–Wiener index of diversity were not significantly different between both seasons.

Key words – Arbuscular mycorrhizal fungal – Colonization – *Eucalyptus camaldulensis* – Rhizosphere – Species diversity

Introduction

The genus *Eucalyptus* (Myrtaceae) is a species–rich (more than 700 species) genus that is widely distributed in Australia. Of the different species, *E. camaldulensis* Dehnh. (river red gum; also known as eucalyptus) has been the most widely used species in plantation forestry. This

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species has many ecologically significant properties, including fast growth rate and its ability to grow on a variety of soil types, to withstand long periods of drought (4 to 8 month), to tolerate salinity and waterlogging. However, the species performs very poorly on limestone soil (Cunningham et al. 1981). Globally, it is the most widely planted hardwood tree in arid and semi–arid areas, especially, in South East Asia, but it is also grown in many other regions. Planted eucalyptus provides key renewable resources for the production of pulp, paper, biomaterials and bioenergy (Bauhus et al. 2010). In Thailand, eucalyptus wood is of considerable economic importance as the most important domestic source of raw materials for the pulp and paper industry. Additionally, eucalyptus trees in Thailand are used for making furniture, and for housing, biomass energy, and charcoal production (Thaiutsa 2002). The species is commonly grown in eastern and north–eastern Thailand because of its ability to endure the locally prevailing weather and soil conditions, its ease for cultivation, and its high growth rate.

Arbuscular mycorrhizal fungi (AMF), belonging to the phylum Glomeromycota, contain three classes, five orders, 15 families, 38 genera and approximately 280 species (Oehl et al. 2011, 2014, Goto et al. 2012, Blaszkowski et al. 2014). AMF are a regular component of rhizosphere microbiota, occurring in soils of the majority of ecosystems. They form symbiotic associations with the roots of approximately 80 % of all terrestrial plant species. AMF improve the growth of individual plants (Smith & Read 2008, Bonfante & Genre 2010), and are crucial to functioning of plant–soil biota–soil ecosystem (Barea et al. 2011). In the mycorrhizal symbiosis, the fungus acquires and subsequently provides to the host plant essential nutrients of low mobility (phosphorus, zinc, copper) and water that are essential for the development of the plant. In exchange, the host plant provides carbon (energy) to the fungus (Carrenho et al. 2007). Benefits for plants from AMF include an increase in the effective absorptive area of roots by formation of an extensive extraradical hyphal network that enhances efficiency in nutrient uptake (George 2000) mainly of phosphorus but also of nitrogen (Koske et al. 2004), and improved soil structure through hyphal exudates that contribute to the formation of water–stable aggregates (Willis et al. 2013), increased drought tolerance (Koide & Mosse 2004), enhanced salt tolerance (Evelin et al. 2009), and enhanced tolerance of heavy metal contamination (Hildebrandt et al. 2007). In addition, AMF also decrease disease incidence (Linderman 1994), protecting the host plant root against pests, pathogens and root and shoot herbivores (Sikes et al. 2009).

Species of the genus *Eucalyptus* can form mycorrhizal associations with both AMF and ectomycorrhizal fungi (ECMF) (Boudarga et al. 1990, Brundrett et al. 1996, Chen et al. 2000). Many studies dealt with the occurrence of AMF on eucalypt seedlings and saplings and their interactions with ECMF, but only few reports dealt with AMF on trees ranging from 15 to over 50 years in the field (Adjoud–Sadadou & Halli–Hargas, 2000). It has been documented that AMF generally are the predominant mycorrhizal form in the early growth stage of plant, being replaced by ECMF with host ageing. Currently, there is limited knowledge of AMF in the rhizosphere of young (less than 5–years old) eucalypt trees planted in agricultural fields outside their natural area. Therefore, the aim of this study was to investigate species identity, richness and diversity of AMF in rhizosphere soil of 2 to 5–years old eucalypts planted at ten different sites located in four provinces of north–eastern Thailand. These sites belong to the research sites of Siam Forestry Co., LTD., Thailand, the main producer and supplier of eucalyptus seedlings to farmers in the north–eastern part of Thailand. The study further aimed to assess changes in AMF in the rainy and dry season. Moreover, the relation between spore density and root colonization in each season was evaluated.

Materials & Methods

Study sites

The diversity of AMF was assessed from rhizosphere soil of 2 to 5–year old eucalypts planted at ten different sites in agricultural fields in the northeast of Thailand. These ten sites occur in four

provinces, viz., Khon Kaen (KKN); Chaiyaphum (CMP); Udon Thani (UDN); and Nongbualamphu (NBP) as shown in Table 1.

Table 1 Geographic location of the ten research sites.

Geography	Study site*									
	KKN-MC1	**KKN-MC2**	**KKN-BH**	**CMP-BK1**	**CMP-BK2**	**CMP-BK3**	**CMP-MC**	**UDN-NW**	**NBP-SB**	**NBP-MN**
Latitude	N16º16'	N16° 15'	N15º45'	N15º49'	N15º49'	N15º49'	N15º49'	N17° 15'	N17º 8'	N17º15'
Longitude	E102º36'	E102°38'	E103º10'	E107º48'	E107º48'	E107º48'	E107º56'	E102°34'	E102º11'	E102º34'

Note:* Khon Kaen: Mancha Khiri site 1 (KKN–MC1), Mancha Khiri site 2 (KKN–MC2), Ban Haet (KKN–BH); Chaiyaphum: Ban Khwao site 1 (CMP–BK1), Ban Khwao site 2 (CMP–BK1), Ban Khwao site 3 (CMP–BK1), Mueang (CMP–MC); Udon Thani: Nong Wua So (UDN–NW); Nongbualamphu: Si Bun Rueang (NBP–SB), Mueang nongbualumphu (NBP–MN).

Soil physic–chemical analysis

Soil pH was determined in soil paste (1:1) using a digital pH meter, Organic matter (OM) content or readily oxidizable carbon was measured by Walkley–Black method (Walkley & Black, 1934), available P by Bray II method (Olsen & Dean, 1965), exchangeable K by 1 N NH_4OAc (Pratt 1965). Soil moisture was determined after drying 10 g fresh soil at 105°C for 24 h in a hot–air oven.

Root and soil samples

Rhizosphere soils and roots of eucalypts were collected from 0–15 cm depth in agricultural eucalyptus fields in the rainy (June to September 2014) and dry (January to April 2015) season. Samples of root–zone soil (each approximately 5 kg) surrounding eucalypt plants, along with fine roots, were collected at the central area (5 m x5 m) of three eucalyptus plots at each of the 10 sites, with internal replicates from each plot. Root and soil samples were kept on plastic bags and further studied at the laboratory.

Estimation of AMF root colonization

The root samples were stained following the methods described by Koske & Gemma (1989). Root samples were washed thoroughly with tap water and cleared in 2.5 % KOH for 1 hour at 90°C, acidified with 1 % HCl and stained overnight in acetic glycerine solution with 0.05 % Trypan blue. The assessment of mycorrhizal colonization was done by the slide method; root segments were selected randomly from the stained samples. The stained roots were then observed under a compound microscope at 40–100x magnification under a Nikon Eclipse50i microscope with an automatic photomicrographic system for the presence of AMF structures. The percentage of root colonization by AMF was determined using the method by Trouvelot et al. (1985).

AMF spore quantification, isolation and identification

Spores of AMF were extracted and separated from soil using the wet–sieving and decanting technique as described by Gerdemann & Nicolson (1963). A suspension of 100 g of dry soil in water was decanted through a series of sieves with the following aperture, 250 µm, 125 µm, 90 µm and 63 µm, and the spores from bottom sieve of each sieve size were collected in Petri dishes and counted under a stereomicroscope (Olympus SZ30). Spores were isolated, mounted on slides with polyvinyl alcohol–lactic acid–glycerol (PVLG), for identification of AMF species. AMF spores were identified based on morphological characteristics such as shape, colour, size, type and number of spore walls, bulbous suspensor, observed under a light microscope ranging from 10–100x magnification. Species identification was performed with the Manual for the identification of VA mycorrhizal fungi of Schenck & Perez (1990) and International culture collection of vesicular and arbuscular mycorrhizal fungi INVAM (http://www.invam.caf.wvu.edu).

Distribution of AMF spores and diversity index

Species richness (SR), relative abundance (RA) and isolation frequency (IF) of AMF were expressed as follows: species richness was determined for each of the ten sites; relative abundance was the number of AMF spores of a species divided by the total spores in a season ×100; and isolation frequency was the number of sites where the AMF species was observed divided by the total number of sites ×100 (Shi et al. 2006). In addition, AMF diversity was evaluated through Simpson's index of dominance: $D = \sum(n_i/N)^2$ (Simpson 1949) and Shannon–Wiener index of diversity: $H' = -\sum_{i=1}^{R}[pi(\ln pi)]$ (Shannon & Wiener 1963), where *i* is the individual species, and *p* is the proportion of spores belonging to the i^{th} species.

Statistical data analysis

Statistical analyses were performed with the Statistical Package for Statistics version 8.0 AMF root colonization and number of AMF spores were analyzed by one–way analysis of variance (ANOVA). Significantly different means were separated by least significant difference (LSD) test, with significant differences set at $P \leq 0.05$. The relationships between number of AMF spores and percentage of root colonization in each season were analyzed by Pearson's correlation coefficient.

Results

Soil characteristics

Results of the soil analyses are provided in Table 2 All sites were acidic (pH ranging from 4.0–5.7). Organic matter (OM) ranged from 0.8 to 4.6 g kg^{-1}, Nitrogen (N) ranged from 0.04 to 0.23 g kg^{-1}, Phosphorus (P) ranged from 3.09 to 39.60 mg kg^{-1} and Potassium (K) ranged from 5.38 to 25 mg kg^{-1}. Moisture in rainy and dry season ranged from 10.13 to 16.73 % and from 1.45 to 8.37 %, respectively.

Table 2 Soil characteristics of soil samples isolated from the 10 different study sites.

Study site	pH	OM (g kg^{-1})	N (g kg^{-1})	P (mg kg^{-1})	K (mg kg^{-1})	Moisture (%)	
						rainy	Dry
KKN–MC1	4.8	2.9	0.15	23.9 ^{M}	8.36	12.08	8.37
KKN–MC2	4.4	3.7	0.19	16.6 ^{M}	14.4	13.21	7.70
KKN–BH	4.0	1.7	0.09	39.6 ^{H}	5.38	14.94	1.45
CMP–BK1	4.7	2.2	0.11	3.09^{L}	9.82	14.90	1.86
CMP–BK2	5.0	1.6	0.08	24.9^{M}	5.45	13.20	3.16
CMP–BK3	5.7	1.1	0.06	20.6^{M}	7.94	12.30	2.23
CMP–MC	5.6	4.6	0.23	4.18^{L}	17.9	10.13	3.77
UDN–NW	4.5	0.8	0.04	3.31^{L}	10.3	14.34	5.70
NBP–SB	4.3	2.4	0.12	8.46^{L}	6.81	16.73	5.72
NBP–MN	4.6	3.9	0.20	30.8^{H}	25	14.72	4.60

Note: According to Land Development, Thailand (Phosri et al. 2010); P<10 mg kg^{-1} is Low (L), P ranging between 11 and 25 mg kg is medium (M), P ranging between 26 and 45 mg kg^{-1} is High (H).

Arbuscular mycorrhizal root colonization

AMF colonization of eucalyptus root showed a large variation between different sites (Table 3). In the dry season, root colonization ranged from 10 to 38 % with the highest colonization in CMP–MC (38 %) and lowest colonization in KKN–BH (10 %). In the rainy season, root colonization ranged from 1 to 15 % with the highest colonization in KKN–MC2 (15 %) and lowest colonization in CMP–BK1 (1 %). AMF root colonization in dry season was significantly higher than in rainy season (P<0.001). Colonization in the dry and rainy season was not correlated (P>0.05). Colonization in the dry season was significantly positively correlated with pH (r = 0.80; P = 0.005).

Number of AMF spores

A higher number of AMF spores in the rhizosphere of eucalyptus was observed in the rainy season than in the dry season (P = 0.02). Highest number of spores in the rainy season was found in the site CMP–MC (82 spores g^{-1} dry soil), and highest number of spores in the dry season was found in site CMP–BK1 (18 spores g^{-1} dry soil). However, in the dry season there were no significant differences in spore numbers between sites (Table 3). Spore numbers in the rainy and dry season were not significantly correlated (P>0.05). Spore numbers in the rainy season were marginally significantly correlated with AMF root colonization in the dry season (r = 0.60; P = 0.07).

Table 3 AMF root colonization (%) and number of AMF spores (spore g^{-1} dry soil) (± standard deviation, n = 3) in rainy and dry season.

Study site	AMF root colonization (%)		Number of AMF spores (spore g^{-1} soil)	
	Rainy	Dry	Rainy	Dry
KKN–MC1	8.71 ± 11.16 ab	31.90 ± 13.18 ab	20.87 ± 13.97 bc	15.33 ± 0.63 a
KKN–MC2	15.02 ± 4.37 a	19.97 ± 22.43 abc	33.13 ± 6.23 b	17.11 ± 4.67 a
KKN–BH	6.58 ± 8.41 ab	9.69 ± 7.84 c	9.73 ± 5.34 c	9.67 ± 2.33 a
CMP–BK1	1.19 ± 0.63 b	19.08 ± 15.18 abc	25.11 ± 15.92 bc	18.40 ± 8.41 a
CMP–BK2	5.80 ± 6.49 ab	17.14 ± 6.87 abc	32.50 ± 4.69 b	18.33 ± 11.27 a
CMP–BK3	3.61 ± 4.81 b	34.16 ± 16.03 ab	22.67 ± 13.86 bc	17.80 ± 5.57 a
CMP–MC	4.63 ± 4.09 ab	38.04 ± 11.64 a	82.44 ± 16.03 a	11.67 ± 3.41 a
UDN–NW	11.61 ± 5.83 ab	17.61 ± 7.94 abc	30.00 ± 10.00 b	9.20 ± 5.57 a
NBP–SB	5.72 ± 4.93 ab	23.59 ± 9.87 abc	26.33 ± 10.15 bc	16.40 ± 2.75 a
NBP–MN	5.79 ± 7.36 ab	15.60 ± 5.25 bc	19.78 ± 3.02 bc	10.47 ± 1.10 a

Note: Values represent mean (n = 3). LSD values as determined by the same letter within each row indicate a significant difference among sites (P≤0.05).

AMF species diversity and distribution of spores

A total of 35 AMF species belonging to 8 genera (*Acaulospora* (10 species), *Dentiscutata* (1 species), *Entrophospora* (1 species), *Glomus* (16 species), *Gigaspora* (3 species), *Racocetra* (1 species), *Scutellospora* (2 species) and *Septoglomus* (1 species)) were identified on the basis of their morphological characteristics (Table 4). The dominant genera were *Glomus*, followed by *Acaulospora*. In both seasons, *Racocetra fulgida* was the most widely distributed, with isolation frequency (IF) in the rainy and dry season of 80 % and 90 %, respectively. Some AMF species were only found in one season, namely, *Acaulospora sporocarpia*, *Acaulospora* sp.3, *Entrophospora nevadensis*, *Glomus macrocarpum*, *G. magnicaule* and *Glomus* sp.4, which were found only in the rainy season. In contrast, *Acaulospora denticulata*, *Acaulospora* sp. 4, *Dentiscutata nigerita*, *Scutellospora heterogama*, *S. persica* and *Septoglomus altomontanum* were found only in the dry season. Based on relative abundance (RA), in the rainy season *G. etunicatum* was most abundant (19.08 %) followed by *A. mellea* (11.17 %) and *A. delicata* (11.14 %). The most abundant taxa in the dry season were *A. mellea* (11.72 %), followed by *R. fulgida* (10.40 %), *G. etunicatum* (8.61 %) and *A. delicata* (7.66 %), respectively (Table 4).

Averaged per site (Table 5), species richness was not different between the rainy season (6.5 species; range 5–10 species) and the dry season (average 6.6 species; range 4–10 species). In the rainy season the highest number of species was found in NBP–MN, in the dry season the highest species number was found in CMP–MC. Species richness in the rainy and dry season were not significantly correlated (P = 0.37). Species richness in the rainy season was significantly negatively correlated with spore number in the dry season (r = –0.83; P = 0.003), whereas species richness in the dry season was significantly positively correlated with spore number in the dry season (r = 0.68; P = 0.03).

Species diversity of AMF expressed by Simpson's index of dominance (*D*) and Shannon–Wiener index of diversity (*H'*) is presented in Table 5. Neither *D* nor *H'* were significantly different between the rainy and dry season (P = 0.14 in both cases). *D* and *H'* were significantly negatively correlated in both seasons (P<0.001 in both cases). Species richness was significantly correlated with *H'* in both seasons (rainy season: r = 0.77, P = 0.01; dry season: r = 0.63, P = 0.05). *H'* in the rainy season was significantly negatively correlated with spore number in the dry season (r = –0.75; P = 0.01).

Table 4 AMF species, Relative abundance (RA), and Isolation frequency (IF) of AMF in rhizosphere soil surrounding root of eucalyptus.

No.	AMF fungal species	Rainy season		Dry season	
		RA (%)	IF (%)	RA (%)	IF (%)
1	*Acaulospora delicata* C. Walker, C.M. Pfeiff. & Bloss	11.14	20	7.66	50
2	*Acaulospora denticulata* Sieverd & S. Toro	0	0	2.69	20
3	*Acaulospora foveata* Trappe & Janos	0.20	10	1.16	10
4	*Acaulospora mellea* Spain & Schenck	11.17	40	11.72	30
5	*Acaulospora scrobiculata* Trappe	0.43	10	2.06	10
6	*Acaulospora sporocarpia* S.M. Berch	0.43	10	0	0
7	*Acaulospora* sp.1	3.43	40	0.95	10
8	*Acaulospora* sp.2	1.70	20	1.95	10
9	*Acaulospora* sp.3	1.79	20	0	0
10	*Acaulospora* sp.4	0	0	2.06	10
11	*Dentiscutata nigerita* Khade SW	0	0	2.48	20
12	*Entrophospora nevadensis* J. Palenzuela, N. Ferrol, Azcón–Aguilar & Oehl	3.32	10	0	0
13	*Glomus albidum* Walker & Rhodes	0.62	10	1.69	20
14	*Glomus badium* Oehl, Redecker & Sieverd.	0.34	10	7.34	40
15	*Glomus delhiense* Mukerji, Bhattacharjee & J.P. Tewari	1.84	10	1.48	10
16	*Glomus etunicatum* W.N. Becker & Gerd	19.08	40	8.61	30
17	*Glomus invermaium* Hall	3.63	20	6.07	30
18	*Glomus macrocarpum* Tul. & C. Tul.	2.18	20	0	0
19	*Glomus magnicaule* I.R. Hall	0.62	10	0	0
20	*Glomus pustulatum* (Nicol.&Gerd.) Walker & Sanders	5.19	20	3.22	20
21	*Glomus* sp.1	1.30	10	2.96	10
22	*Glomus* sp.2	6.18	10	0.37	10
23	*Glomus* sp.3	2.24	20	1.48	10
24	*Glomus* sp.4	1.67	20	0	0
25	*Glomus* sp.5	1.13	20	1.00	10
26	*Glomus* sp.6	2.44	40	0.84	10
27	*Glomus* sp.7	2.15	40	1.85	20
28	*Glomus* sp.8	1.81	20	5.54	20
29	*Gigaspora decipiens* I.R. Hall & Abbott	2.24	20	3.01	30
30	*Gigaspora* sp.1	0.96	20	5.54	50
31	*Gigaspora* sp.2	2.58	20	2.53	30
32	*Racocetra fulgida* Koske & C. Walker	8.19	90	10.40	80
33	*Scutellospora heterogama* T.H. Nicolson & Gerd.	0	0	1.16	20
34	*Scutellospora persica* Koske & C. Walker	0	0	1.69	20
35	*Septoglomus altomontanum* Palenz., Oehl, Azcón–Aguilar & G.A. Silva	0	0	0.48	10

Table 5 AMF species richness (SR), Simpson's index of dominance (*D*) and Shannon–Wiener index of diversity (*H'*) in each site.

Soil sample	Rainy season			Dry season		
	SR	***D***	***H'***	**SR**	***D***	***H'***
KKN−MC1	5	0.27	1.47	6	0.18	1.76
KKN−MC2	5	0.39	1.21	4	0.52	0.92
KKN−BH	7	0.33	1.36	6	0.20	1.69
CMP−BK1	6	0.66	0.76	5	0.21	1.58
CMP−BK2	5	0.43	1.15	7	0.17	1.86
CMP−BK3	5	0.36	1.23	7	0.55	1.04
CMP−MC	7	0.37	1.42	10	0.17	2.04
UDN−NW	9	0.24	1.86	7	0.16	1.87
NBP−SB	6	0.25	1.50	7	0.16	1.88
NBP−MN	10	0.17	2.03	7	0.16	1.88
Average	**6.5±1.78**	**0.35±0.14**	**1.40±0.36**	**6.6±1.58**	**0.25±0.15**	**1.65±0.38**

Discussion

In our study, AMF colonization of eucalyptus roots showed a wide range in different seasons and different sites. AMF root colonization was higher in the dry season than in the rainy season. In contrast, the number of AMF spores in the rhizosphere of eucalyptus was higher in the rainy season and lower in the dry season (Table 3). Our result was in agreement with the findings of Ramos–Zapata et al. (2011) who reported higher AMF colonization during the dry season compared to the rainy season and a higher AMF spore number in the rainy season than in the dry season. Urcoviche et al. (2014) reported higher colonization in winter and higher number of AMF spores in summer in Paraná state, Brazil. Adjoud–Sadadou & Halli–Hargas (2000) equally showed higher AMF root colonization in roots of old eucalyptus (over 15–years old) in the dry season than in the rainy season. However, our results were obtained from young eucalypt trees (2–5–years old).

Siqueira (1993) stated that cyclical changes in soil moisture stimulated AMF sporulation. In pot cultures drought induces sporulation by AMF. Our field data showed the opposite pattern. Our data agree with those from Radhika & Rodrigues (2010), who also reported a higher number of spores in August (rainy season) than in January (dry season). Similar seasonal patterns in spore numbers were observed in other studies (Guitton 1996, Martins et al. 1999, Miranda & Miranda 1997), with gradual increases in the spore numbers during the rainfall period, followed by decreases during the dry period. A potential explanation for this discrepancy could be that pot cultures impose an annual life cycle on AMF, whereas in the field a perennial life cycle of AMF may be maintained.

Variation in number of spores and percentage of root colonization between sites could be explained by different soil chemical and physical properties (Fitzsimons et al. 2008, Bainard et al. 2014). In our study we observed a significantly positive correlation between soil pH and mycorrhizal colonization in the dry season. The effect of soil pH can be even more important than the host plant for selection of AMF species (Bainard et al. 2014). However, there were no significant relationships between P–availability, which ranged from low to high (Phosri et al. 2010) and either spore number or root colonization, even though P availability usually has a major influence on mycorrhizal functioning.

There was no relationship between the percentage of AMF root colonization and number of AMF spores in dry season. The literature provides contrasting outcomes in this regard, with some studies reporting no significant relation between both mycorrhizal parameters (Brundrett 1991, Zahka et al. 1995, Brundrett et al. 1996, Clapp et al. 1995, Merryweather & Fitter 1998, Paula & Siqueira 1987), but others reporting a significantly positive correlation (Muthukumar & Udaiyan

2000, Muthukumar et al. 2003). He et al. (2002) and Ragupathy & Mahadevan (1993) reported that when soil conditions are suitable for spore germination, mycorrhizal colonization increases and spore numbers decrease.

A total of 35 AMF species was obtained in the present study, belonging to 8 genera including *Acaulospora* (10 species), *Dentiscutata* (1 species), *Entrophospora* (1 species), *Glomus* (16 species), *Gigaspora* (3 species), *Racocetra* (1 species), *Scutellospora* (2 species) and *Septoglomus* (1 species). Similar results were obtained by Charoenpakdee et al. (2010) who reported, also on the basis of morphological identification of spores, 34 AMF species that were associated with physic nut (*Jatropha curcas*) in ten different sites in Thailand. The dominant genera were *Glomus* and *Acaulospora*. Rajkumar et al. (2012) reported that the genera *Glomus* and *Acaulospora* were dominant in the roots of medicinal plants in Karnataka, India. Urcoviche et al. (2014) reported that *Glomus* was the dominant genus in rhizosphere of medicinal and spice plants in Parana, Brazil, followed by *Acaulospora*. Our data also agree with the studies of Deotare et al. (2014), Songachan et al. (2015) and Charoenpakdee et al. (2010) of the dominant AMF genera on *Opuntia humifusa*, *Clerodendron* and *Jatropha curcas*. *Glomus* mainly dominates in disturbed environments, such as agro–ecosystems, because their high sporulation rate enables rapid colonization of these environments (Caproni et al. 2003). *Acaulospora* species are well adapted to low–pH soils in tropical regions (Sieverding 1991). Next to their adaptation to the soil conditions (disturbance, low pH), two further factors explain their dominance. Bever et al. (1996) documented that *Glomus* and *Acaulospora* species produce smaller–sized and therefore more spores than *Gigaspora* and *Scutellospora* species in the same environment. Furthermore, *Glomus* and *Acaulospora* species require a shorter time to produce spores than *Gigaspora* and *Scutellospora* species (Hepper 1984).

Simpson's index of dominance (*D*) and Shannon–Wiener index of diversity (*H'*) were highly significantly negatively correlated. The result of the present study was not supported by the findings of Kavitha & Nelson (2013) whose data show a non–significant negative correlation between both diversity assessments in ten rhizosphere soils of sunflower (*Helianthus annuus*). Surprisingly, calculation of the correlation between *D* and *H'* in the study by Charoenpakdee et al. (2010) from ten rhizosphere soils of physic nut (*Jatropha curcas*) in Thailand showed a highly significant positive correlation ($P<0.001$), however, we cannot exclude calculation errors in that latter study as suggested by the fact that all *H'* values were below 1.

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References

Adjoud–Sadadou D, Halli–Hargas R. 2000 – Occurrence of arbuscular mycorrhiza on aged *Eucalyptus*. Mycorrhiza 9, 287–290.

Bainard LD, Bainard JD, Hamel C, Gan Y. 2014 – Spatial and temporal structuring of arbuscular mycorrhizal communities is differentially influenced by abiotic factors and host crop in a semi–arid prairie agroecosystem. FEMS Microbiology Ecology 88, 333–344.

Barea JM, Palensuela J, Cornejo P, Sánchez–Castro I et al. 2011 – Ecological and functional roles of mycorrhizas in semi–arid ecosystems of Southeast Spain. Journal of Arid Environments 75, 1292–1301.

Bauhus J, Van der Meer P, Kanninen M. 2010 – Ecosystem Goods and Services from Plantation Forests. Earthscan, London, UK.

Bever JD, Morton JB, Antonovics J, Schultz PA. 1996 – Host–dependent sporulation and species diversity ofarbuscular mycorrhizal fungi in a mown grassland. Journal of Ecology 84**,** 71–82.

Blaszkowski J, Chwat G, Goto BT. 2014 – *Rhizophagus natalensis*, a new species in the Glomeromycota. Mycotaxon 129, 97–108.

Bonfante P, Genre A. 2010 – Mechanisms Underlying Beneficial Plant–Fungus Interactions in Mycorrhizal Symbiosis. Nature Communications 1, 48.

Boudarga K, Lapeyrie F, Dexheimer J. 1990 – A technique for dual versicular arbuscular endomycorrhizal and ectomycorrhizal infection of *Eucalyptus in vitro*. New Phytologist 114, 73–76.

Brundrett MC. 1991 – Mycorrhizas in natural ecosystems. Advances in Ecological Research 21, 171–313.

Brundrett MC, Ashwath N, Jasper DA. 1996 – Mycorrhizas in the Kakadu region of tropical Australia. I. Propagules of mycorrhizal fungi and soil properties in natural habitats. Plant Soil 184, 159–171.

Caproni AL, Franco AA, Berbara RLL, Trufem SFB et al. 2003 – Ocorrência de fungos micorrízicos arbusculares em áreas revegetadas após mineração de bauxita em Porto Trombetas, Pará. Pesquisa Agropecuária Brasileira 38, 1409–1418.

Carrenho R, Trufem SFB, Bonoi VLR, Silvae S. 2007 – The effect of different soil properties on arbuscular mycorrhizal colonization of peanuts, sorghum and maize. Acta Botanica Brazilica 21, 723–730.

Charoenpakdee S, Phosri C, Dell B, Lumyong S. 2010 – The mycorrhizal status of indigenous arbuscular mycorrhizal fungi of physic nut (*Jatropha curcas*) in Thailand. Mycosphere 1(2), 167–181.

Chen YL, Brundrett MC, Dell B. 2000 – Effects of ectomycorrhizas and vesicular–arbuscular mycorrhizas, alone or in competition, on root colonization and growth of *Eucalyptus globulus* and *E. urophylla*. New Phytologist 146, 545–556.

Clapp JP, Young JPW, Merryweather J, Fitter A. 1995 – Diversity of fungal symbionts in arbuscular mychorrizas from a natural community. New Phytologist 130, 259–265.

Cunningham GM, Mulham WE, Milthorpe PE, Leigh JH. 1981 – Plants of Western New South Wales, Soil Conservation Service of New South Wales, Australia.

Deotare PW, Metkar SS, Maggirwar RC, Khodke SP. 2014 – AMF status of naturally growing *Opuntia humifusa*. International Journal of Life Sciences 2(1), 63–66.

Evelin H, Kapoor R, Giri B. 2009 – Arbuscular mycorrhizal fungi in alleviation of salt stress: a review. Annals of Botany 104**,** 1263–1280.

Fitzsimons MS, Miller RM, Jastrow JD. 2008 – Scale–dependent niche axes of arbuscular mycorrhizal fungi. Oecologia 158, 117–127.

George E. 2000 – Nutrient uptake – Contributions of arbuscular mycorrhizal fungi to plant mineral nutrition. In: Kapulnik Y, Douds DD. (eds), Arbuscular mycorrhizas: Physiology and function, p. 307–343, Kluwer Academic Publishers. Netherlands.

Gerdemann JW, Nicolson TH. 1963 – Spores of mycorrhizal *Endogone* species extracted from soil by wet sieving and decanting. Transactions of the British Mycological Society 46**,** 235–244.

Goto BT, Silva GA, Assis DMA, Silva DKA et al. 2012 – *Intraornatosporaceae (Gigasporales)*, a new family with two new genera and two new species. Mycotaxon 119, 117–132.

Guitton TL. 1996 – Micorrizas vesículo–arbusculares em oito espécies florestais da Amazônia: Efeitos de fatores sazonais e edáficos em plantios experimentais de terra firme na região de Manaus – AM. Manaus, 81p. (Dissertação de Mestrado. Instituto Nacional de Pesquisas da Amazônia/ Universidade Federal do Amazonas. INPA/ UFAM).

Hildebrandt U, Regvar M, Bothe H. 2007 – Arbuscular mycorrhiza and heavy metal tolerance. Phytochemistry 68**,** 139–146.

He X, Mouratov S, Steinberger Y. 2002 – Temporal and spatial dynamics of vesicular–arbuscular mycorrhizal fungi under the canopy of *Zygophyllum dumosum* Boiss. in the Negev Desert. Journal of Arid Environments 52, 379–387.

Hepper CM. 1984 – Isolation and culture of VA mycorrhizal (VAM) fungi. In: VA Mycorrhizae. (eds. CL Powell, DJ Bagyaraj) CRC Press, Florida, USA. pp 95–112.

Kavitha T, Nelson R. 2013 – Diversity of Arbuscular Mycorrhizal Fungi (AMF) in the Rhizosphere of *Helianthus annuus* L. American–Eurasian Journal of agricultural & environmental Sciences 13(7), 982–987.

Koske RE, Gemma JN. 1989 – A modified procedure for staining roots to detect VA–mycorrhizas. Mycological Research 9, 486–505.

Koske RE, Gemma JN, Corkidi L, Sigüenza C et al. 2004 – Arbuscular mycorrhizas in coastal dunes. In: Martínez ML, Psuty NP. (eds), Coastal dunes: ecology and conservation, p. 173–187, Springer, Heidelberg.

Koide RT, Mosse B. 2004 – A history of research on arbuscular mycorrhiza. Mycorrhiza 14, 145–163.

Linderman RG. 1994 – Role of VAM in Biocontrol. In: Mycorrhizae and Plant Health. (eds. FL Pfleger, RG Linderman) APS Press, St. Paul, Minnesota, USA. pp 1–26.

Martins CR, Miranda JCC, Miranda LN. 1999 – Contribuição de fungos micorrízicos arbusculares nativos no estabelecimento de Aristida setifolia Kunth em áreas degradadas do Cerrado. Pesquisa Agropecuária Brasileira 34, 665–674.

Merryweather J, Fitter A. 1998 – The arbuscular mycorrhizal fungi of *Hyacinthoides non-scripta*. II. Seasonal and spatial patterns of fungal populations. New Phytologist 138, 131–142.

Miranda JCC, Miranda LN. 1997 – Micorriza arbuscular. In: Vargas MAT, Hungria M. (eds), Biologia dos Solos dos Cerrados, p. 69–123. Embrapa Cerrados, Planaltina.

Muthukumar T, Udaiyan K. 2000 – Arbuscular mycorrhizas of plants growing in the Western Ghats region, Southern India. Mycorrhiza 9, 297–313.

Muthukumar T, Sha LQ, Yang XD, Cao M et al. 2003 – Mycorrhiza of plants in different vegetation types in tropical ecosystems of Xishuangbanna, southwest China. Mycorrhiza 13, 289–297.

Oehl F, Sieverding E, Palenzuela J, Ineichen K et al. 2011 – Advances in *Glomeromycota* taxonomy and classification. IMA Fungus 2, 191–199.

Oehl F, Sánchez–Castro I, Palenzuela J, Silva GA. 2014 – *Palaeospora spainii*, a new arbuscular mycorrhizal fungus from Swiss agricultural soils. Nova Hedwigia 101, 1–2.

Olsen SR, Dean LA. 1965 – Phosphorus. In: Methods of Soil Analysis. Part 2: (eds. CA Black, DD Evans, JL White, LE Ensminger, PE Clark). Chemical and Microbiological Properties. Madison, Wisconsin, USA. pp. 1035–1049.

Paula MA, Siqueira JO. 1987 – Efeitos da umidade do solo sobre a simbiose endomicorrízica em soja: I. Colonização radicular, esporulação, nodulação e acúmulo de nitrogênio. The Revista Brasileira de Ciência do Solo 11, 283–287.

Phosri C, Rodriguez A, Sander IR, Jeffries P. 2010 – The role of mycorrhizas in more sustainable oil palm cultivation. Agriculture, Ecosystems and Environment 135, 187–193.

Pratt PF. 1965 – Potassium. In: Methods of Soil Analysis, Part 2. (eds. CA Black, DD Evans, JL White, LE Ensminger, PE Clark). Chemical and Microbiological Properties. Madison, Wisconsin, USA. pp. 1022–1034.

Radhika KP, Rodrigues BF. 2010 – Arbuscular mycorrhizal fungi density in some commonly occurring medicinal plants of Western Ghats, Goa region. Journal of Forest Research 21, 45–52.

Ragupathy S, Mahadevan A. 1993 – Distribution of vesicular–arbuscular mycorrhizae in the plants and rhizosphere soils of the tropical plains, Tamil Nadu, India. Mycorrhiza 3, 123–136.

Rajkumar HG, Seema HS, Sunil Kumar CP. 2012 – Diversity of arbuscular mycorrhizal fungi associated with some medicinal plants in Western Ghats of Karnataka region, India. World Journal of Science and Technology 2(1), 13–20.

Ramos–Zapata JA, Zapata–Trujillo R, Ortiz–Diaz JJ, Guadarrama P. 2011 – Arbuscular mycorrhizas in a tropical coastal dune system in Yucatan Mexico. Fungal Ecology 4, 256–261.

Schenck NC, Perez Y. 1990 – Manual for identification of VA mycorrhizal fungi. (eds. NC Schenck, Y Perez Gainesville), Florida, USA. pp 241.
Sieverding E. 1991 – Vesicular-arbuscular mycorrhiza management in tropical agrosystems. Deutsche Gesellschaft für Technische Zusammenarbeit, Eschborn.
Shannon CE, Wiener W. 1963 – The Mathematical Theory of Communication. University of Illinois Press, Urbana, USA.
Shi ZY, Chen YL, Feng G, Liu RJ et al. 2006 – Arbuscular mycorrhizal fungi associated with the Meliaceae on Hainan island, China. Mycorrhiza 16, 81–87.
Siqueira JO. 1993 – Biologia do Solo. ESAL/FAEPE, Lavras, Minas Gerais, Brazil.
Simpson EH. 1949 – Measurement of diversity. Nature 163, 688.
Sikes BA, Cottenie K, Klironomos JN. 2009 – Plant and fungal identity determines pathogen protection of plant roots by arbuscular mycorrhizas. Journal of Ecology 97**,** 1274–1280.
Songachan LS, Kayang H, Moinao P. 2015 – Diversity and species composition of arbuscular mycorrhizal fungi in *Cleroderum* species. Mycosphere 6(2), 150–158.
Smith SE, Read DJ. 2008 – Mycorrhizal Symbiosis, 3rd edn. Academic, London.
Thaiutsa B. 2002 – Silvicultural management of private Eucalyptus plantations for wood chips in Thailand. Proceedings of the International Symposium, Guangzhou, China.
Trouvelot A, Kough JL, Gianizai–Pearson V. 1985 – Mesure du Taux de Mycorrhization VA d'un Systeme Radiculaire. In Gianinazzi–Pearson V, Gianinazzi S. (eds), Aspects Physiologiques et Genetiques des Mycorrhizes. Actes du 1er Symposium Europeen sur les Mycorrhizes, p. 217–221). INRA Press, Paris.
Urcoviche RC, Castelli M, Gimenes RMT, Alberton O. 2014 – Spore density and diversity of Arbuscular mycorrhizal fungi in medicinal and seasoning plants. African Journal of Agricultural Research 9(16), 1244–1251.
Walkley A, Black IA. 1934 – An examination of the Degtjareff method for determining organic carbon in soils: Effect of variations in digestion conditions and of inorganic soil constituents. Soil Science 63, 251–263.
Willis A, Rodrigues BF, Harris PJC. 2013 – The ecology of arbuscular mycorrhizal fungi. Critical Reviews in Plant Sciences 32**,** 1–20.
Zahka GA, Baggett KL, Wong BL. 1995 – Inoculum potential and other VAM fungi parameters in four sugar maple forests with different levels of stand dieback. Forest Ecology and Management 75, 123–134.