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Diversity of Soil Fungi in Different Land Use Types in Tha Kum-Huai Raeng Forest Reserve, Trat Province

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ABSTRACT

The soils collected from five land use types located in the Tha Kum-Huai Raeng Forest Reserve, Trat province were studied with two objectives: 1) to assess and compare the composition, abundance and diversity of soil fungi; and 2) to relate the fungal diversity with leaf litter dry weight and soil physical and chemical properties. The five land use types were: secondary dry evergreen forest (DEF), Phayoong (*Dalbergia cochinchinensis*) plantation (PP), grassland (GL), rubber (*Hevea brasiliensis*) plantation (RP) and a pineapple field (PF). The soil collection was carried out in the rainy season by establishing a sample plot of 100×100 m in each land use type and nine soil samples were taken from 0-10 cm soil depth. Before a soil sample was taken, the leaf litter covering the soil in a plot 1×1m was scraped and put into a plastic bag. The litter samples were dried and measured for dry weight. Each soil sample was analyzed for soil physical and chemical properties and isolated for soil fungi using the soil dilution plate method. The emerging fungal colonies were counted for numbers and identified to the species level based on morphological characteristics.

A total of 28 genera and 71 species of soil fungi were identified from the DEF, PP, GL, RP and PF soils. They belonged to the Class Zygomycetes (4 genera and 4 species), the Class Ascomycetes (4 genera and 5 species) and the Class Deuteromycetes (20 genera and 62 species) and most of them were organic matter decomposers. Some soil fungi appeared in all land use types, but some appeared only in a few land use types or in as little as one. The land use type which had the highest Shannon-Wiener's diversity index of soil fungi was PF, followed by DEF, PP, GL and RP, respectively. The highest similarity of fungal community composition between two land use types calculated by Sorensen's index of similarity occurred between DEF and PP, followed by PP and GL, and then DEF and GL, while the lowest similarity index value was between PF and RP. The analysis of variance of mean values of soil environmental factors, including leaf litter dry weight and soil chemical and physical properties, among the five land use types revealed that the soils were significantly different from each other. The relationships of soil fungi with all the mentioned soil environmental factors analyzed by the ordination method with canonical correspondence analysis (CCA) showed that some soil fungi related positively with the P, Ca and Mg contents in soils**.** It is recommended that the five land use types should be properly managed, so that there are no abrupt changes in their soil environmental conditions, in order to keep the existing diversity of soil fungi.

Keywords: diversity, soil fungi, land use type, relationship, environmental factors

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INTRODUCTION

Soil fungi play an important role as major decomposers in the soil ecosystem. They also provide mankind with very useful pharmaceutical products, such as antibiotics and other valuable substances, including organic acids, enzymes, pigments and secondary metabolites used in the food industry and fermentation. In addition, many soil fungi are biological control agents for plant pathogens and insect pests. On the other hand, some of them are very harmful causing food spoilage and diseases to plants, animals and humans with significant economic losses and produce mycotoxins in certain products (Manoch, 1998).

There are about 75,000 species of soil fungi in the world (Finlay, 2007) but in Thailand, soil fungi, identified up until 1998, numbered only 89 genera and 95 species (Manoch, 2004). Many studies of soil fungi in Thailand in the past emphasized species diversity in soil samples collected from various agricultural areas and forest types (Manoch, 1993, 1998; Kosol, 1999; Manoch *et al*., 2000; Dethoup *et al*., 2007). However, relatively few studies have tried to compare quantitatively the fungal diversity among different habitats. There have been also very few studies reported on the relationships of soil fungal diversity with environmental factors. Wongseenin (1971) reported that the soil fungal population and diversity were higher in the dry evergreen forest than in the dry dipterocarp forest on the Sakaerat Environmental Research Station, Nakhon Ratchasima province. These higher numbers corresponded with the higher moisture content, organic matter content, mineral levels and acidity of the soil in the former forest type than in the latter one. Wongvutiyan (1993) compared the number of microorganisms (fungi and bacteria) in undisturbed natural forest and disturbed natural forest following selection cutting in Kanchanaburi province and found that two years after the cutting, there was no difference in the numbers of microorganisms between the two sites, because there had not been enough time for disturbance to change the environment of the microorganisms. However, three years after the cutting, the number of microorganisms in the disturbed site showed a decreasing trend, as the soil there had a higher pH and lower organic matter and mineral P than the undisturbed natural forest soil. Since forest soil microbial biomass is dominated by fungi (Houston *et al*., 1998), studying fungal diversity in relation to soil properties may provide useful information on soil fungal diversity management of the areas.

The present study was undertaken to: 1) assess and compare the composition, abundance and diversity of soil fungi in five land use types in the Tha Kum-Huai Raeng Forest Reserve, Trat province; and 2) relate the fungal diversity with leaf litter dry weight and soil physical and chemical properties. Results from the study would identify which fungal species occurred in which land uses and at what levels, as well as indicating similarities or differences in the fungal species associated with each land use and which environmental factors influenced fungal diversity.

MATERIALS AND METHODS

Site descriptions

The Tha Kum-Huai Raeng Forest Reserve is located in Trat province, eastern Thailand and is about 400 km from Bangkok. The total area of the Forest Reserve is 123 km^2 and covers the Khao Saming sub-district, Khao Saming district, the Dan Chumphon sub-district, Bau Rai district and the Huai Raeng and Tha Kum sub-districts, Muang district (12.28-12.44° N and 102.49-102.72 °E). Commencing in 1961, the Forest Reserve was under logging concessions and suffered from illegal encroachment by nearby villagers and concessionaire workers. Though all the logging concessions in Thailand were banned by the government in 1989, the illegal encroachments have not stopped. Therefore, the natural forests have changed into agricultural land,

tree plantations and grasslands. These land use changes have had direct and indirect impacts on the soil physical and chemical properties and more importantly, on soil microorganisms (Grishkan *et al*., 2005).

The five land use types located in the Forest Reserve and selected for this study were: a secondary dry evergreen forest (DEF), a 25- year-old Phayoong (*Dalbergia cochinchinensis*) plantation (PP), a grassland (GL) dominated with *Arundo donax* grass 3-5 m in height, a 14-year-old rubber (*Hevea brasiliensis*) plantation (RP), and a pineapple field (PF) that had been planted for the last three years consecutively with pineapples (Figure 1).

Soil and leaf litter samples

Soil samples were collected in August 2008, during the rainy season, since Kosol (1999) reported that the maximum species and numbers of soil fungi were found in soil collected in the rainy season. In each of the selected land use types, a sample plot of 100×100 m was established and nine soil samples (four from the corners, four from the middle of the four sides of the rectangular plot and one from the center of the plot), each totaling approximately 500 g were taken from 0-10 cm soil depth and put into individual plastic bags. Before a soil sample was taken, the leaf litter covering the soil (1×1 m) was scraped and put into a plastic bag. Therefore, there were, altogether, 45 soil samples and 45 leaf litter bags collected from the five land use types.

Each leaf litter sample was dried at 80°C for 24 h and measured for dry weight. Each soil sample was divided into two parts; the first part was sent to be analyzed for soil physical properties (sand, silt and clay percentages) and chemical properties (pH, organic matter percentage and phosphorus, potassium, calcium, and magnesium contents) at the Forest Soil Laboratory, Department of Silviculture, Faculty of Forestry, Kasetsart University. The second part of the soil sample was isolated to obtain pure soil fungal cultures at the Forest Pathology Laboratory, Department of Forest Biology, Faculty of Forestry, Kasetsart University.

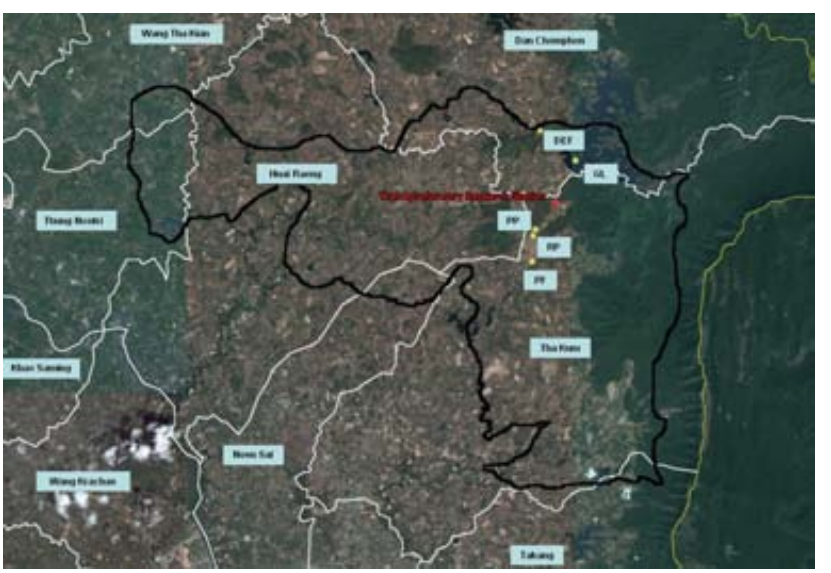

Figure 1 Location of the five land use types: secondary dry evergreen forest (DEF), Phayoong plantation (PP), grassland (GL), rubber plantation (RP) and pineapple field (PF) in the Tha Kum-Huai Raeng Forest Reserve.

Isolation and identification of soil fungi

The soil dilution plate method (Manoch, 1998) was used to isolate fungi from the soil samples. Each soil sample was diluted to 1×10^{-4} concentration suspension. Then, 1 mL of the soil suspension (containing 0.0001 g wet weight soil) was drawn by pipette into a Petri dish 90 mm in diameter. A mixture of 25 mL of warm, melted glucose-ammonium nitrate agar (GAN) added with Rose Bengal and streptomycin was poured over the soil suspension and the Petri dish was rotated gently to let the soil suspension mix well with the agar medium. Five replications were completed for each soil sample (0.0005 g wet weight soil/sample). Since nine soil samples/land use type were collected, there were 45 Petri dishes/ land use type (0.0045 g wet weight soil/land use type). All the Petri dishes were incubated at room temperature (26-28°C) in darkness for 3-5 d or longer. After incubation, the emerging fungal colonies were examined. Hyphal tips of the different colonies were transferred to potato dextrose agar (PDA) slants using a transfer needle. The tube slants were incubated in indirect light at room temperature for 2 w, then grouped into presumed entities on the basis of the morphological characters of the colonies. The total number of colonies of each entity was recorded. Identification of fungal species was based on morphological characteristics in plate cultures on suitable media and observation under compound and dissecting microscopes. The basic identification keys used were: Raper and Thom (1949); Raper and Fennell (1965); Berron (1968); Ellis (1971, 1976); Domsh *et al*. (1993) and Barnett and Hunter (1998).

For future reference, pure cultures of all the fungi in this study have been maintained in the Mycology Laboratory, Department of Plant Pathology, Faculty of Agriculture, Kasetsart University.

Data analysis

1. The number of colonies of a soil fungus/0.0045 g wet weight soil/land use type was converted to the number of colony forming units (CFU)/g wet weight soil/land use type or the abundance of a soil fungus. By summing up all the individual abundance records of a soil fungus in a land use type, the total abundance or total CFU/g wet weight soil for a land use type was obtained.

2. The Shannon-Wiener's index (H′) was chosen to measure the fungal species diversity in a land use (Magurran, 1988), using Equation 1:

$$H' = -\sum_{i=1}^{n} p_i \ln p_i \quad (1)$$

Where: p_i is the proportion of number of colonies of the i th species to the total number of colonies when $i = 1, 2, 3, \ldots, n$

3. The Sorensen's index of similarity (IS) was used to compare fungal community composition between two land uses (Krebs, 1989), using Equation 2:

$$IS = [2C/(A+B)]\times100\% \quad (2)$$

Where: A is the total abundance of soil fungi in the first land use
B is the total abundance of soil fungi in the second land use
C is the total (summation) of the minimum abundances of the soil fungi found both in land use

4. Soil environmental factors, measured as leaf litter dry weight and soil physical properties (percentages of sand, silt and clay) and soil chemical properties (pH, percentage of organic matter, and the contents (mg/kg soil) of phosphorus, potassium, calcium and magnesium) in the five land use types, were analyzed statistically to identify any differences among the means by analysis of variance (ANOVA). Duncan's new multiple range test was performed to compared means where necessary.

5. The relationships of soil fungi with

leaf litter dry weight and the soil physical and chemical properties mentioned were performed using the ordination method with canonical correspondence analysis (CCA).

RESULTS

Soil fungal diversity

A total of 3,910 colonies of soil fungi were isolated from the five land use types. They were identified to 28 genera and 71 species, which belonged to the Class Zygomycetes (4 genera and 4 species), the Class Ascomycetes (4 genera and 5 species) and the Class Deuteromycetes (20 genera and 62 species). The numbers of fungal species identified from the soils in DEF, PP, GL, RP and PF were 33, 29, 38, 27 and 36, respectively. Table 1 lists the fungal classes, species and abundance levels of soil fungi (CFU/g wet weight soil) identified from each land use type**.** There was not only variation in the total number of soil fungi, but also in the variation of soil fungal species and abundance from one land use type to another. Some soil fungi appeared in all land use types (*Cuninghamella elegans*, *Gongronella butleri*, *Penicillium* sp.3, *Penicillium* sp.6, *Trichoderma hamatum*, *T. harzianum, T. oblongisporum* and unidentified sp. 1), but some appeared only in a few land use types (for example, *Mucor* sp., *Eupenicillium* sp., *Talaromyces* sp., *Aspergillus candidus*, *A. cervinus*, *A. japonicas*, *A. niveus*, *Cephalosporium* sp., *Chaetomella* sp.) or in as little as one (for example, *Mortiella pavisporum*, *Nectria* sp., *Aspergillus niger*, *Aspergillus* sp., the *Aspergillus wentii* group, *Geotrichum* sp., *Gliocladium roseum*, *Heterocephalum* sp., *Leptographium* sp., *Penicillium oxalicum, P. striatrisporum* and *Penicillium* sp.16).

The appearance of soil fungi in all or some land use types was used to categorize the soil fungi in Table 1 into species that had a distribution that was: very broad, broad, moderate, narrow or very narrow, when the fungi appeared in five, four, three, two and one land use type(s), respectively. In addition to this category, the soil fungi with abundance values less than or equal to 500 CFU/g wet weight soil were considered as rare species, while those having more than 500 CFU/g wet weight soil were considered as common species. For example, *Cuninghamella elegans* was a rare species in DEF, PP and PF and a common species in GL and RP (modified from Keller and Bidochka, 1998).

Diversity index and similarity index

From Table 2, the land use that had the highest diversity of soil fungi based on the Shannon-Wiener's diversity index was PF (2.9), followed by DEF (2.19), PP (2.12), GL (1.65) and RP (1.56).

The similarities in fungal community composition between two land uses calculated by Sorensen's index of similarity are shown in Table 3. The results revealed that the greatest similarity in soil fungi occurred between DEF and PP (71.73%), followed by PP and GL (61.75%) and DEF and GL (55.72%). The similarities of soil fungi between these three pairs of land uses were considered high (> 50%). The pairs between which the similarity in soil fungi was considered medium were RP and DEF (44.93%), RP and PP (45.32%) and RP and GL (41.67%). There was a low similarity in soil fungi between PF and DEF (21.19%), PF and PP (27.54%), PF and GL (25.02%) and PF and RP (19.44%).

Statistical analyses of leaf litter dry weight and soil properties among land uses

The analysis of variance of the mean values of the leaf litter dry weight and the soil properties among the five land use types are shown in Table 4. Only the means of two soil physical properties (% sand and % silt) were not significantly ($P > 0.05$) different. The means of leaf litter dry weight, a soil physical property (% clay), and soil chemical properties including, pH,

Table 1 Classes and species and abundance of soil fungi identified from: secondary dry evergreen forest (DEF); Phayoong plantation (PP); grassland (GL); rubber plantation (RP); and pineapple field (PF).

No.	Fungal species	Abundance (CFU/g wet weight soil × 10^3)				
		DEF	PP	GL	RP	PF
	Class Zygomycetes					
s1	*Cuninghamella elegans******	0.4	0.2	1.3	0.9	0.2
s2	*Gongronella butleri******	78.7	80.9	113	57.1	12.7
s3	*Mortiella pavisporum**	0.2				
s4	*Mucor* sp.***		0.2		98	0.4
	Class Ascomycetes					
s5	*Eupenicillium* sp.***		0.7	1.1		0.4
s6	*Nectria* sp.*	0.2				
s7	*Talaromyces* sp.***			1.6	0.2	4.2
s8	*Thielavia terricola**					1.1
s9	Unidentified ascomycetes *			0.4		
	Class Deuteromycetes					
s10	*Aspergillus candidus***		2.2	0.4		
s11	*Aspergillus cervinus****		2.2	0.4		0.2
s12	*Aspergillus japonicus****	0.4		0.2		0.2
s13	*Aspergillus niger**				0.2	
s14	*Aspergillus niveus***				0.2	1.6
s15	*Aspergillus paradoxus***			0.2		0.7
s16	*Aspergillus* sp.*	0.2				
s17	*Aspergillus wentii* group*	0.2				
s18	*Aspergillus zonatus**	0.7				
s19	*Cephalosporium* sp.**	0.9		0.4		
s20	*Chaetomella* sp.**			0.7		1.1
s21	*Chloridium* sp.**	2.7		0.4		
s22	*Fusarium oxysporum****			0.2	0.2	1.3
s23	*Geotrichum* sp.*				0.4	
s24	*Gliocladium roseum**	0.2				
s25	*Heterocephalum* sp.*					0.2
s26	*Humicola fuscoatra*****	2.2	1.3		1.6	2.4
s27	*Leptographium* sp.*				0.2	
s28	*Mariannaea elegans****		2.2	0.9	0.4	
s29	*Monocillium* sp.**		1.8			0.4
s30	*Penicillium adametzi****		0.2	0.4	0.7	
s31	*Penicillium ochro-chloron*****	3.8	14.2	0.7		1.1
s32	*Penicillium oxalicum**			0.2		
s33	*Penicillium rubrum****	0.2	0.7	2.7		
s34	*Penicillium rugulosum****	2.9	0.7			0.2
s35	*Penicillium simplicissimum***	2			0.7	

Table 1 (continued).

No.	Fungal species	Abundance (CFU/g wet weight soil × 10^3)				
		DEF	PP	GL	RP	PF
s36	*Penicillium striatisporum**	0.4				
s37	*Penicillium verruculosum****	13.6	2.7	0.2		0.4
s38	*Penicillium* sp.1*		0.9			
s39	*Penicillium* sp.2***	2.2		0.2		0.2
s40	*Penicillium* sp.3*****	1.8	2	1.1	4.7	0.7
s41	*Penicillium* sp.4*					2.2
s42	*Penicillium* sp.5*					0.4
s43	*Penicillium* sp.6*****	18.9	13.8	0.2	0.2	2.2
s44	*Penicillium* sp.7***	0.2	2.4	0.4		
s45	*Penicillium* sp.8**		0.4	0.4	0.9	7.1
s46	*Penicillium* sp.9*		1.3			
s47	*Penicillium* sp.10*	4.2				
s48	*Penicillium* sp.11***			2.7	0.4	7.1
s49	*Penicillium* sp.12**			3.3		0.2
s50	*Penicillium* sp.13*					0.4
s51	*Penicillium* sp.14*	0.7				
s52	*Penicillium* sp.15*		0.4			
s53	*Penicillium* sp.16*	0.2				
s54	*Penicillium* sp.17*			0.9		
s55	*Penicillium* sp.18*		0.7			
s56	*Penicillium* sp.19**		4			11.3
s57	*Pestalotiopsis* sp.**	0.2	0.4			
s58	*Phialophora* sp.**				0.7	2.2
s59	*Phoma* sp.**			1.1	0.2	
s60	*Scopulariopsis brumptii**	0.9				
s61	*Scytalidium* sp.***	0.4		0.7		1.1
s62	*Trichoderma artroviride****		5.6	6.4	0.9	2.4
s63	*Trichoderma hamatum*****	22.4	3.8	0.2	2	0.2
s64	*Trichoderma harzianum*****	22	20.7	13.1	43.8	1.1
s65	*Trichoderma oblongisporum*****	10.9	26.9	4.7	7.1	8
s66	*Trichoderma virens****	1.6			0.4	0.4
s67	*Verticillium lecanii***			2.4	0.2	
s68	unidentified hyphomyces*			2.4		
s69	unidentified sp.1*****	2.2	1.8	0.7	1.8	7.6
s70	unidentified sp.2**			0.2	0.2	
s71	unidentified sp.3*			1.1		
	total	198.9	193.3	168	224.4	84.2

Note: Species distribution: ***** = very broad; **** = broad; *** = moderate; ** = narrow; * = very narrow.

Table 2 Total species, total abundance and Shannon-Wiener's diversity index of soil fungi in five land use types.

Land use type	Total species	Total abundance (CFU/g wet weight soil)	Shannon-Wiener's diversity index
DEF	33	198.9×10^3	2.19
PP	29	193.3×10^3	2.12
GL	38	168.0×10^3	1.65
RP	27	224.4×10^3	1.56
PF	36	84.2×10^3	2.90

Note: DEF = secondary dry evergreen forest; PP = Phayoong plantation; GL = grassland; RP = rubber plantation; PF = pineapple field.

Table 3 Sorensen's index of similarity of soil fungi (%) between land use types.

	DEF	PP	GL	RP	PF
DEF		71.73	55.72	44.93	21.19
PP			61.75	45.32	27.54
GL				41.67	25.02
RP					19.44
PF					

Note: DEF = secondary dry evergreen forest; PP = Phayoong plantation; GL = grassland; RP = rubber plantation; PF = pineapple field.

Table 4 Means of leaf litter dry weights (LDW) and physical and chemical properties of the soils collected from five land use types and their F values resulting from analysis of variance (ANOVA).

Environmental factor	DEF	PP	GL	RP	PF	F value
LDW (g/m^2)	43.42 cd	126.51 a	68.02 b	61.47 bc	27.38 d	27.90**
Soil physical properties						
% sand	42.90	49.29	47.12	55.46	44.46	2.20^{ns}
% silt	29.11 a	22.11 b	24.56 b	24.56 b	24.84 b	5.39**
% clay	27.93	28.48	28.67	19.93	30.64	2.32^{ns}
Soil chemical properties						
pH	4.59 cd	4.42 d	5.26 b	5.67 a	4.80 c	28.00**
% organic matter	7.00 a	5.35 a	6.08 a	3.35 b	3.03 b	8.11**
Phosphorus (mg/kg)	4.99 b	2.24 c	2.44 c	1.82 c	7.58 a	9.01**
Potassium (mg/kg)	111.27 a	46.12 b	86.08 a	39.64 b	46.80 b	7.46**
Calcium (mg/kg)	326.15 ab	185.02 b	436.36 a	228.16 ab	126.83 b	2.82*
Magnesium (mg/kg)	80.07 a	34.93 bc	65.50 ab	19.47 c	11.51 c	6.60**

Note: DEF = secondary dry evergreen forest; PP = Phayoong plantation; GL = grassland; RP = rubber plantation; PF = pineapple field.

* = Values followed by different letters within a row are significantly different ($P<0.05$).

** = Values followed by different letters within a row are highly significantly different ($P<0.01$).

^{ns} = not significantly different ($P > 0.05$).

% organic matter, and phosphorus, potassium and magnesium contents were highly significantly ($P < 0.01$) different, while the means of calcium content were significantly ($P < 0.05$) different. Multiple comparisons of means by Duncan's new multiple range test are also shown in Table 4. All of the analyses indicated that the soils in the five land use types were significantly different from each other.

Relationships of soil fungi with leaf litter dry weight and soil properties

Analysis of the relationships of soil fungi with leaf litter dry weight and soil physical and chemical properties by the ordination method using CCA showed that the presence of some soil fungi was related to the P, Ca and Mg contents in the soil, but was not related to the leaf litter dry weight or the percentage of sand, silt or clay, pH, percentage of organic matter and the potassium content (Figure 2).

Figure 2 shows that the soil fungi that related positively and more closely with the P content in the soils were: *Eupenicillium* sp. (s5), *Aspergillus cervinus* (s11), *Aspergillus japonicas* (s12), *Cephalosporium* sp. (s19), *Chloridium* sp.

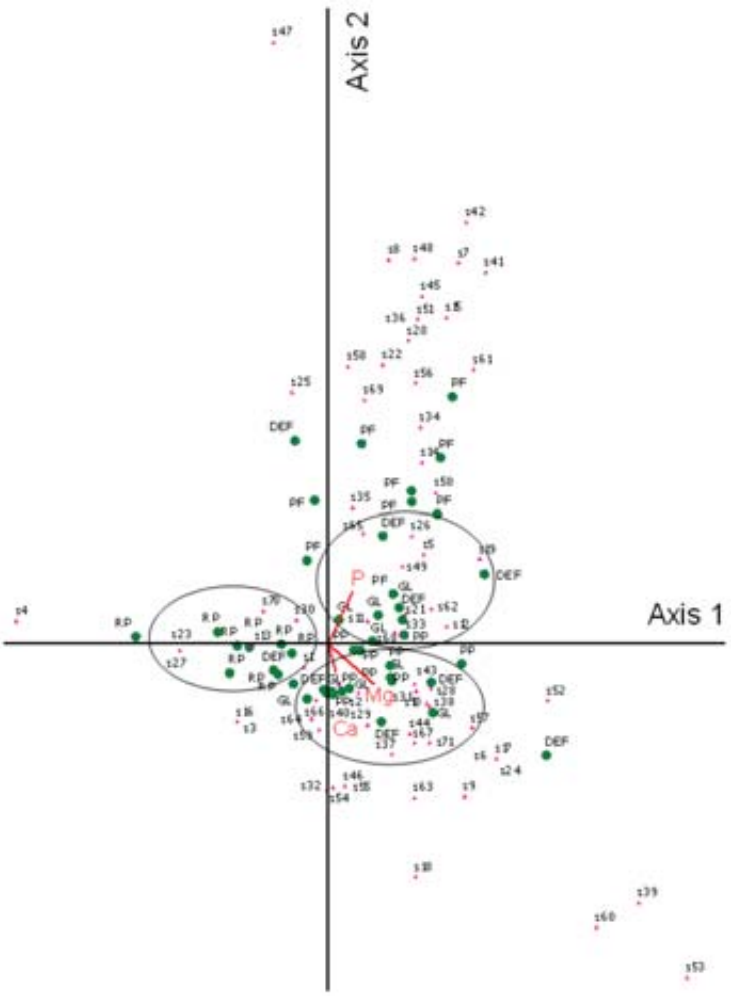

Figure 2 The relationship of soil fungi with phosphorus (P), calcium (Ca) and magnesium (Mg) contents analyzed by the ordination method using canonical correspondence analysis (CCA).

(s21), *Humicola fuscoatra* (s26), *Penicillium rubrum* (s33), *Penicillium* sp.12 (s49), *Trichoderma artroviride* (s62), *Trichoderma oblongisporum* (s65) and an unidentified hyphomycetes (s68). These fungi appeared in soils from DEF, GL and PF that had a P content of 4.99, 2.44 and 7.58 mg/kg, respectively, or 0.33×10^{-3}, 0.16×10^{-3} and 0.51×10^{-3} M, respectively.

The soil fungi that related positively and more closely with the Ca and Mg content in the soils were from the same group. They were: *Gongronella butleri* (s2),*Aspergillus candidus* (s10), *Mariannaea elegans* (s28), *Monocillium* sp. (s29), *Penicillium ochro-chloron* (s31), *Penicillium verruculosum* (s37), *Penicillium* sp.1 (s38), *Penicillium* sp.3 (s40), *Penicillium* sp.6 (s43), *Penicillium* sp.7 (s44), *Pestalotiopsis* sp. (s57), *Phoma* sp. (s59), *Trichoderma hazianum* (s64), *Trichoderma virens* (s66), *Verticillium lecanii* (s67) and an unidentified sp.3 (s71). These fungi appeared in soils from DEF, PP and GL that had a Ca content of 326.15, 185.02 and 436.36 mg/kg, respectively, or 16.3×10^{-3}, 9.25×10^{-3} and 21.82×10^{-3}M, respectively, and an Mg content of 80.07, 34.93 and 65.50 mg/kg, respectively, or 6.67×10^{-3}, 2.91×10^{-3} and 5.46×10^{-3} M, respectively.

There was a group of soil fungi which clearly had no relationship with the P, Ca and Mg contents in soils. They were: *Cunninghamella elegans* (s1), *Aspergillus niger* (s13), *Geotrichum* sp. (s23), *Leptographium* sp. (s27), *Penicillium adametzi* (s30) and an unidentified sp.2 (s70). These fungi had high abundance values and were only found in soil from RP.

DISCUSSION

Soil fungal diversity

Most of the soil fungi in all five land use types, which belonged to the classes Zygomycetes and Deuteromycetes, are very common soil fungi that have been reported in various agricultural and forest soils in Thailand (Wongseenin, 1971; Wongvutiyan, 1993; Manoch, 1998, 2000, 2004; Kosol, 1999; Ramanwong *et al*., 2000). The soil dilution plate method used for the isolation of soil fungi in the current study is a suitable method for isolating fungi in these two classes, as it supports releasing and mixing of numerous spores produced by the fungi into the diluted soil suspension (Manoch *et al.,* 1998; Kosol, 1999). A reason that may explain why only five fungi in the class Ascomycetes were obtained is that the suitable methods for their isolation were not used, namely, heat treatment and alcohol treatment methods (Manoch *et al*., 1998). The lists of soil fungi in Table 1 may be an underestimation of the total number of species in the five land use types, because only one isolation method was used. However, the dilution plate method, logically, remains the simplest, as well as the most judicious, method by which soil fungi assemblages may be screened (Keller and Bidochka, 1998).

Most of the identified soil fungi have been reported to be organic matter decomposers, with the exception of *Fusarium oxysporum*, a phytopathogenic species. *Penicillium* spp, *Gliocladium roseum* and *Trichoderma* spp. can be antagonists against other fungal species, especially phytopathogenic species (Elad and Freeman, 2002; Gomez *et al*., 2007). The antagonistic fungi in the current study may be isolates that have a high potential for use in the biological control of phytopathogenic species, if screening tests are carried out (Intana, 2003).

Though the moisture content of the collected soil samples was not measured, it was known that the soils had high moisture content. An indicator of the high moisture content was the isolation of *Gongronella butleri* (Zygomycetes) at very high abundance levels from all land use type soils. This fungus and other fungi in the Zygomycetes, such as *Cunninghamella elegans* and *Mucor* sp., prefer high moisture content habitats (Grishkan *et al*., 2005). *G. butleri* has been

reported as having a worldwide distribution with apparently higher frequencies in the sub-tropical region (Domsh *et al*., 1993). Furthermore, some isolates of *G. butleri* can produce high chitosan (Tan *et al*., 1996; Nwe and Stevens, 2002), the substance used in biomedical applications, such as wound dressings, drug delivery systems and space-filling implants. Therefore, *G. butleri* and, according to Beran *et al*. (2005), the other chitosan-producing fungi found in the current study (*Aspergillus niger* and *Penicillium oxalicum*), should be further researched for their ability to produce chitosan.

The purpose in categorizing soil fungi based on their appearances in all or some land use types and on their abundance values is to indicate their chances of disappearing from the areas. The soil fungi with a species distribution that was categorized as very broad or broad, but not rare, will not disappear easily from Tha Kum-Huai Raeng Forest Reserve, while those that distribution was categorized as very narrow or rare will disappear easily from the Forest Reserve. The latter category of soil fungi should receive much more attention from mycologists. It should be screened to see if it contains any promising species for industrial, medicinal or agricultural purposes and, in addition, all of the fungi in this category should be well preserved before they disappear from the area should any abrupt changes in land use type occur.

Diversity index and similarity index

The Shannon-Wiener's diversity index was used instead of the direct number of soil fungal species to indicate the fungal diversity of each land use and the index value was compared with other land use diversity indices, because: 1) it is logarithmic, thus preventing an overestimation of heavily sporulating species; and 2) it takes into account not only the number of species, but also the number of isolates (colonies) of all species (Grishkan *et al*., 2005). When comparing GL soil, which had the highest number of soil fungal species, with PF soil, which had the second highest number of soil fungal species, Table 1 indicates that there was much greater variation in the abundance values for soil fungi in GL (range 0.2 $\times 10^3$ to 113 $\times 10^3$ CFU/g wet weight soil) than in PF (range 0.2 $\times 10^3$ to 12.7 $\times 10^3$ CFU/g wet weight soil). This caused the total abundance of soil fungi in GL to be very high (168 $\times 10^3$ CFU/g wet weight soil) compared to that of PF (84.2 $\times 10^3$ CFU/g wet weight soil). The total abundance of soil fungi was used as a divisor in the Shannon-Wiener's diversity index. Therefore, it was not surprising that the PF soil had the highest fungal diversity index calculated using the Shannon-Wiener's index. The lowest fungal diversity index was for the RP soil because the soil had the lowest number of fungal species and very high variation in fungal abundances. It may have had the lowest number of fungal species as a result of bi-annual herbicide applications based on information from the owner of the rubber plantation. The finding that the highest fungal diversity index was recorded in PF deviated from the general rule suggesting that completely undisturbed sites or sites that have not been disturbed for a long time, like DEF and PP, should have higher soil fungal diversities. This may have been due to the fact that the PF soil had been ploughed and supplemented with some chemical fertilizers (composed of 13N:13P:21K and 21N:0P:0P). The nutrients released into the soil from these fertilizers might have benefited the growth of soil fungi. In addition, the PF soil was more exposed to sunlight than the other land use soils, so that dark-colored soil fungi were isolated with comparatively high abundance levels, including, *Thielavia terricola*, *Chaetomella* sp., *Phialophora* sp. and *Scytalidium* sp. These fungi produce melanin in response to sunlight (Grishkan *et al*., 2005).

Similarity of soil fungi

The high similarity indices of soil fungi

between DEF and PP, between PP and GL, and between DEF and GL should indicate the high similarity between the soil environmental factors in these three pairs of land uses, as was shown in the work of Houston *et al*. (1998). They reported that the fungal community composition was more similar in soils having similar soil moisture, organic matter, pH and electrical conductivity. However, the analysis of soil environmental factors in the current study did not support the high fungal similarity indices, perhaps due to the high variation among the mean values of the soil environmental factors. Therefore, in future research of this kind, the number of soil samples per land use type should be increased, and many other soil environmental factors, such as moisture content, temperature and electrical conductivity should be measured, to help improve the statistics available for analysis.

Relationships of soil fungi with leaf litter dry weight and soil properties

P, Ca and Mg are trace elements necessary for fungal growth. They are required by fungi in very low concentrations, in the order of 1×10^{-3} M for P and Mg, and 1×10^{-4} M for Ca (Griffin, 1994). The fungi that had close positive relationships with P in the current study grew in the soils having P concentrations (0.16×10^{-3}-0.51×10^{-3} M) much lower, and Ca (9.25×10^{-3}-21.82×10^{-3}M) and Mg (2.91×10^{-3}-6.67×10^{-3} M) concentrations much higher than reported by Griffin (1994). Most of the land uses where these fungi appeared (DEF, PP and GL) have been completely undisturbed or not disturbed for a long time, so the soils should be naturally balanced to support fungal growth. If there is no abrupt land use change, natural soil fungi will exist. In the current study, it seems that the common agricultural practices of the local farmers in PF did not affect the diversity of soil fungi, because PF soil had the highest diversity index of soil fungi. The farmers should be advised to continue their agricultural practices with careful application of fertilizers at the proper rates and times, to maintain both good growth rates of their pineapples and diversity of soil fungi.

CONCLUSION

A total of 28 genera and 71 species of soil fungi were isolated from DEF, PP, GL, RP and PF soils. They belonged to the Class Zygomycetes (4 genera and 4 species), the Class Ascomycetes (4 genera and 5 species) and the Class Deuteromycetes (20 genera and 62 species). Some soil fungi appeared in all land uses, but some appeared in only a few land uses or in as little as one. The land use that had the highest Shannon-Wiener's diversity index of soil fungi was PF, followed by DEF, PP, GL and RP, respectively. The highest similarity of fungal community composition between two land uses calculated by Sorensen's index of similarity occurred between DEF and PP, followed by PP and GL, and DEF and GL, while the lowest similarity index of soil fungi was between PF and RP. Among the five land uses, the analysis of variance of mean values of the soil environmental factors, including leaf litter dry weight and soil chemical and physical properties, revealed that the soils were different from each other. The relationships of soil fungi with all the mentioned environmental factors analyzed using the ordination method and canonical correspondence analysis showed that some soil fungi related positively with the P, Ca and Mg contents in the soil. Since the five different land use types in the Tha Kum- Huai Raeng Forest Reserve vary in the number and the species of soil fungi that contribute to the soil fungal diversity of the Forest Reserve, it is recommended that the five land use types should all be managed using methods which will not allow any abrupt changes in the soil environmental conditions of the land uses in order to keep the existing diversity of soil fungi.

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LITERATURE CITED

Barnett, H.L. and B.B. Hunter. 1998. **Illustrated Genera of Imperfect Fungi.** 4th ed. Aps Press, USA. 218 pp.

Beran, M., P. Hanak, P. Molik, M. Urban, L. Adamek and M. Krajicek. 2005. **Applications of Fungal Chitosans in Biomaterials.** PUBLIKACE, [cited 2010 Apr 20]. Available from: http: //www.vupp.cz/czvupp/publik/05poster/05ShannghaiPoster2005b.pdf.

Berron, G.L. 1968. **The Genera of Hyphomycetes from Soil.** The William & Wilkins Company, Baltimore. 364 pp.

Dethoup, T., L. Manoch, N. Visarathanonth, C. Chamswarng, S. Chawpongpang, S. To-Anun and A. Kijjoa. 2007. Diversity of Talaromyces from soils and their effects on plant pathogenic fungi *in vitro*, pp. 563-570. *In* **The Proceedings of 45th Kasetsart University Annual Conference**, Bangkok.

Domsh, K.M., W. Gams and T. Anderson. 1993. **Compendium of Soil Fungi.** Vol 1, 2nd ed. Academic Press, London. 860 pp.

Elad, S. and S. Freeman, 2002. Biological control of fungal plant phathogen, pp. 93-110. *In* K. Esser and J.W. Bannett (eds.). **The Mycota: A Comprehensive Treatise on Fungi as Experimental Systems for Basic and Applied Research, Agricultural Applications.** Springer, New York.

Ellis, M.B. 1971. **Dematiaceous Hyphomycetes.** Commonwealth Mycological Institute, England. 608 pp.

_____. 1976. **More Dematiaceous Hyphomycetes.** Commonwealth Mycological Institute, England. 506 pp.

Finlay, R.D. 2007. The fungi in soil, pp. 107-146. *In* J.D. Van Elsas, J.K. Jansson and J.T. Trevors (eds.). **Modern Soil Microbiology.** 2nd ed. CRC Press, New York.

Gomez, E., R. Pioli and M. Conti. 2007. Fungal abundance and distribution as influenced by clearing and land use in a vertic soil of Argentina. **Bio Fertil Soils** 43: 373-377.

Griffin, D.H. 1994. **Fungal Physiology.** 2nd ed. Wiley-Liss, Inc., USA. 458 pp.

Grishkan, I., A. Tsatskin and E. Nevo. 2005. Diversity of cultured microfungal communities in surface horizons of soils on different lithologies in upper Galilee, Israel. **European Journal of Soil Biology** 44: 180-190.

Houston, A.P.C., S. Visser and R.A. Lautenschager. 1998. Microbial process and fungal community structure in soils form clear-cut and unharvested areas of two mixed wood Forest. **Can. J. Bot.** 76: 630-640.

Intana, W. 2003. **Selection and Development of *Trichoderma* spp. for High Glucanase, Antifungal Metabolites Producing and Plant Growth Promoting Isolates for Biological Control of Cucumber Damping-off Caused by *Pythium* spp.** Ph.D. dissertation. Kasetsart University, Bangkok.

Keller, L. and M.J. Bidochka. 1998. Habitat and temporal differences among soil microfungi among soil microfungi assemblages in Ontario. **Can. J. Bot.** 76: 1798-1805.

Kosol, S. 1999. **Biodiversity of Micro-fungi in Soil, Water and Plant under Teak Plantation of Linthin Watershed, Kanchanaburi Province.** M.Sc. Thesis, Kasetsart University. Bangkok.

Magurran, A.E. 1988. **Ecological Diversity and**

Its Measurement. Princeton University Press, N.J. 620 pp.

Manoch, L. 1993. Some Pythiaceae, Zygomycetes, Ascomycetes and Hyphomycetes from Thailand soils, pp. 739-752. *In* **The Proceedings of 30th Kasetsart University Annual Conference**, Bangkok.

Manoch, L. 1998. Biodiversity of soil fungi in Thailand, pp. 126-140. *In* **Proceedings of the Asia-Pacific Mycological Conference on Biodiversity and Biotechnology**, Hua Hin.

_____. 2000. Microfungi from different forest types of Huay Kha Khang Wildlife Sanctuary, pp. 436-444. *In* **The Proceedings of 37th Kasetsart University Annual Conference**, Bangkok.

_____. 2004. Soil fungi, pp. 141-154. *In* E.B.G. Jones, M. Tanticharoen and K.D. Hyde (eds.). **Thai Fungal Diversity.** BIOTECH, Thailand.

Nwe, N. and W.F. Stevens. 2002. Production of fungal chitosan by solid substrate fermentation followed by enzymatic extraction. **Biotechnology Letters** 24: 131-134.

Ramanwong, K., W. Himamarn, J. Ayawong and S. Maneerat. 2000. Preliminary survey on soil fungi at eastern Thungyai Naresuan Wildlife Sanctuary. **Journal of Thai Forestry Research** 2(2): 117-124.

Raper, K.B. and C. Thom. 1949. **Manual of the Penicillia.** Hafner Publishing Company, N.Y. 875pp.

_____and D.I. Fenell. 1965. **The Genus *Aspergillus*.** Willium & Wilkins Co., Baltimore. 686 pp.

Tan, S.C. , T.K. Tan, S.M. Wong and E. Khor. 1996. The chitosan yield of zygomycetes at their optimum harvesting time. **Carbohydr. Polym.** 30: 239-242.

Wongseenin, P. 1971. **Studies on Soil and Root Fungi at Sakaerat Forest.** M.Sc. thesis, Kasetsart University. Bangkok.

Wongvutiyan, K. 1993. **The Impact on Soil Microbiology in Mixed Deciduous Logged Area at Huay Linthin Changwat Kanchanaburi.** M.Sc. thesis, Kasetsart University. Bangkok.