Phosphorus Fractions and Arbuscular Mycorrhizal Fungi Communities in a Tropical Coarse-Textured Soil under Natural Forest and Para Rubber Ecosystems

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ABSTRACT

Soil phosphorus (P) plays an essential role in rubber tree plantations that are rapidly and extensively being established in Southeast Asia. However, available information is quite limited on soil P fractions and arbuscular mycorrhizal fungi (AMF) in the tropical region. Herein, we investigated P fractions and AMF community under natural forest and rubber plantations at different ages of 5 years, 11 years, and 22 years in tropical coarse-textured soils from Thailand. The studied loamy sand soils were acidic (pH=5.0-5.7) with low available P concentrations (1.73-6.48 mg/kg). Data on the P fractions data revealed that the labile P (water-extractable P_i and $NaHCO_3$-extractable P_i) and moderately labile P ($NaOH_{0.1}$-extractable P_o and HCl-extractable P_i) pools in rubber-growing soils were higher than those in the natural forest soil. Elevated values of these properties were substantial with increasing stand ages. The rubber monocropping systems declined in the density and diversity of AMF spores compared to the natural forest site. *Glomus badium*, *Rhizophagus fasciculatus*, *Acaulospora Laevis*, and *Ambispora appendiculata* were the most dominant and tolerant AMF species across the rubber stands (>50% of the total species). The P fractions and AMF were correlated with soil labile-P forms. Soil labile and moderately P fractions were the important factors affecting the difference in AMF community. This study highlighted that long-term rubber plantations in tropical ecosystems promoted labile P fraction but demoted AMF density and diversity.

1. INTRODUCTION

Rubber tree plantations have been established more rapidly and extensively than any other tree crops in Southeast Asia, where over 85% of the world's rubber tree plantations are located (IRSG, 2021; WRS, 2021). Thailand has been ranked as the top natural rubber producer, with its expansion lasting for more than a century. In 2021, the rubber plantation area in Thailand covered over 22 million ha, representing the second-largest area of such plantations in the world (FAO, 2019; IRSG, 2021). The expansion of rubber plantations in Thailand formerly replaced natural forests; however, more recently, its expansion has substituted much existing agriculture or intensive annual cash crops such as sugarcane or cassava, because of native forest depletion and protection (Chambon et al., 2016). Studies have reported that monocropping rubber plantation exacerbated soil chemical fertility through acidification and caused a severe decline in P availability, presumably due to the abundant amounts of Fe/Al (hydr)oxides and clay-sized particles that accumulated with increasing rubber stand age (Fox et al., 2014).

Phosphorus (P) is the primary limiting essential plant nutrient for agricultural productivity in tropical regions (Cherubin et al., 2016; Maranguit et al., 2017; Wagg et al., 2014). Highly weathered acidic soils and abundant Fe/Al (hydr)oxides can retain and

chemically fix P, leading to P restrictions in tropical agricultural production (Holford, 1997; Rausch and Bucher, 2002). Parent materials, organic matter decomposition and P fertilizer addition contribute available P to soils; however, different pathways of P resupply may cause different P pool sizes. The P replenishment from diverse P pools with differing solubilities becomes vital when available P is depleted (Henriquez, 2002; Maranguit et al., 2017). Many studies have indicated that the cycling of various P pools is influenced by plant-soil-microbial interactions, which are, in turn, dependent on agricultural practices, stand age, seasonal changes, ecosystem types, and environmental factors (Chen et al., 2008; Liu et al., 2018; Stutter et al., 2015). In addition to soil-based P nutrition, rubber trees could also retranslocate or redistribute a substantial portion of nutrients before senescence of plant parts which could reduce the dependency of rubber trees on soil nutrients (Li et al., 2016). Soil P exists in inorganic and organic forms, such as dissolved inorganic and organic P in aqueous solution or adsorbed P in solid minerals (Negassa and Leinweber, 2009). Inorganic P (P_i) typically includes primary mineral-P (such as apatite: $Ca_5(PO_4)_3(OH,F,Cl)$) and secondary crystalline and amorphous precipitates of Al/Fe (hydr)oxides and P absorbed onto silicate clay minerals (Costa et al., 2016). Organic P (P_o) is primarily associated with microbial biomass and recalcitrant compounds of soil organic matter, such as stabilized inositol phosphates and active orthophosphate diesters (Nash et al., 2014; Steidinger et al., 2015). Various P fractions with different levels of mobility and solubility can be assessed using sequential extractions, which provide information about labile and non-labile P fractions that can act as sources or sinks of available P to plants (Costa et al., 2016; Neufeldt et al., 2000). Non-labile P was the most dominant form of P fractions in tropical acid soils with less contribution from labile and moderately labile P fractions (Lustosa Filho et al., 2020).

Chemical fractions and bioavailability of soil P have been reported to substantially decrease with increasing stand age of rubber tree plantations in the temperate zone of China (Liu et al., 2018). Several microbial organisms can enhance soil P availability (Wagg et al., 2014). Arbuscular mycorrhizal fungi (AMF) are an essential group of soil microorganisms that play a vital role in soil fertility, plant nutrition, and changes in plant physiology and secondary metabolisms (Cervantes-Gámez et al., 2016;

Schweiger and Müller, 2015). In addition, AMF can assimilate P from the pools often regarded as unavailable to plants (Cardoso et al., 2006; Liu et al., 2018; Moreira et al., 2013). Rubber monoculture plantations have been shown to harbor lower AMF spore diversity than natural rubber tree stands, possibly due to management practices in the plantations, such as pesticide utilization or extensive weeding (Feldmann et al., 2000). Some studies indicated that the cycling of various P fractions was influenced by plant-soil-microbial interactions and soil management and environmental-related factors (Chen et al., 2015; Stutter et al., 2015). However, information on the effects of rubber tree plantation age on soil P fractionations and AMF diversity remains poorly understood.

The main objectives of this study were to (i) investigate the P fractions and AMF diversity in different ages of rubber plantations from young rubber (5 years old: YR), mature rubber (11 years old: MR), and old rubber (22 years old: OR) compared to a natural forest (FR) and (ii) examine the relationship of the P fractions with AMF diversity in the soils. The result of this study should improve the understanding of soil P forms and the diversity of AMF communities in tropical soil under different ages of rubber tree plantations.

2. METHODOLOGY
2.1 Study sites
The study was carried out in a rubber tree plantation (*Hevea brasiliensis*) compared to the natural forest in the Don Chang Sub-district, Mueang District, Khon Kaen Province, Northeast Thailand (16°21′N, 102°45′E; Figure 1). This area has a tropical savanna environment. Temperature and precipitation data have been monitored for several years in the Khon Kaen Province. Based on the records, the temperature varied between 18 and 35°C throughout the year, with a mean annual temperature of 30°C and average daily low and high temperatures of 22 and 34°C, respectively. The mean annual precipitation was 1,250 mm (Thai Meteorological Department, 2018). The studied sites were covered by natural deciduous dipterocarp forest (FR), young rubber plantation (YR), mature rubber plantation (MR) and old rubber plantation (OR). The rubber sites had been used for cassava monocropping before being converted to rubber ecosystems. The rubber plantations had been tilled between the tree lines (7 m) twice a year at the beginning and the end of the rainy season during the

first 5 years of the rubber tree growth. There was no additional control of the undergrowth in rubber plantations after the initial 5 years period. Chemical fertilizer (N:P:K, 20:10:17) and manure had been applied mostly in July during the first 5 years, depending on materials and availability. On average, these farmers used chemical fertilizer at the rates of 44 kg/ha/year for YR plantations, respectively. The

fertilizers were applied at a distance of 0.5 m around the tree base. Local grass could be observed in the FR and YR sites, whereas there were no under growths in the MR and OR sites. Some shrubs could also be found in the FR site. The studied soils were Ban Phai (Bpi) series (Marlairotsiri et al., 2004), which was classified as Lixisols or Alfisols based on World Reference Base for Soil Resources or USDA Soil Taxonomy.
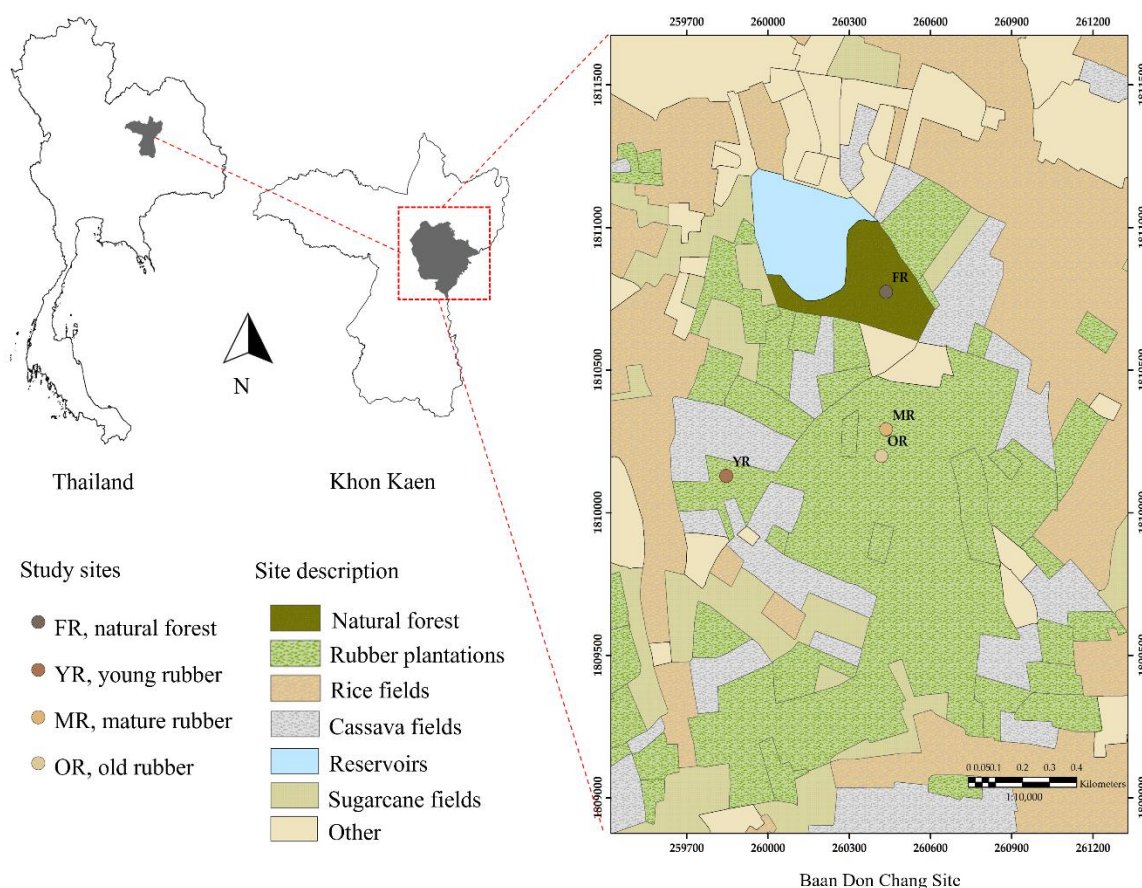
Figure 1. Location of four study sites in Don Chang, Khon Kaen District, Thailand

2.2 Soil sampling

Soil samples were collected from three representative blocks on each of the rubber stand sites of each of the three stand ages and in the natural forest in dry season (June 2018). This sampling period was undertaken before the annual fertilization period in July. The experimental design was Randomized Complete Block Design. For each block on each the rubber sites, three soil samples were taken and composited: three at mid-distances between the 7 m inter-rows. For the natural forest site, three samples were collected and composited from the plot diagonal. About 2 kg of the composite sample were obtained from each site. The samples were obtained at the topsoil from 0-15 cm depth, which was expected to

have high microbial activity. Each sample replicate was passed through a 2 mm diameter mesh. The soil was divided into two parts. One part was used for the analysis of physicochemical properties and the other for microbial community analysis. A sub-sample was preserved on ice during the sampling procedure and stored at -4°C before microbial analyses. In addition, triplicates of soil cores were collected for bulk density analysis.

2.3 Physical and chemical properties analyses

Soil texture was examined using the pipette method (Gee and Bauder, 1986). Bulk density (BD) was calculated as the ratio of the dry mass of fine soil (<2 mm) to the soil core volume (Blake and Hartge,

1986). The pH was determined in distilled water using a soil-to-water ratio of 1:1. Total nitrogen (TN) was determined using the Kjeldahl method (Bremner, 1965). Organic matter content (OM) was determined using the Walkley and Black method (Walkley and Black, 1934). Available phosphorus (Avail. P) was extracted using the Bray-II method (Bray and Kurtz, 1945). Total phosphorus (TP) was determined using perchloric acid ($HClO_4$) digestion (Kuo, 1996). The available potassium (Avail. K), available magnesium (Avail. Mg), and available calcium (Avail. Ca) of soils 1 mol/L ammonium acetate at pH 7 were measured using atomic absorption spectroscopy (Chapman, 1965). Soil microbial biomass P (MBP) was analyzed the chloroform fumigation extraction method. In brief, aliquots of the fresh soil corresponding to 15 g dry weight equivalent were fumigated for 24 h using $CHCl_3$, and then the amount of inorganic P was extracted using 0.5 mol/L $NaHCO_3$ (pH 8.5). Nonfumigated soil was treated using the same method as the control. The microbial biomass P was calculated based on the difference in available P extracted with 0.5 mol/L $NaHCO_3$ between the fumigated and unfumigated soil divided by a correction factor of 0.4 (Brookes et al., 1982).

2.4 Phosphorus sequential fractions

Soil P fractions were determined using the sequential extraction scheme of Hedley et al. (1982) as modified (Zhang and Kovar, 2009; Liu et al., 2018). Details of the extraction procedures, including extracting solution, target pool, extraction conditions and solid-to-solution ratio, are summarized in Table 1. In each step, 30 mL of the extractant was added to 1 g of soil sample in a 50 mL centrifuge tube (1:30 soil-to-solution ratio), with the centrifuge tubes being shaken end-over-end for 16 h at 25°C. The soil extractions were centrifuged at 2,054 g (3,500 rpm) for 15 min and filtered through a Whatman No. 42 membrane to collect a clear solution for P analysis. The extracted P_i was measured using the molybdate colorimetric method at 882 nm (Tiessen and Moir, 1993). The total P in the extract was determined after digestion using H_2SO_4 and potassium persulfate in a heat block at 121°C (Hedley et al., 1982). The P_o was calculated as the difference between total P and P_i (Zhang and Kovar, 2009). The P_o in the water and HCl-extractable fractions was not excluded from the analysis because several studies have shown that the P concentrations in both extracts were below the method detection limit (Costa et al., 2016; Maranguit et al., 2017). Finally, soil residual P was digested using concentrated H_2SO_4 and 30% H_2O_2 extraction at 225°C for 30 min, with a step of digestion at 360°C for 1 h. To reflect ecological relevance, the P fractions were classified into three groups: labile P (water-P_i+$NaHCO_3$-P_i+$NaHCO_3$-P_o), moderately labile P ($NaOH_{0.1}$-P_i+$NaOH_{0.1}$-P_o+HCl-P_i) and non-labile P ($NaOH_{0.5}$-P_i+$NaOH_{0.5}$-P_o+residual-P_o), according to previous studies (Costa et al., 2016; Crews and Brookes, 2014; Hu et al., 2016; Liu et al., 2018).

Table 1. Summarized six-step sequential extraction of inorganic P and three-steps of organic P procedure and their hypothetical interpretation

P fraction	Extracting solution	Target pool	Extraction condition	SSR (g/mL)
F_i-1[a]	Deionized water	Labile P_i or mobile P	16 h	1:30
F_i-2	0.5 mol/L $NaHCO_3$ (pH 8.5)	Labile P_i and adsorbed onto soil surfaces	16 h	1:30
F_o-2[b]	0.5 mol/L $NaHCO_3$ (pH 8.5)/ $(NH_2)_2S_2O_8$+H_2SO_4	Labile P_o and adsorbed onto soil surfaces	16 h/ 0.5-1 h at 121°C	1:20
F_i-3	0.1 mol/L NaOH	Poorly crystalline Fe and Al (hydr)oxides bound P_i	16 h	1:30
F_o-3[b]	0.1 mol/L NaOH/ $(NH_2)_2S_2O_8$+H_2SO_4	Poorly crystalline Fe and Al (hydr)oxides bound P_o	16 h/ 0.5-1 h at 121°C	1:20
F_i-4	1 mol/L HCl	Insoluble Ca-P and apatite minerals	16 h	1:30
F_i-5[c]	0.5 mol/L NaOH	P_i strongly bound Fe and Al (hydr)oxides	16 h	1:30
F_o-5[b]	0.5 mol/L NaOH/ $(NH_2)_2S_2O_8$+H_2SO_4	P_o strongly bound Fe and Al (hydr)oxides	16 h/0.5-1 h at 121°C	1:20
F-6	(H_2SO_4)+30% (H_2O_2)	Residual fractions	0.5 h at 225°C 1 h at 360°C	1:20

SSR=solid-to-solution ratio; [a]Tiessen and Moir (1993); [b]Zhang and Kovar (2009); [c]Condron et al. (2005)

2.5 Arbuscular mycorrhiza fungi spore extraction and identification

Arbuscular mycorrhiza fungi (AMF) were extracted from 100 g of soil samples using modified wet sieving and the 50% sucrose centrifugation method (Daniels and Skipper, 1982). The retained spores after passing through a 45 µm sieve were collected using a micropipette under a microscope (Primo Star, ZEISS P95-T2,1,6X DSLR 415500-1825). Spore characterization was determined by mounting samples on glass slides in polyvinyl-lactoglycerol (PVLG). Spores were examined microscopically and identified to the species level whenever possible, based on morphological characters using descriptions provided from INVAM (International Culture Collection of (Vesicular) Arbuscular Mycorrhizal Fungi, Morgantown, WV, United States). Relative abundance (RA) was measured based on the following formula:

$$RA = \frac{\text{Spore numbers of a species (genus)}}{\text{Total number of identified spore sample}} \times 100$$

The equations for the Shannon diversity index (H) and Evenness (E) index were:

$$\text{Shannon diversity index (H)} = -\sum (n_i/N) \ln(n_i/N)$$

$$\text{Evenness index (E)} = H/\ln S$$

Where; n_i is the total number of spores of a species, N is the total number of identified spore samples, and S is the total number of identified species per sampling site (Zak et al., 1994).

2.6 Statistical analyses

Differences in the P fractions and in the soil properties between the different ages of rubber tree plantations and the natural forest were tested using analysis of variance and least significant difference (LSD) at $p=0.05$. The data showed that there was no statistical between each block, which indicated the area homogeneity. All statistical analyses were tested using the SAS statistical analysis software version 10.0.

Principal component analysis (PCA) was performed to identify associations of P fractions and soil properties with the relative abundance of AMF in the rubber tree and forest systems. Only variables with factor loadings lower than 0.70 were excluded from the PCA analysis to identify the importance of P fractions, soil properties and the AMF species (Krailertrattanachai et al., 2019).

3. RESULTS

3.1 Soil physical and chemical properties

Of the selected soil physical properties, the soil textures of FR, YR, MR, and OR were loamy sands and the bulk density increased with increasing stand age (Table 2). The lowest bulk density was in the YR site. The pH of the soils was strongly acidic to moderately acidic, with a significant difference among the sites. The concentrations of plant nutrients, (TP, Avail. P, and Avail. Mg) increased with increasing stand age from 5 years to 11 years to 22 years, respectively. The TP and Avail. P concentrations for the OR site were highest among the locations. The natural forest site had a higher levels of organic matter and Avail. Ca, Avail. K, and TN than the rubber tree plantation sites (Table 3).

Table 2. Physical properties of studied soils from natural forest (FR), young rubber plantation (YR), mature rubber plantation (MR), and old rubber plantation (OR)

Site	Sand (%)	Silt (%)	Clay (%)	Bulk density (g/cm³)
FR	77[c]±0.2	13[b]±0.2	10[a]±0.3	1.72[b]±0.04
YR	81[b]±0.8	10[c]±0.4	9[a]±0.4	1.66[c]±0.02
MR	77[c]±0.1	18[a]±0.3	5[c]±0.3	1.76[ab]±0.04
OR	84[a]±0.6	10[c]±0.8	7[b]±0.2	1.79[a]±0.01
p-value	*	*	*	*
CV (%)	0.42	3.23	4.22	1.70

Values (mean±SD) with different lowercase superscripts in each column are significantly ($p<0.05$) different.
* indicates statistical different based on LSD ($p<0.05$)

Table 3. Chemical properties of studied soils from natural forest (FR), young rubber plantation (YR), mature rubber plantation (MR) and old rubber plantation (OR)

| Site	pH	OM (g/kg)	TN (g/kg)	TP (mg/kg)	Avail. P (mg/kg)	Avail. K (mg/kg)	Avail. Mg (mg/kg)	Avail. Ca (mg/kg)
FR	5.0[b]	7.93[a]	0.35[a]	138.21[b]	2.96[c]	74.6[a]	35.6[a]	139.2[a]
	±0.1	±0.8	±0.1	±2.9	±0.7	±7.9	±9.4	±33.2
YR	5.3[ab]	3.82[b]	0.19[b]	122.41[c]	1.73[d]	38.5[c]	21.5[ab]	50.9[b]
	±0.1	±0.3	±0.0	±2.0	±0.2	±5.0	±2.3	±12.1
MR	5.4[ab]	3.30[b]	0.15[b]	134.83[b]	4.94[b]	59.0[b]	16.9[b]	82.6[b]
	±1.0	±0.6	±0.1	±1.0	±0.6	±3.7	±2.0	±15.6
OR	5.7[a]	3.87[b]	0.19[b]	144.73[a]	6.48[a]	52.9[bc]	32.5[a]	68.2[b]
	±0.2	±0.2	±0.0	±1.4	±0.7	±9.5	±10.8	±17.1
p-value	*	*	*	*	*	*	*	*
CV (%)	5.84	7.17	32.6	1.48	15.3	13.1	26.9	27.1

OM=organic matter; TN=Total nitrogen; TP=Total phosphorus; Avail. P=available phosphorus; Avail. K=available potassium; Avail. Mg=available magnesium; Avail. Ca=available calcium

Mean values (±SD) with different lowercase superscripts in each column are significantly different at $p<0.05$ (*).

3.2 Soil phosphorus fractions

The absolute and relative concentrations of P extracted in each sequential extraction step are presented in Figure 2 and Table S1. There were evident effects of rubber age on changes in both inorganic and organic P fractions. The labile P, moderately labile P, and non-labile P pools contributed 13%, 43%, and 44%, respectively, to total soil P for the natural forest, and 16-17%, 32-42%, and 42-52%, respectively for young rubber, mature rubber, and old rubber stands. Of the P fractions within the labile P pool, the water-extractable P_i fraction for the natural forest (1.5% of total soil P) was smaller than for the rubber plantations (1.8-2.4% of total soil P). The $NaHCO_3$-P_i fraction increased from 2.6% to 3.8% to 6.6% of total soil P for the respective rubber tree ages of 5 years, 11 years, and 22 years, respectively. However, the $NaHCO_3$-P_o fraction for the corresponding-aged rubber stands showed a decreasing trend from 12.4% to 10.7% to 6.6% of total soil P, respectively.
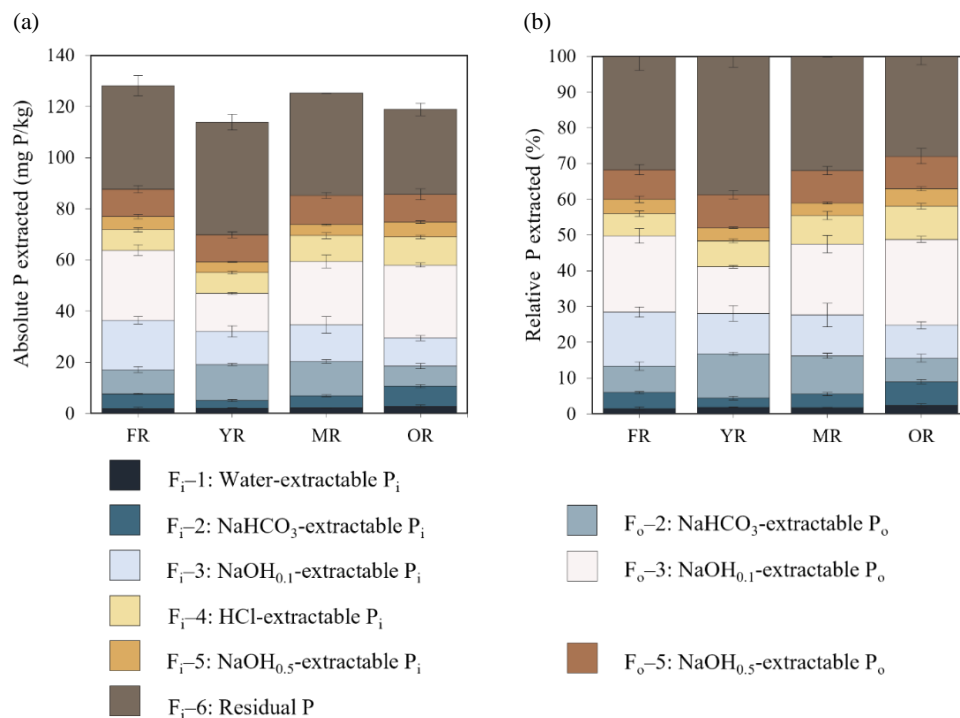
Figure 2. P fractionations in tropical sandy soils under natural forest (FR), young rubber (YR), mature rubber (MR), and old rubber (OR) plantations: (a) absolute P extracted; (b) relative P extracted, where data are means of triplicate with error bars denoting SD and statistical differences between fractions and sites are provided in Table S1.

For the moderately labile P pool, the $NaOH_{0.1}$-P_i fraction (9.2-11.4% of total soil P) was highest in the natural forest. The fractions of $NaOH_{0.1}$-P_o (13.1%, 19.8%, and 24.0% of total soil P) and of HCl-P_i (7.2%, 8.1%, and 9.3% of total soil P) for the stands aged 5 years, 11 years, and 22 years, respectively, increased with the rubber stand age. Both the $NaOH_{0.1}$-P_o and HCl-P_i pools in the natural forest were comparable to those in the rubber ecosystems (21.3% and 6.3% of total soil P, respectively) were comparable to those in the rubber ecosystems.

For the non-labile P pool, the $NaOH_{0.5}$-P_i fraction in the natural forest (4.0% and 3.6-4.8% of total soil P, respectively), was similar to those for the rubber plantations. The $NaOH_{0.5}$-P_o fraction in the natural forest and rubber ecosystems were also similar, corresponding to 8.3% and 9.1-9.3% of total soil P, respectively. However, the residual P pool in the young rubber, mature rubber, and old rubber stands declined with the plantation age to 38.7%, 31.9%, and 27.9% of total soil P, respectively, for ages 5 years, 11 years, and 22 years, while that in the natural forest (31.7%) was similar to the mature rubber.

The organic P pool calculated from the sum of the $NaHCO_3$-, $NaOH_{0.1}$-, and $NaOH_{0.5}$-P_o fractions varied between 35% and 40% of total soil P across the sites, suggesting that the inorganic P pools (60-65% of the total soil P) were the dominant P pool in both the natural forest and rubber plantations. There were no substantial differences between the inorganic and organic P observed for the forest and rubber tree plantations.

3.3 Soil microbial biomass phosphorus and abuscular mycorrhizal fungal spore distribution

The microbial biomass P (MBP) in the natural forest was similar to that for the young rubber and old. However, it was lower in mature rubber plantations (Figure 3(a)). Consequently, this parameter was affected by the rubber stand age.

The levels of AMF spores for the old rubber and natural forest sites ranged from 3.64 to 16.28 spores per g soil collected, respectively (Figure 3(b)). The highest level of spore abundance was recorded in the natural forest and the lowest was in the old rubber stand. In total, 10 species were detected based on morphological spore identification (Table 4), belonging to 2 genera of AMF, namely *Glomus* (64%) and *Acaulospora* (31%) that were the most abundant and were identified in all soils. Figure 4 shows the spores of some important AMF.
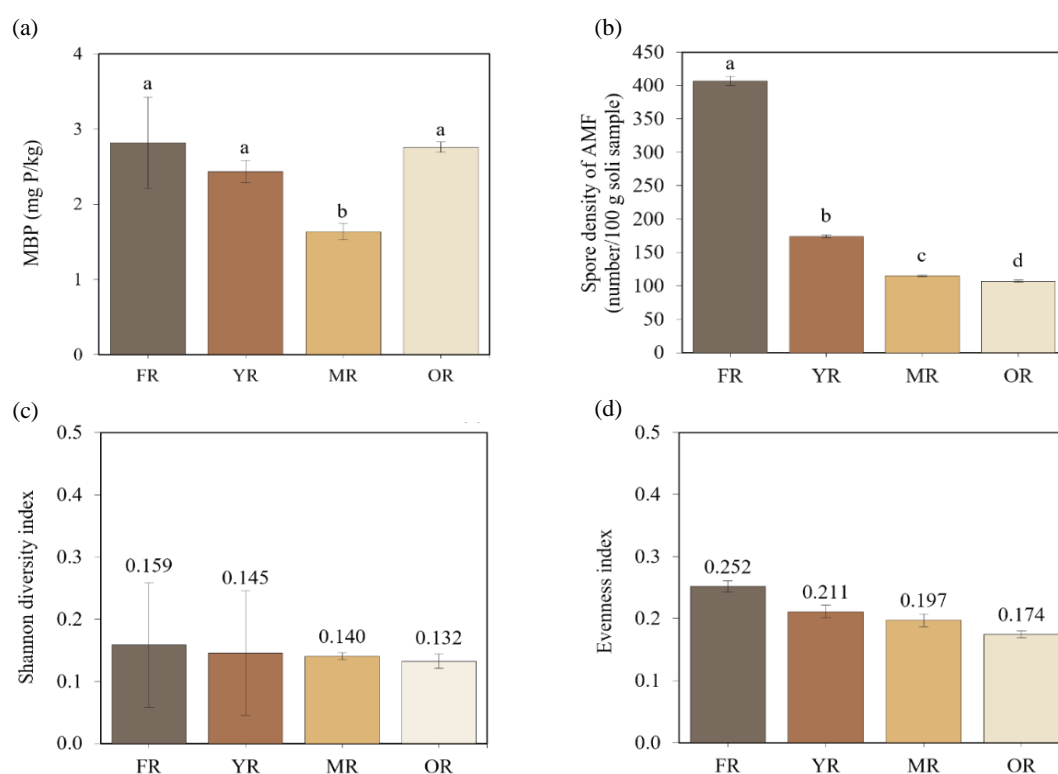

Figure 3. Microbial biomass P (a), AMF spore density (b), Shannon diversity index (c), and Evenness index (d) in natural forest (FR), young rubber (YR), mature rubber (MR), and old rubber (OR) plantations. The data were means of triplicate (±SD) and different lowercase letters indicate significant ($p<0.05$) difference.

Table 4. Relative abundance of arbuscular mycorrhizal fungi isolated from soil at study sites

Arbuscular mycorrhizal fungi (AMF)	Relative abundance (%)			
	FR	YR	MR	OR
Acaulospora scrobiculata	4.91	4.02	6.96	3.74
Ambispora appendiculata	7.62	2.30	0.87	0.93
Acaulospora laevis	12.78	11.49	6.96	9.35
Acaulospora mellea	3.19	5.17	5.22	4.67
Acaulospora sp.	5.65	5.75	13.91	10.28
Glomus badium	14.99	14.37	12.17	11.21
Rhizophagus fasciculatus	7.86	5.75	7.83	6.54
Claroideoglomus etunicatum	4.18	4.02	5.22	6.54
Rhizophagus manihotis	1.23	1.72	0.87	0.93
Funneliformis verruculosus	3.69	4.60	3.48	2.80
Rhizophagus intraradices	1.97	1.15	1.74	0.93
Glomus sp.	28.50	32.18	34.78	35.51
Unidentified species	3.44	7.47	0.00	6.54

FR=natural forest; YR=young rubber plantation; MR=mature rubber plantation; OR=old rubber plantation
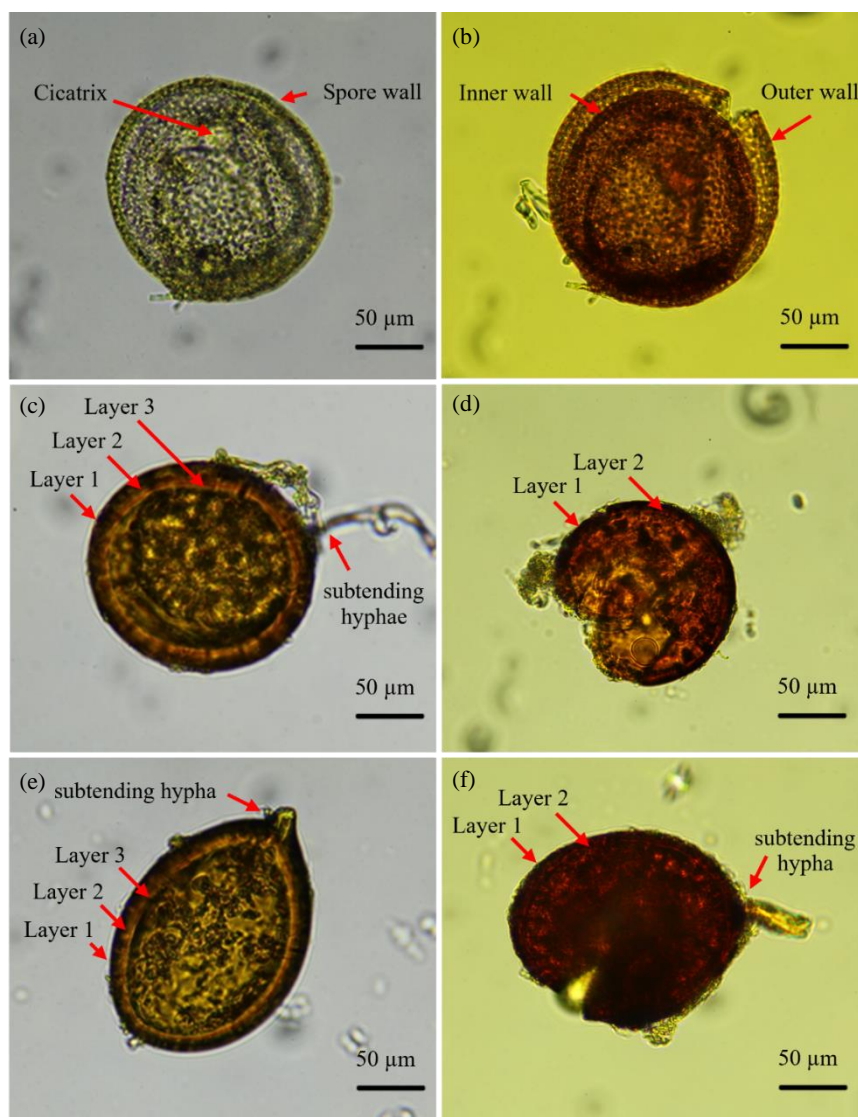
Figure 4. Some arbuscular mycorrhizal fungi spore morphotypes detected in topsoil in study pots for spore characterization in water (a, b, and e) and polyvinyl-lactoglycerol (b, c, and f) for: (a, b) *Acaulospora scrobiculata*; (c, d) *Glomus badium*; (e, f) *Rhizophagus fasciculatus*
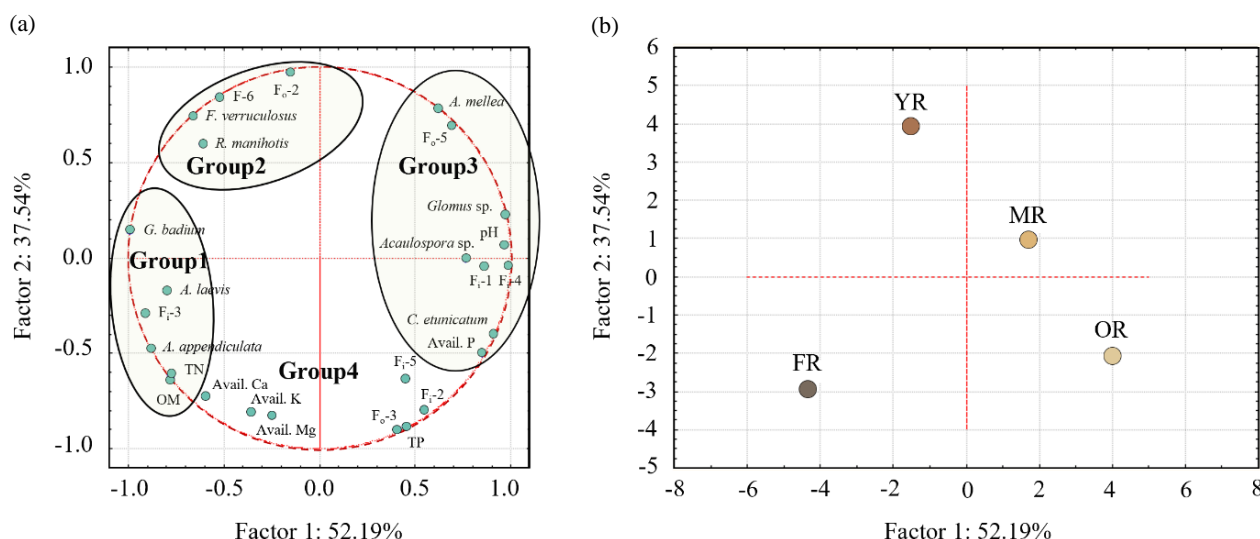
3.4 Arbuscular mycorrhizal fungal diversity

The AMF diversity indices differed significantly between natural forest and rubber tree age. Therefore, the Shannon-Wiener diversity index and Evenness index were highest in the natural forest, but they were similar in all rubber plantations (Figure 3(c) and 3(d)). The AMF communities were dominated by *Glomus badium* (14.99-11.21% of total) at all sites, followed by *Acaulospora Laevis* (12.78-9.35% of total), as shown in Table 4.

3.5 Relationships between phosphorus fractions and abuscular mycorrhizal fungal species

PCA of the soils revealed that land-use types (rubber trees with differing stand ages and natural forests) affected soil AMF species, P fractions, and relevant soil properties. The first two axes in the PCA analysis contributed 90% of the variation in soil attributes and microbial communities, indicating substantial diversity in the nature of the studied soils (Figure 5, Figure S1). Four groups were recognized based on the positive and negative values of factor loadings: OM group 1 (OM, TN, F_i-3, *A. scrobiculata*, *A. appendiculata*, and *G. badium*), F-6 group 2 (F-6, F_o-2, *R. manihotis*, and *F. verruculosus* and pH group 3 (pH, F_i-1, F_i-4, Avail. P, F_o-5, *C. etunicatum*, *A. mellea, Glomus* sp., and *Acaulospora* sp.) with some in an outlier group 4 (TP, F_i-2, F_i-5, F_o-3, Avail. K, Avail. Ca, and Avail. Mg). The soil properties and AMF species in group 1 and group 2 separated the natural forest from the young rubber, whereas those in group 3 divided the mature and old rubber tree soils. High Avail. K and Avail. Mg contents were present in soils of the natural forest and the old rubber tree stand ecosystems.

Figure 5. Principal component analysis of P fractions, soil properties, and arbuscular mycorrhizal fungal species in soils under natural forest (FR), young rubber (YR), mature rubber (MR), and old rubber (OR) plantations: (a) distribution of soil parameters (variables); (b) distribution of forest and rubber systems (cases), where only factor loadings with absolute values lower than 0.70 are included and Group abbreviations for P-fractions are provided in Table S1.

4. DISCUSSION

4.1 Changes in P fractions under natural forest and rubber ecosystems

Our data highlighted that rubber stand age had a profound impact on total P, the available P content, and the corresponding P fractionation (Table 1 and Figure 1). The P sequential extraction data also demonstrated the transformation of diverse P pools in the studied soils induced by rubber plantation management. The conversion from natural forest to young rubber plantations decreased the moderately labile P ($NaOH_{0.1}$-P_i and $NaOH_{0.1}$-P_o) and non-labile pools ($NaOH_{0.5}$-P_i) but increased the labile P pools (water-extractable P_i, and $NaHCO_3$-extractable P_o) and the soil pH. This information could reflect the transformation of the P pools associated with poorly crystalline Fe/Al (hydr)oxides ($NaOH_{0.1}$-P_i and P_o) and P associated with crystalline Fe/Al (hydr)oxides ($NaOH_{0.5}$-P_i) to the more labile P pool that is the most relevant for the readily available P fraction for plant utilization (Lustosa Filho et al., 2020). It was also possible that the elevation of the labile P pool could have been enhanced by inorganic P fertilizer freshly used as part of the management of the young rubber

plantation (Neufeldt et al., 2000), whereas the effects of inorganic P fertilizers on the labile P were less pronouced as the P fertilization were stoped after about 8 years of the rubber plantation. Several studies have documented consistent data indicating the enhancement of water-extractable P and $NaHCO_3$-P_i fractions in different rubber plantation stands (Henriquez, 2002; Maranguit et al., 2017), attributable to inorganic P fertilizer use and subsequent accumulation of newly adsorbed P on soil constituents that could remain readily available for plant use. The depletion of moderately labile P_o ($NaOH_{0.1}$-P_o) in the young rubber plantation may have been due to the higher rate of organic matter decomposition and reduction of P input from litterfall. Conversely, the incremental increase of labile P_o ($NaHCO_3$-P_o) in the young rubber plantation could not be attributed to a decrease in the moderately labile P_o. In addition, soil microorganisms are an important source of soil organic phosphorus; thus, the decrease in organic phosphorus may have been related to the decrease in microbial biomass phosphorus.

With the increasing age of the rubber plantation stands from 5 years to 22 years, some clear trends were observed in the P fractionation transformation: i) an increase in the total P, Bray-II-extractable P, water-P_i, $NaHCO_3$-P_i, $NaOH_{0.1}$-P_o, and HCl-P_i and ii) a decrease in the $NaHCO_3$-P_o and residual P. The change in soil labile P have been due to the fertilizer quantity and the higher canopy cover in the mature rubber and old rubber plantation, causing a lower degree of leaching by drainage and possibly decreasing basic cation loss and soil acidification (Haynes and Swift, 1986). Progressive P fertilization could build up both the total P content and labile P pools (water-P_i, $NaHCO_3$-P_i) in the studied soils. Moreover, the alleviation of soil acidification in the mature rubber and old rubber could decrease P adsorption onto Fe and Al (hydr)oxides (Arai and Sparks, 2001), resulting in a higher level of labile P pools. These data were consistent with studies in temperate and tropical soils showing that soil acidification caused a rapid transformation of the labile P pool into moderately labile, occluded and recalcitrant P through precipitation of Fe/Al (hydr)oxides with the labile P (Costa et al., 2016; Yang and Post, 2011). Furthermore, the decreases in the residual P and the $NaHCO_3$-P_o fractions with increasing water-P_i and $NaHCO_3$-P_i suggested that these P pools could act as both a source and sink of bioavailable P, which could be manageable by modifying chemical conditions.

4.2 Soil microbial biomass under natural forest and rubber ecosystems

The soil microbial biomass phosphorus was significantly different between natural forest and rubber plantation. The soil MBP significantly decreased in the mature rubber plantations compared to the young and old rubber plantations and natural forest (Figure 3(a)). MBP was highly dependent on the quantity and quality of organic matter and on the plant litter content returned to the soil. Further detailed investigation is required of the functional groups in the organic matter. However, as the rubber trees grew, the soil MBP was significantly increased in the old rubber stand to reach a similar level to that in the natural forest because more carbon and other nutrients released by rhizodeposition from the larger roots of rubber trees would stimulate microbial growth and activity in soils (Moreira et al., 2013).

4.3 Arbuscular mycorrhizal fungal spore distribution under natural forest and rubber ecosystems

PCA showed that the conversion of natural forests to rubber plantations contributed to variations in the AMF diversity and the concentrations of the soil P fractions (Figure 5). The rubber tree plantation chronosequence affected the concentrations of available P and the inorganic and organic P. The AMF diversity was correlated with soil P forms (labile P, moderately labile P, and residue P) involved in P status in the rubber ecosystem. Furthermore, the increased concentrations of available P in the rubber tree plantation chronosequence were related to soil pH, *C. etunicatum, A. mellea, Glomus* sp., and *Acaulospora* sp. in the old rubber plantation. The results indicated that AMF diversity was likely to be altered by the rubber trees at different ages. In addition, the distribution of AMF species could have been driven by soil P in the natural forests and rubber plantations ecosystems. Rubber ecosystems declined in term of *G. badium* and *A. appendiculata* and were enhanced in *C. etunicatum* compared to the natural forest, which could have been due to the AMF could thriving under the native forest conditions compared to in the tree plantations. Overall, the old rubber stand had a negatively impact on AMF diversity the most. In addition, AMF revealed the colonization, communities and diversity of AMF associated with tree species having different functions to hosts and AMF species being slightly sensitive to fertilization are important influences on the distribution, diversity

and regeneration of plant communities (van der Heijden et al., 1998; Bhadalung et al., 2005; Wang et al., 2019).

5. CONCLUSION

Our study showed that rubber plantation increased total P, available P, and available magnesium in tropical sandy soils. Furthermore, these soil properties were more substantially affected with increasing stand age. The P fractions data demonstrated that labile pools, P_o adsorbed to Fe/Al (hydr)oxides and P_i associated with Ca compounds were enhanced with increasing stand age, suggesting the possible transformation of these fractions from the residual P, P_i adsorbed to Fe/Al (hydr)oxides, and exchangeable P_o. The AMF diversity tended to decrease in rubber plantations compared to the natural forest. The diverse P fractions were variously associated with different AMF species. Based on the current study, natural forest conversion to rubber plantation could improve soil P availability but it could deteriorate AMF diversity. The data suggested that ecological-based management should also be undertaken to improve both P availability and microbial diversity for the sustainability of rubber plantation in tropical sandy soil environments.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

REFERENCES

Arai Y, Sparks DL. ATR-FTIR Spectroscopic investigation on phosphate adsorption mechanisms at the ferrihydrite-water interface. Journal of Colloid and Interface Science 2001; 241:317-26.

Bhadalung NN, Suwanarit A, Dell B, Nopamornbodi O, Thamchaipenet A, Rungchuang J. Effects of long-term NP-fertilization on abundance and diversity of arbuscular mycorrhizal fungi under a maize cropping system. Plant and Soil 2005;270(1):371-82.

Blake GR, Hartge KH. Bulk density. In: Klute A, editor. Methods of Soil Analysis. Madison, WI, USA: American Society of Agronomy; 1986. p. 363-82.

Bray RH, Kurtz LT. Determination of total, organic, and available forms of phosphorus in soils. Soil Science 1945;59:39-46.

Bremner JM. Total nitrogen. In: Black CA, editor. Methods of Soil Analysis. Madison, WI, USA: American Society of Agronomy; 1965. p. 1049-178.

Brookes PC, Powlson DS, Jenkinson DS. Measurement of microbial biomass phosphorus in soil. Soil Biology and Biochemistry 1982;14:319-29.

Cardoso I, Boddington C, Janssen B, Oenema O, Kuyper T. Differential access to phosphorus pools of an oxisol by mycorrhizal and nonmycorrhizal maize. Communications in Soil Science and Plant Analysis 2006;37:1537-51.

Cervantes-Gámez R, Bueno-Ibarra MA, Cruz-Mendívil A, Calderón-Vázquez CL, Ramírez-Douriet CM, Maldonado-Mendoza IE, et al. Arbuscular mycorrhizal symbiosis-induced expression changes in *Solanum lycopersicum* leaves revealed by RNA-seq analysis. Plant Molecular Biology Reporter 2016;34:89-102.

Chambon B, Ruf F, Kongmanee C, Angthong S. Can the cocoa cycle model explain the continuous growth of the rubber (*Hevea brasiliensis*) sector for more than a century in Thailand? Journal of Rural Studies 2016;44,187-97.

Chapman HD. Cation-exchange capacity. In: Norman AG, editor. Methods of Soil Analysis. Madison, WI, USA: American Society of Agronomy; 1965. p. 891-901.

Chen CR, Condron LM, Xu ZH. Impacts of grassland afforestation with coniferous trees on soil phosphorus dynamics and associated microbial processes: A review. Forest Ecology and Management 2008;255:396-409.

Chen CR, Hou EQ, Condron LM, Bacon G, Esfandbod M, Olley J, et al. Soil phosphorus fractionation and nutrient dynamics along the Cooloola coastal dune chronosequence, Southern Queensland Australia. Geoderma 2015;(257-258):4-13.

Cherubin MR, Franco ALC, Cerri CEP, Karlen DL, Pavinato PS, Rodrigues M, et al. Phosphorus pools responses to land-use change for sugarcane expansion in weathered Brazilian soils. Geoderma 2016;265:27-38.

Condron LM, Turner BL, Cade-Menun BJ. Chemistry and dynamics of soil organic phosphorus. In: Sims JT, Sharpley AN, editors. Phosphorus: Agriculture and the Environment. Kimberly, ID, USA: American Society of Agronomy; 2005. p. 87-121.

Costa MG, Gama-Rodrigues AC, Gonçalves JL, de M Gama-Rodrigues EF, Sales MV, et al. Labile and non-labile fractions of phosphorus and its transformations in soil under eucalyptus plantations Brazil. Forests 2016;7:1-15.

Crews TE, Brookes PC. Changes in soil phosphorus forms through time in perennial versus annual agroecosystems. Agriculture, Ecosystems and Environment 2014;184:168-81.

Daniels BA, Skipper HA. Methods for the recovery and quantitative estimation of propagules from soil. In: Schenck, NC, editor. Methods and Principles of Mycorrhizal Research. St. Paul, MN, USA: American Phytopathological Society; 1982; p. 29-35.

Food and Agriculture Organization (FAO). FAOSTAT [Internet]. 2019 [cited 2020 Aug 1]. Available from: http:// www.fao.org /go/to/home/E.

Feldmann F, da Silva Jr JP, Idczak E, Lieberei R. AMF spore community composition at natural and agricultural sites in Central Amazonia-a long term study. Proceedings of German-Brazilian Workshop on Neotropical Ecosystems-Achievements and Prospects of Cooperative Research; 2000 Sep 3-8; Hamburg: Germany; 2000.

Fox J, Castella JC, Ziegler AD, Westley SB. Rubber plantations expand in mountainous Southeast Asia: What are the consequences for the environment? Asia Pacific Issues 2014; 114:1-8.

Gee GW, Bauder JW. Particle-size analysis. In: Klute A, editor. Methods of Soil Analysis. Madison, WI, USA: American Society of Agronomy; 1986. p. 383-411.

Haynes RJ, Swift RS. Effects of soil acidification and subsequent leaching on levels of extractable nutrients in a soil. Plant Soil 1986;95:327-36.

Hedley MJ, Stewart JWB, Chauhan BS. Changes in inorganic and organic soil phosphorus fractions induced by cultivation practices and by laboratory incubations. Soil Science Society of America Journal 1982;46:970-6.

Henriquez C. Assessing Soil Phosphorus Status Under Different Agronomic Land Use [dissertation]. Iowa State University; 2002.

Holford ICR. Soil phosphorus: Its measurement, and its uptake by plants. Australian Journal of Soil Research 1997;35:227-39.

Hu B, Yang B, Pang X, Bao W, Tian G. Responses of soil phosphorus fractions to gap size in a reforested spruce forest. Geoderma 2016;279:61-9.

International Rubber Study Group (IRSG). Rubber statistical bulletin, database [Internet]. 2021 [cited 2021 Aug 1]. Available from: https://www.rubberstudy.org/welcome.

Krailertrattanachai N, Ketrot D, Wisawapipat W. The distribution of trace metals in roadside agricultural soils Thailand. International Journal of Environmental Research and Public Health 2019;16(5):Article No. 714.

Kuo S. Phosphorus. In: Sparks DL, Page AL, Helmke PA, Loeppert RH, Soltanpour PN, Tabatabai MA, Johnston CT, Sumner ME, editors. Methods of Soil Analysis. Madison, Wisconsin, USA: Soil Science Society of America; 1996. p. 869-919.

Li Y, Lan G, Xia Y. Rubber trees demonstrate a clear retranslocation under seasonal drought and cold stresses. Frontiers in Plant Science 2016;7:Article No. 1907.

Liu C, Jin Y, Liu C, Tang J, Wang Q, Xu M. Phosphorous fractions in soils of rubber-based agroforestry systems: Influence of season, management and stand age. Science of the Total Environment 2018;(616-617):1576-88.

Lustosa Filho JF, de Silva Carneiro JS, Barbosa CF, de Lima KP, do Amaral Leite A, Melo LCA. Aging of biochar-based fertilizers in soil: Effects on phosphorus pools and availability to *Urochloa brizantha* grass. Science of the Total Environment 2020;709:Article No. 136028.

Maranguit D, Guillaume T, Kuzyakov Y. Land-use change affects phosphorus fractions in highly weathered tropical soils. Catena 2017;149:385-93.

Marlairotsiri K, Suchinai A, Hoontakul K. Characterization of Established Soil Series in the Northeast Region of Thailand Reclassified According to Soil Taxonomy 2003. Bangkok, Thailand: Land Development Department; 2004. p. 6-7.

Moreira A, Moraes LAC, Zaninetti RA, Canizella BT. Phosphorus dynamics in the conversion of a secondary forest into a rubber tree plantation in the amazon rainforest. Soil Science 2013; 178:618-25.

Nash DA, Friedman JW, Mathu-Muju KR, Robinson PG, Satur J, Moffat S, et al. A review of the global literature on dental therapists. Community Dentistry and Oral Epidemiology 2014;42:1-10.

Negassa W, Leinweber P. How does the Hedley sequential phosphorus fractionation reflect impacts of land use and management on soil phosphorus: A review. Journal of Plant Nutrition and Soil Science 2009;172:305-25.

Neufeldt H, da Silva JE, Ayarza MA, Zech W. Land-use effects on phosphorus fractions in Cerrado oxisols. Biology and Fertility of Soils 2000;31:30-7.

Rausch C, Bucher M. Molecular mechanisms of phosphate transport in plants. Planta 2002;216:23-37.

Schweiger R, Müller C. Leaf metabolome in arbuscular mycorrhizal symbiosis. Current Opinion in Plant Biology 2015;26:120-6.

Steidinger BS, Turner BL, Corrales A, Dalling JW. Variability in potential to exploit different soil organic phosphorus compounds among tropical montane tree species. Functional Ecology 2015;29:121-30.

Stutter MI, Shand CA, George TS, Blackwell MSA, Dixon L, Bol R, et al. Land use and soil factors affecting accumulation of phosphorus species in temperate soils. Geoderma 2015;(257-258):29-39.

Thai Meteorological Department. Climate data [Internet]. 2018 [cited 2021 Dec 7]. Available from: https://www.tmd.go.th/programs/uploads/tempstat/max_stat_latest_en.pdf.

Tiessen H, Moir JO. Characterization of available P by sequential extraction. In: Carter MR, editor. Soil Sampling and Methods of Analysis. Canadian Society of Soil Science; 1993. p. 75-86.

van der Heijden MGA, Klironomos JN, Ursic M, Moutoglis P, Strietwolf Engel R, Boller T, et al. Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. Nature 1998;74:69-72.

Wagg C, Bender SF, Widmer F, van der Heijden MGA. Soil biodiversity and soil community composition determine ecosystem multifunctionality. Proceedings of the National Academy of Sciences of the United States of America 2014;111:5266-70.

Walkley A, Black IA. An examination of the Degtjareff method for determining soil organic matter, and a proposed modification of the chromic acid titration method. Soil Science 1934;37:29-38.

Wang J, Wang GG, Zhang B, Yuan Z, Fu Z, Yuan Y, et al. Arbuscular mycorrhizal fungi associated with tree species in a planted forest of Eastern China. Forests 2019;10:1-14.

World Rubber Summit (WRS). Facing the future: Inclusiveness, sustainability and growth for the next normal [Internet]. 2021 [cited 2021 Aug 1]. Available from: https://www.wrs 2021. rubberstudy.org/.

Yang X, Post WM. Phosphorus transformations as a function of pedogenesis: A synthesis of soil phosphorus data using Hedley fractionation method. Biogeosciences 2011;8:2907-16.

Zak JC, Willig MR, Moorhead DL, Wildman HG. Functional diversity of microbial communities: A quantitative approach. Soil Biology and Biochemistry 1994;26:1101-8.

Zhang H, Kovar JL. Fractionation of soil phosphorus. In: Kovar JL, Pierzynski GM, editors. Methods for Phosphorus Analysis for Soils, Sediments, Residuals, and Waters. Southern Cooperative Series Bulletin; 2009. p. 50-60.