STATUS OF SOIL MICROBIAL POPULATION, ENZYMATIC ACTIVITY AND BIOMASS OF SELECTED NATURAL, SECONDARY AND REHABILITATED FORESTS

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ABSTRACT

Substantial clearance of forests and conversion of forest into various land use types contribute to deterioration of soil fertility and associated nutrients loss. Soils from natural and rehabilitated forest in Chikus Forest Reserve and also enrichment planting forest and secondary forest of Tapah Hill Forest Reserve, Perak, Malaysia were selected in order to assess the influence of land use change on biological properties. This study was carried out to provide fundamental information on soil biological properties and also to compare the differences between natural forest, mono-rehabilitated forest, mixed planting forest and natural regenerated forest (secondary forest). Six subplots (20×20 m) were established at each study plot and soil samples were collected at the depths of 0-15 cm (topsoil) and 15-30 cm (subsoil). Soil microbial population was determined using spread-plate technique. Fluorescein Diacetate (FDA) hydrolysis was used to assess the amount of microbial enzymatic activity for each forest plot. Soil Microbial Biomass C (MBC) and N (MBN) were extracted using chloroform fumigation extraction technique and the amount of MBC was determined by dichromate digestion, while MBN via Kjeldahl digestion technique. Soil acidity was determined by pH meter and moisture content was elucidated using gravimetric method. The levels of microbial population of bacterial and fungal at natural significantly exceeded the corresponding values of rehabilitated and secondary forest. However, microbial population is much higher in rehabilitated forest of Tapah Hill compared to that of secondary forest and also Chikus Forest Reserve planted forest which proves that rehabilitation activities do help increase the level of microbial community in the soils. Longer period of time after planting as in enrichment planting compared to mono planting of *S. leprosula* plantation showed that restoring and recovery of the planted forest needed time. Deforestation activities decrease soil biological activities; however, proper forest management and rehabilitation activities are able to restore the condition of degraded forest land to its original state.

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1. INTRODUCTION

Malaysia is one of the mega-biodiversity countries in the world known for its tropical rainforest (Arifin *et al.*, 2008; Hamzah *et al.*, 2009; Zaidi *et al.*, 2010; Karam *et al.*, 2011; 2012). Tropical rainforest is a system that is in constant flux where it is continuously restructuring itself (Gonzalez-Iturbe *et al.*, 2002; Aquilar-Amuchastequi and Henebry, 2007). It acts as animportant regulator of carbon cycle, biological conservation, woody and non-woody production, soil and water conservation and acts as protection from flood, drought and erosion Food and Agriculture Organization, 1995 (Karam *et al.*, 2012). However, tropical forest also being the subject of over-logging and deforestation activities due to the demand of high timber quality. Many deforested areasare left without proper forest treatment and silvilculture, leading to massive soil erosion and degradation. To overcome this problem, efforts to rehabilitate plantations on degraded forestland have been carried out in order to overcome the problem of over exploitation of tropical rainforest product and to restore soil fertility.

Arifin *et al.* (2008) stated that in the past forest rehabilitation activity in Malaysia was started with natural regeneration approach. It was later converted into artificial regeneration planting of exotic species like *Acacia mangium* where it can grow and adapt faster on degraded forest land (Arifin *et al.*, 2008; Karam *et al.*, 2012). Besides, indigenous and exotic tree species which exhibit superior survival and growth characteristics and can grow on poor nutrient soils are chosen. This is because these tree species might be able to increase the soil fertility (Otsamo *et al.*, 1996; Otsamo, 1998; Akbar *et al.*, 2010).

Forest which is left for natural regeneration without any forest treatment is known as secondary forest. Akbar *et al.* (2010) and Karam *et al.* (2011) defined secondary forest as the forest that regenerate after improper and misused years. Karam *et al.* (2012) carried out studies on the biological properties of soils between secondary forest and enrichment planting in Tapah Hill Forest Reserve, Perak found that the secondary forest used to be forest that wasover-exploited for its woody and non woody products. The majority of secondary forest tree species are thought to be fast growing and light demanding species such as Macaranga spp. as compared to primary forest species because light intensity is higher than primary forest (Saga *et al.*, 2010; Singh *et al.*, 2012; Arifin *et al.*, 2008).

One of the artificial planting techniques utilized for forest rehabilitation activity in Malaysia is enrichment planting. Montagnini *et al.* (1997) stated that introduction of valuable and high quality tree species to degraded forest land without elimination of other existing valuable individual trees is known as enrichment planting. Johnson and Lapadat (2002) described enrichment planting as an artificial regeneration technique designed to improve the proportion of a desired species. Costa *et al.* (2000) concluded that enrichment planting is more effective in lowering negative impacts on soil quality compared to monoculture technique because it results in less susceptibility to pest and diseases, higher biodiversity, better water conservation and less soil erosion.

Multi-storied forest management is a technique of forest rehabilitation in which high quality timber trees are employed to create two or more layers of canopies (Arifin *et al.*, 2008; Karam *et al.*, 2012). The upper canopies are secondary forest, while the lower canopy consists of the planted or introduced dipterocarps trees species. In Malaysia, Multi-Storied Forest Management Project was carried out from 1991 to 1999. Multi-storied planting technique is a method of replanting trees by using fast growing trees to act as a canopy sheltering shade tolerant tree species. The Forestry Department Peninsular Malaysia (FDPM, 2003) stated that multi-storied forest management has gained significant attention as an ideal forest management technique for conserving biodiversity, preserving the environment and producing timber. Chikus and Bukit Kinta Forest Reserves in Perak, Malaysia, have been subjected to this multi-storied planting technique where *Acacia mangium* and indigenous high quality timber species including *Shorea* and *Hopea* spp. were planted during the early stage of the project.

Forest soil is home to bulk of forest biodiversity and its importance cannot be neglected (Arpin *et al.*, 1998; Caravaca *et al.*, 2002; Decaens *et al.*, 2006). It is a heterogeneous and structured environment dominated by a solid phase and wherein microbial life exists in discrete microhabitats, physical, chemical and biological characteristics of which differ in both time and space (Aiko *et al.*, 2000; Beylich *et al.*, 2010; Susyan *et al.*, 2011). Gregorich *et al.* (1998) investigated the biological attributes of soil which include living organisms and material derived from living organisms namely plants, animals and microbes, ranging in various size and function. Dinesh *et al.* (2003) reported that soil possessed the ability to supply nutrients to plants and have the potential capacity to perform as essential key of ecological function where the soil structure can be easily

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disturbed when affected by disturbances, physically, chemically or biologically.

Soil microorganisms are the driving force for the nutrient cycling processes in the soils because it is able to carry and transfer this substrate through biochemical process (Lima *et al.*, 1996; Jordan *et al.*, 1999; Garau *et al.*, 2007). Barabasz *et al.* (2002) claimed that soil microorganisms play a crucial role in the functional process of the entire ecosystems since they can exert essential effects on the dynamics of multi-directional microbiological processes.

Soil microbial enzymatic activity is central to the soil biological processes as its associated with organic matter breakdown and nutrient cycling which is being mediated by soil microorganism (Karam *et al.*, 2012). Research on soil enzymes provides insights into biogeochemical cycling of carbon and other nutrients and on microbial community functions in space and time. Moussa and Abdel-Aziz (2008) reported that high levels of microbial activities are fundamental in maintaining soil quality. Thus, soil microbial activities can be considered to have a direct influence on ecosystem stability and fertility.

Microbial biomass is defined as the living microbial component of the soil consisting largely of pools of bacteria and fungi (Gregorich *et al.*, 1998). Microbial biomass is considered to serve as the primary catalyst of bio-geochemically processedproduct as well as energy and nutrient reservoir in soil. Soil microbial biomass can also react quickly to the changes occurring in nutrient cycling, humidity, temperature, organic matter levels and soil cation availability (Peacock *et al.*, 2001). Insam *et al.* (1991) studied that soil Microbial Biomass Carbon (MBC) and Nitrogen (MBN) are the recycling and store pools of soil C and N. Viable soil microbial biomass is integral for aggregate formation, both a source and sink of available nutrients for plants and regulating ecosystems process such as decomposition, energy flow and trace gas fluxes in soil (Peacock *et al.*, 2001; Haubensak *et al.*, 2002; Mendham *et al.*, 2002).

Evaluation on soil fertility and quality has been made every year at different forest types; however, there islack of information on soil biological properties as many research focus more on physical and chemical aspects of the forest soil properties. Furthermore, data on the effects of rehabilitating forest on soil fertility focusing on establishment of enrichment planting are also lacking because many individual and organization are more interested on the tree growth performance without knowing that the success of forest rehabilitation are based on the ability on the soil to provide adequate supply of nutrients for the plants to grow well. The objectives of the current research studies were: (1) to provide fundamental information on soil biological properties; and (2) to determine the differences in biological properties level at natural, secondary and rehabilitated and enrichment planting forests atChikus and Tapah Hill Forest Reserves, Perak, Malaysia.

2. MATERIALS AND METHODS

2.1. Description of the Study Sites

Ths study was carried out in a natural forest (N 04.10076°C E 101.19411°, ±28 m a.s.l) and an 18-year-old stand of *Shorea leprosula* (N 04.09197° E 101.19499°, ±28 m a.s.l) in Chikus Forest Reserves, Perak, Malaysia. Planted forest (N 04.179394°E 101.31998°, ±46 m a.s.l) and secondary forest (N 04.17336°E 101.31974°, ±32 m a.s.l) in Tapah Forest Reserves, Perak were also selected as study sites. Sampling was done on December 10th 2010 to January 14th, 2013.

In Chikus Forest Reserve, the area has an average annual precipitation of 3 223 mm and a mean temperature of 27.7°C. The *S. leprosula* was planted in 1992 through collaborative effort of the Multi-Storied Forest Management System involving the Forestry Department Peninsular Malaysia, Perak State Forestry Department and Japan International Cooperation Agency (JICA). The planting distance for each tree was 10×3 m.

As for planted forest atTapah Hill Forest Reserve, the tree was planted in 1968 using enrichment planting technique where new tree species were planted without distrupting the growth of other existing trees. The adjacent secondary forest was left idle without any forest silviculture treatment to allow natural regeneration. *S. leprosula*, *S. parvifolia*, *S. bracteolata* and *S. macroptera* were the main species of Dipterocarpaceae planted in the compartment 13 of the enrichment planting plots. The distance between each tree was also 10×3 m.

2.2. Sampling Design and Soil Sampling

This study used a completely randomized design. Six subplots were demarcated in each plot in order to serve as replicates. Six soil samples were randomly collected at the depths of 0-15 cm and 15-30 cm in each subplot. The samples were then mixed together to form a composite sample for each soil depth range based on subplot. The composite sample collected for biological properties analyses was kept in a UV-sterilized polyethylene bags at 0-4°C prior to immediate analyses.

Another part of the soil composite samples were air-dried for 48 h and kept in polyethylene bags for physical and chemical properties analyses.

2.3. Soil Analyses

A spread-plate technique was used to estimate the microbial population (Sleytr *et al.*, 2007; Cycon and Piotrowska-Seget, 2009). Nutrient agar was employed for bacterial culture where dilution factor of 10^{-2}, 10^{-3} and 10^{-4} were found to be suitable for colony calculation after few pilot tests were carried out to standardize the dilution factor for every population count. The Fluorescen Diacetate (FDA) hydrolysis assay illustrated by Sanchez-Monedero *et al.* (2008) and Gagnon *et al.* (2007) was used to evaluate microbial enzymatic activity. Soil Microbial Biomass C (MBC) and N (MBN) were extracted using rapid chloroform fumigation extraction procedure as described by Witt *et al.* (2000). Soil MBC was analysed by wet dichromate oxidation (Vasquez-Murrieta *et al.*, 2007) and calculation for biomass C was as follow (Equation 1):

$$MBC = \left(C_{fumigated} - C_{un-fumigated}\right) / kEC \qquad (1)$$

The chloroform-labile C pool was calculated as the difference between samples of fumigated and fumigated which is proportional to MBC, where kEC is soil specifically estimated as 0.38 (Vance *et al.*, 1987).

Soil MBN was determined using the Kjeldahl digestion and distillation technique. The calculation for biomass N was as follows (Equation 2):

$$MBN = \left(N_{fumigated} - N_{un-fumigated}\right) / kEN \qquad (2)$$

The chloroform-labile N pool was calculated as the difference between samples of unfumigated and fumigated N which is proportional to MBN, where kEN is soil specifically estimated at 0.54 (Brookes *et al.*, 1985).

Bulk density was determined using disturbed soil technique, while gravimetric method was used for soil moisture content determination (Gupta, 2009). Soil acidity was extracted in 1:5 soil to water ratio and the degree of active hydrogen ions (H^+) was read using glass-electrode meter (Akbar *et al.*, 2010; Saga *et al.*, 2010; Karam *et al.*, 2012).

2.4. Statistical Analyses

A one way Analysis of Variance (ANOVA) and post hoc test of Duncan Multiple Range Test (DMRT) was used to compare the differences between mean values for each parameter analysed including microbial population, microbial enzymatic activity, microbial biomass C, microbial biomass N and selected soil physic-chemical properties analysed. SPSS version 16.00 was used for the data analyses and arrangement.

3. RESULTS

3.1. Acidity, Moisture Content and Biological Properties of the Soils at Selected Forests in Chikus and Tapah Hill Forest Reserves

Soil acidity and moisture content values for each natural, planted *S. leprosula*, secondary forest and enrichment planting plot are presented in **Table 1**. All of the forest plots exhibited acidic soil reactioneither in the topsoil or subsoil. The pH of the forests studied ranged between 4.00 and 4.63 with values lower in the topsoil. This is because of the presence of more organic matter in the topsoil; organic matter contains organic acids. Natural forest of Chikus Forest Reserves showed the highest amount of moisture content, followed by enrichment planting plot. The moisture content for each forest plot soil decreased from topsoil to the subsoil.

Table 2 shows the results of selected soil biological properties analysed of selected forests studied. Natural forest had the higher amount of microbial population compared to the other forest plots. The topsoil in secondary forest and enrichment planting of Tapah Hill Forest Reserves showed the lowest significant differences in total mean of bacteria population.

Table 1. Soil pH and moisture content in selected forests in Chikus and Tapah Hill Forest Reserves

Plot	pH	Moisture content (%)
Topsoil (0-15 cm)		
Natural forest	4.21 ± 0.09^a	52.83^a
Planted *S. leprosula*	4.24 ± 0.09^a	19.50^c
Secondary forest	4.26 ± 0.03^a	20.50^c
Enrichment planting	4.00 ± 0.01^a	26.33^b
Subsoil (15-30 cm)		
Natural forest	4.63 ± 0.01^a	50.50^a
Planted *S. leprosula*	4.54 ± 0.03^b	17.67^b
Secondary forest	4.30 ± 0.02^c	19.17^b
Enrichment planting	4.23 ± 0.03^d	23.33^b

Note: Different letters within row indicate significant differences between means of the same soil depths at selected forests in Chikus and Tapah Hill Forest Reserves

Table 2. Selected soil biological properties of selected forests in chikus and tapah hill forest reserves

Parameters	Natural forest	Planted S. lerosula	Secondary forest	Enrichment planting
Topsoil (0-15 cm)				
Bacterial count ($\log_{10} g^{-1}$ soil)	5.37 ± 0.10^a	4.91 ± 0.08^b	4.04 ± 0.10^c	4.21 ± 0.07^c
Fungal count ($\log_{10} g^{-1}$ soil)	4.37 ± 0.05^a	4.06 ± 0.03^{bc}	3.99 ± 0.06^c	4.16 ± 0.04^b
Enzymatic activity ($\mu g\ g^{-1}\ 0.5\ h^{-1}$)	58.54 ± 1.19^{ab}	52.83 ± 4.15^b	54.92 ± 5.24^b	64.34 ± 1.72^a
Biomass C ($\mu g\ g^{-1}$ soil)	198.08 ± 46.01^a	118.68 ± 28.00^a	133.44 ± 70.99^a	134.90 ± 30.45^a
Biomass N ($\mu g\ g^{-1}$ soil)	149.22 ± 19.67^b	37.17 ± 4.48^c	161.64 ± 17.62^b	239.04 ± 7.99^a
Subsoil (15-30 cm)				
Bacterial count ($\log_{10} g^{-1}$ soil)	4.52 ± 0.07^a	4.09 ± 0.04^b	3.99 ± 0.07^b	4.11 ± 0.12^b
Fungal count ($\log_{10} g^{-1}$ soil)	4.36 ± 0.07^a	4.09 ± 0.07^b	2.98 ± 0.03^c	4.06 ± 0.05^b
Enzymatic activity ($\mu g\ g^{-1}\ 0.5\ h^{-1}$)	57.67 ± 1.66^a	51.22 ± 3.65^a	55.88 ± 5.53^a	56.76 ± 2.83^a
Biomass C ($\mu\ g^{-1}$ soil)	84.99 ± 25.08^a	74.83 ± 23.86^a	105.81 ± 3864^a	164.95 ± 38.67^a
Biomass N ($\mu\ g^{-1}$ soil)	113.49 ± 29.05^{ab}	9.00 ± 4.12^c	78.48 ± 11.24^b	134.28 ± 12.09^a

Note: Different letters within same row indicate significant differences (p<0.05) between means of the same soil depths at selected forests in Chikus and Tapah Hill Forest Reserves

In contrast, there were no significant differences detected at subsoil between planted *S. leprosula* forest of Chikus Forest Reserve, enrichment planting and secondary forest plots of Tapah Hill Forest Reserve. Natural forest also possessedhigher fungal count compared to that of the planted forest of *S. leprosula*, secondary forest and enrichment planting plots.Nevertheless, the secondary forest showed the lowest total microbial population of fungi. There is no much difference that could be found in total fungi microbial population in between planted *S. leprosula* and enrichment planting plots.

Besides, topsoil at enrichment planting showed the higher total amount of microbial enzymatic activities compared with natural, rehabilitated and secondary forests. Furthermore, there was no significant difference detected between natural forest and enrichment planting plots and alsobetween planted *S. leprosula* plot and secondary forest. There were no significant differences among four types of forests at subsoil level and the microbial enzymatic rate in subsoil level was generally lower compared to that of the topsoil.

There were no significant differences of MBC observed at Chikus and Tapah Forests Reserves at the same soil depths.The MBN in the soils of enrichment planting plot showed the highest significant difference. There were no significant differences detected forMBN level at natural forest and secondary forest plots. MBN of planted *S. leprosula* plots was relatively low at both soil depths.

4. DISCUSSION

High amount of fungi communities in the natural forest is due to the natural forest condition which is undisturbed and contain abundance of organic matter on the ground (Karam *et al.*, 2011; 2012). Organic matter serves as an ideal medium for fungal growth (Beare *et al.*, 1997; Banning *et al.*, 2008). This condition occurs because the natural forest is in equilibrium state and has an abundant plant dead material which is the main source of organic matter in the soils (Kourtev *et al.*, 2002; Bird *et al.*, 2011). Hence, it promotes microbial decomposing activity and increases soil fertility (Meriles *et al.*, 2009; Tang *et al.*, 2011). Besides, the well root growth condition in natural forest stimulates the growth of bacterial and fungal populations by increasing root exudates (Barbhuiya *et al.*, 2004). In addition, high soil moisture in natural forest might affect the amount of root exudation which contributes to increase in microbial population (Paradelo and Barral, 2009; Karam *et al.*, 2011). Nevertheless, decrease in fine root production especially in compacted soil might result in changes in microbial population with fewer fungi and reduced carbohydrates in soil (Xiao *et al.*, 2005).

Microbial enzymatic activity was found to be higher in the topsoil ofthe natural and enrichment planting compared to that of the rehabilitated and secondary forest because natural forest is diverse in vegetation, rich soil nutrients and high in soil litter inputs (Barbhuiya *et al.*, 2004; Karam *et al.*, 2011; 2012). Similarly, the indigenous and exotic species planted in enrichment planting plot contributed to a high accumulation of leaf litter on the forest floor after 42 years. Saswati and Vadakepuram (2010) justified that microbial enzyme activities were significantly correlated with nutrient parameters. Moreover, process of litter decaying has been proven to be increased the enzymatic

activities (Kourtev *et al*., 2002; Li *et al*., 2004). Nevertheless, the respectively low rate of microbial enzymatic activities at planted *S. leprosula* plot and secondary forest might have incurred due to previous logging and cultivation activities (Kobayashi, 2004).

The levels of MBC and MBN at the plantation site showed higher values compared to other three plots, indicating greater microbial activity under the plantation sites. The very low microbial biomass N at rehabilitated and secondary forest may be described as soil may become compacted through heavy traffic created by logging. Compaction might decrease soil microbial biomass due to lower availability of organic substrates and nutrients, aeration, soil moisture content and oxygen deficiency (Bouwman and Arts, 2000; Hakansson and Lipiec, 2000; Xiao *et al*., 2005). Nonetheless, some studies show that soil compaction did not affect MBC (Xiao *et al*., 2005) in both the forest floor and mineral soil. On the other hand, Barbhuiya *et al.* (2004) investigated abundance of forest floor litter which favoured the growth of microbial biomass C and N in the natural forest and enrichment planting plots where it proved that the diversity of the organic substrate production in enrichment planting which improved the quality of soil nutrients, contributing to a high level of microbial biomass in the soils.

5. CONCLUSION

Bacterial and fungal population was found to be comparatively high in undisturbed natural forest soil compared to that of the disturbed, deforested, degraded land or converted forest. Natural forest and planted forest inTapah Hill Forest Reserve showed higher microbial enzymatic activity due to high amount of organic matter which serves as suitable medium for soil microorganism growth and decomposition activities to take place. Topsoil contains higher amount of microbial population, enzymatic activity and microbial biomass compared to subsoil because the uppermost layer of the soil is usually concentrated with organic matter and plant roots. Enrichment planting technique is better compared to other techniques in terms of soil biological properties. This is because the variety of species planted in enrichment planted forest gave variety of substrate medium for soil microbial decomposition activities. It is recommended that soil biological properties of rehabilitated and planted forest in tropical regions should be further studied because microbial indices of forest soils subjected to long-term disturbance would provide a valuable insight into the extent of soil deterioration and help enhance management practices and strategies in improving forest soil quality.

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