J. Trop. Agric. and Fd. Sc. 41(1)(2013): 95–108

Characterization of soil microbial functional diversity in Pulau Tekak Besar, Tasik Kenyir

(Pencirian kepelbagaian fungsi mikroorganisma tanah di Pulau Tekak Besar, Tasik Kenyir)

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Keywords: microbial activity, microbial richness, catabolic diversity, average well-colour development (AWCD)

Abstract

Pulau Tekak Besar is one of the 340 islands found in the biggest man-made lake in Southeast Asia, Tasik Kenyir. This study was conducted to evaluate the effect of land clearing on soil bacterial functional diversity of Pulau Tekak Besar. The study was conducted on 6 sampling areas, 3 disturbed (land clearing) and 3 non-disturbed soils, using the Biolog EcoPlate™ system which contained 31 useful carbon sources for soil bacterial functional diversity with characteristic reaction patterns known as metabolic fingerprints. Bacterial functional diversity was evaluated through microbial activity {colony forming unit (CFU) and average well-colour development (AWCD)} and community level physiological profile (richness and Shannon Weaver Index) using the metabolic fingerprint produced in 48 and 72 h. In general, AWCD values for undisturbed areas were higher compared to the disturbed areas. This indicated that soil microbial communities from these areas have higher metabolic diversity and carbon-mineralization compared to disturbed areas. The species richness for both areas was not significant which indicated that the microorganisms in the undisturbed and disturbed areas oxidized relatively similar number of carbon substrates. This showed that both areas had an equivalent number of different groups of microorganisms occuring together. The microbes in undisturbed areas utilized high carbon sources as compared to disturbed areas. Substrates such as D-galacturonic acid, D-mannitol, D-galactonic acid γ-lactone and N-acetyl-D-glucosamine were highly metabolized from both areas. It can be concluded that land clearing (disturbed areas) may influence microbial metabolic diversity and carbon-mineralization. However, microbial function from both areas did not change significantly.

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Introduction

Microbial diversity is fundamental to maintenance and conservation of global genetic resources (Colwell 1997). Measures must be taken to estimate, record and conserve microbial diversity for future application through wise use and conservation of genetic resources of the microbial world. This is because soil microbes contribute to a wide range of processes such as basic indicators of soil fertility, soil carbon storage, cycling of nutrients, degradation of pollutants and maintenance of soil structure. Hence, soil microbes are very important for a balanced ecosystem and the decline of these microbes may give negative impacts to soil health, which in the long run will affect the ecosystem. Thus, soil microbes need to be managed properly and one of the ways of doing this is by estimating the microbial functional diversity.

The estimation of microbial functional diversity is one of the approaches to detect changes due to soil management. According to Gomez et al. (2004), microbial functional diversity can be measured using the community level physiological profiles (CLPP), which is based on carbon sources utilization profiles from metabolic fingerprint produced by Biolog Ecoplate™ . This fingerprint reflects the diversity of carbon-oxidation pathways and therefore, functional diversity of soil microbial communities (Liu et al. 2010).

Tasik Kenyir is the largest man-made lake in Southeast Asia. The lake covers 260 km^2 and contains 340 small islands, which were once hilltops and highlands. Among the islands is Pulau Tekak Besar, which is about 200 – 500 m long and 200 m wide. The highest peak is about 160 m from sea level and the temperature here ranges from 23 – 30.4 °C. This island is located in the north of Tasik Kenyir (50 10' 07"U and 1020 44' 16"T) covering an area of 5 ha and will be developed into an eco-tourism and educational park based on agroforestry approach. Although land clearing was very minimal, it will influence the microbial diversity and activity in the ecosystem (Gomez et al. 2000). Thus, this study was carried out to evaluate the effect of land clearing on soil microbial functional diversity of Pulau Tekak Besar so that interim measures can be planned for better soil management and conservation of the island.

Materials and methods

Study area

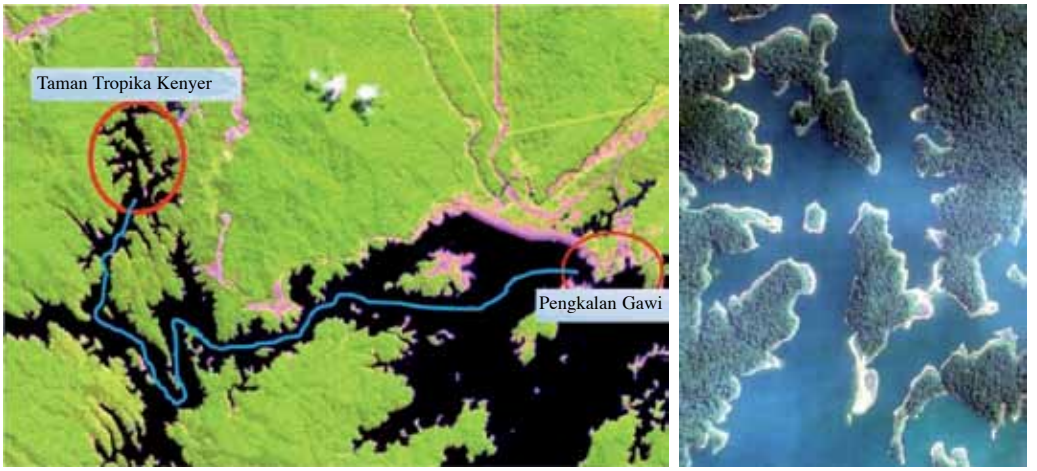
The study was carried out on Pulau Tekak Besar, which is located north of Tasik Kenyir, Terengganu, Malaysia (*Figure 1*). This island will be developed as an eco-tourism and educational park known as Taman Tropika Kenyir. The soil type was classified as Renggam series and the temperature ranged between 23 and 31 °C with average evaporation rate at 5 mm/day. The rainy season started usually in October and ended in January. The mean annual rainfall is 4,100 mm of which 54% is precipitated during the months of November till January. The dry season is from February till September.

Soil sampling

Soil samples were collected at a depth of 20 cm from 6 different areas at Pulau Tekak Besar, based on 3 disturbed (land clearing) and 3 non-disturbed areas (*Figure 1*). For each area, samples were taken from 3 locations and at each location, 3 replicates were taken within an area of 8 x 2 m. Data on microclimate such as temperature and humidity were also taken from the sampling sites.

Soil physico-chemical properties

Soils were analyzed for physico-chemical properties which included moisture, pH, organic carbon, total nitrogen, sulphur, C: N ratio, total P, available P, K, Ca, Mg, Na, Cation Exchange Capacity (CEC) and base saturation. Soil pH was determined by mixing soil sample in water at 1:2.5 soil:water ratio (Chapman and Pratt 1978).



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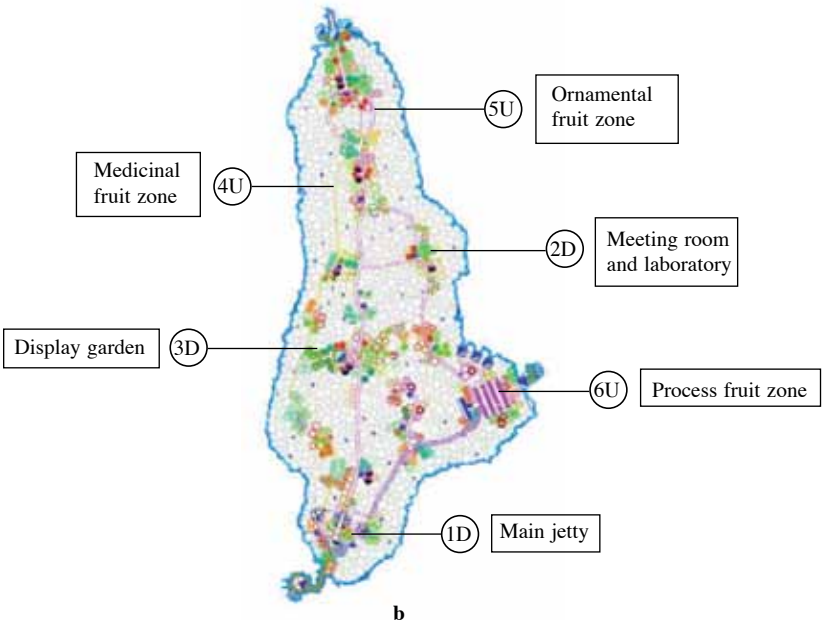


b

Figure 1. Location of Taman Tropika Kenyir (a) and sampling sites on Pulau Tekak Besar, Tasik Kenyir (b)

The total nitrogen, organic carbon and sulphur were analyzed using the CHNS Elemental Analyzer. Readily available P was determined colorimetrically by a spectrophotometer (Olsen and Sommers 1982). Exchangeable cations (K, Ca, Mg and Na) were extracted with 1M CH3COONH4 and determined by Inductively Coupled Plasma (Perkin Elmer Analyst 400). The CEC was determined by saturation with CH3COONH4 at pH 7, ethanol washing, NH4+ displacement with acidified 10% NaCl and subsequently analysed by steam distillation (Chapman 1965).

Bacterial functional diversity analysis

Sample preparation The Biolog EcoPlate™ system was used to assess the microbial activity and community level physiological profile. The 96-well microplate consisted of 3 replicates, each one comprising 31 most useful sole carbon

sources for soil bacterial functional diversity and water blank. Communities of organisms would give characteristic reaction patterns called metabolic fingerprints (Garland 1997). Soil suspensions (soil 100 g and distilled water 1 litre) were shaken for 30 min, then filtered using filter paper (pore size – 0.45 µm). Aliquots of 100 µl were inoculated in the microplates and incubated at 27 °C. Colour development in each well was recorded as optical density (OD) at 590 nm wavelength. The OD was recorded at 48 and 72 h to allow microbial utilization of any soluble organic carbon derived from the soil that could interfere in the sole carbon source-use response (Gomez et al. 2004).

Microbial activity Microbial activity was measured using colony forming unit (CFU) and average well-colour development (AWCD) in accordance with Gomez et al. (2006). For CFU, tenfold dilution series from soil suspensions (soil 1 g; sterile distilled water 10 ml; shaken for 1 h) were performed and aliquots of 1µl were plated on nutrient agar. Colony forming unit counts were taken after 2 days of incubation at 25 °C and results expressed as $\log_{10}$ CFU/ml. AWCD was determined from OD values recorded at 590 nm wavelength from incubated (48 and 72 h) Biolog EcoPlate™ wells. It measures the colour development in each well after the plate incubation. AWCD was calculated as follows:

$$AWCD = \sum OD_i/31$$

where OD_i is the optical density value from each Biolog EcoPlate™ well.

Community level physiological profile (CLPP) This profile was measured using Shannon Weaver Index (H) and richness (R) according to Garland (1997). The values were calculated from the OD values of Biolog EcoPlate™ wells incubated at 48 and 72 h. R was determined as the total number of oxidized carbon substrates (Biolog EcoPlate™ well with OD ≥ 0.25 as threshold for positive response of oxidized carbon substrates).

Shannon Weaver Index (H) was calculated as follows:

$$H = \sum p_i (\ln p_i)$$

where p_i is the ratio of the activity on each substrate (OD_i) to the sum of activities on all substrates ($\sum OD_i$) in Biolog EcoPlate™ well while ln is the natural logarithm.

Statistical analysis

AWCD, R and Shannon Weaver Index were analyzed using ANOVA and multiple comparisons of means using Fisher's Least Significance Difference (LSD) test. All statistical analyses were performed with SAS (9.1) 2002 – 2003 for Windows.

Results and discussion

Soil microbial activity

The study showed that the AWCD values increased at 48 and 72 h of incubation (*Figure 2*). The AWCD reflects the oxidative capacity of soil microorganisms developing in Biolog EcoPlate™ and maybe used as an indicator of microbial activity (Gomez et al. 2006). The undisturbed area 4U was observed to have the highest AWCD within 48 (1.44) and 72 (2.27) h. It was also demonstrated that the disturbed area 2D had the lowest AWCD (1.00) followed by area 3D (1.08) in 48 h.

According to Garland and Lehman (1999), CFU counts are the most suitable enumeration technique to relate with CLPP because CLPP is a method based on culturability. However, there was no significant difference in CFU from all areas. Kirk et al. (2004) reported that only 1% of the soil bacterial population can be cultured by standard laboratory practices. On the other hand, AWCD values maybe influenced by the activity of both culturable and unculturable microbes. Furthermore, AWCD measures the overall rate of inoculums density (Garland 1997). In

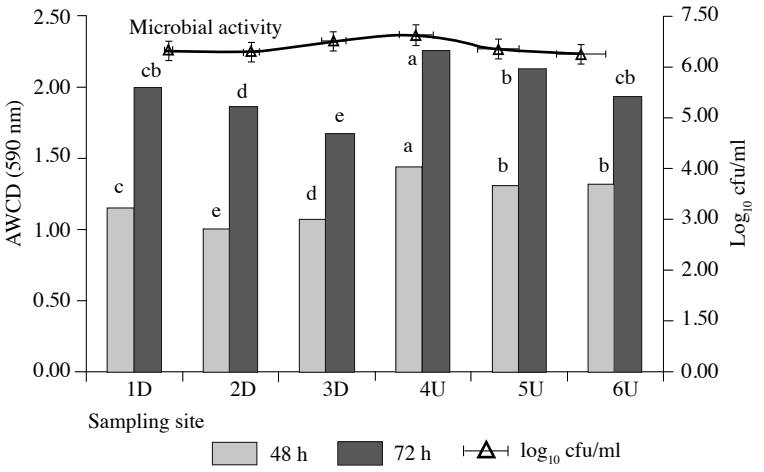


Figure 2. Average well-colour development (AWCD) of metabolized carbon substrate used in Biolog EcoPlate™ for disturbed and undisturbed areas read at 48 and 72 h of incubation. Mean separations was carried out using Fisher's Least Significance Difference (LSD) test. Means with the same letters are not significantly different

general, AWCD values for undisturbed areas was higher compared to the disturbed areas which indicated higher microbial metabolic diversity and carbon-mineralization. In agreement with our results, reports by Gomez et al. (2006), Widmer et al. (2006) and Chong et al. (2007) also demonstrated high potential of carbon source utilization in microbial communities from native sites. From this study, it was found that area 4U had higher moisture content (44.8%), soil organic carbon (5.59%), organic matter (9.64%), nitrogen (0.31%), C/N ratio (18%) and Cation Exchange Capacity {21 cmol (+)/kg} compared to the other areas (*Table 1*). These physico-chemical properties may contribute to high microbial activities in that area.

More native trees found in undisturbed area 4U caused naturally less intense light and hence, increased the soil moisture (*Table 1*). High moisture content in this area could increase the release of organic matter from woody debris and stimulate an increase in microbial activity (Eaton et al. 2011). Liu et al. (2010), in their study of soil microbial functional diversity in temperate steppe at a regional scale, showed that soil water content was a major abiotic factor influencing the functional diversity. According to Grayston et al. (1998), carbon is a key factor governing soil microorganism growth. Subsequently, Gomez et al. (2006), also suggested that the increase in microbial community functional potential maybe influenced by an increase in carbon availability which could explain the high microbial activity in undisturbed area 4U which was also influenced by high value of soil organic carbon (*Table 1*). More native trees such as *Barringtonia* and *Dipterocarpus lowii* in the undisturbed area had encouraged the growth of root-parasitic nematodes and their existence on these native trees could have increased the soil nitrogen in undisturbed area 4U (*Table 1*), thus enhancing the activity of microbes. A study by Tu et al. (2003) on root-parasitic nematodes indicated that soil nitrogen enhanced microbial activities and nitrogen mineralization through the plant roots infection by the obligate root-parasitic nematodes. On the other hand, high cation exchange capacity observed in this area

Table 1. Sampling location, microclimate and soil physico-chemical properties of sampling areas

Parameters	1D	2D	3D	4U	5U	6U
Sample Type	D	D	D	U	U	U
Longitude	05° 09.794	05° 09.856	05° 09.859	05° 09.929	05° 09.973	05° 09.917
Latitude	102° 44.674	102° 44.706	102° 44.689	102° 44.685	102° 44.675	102° 44.669
Temperature (°C)	28.0	31.0	31.0	30.0	31.0	26.0
Relative Humidity (%)	82.0	74.0	70.0	71.0	70.0	98.0
Moisture (%)	25.9	22.1	31.0	44.8	26.7	22.6
Organic carbon (%)	2.12	2.05	2.82	5.59	1.97	1.45
Organic matter (%)	3.65	3.53	4.86	9.64	3.40	2.50
Total nitrogen (%)	0.26	0.26	0.26	0.31	0.25	0.24
Sulphur (%)	0.49	0.5	0.5	0.49	0.51	0.52
C/N ratio (%)	8.0	8.0	11.0	18.0	8.0	6.0
Total P (ppm)	109.0	146.0	117.0	131.0	137.0	145.0
Available P (ppm)	3.0	4.0	3.0	3.0	3.0	3.0
Pottasium (K) {cmol (+)/kg}	0.07	0.04	0.08	0.06	0.05	0.04
Calcium (Ca) {cmol (+)/kg}	0.07	0.13	0.09	0.02	0.07	0.1
Magnesium (Mg) {cmol (+)/kg}	0.12	0.16	0.16	0.1	0.12	0.1
Sodium (Na) {cmol (+)/kg}	0.02	0.03	0.02	0.02	0.02	0.02
Cation Exchange Capacity (CEC) {cmol (+)/kg}	7.4	13.7	13.7	21.0	11.8	7.5
pH	4.6	4.1	4.3	4.0	4.8	4.3
Base saturation	3.8	2.6	2.6	1.0	2.2	3.4

D = disturbed; U = undisturbed

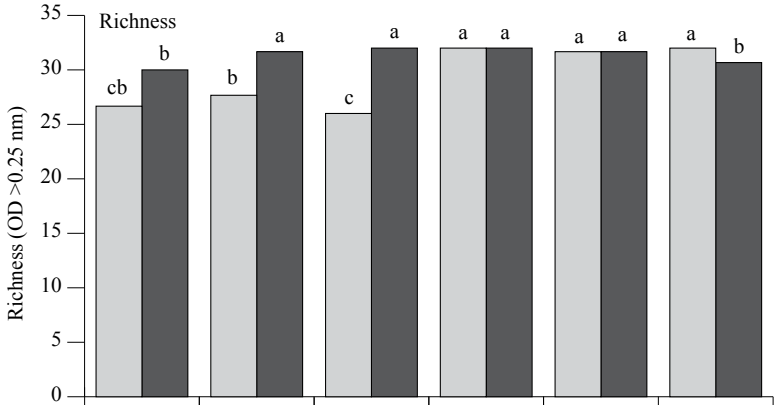
provided an effective soil ability to hold applied nutrient and thus increased microbial activities (*Table 1*).

Minimal land clearing was conducted in the disturbed area 2D in order to build laboratories and meeting rooms. Previously, this area was naturally vegetated with shade tolerance plant colonized with timber trees (trunk diameter 15 – 30 cm) and non-timber trees such as Bertam palm. Due to land clearing, the disturbed area has less plant coverage and thus affect the soil chemical properties and also reduced the plant litter. The activities of tree-associated microbes could diminish because of land clearing, for instance, the yeast community that was involved in the accumulation of ethanol by *in-situ* fermentation of Bertam palm nectar (Camarasa et al. 2011).
Soil chemical properties and plant litter influenced microbial activities as shown in the previous studies. According to Nguyen (2000) and Brodie et al. (2002), physico-chemical properties might be important in driving the change of microbial community parameters. Changes in litter C:N ratios can have different effects on concentrations and dynamics of denitrifying enzymes which altered soil chemical properties (Menyailo and Huwe 1999).

Community level physiological profile

Species richness was measured from the number of oxidized carbon substrates with OD at 0.25 nm as threshold for positive response (Garland 1997). The study showed that the disturbed areas had lower richness as compared with undisturbed areas in 48 h (*Figure 3*). It was noted that undisturbed areas 4U and 5U had oxidized all carbon substrates in 48 h and 72 h. These observations indicated that disturbed areas have less number of different groups of microorganisms found occuring together resulting in less carbon substrates oxidation.

Disturbances can cause changes in resources or in the physical environment and affect the bacterial communities (Berga et al. 2012). However, it was observed that disturbed area 1D and undisturbed area 6U have a low richness compared to other areas in 72 h. Allison and Martiny (2008) reported



	1D	2D	3D	4U	5U	6U
48 h	26.67	27.67	26.00	32.00	31.67	32.00
72 h	30.00	31.67	32.00	32.00	31.67	30.67

Figure 3. Richness of metabolized carbon substrate used in Biolog EcoPlate™, for disturbed and undisturbed areas read at 48 h and 72 h of incubation. Mean separation was carried out using Fisher's Least Significance Difference (LSD) test. Means with the same letters are not significantly different

that soil microbial community composition is generally sensitive to disturbances. Nevertheless, microorganisms in disturbed area 1D might have changed functions but not their community composition which can be described as having high functional plasticity (Agrawal 2001).

According to Griffiths et al. (2001), microbial function will not be eliminated even if there are changes in microbial composition. As long as there is a broad spectrum of species available, others can take advantage of the changed conditions of altered land use and the process of decomposition can continue. Perhaps these could explain why the values of soil sulphur, available phosphorus and sodium for all areas were nearly the same (*Table 1*) since microorganisms are involved in the immobilization of inorganic sulphur and mobilization of organically bound sulphur in the soil and these processes are linked to the microbial biomass present in the soil (Kertesz and Mirleau 2004).

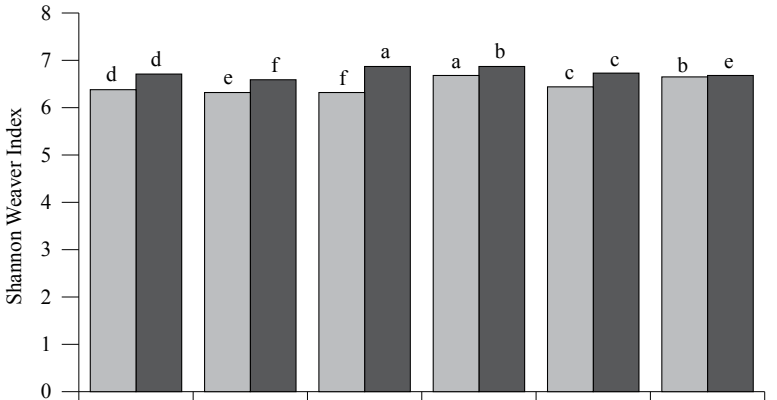
Richardson and Simpson (2011) reported that microorganisms play an important role in soil phosphorus cycle by enhancing the capacity of plants to acquire phosphorus from soil through various mechanisms. Microorganisms in the undisturbed areas may increase root growth through an extension of existing root systems or by hormonal stimulation (Richardson et al. 2009; Hayat et al. 2010) while in the disturbed areas, microorganisms may facilitate the mobility of organic phosphorus through induction of metabolic processes that are effective in solubilizing and mineralizing phosphorus from sparingly available form of soil inorganic and organic phosphorus (Richardson et al. 2009).

Acidic soils (pH 4.0 – 4.8) in both areas contributed to the equivalent number of different groups of microorganisms found occuring together since pH influences the microbial community in soil as reported by Rousk et al (2009). A study by Kemmitt et al. (2006) showed that pH strongly influences abiotic factors such as carbon and nutrient availability while Bååth and Anderson (2003) discovered that soil pH may control the biomass composition of fungi and bacteria.

The Shannon Weaver Index specifically measures the richness and eveness of response by using an OD 0.25 as a positive threshold (Garland 1997). The index value for undisturbed areas was significantly higher than disturbed areas in 48 h (*Figure 4*). Higher index value (6.69) was observed after 48 h for undisturbed area 4U than other areas while disturbed area 3D had significantly lower index value (6.32) compared to the other areas after 48 h. In contrast, disturbed area 3D had a high index value (6.87) which was similar with undisturbed area 4U in 72 h while area 2D had the lowest value (6.59) in the same time.

It was observed that there was higher catabolic diversity from undisturbed area 4U in 48 h based on Shannon Weaver Index. Maybe this could be explained by the presence of more plant coverage in the undisturbed soil which promotes higher microbial population. More plant coverage means higher plant and litter diversity which supports a greater diversity of decomposers (Hansen 2000). In addition, specific plant functional groups can positively influence microbial biomass due to enhanced litter quality (Scherer-Lorenzen et al. 2003). Furthermore, as shown previously, undisturbed area 4U had the highest AWCD and also oxidized all carbon substrates within 48 h.

However, it was noted that the disturbed area 3D and undisturbed area 4U had high and similar values of catabolic diversity after 72 h (*Figure 4*). This scenario could be explained by the equal oxidative capacity of both areas as displayed by species richness in 72 h. Minimal land clearing was done in disturbed area 3D to transform it into a display garden which involved the planting of selected rare fruit trees such as *Lepisanthes fruticosa*. Therefore, this area had also plants which



	1D	2D	3D	4U	5U	6U
48 h	6.38	6.32	6.32	6.68	6.44	6.65
72 h	6.71	6.59	6.87	6.87	6.73	6.68

Figure 4. Shannon Weaver Index of metabolized carbon substrate used in Biolog EcoPlate™, for disturbed and undisturbed areas read at 48 and 72 h of incubation. Mean separation was carried out using Fisher's Least Significance Difference (LSD) test. Means with the same letters are not significantly different

could enhance the catabolic diversity. Plant species influence soil bacterial community structures as reported from several studies (Smalla et al. 2001; Larkin 2003; Reynolds et al. 2003). Reynolds et al. (2003) suggested that a particular plant species promotes a particular soil-borne microbial population in the rhizosphere. Plant diversity may also enhance net primary productivity (Bartelt-Ryser et al. 2005) which is expected to increase the soil-carbon input via enhanced turnover of plant biomass and enhanced root exudation. This may, therefore, influence carbon limited microbial communities in the soil (Zak et al. 2003).

Carbon substrates group

Different groups or different species of microbes may utilize different carbon substrates for their growth. Analysis of the microbial community based on their ability in utilizing different groups of carbon within 48 and 72 h, indicated that soil microorganisms in area 4U had significantly highest (p <0.05) average utilization intensity of carbohydrates, carboxylic acids, amides/amines and polymers while 5U had significantly higher (p <0.05) average utilization intensity of amino acids (*Figures 5* and *6*). Miscellaneous substrate (glucose-1-phosphate and D,L-α-glycerol phosphate) was also intensely utilized in areas 4U and 6U in 48 and 72 h respectively. This demonstrates that over incubation time, the undisturbed areas utilized high carbon sources as compared to disturbed areas. The undisturbed areas have more native trees compared to disturbed areas.

Stephan et al. (2000) reported that there was positive influence of plant diversity on carbon source utilization patterns in the activity and functional diversity of culturable soil bacteria. There could be a mutual relationship which plants may also profit from diverse soil bacterial communities, for example, mediated by better nutrient mineralization, growth stimulation and enhanced antibiosis to pathogens (Kim et al. 1998; Shah et al. 1998). Epiphytic microflora is associated with all plant species in the natural habitat (Beattie and Lindow 1999).

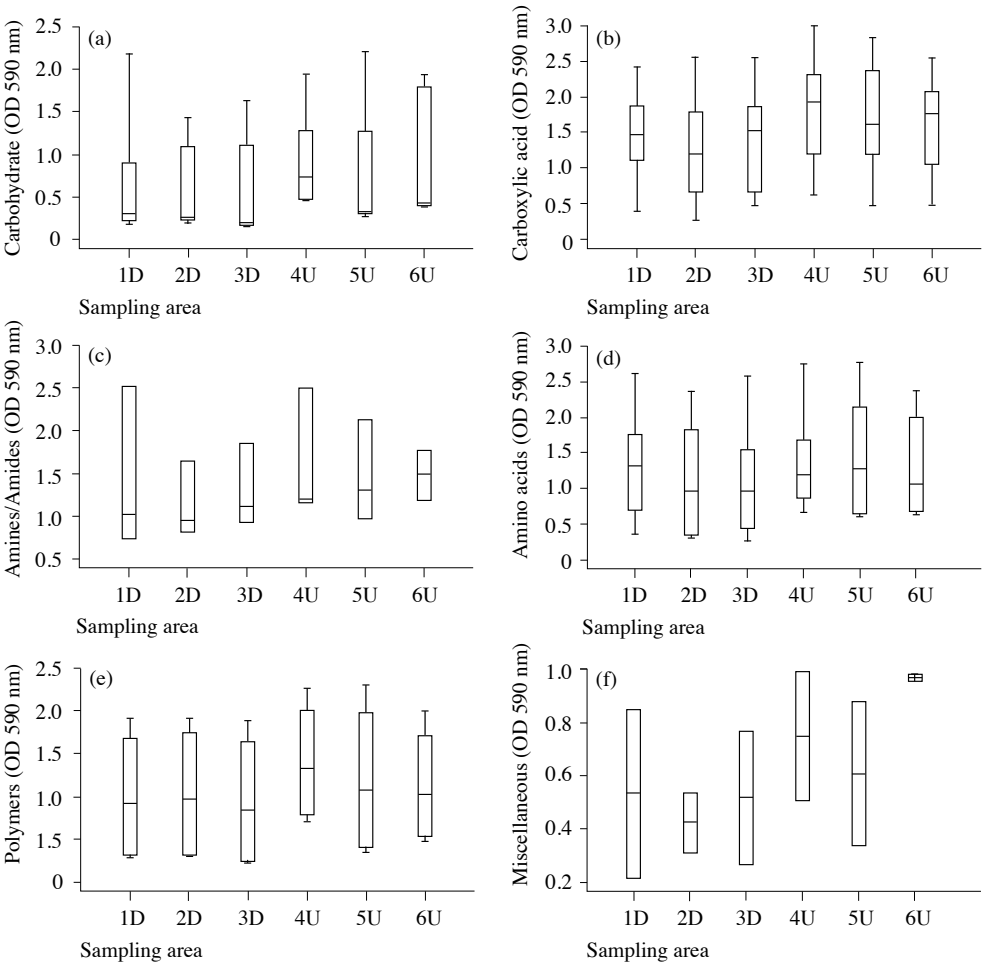


Figure 5. Carbon substrates groups utilized by all areas in 48 h using box-whisker diagrams. Carbon substrates were divided into 6 groups, i. e. carbohydrate (a), carboxylic acids (b), amines/amides (c), amino acids (d), polymers (e) and miscellaneous (f). The lower and upper boxes show the lower and upper quartiles respectively

Hallmann et al. (1997) reported that the composition and quantity of nutrients, including carbohydrates, organic acids and amino acids that support the growth of epiphytic bacteria, are affected by the plant species, leaf age, leaf physiological status and the presence of tissue damage. Correspondingly, host plants, leaf age, leaf position, physical environmental condition and availability of immigrant inoculum have also been suggested to be involved in determining species of microbes (Wilson and Lindow 1994).

From the observation, the interquartile range for amino acids consumption was the highest (1.488) among the carbon substrates group in 48 h. However, after 72 h, carbohydrates displayed the highest interquartile range (2.106) as compared with other groups. Substrates such as D-galacturonic acid, D-mannitol, D-galactonic acid γ-lactone and N-acetyl-D-glucosamine were highly metabolized from both areas. These results coincide with Widmer et al. (2006) for all substrates except for N-acetyl-D-glucosamine.

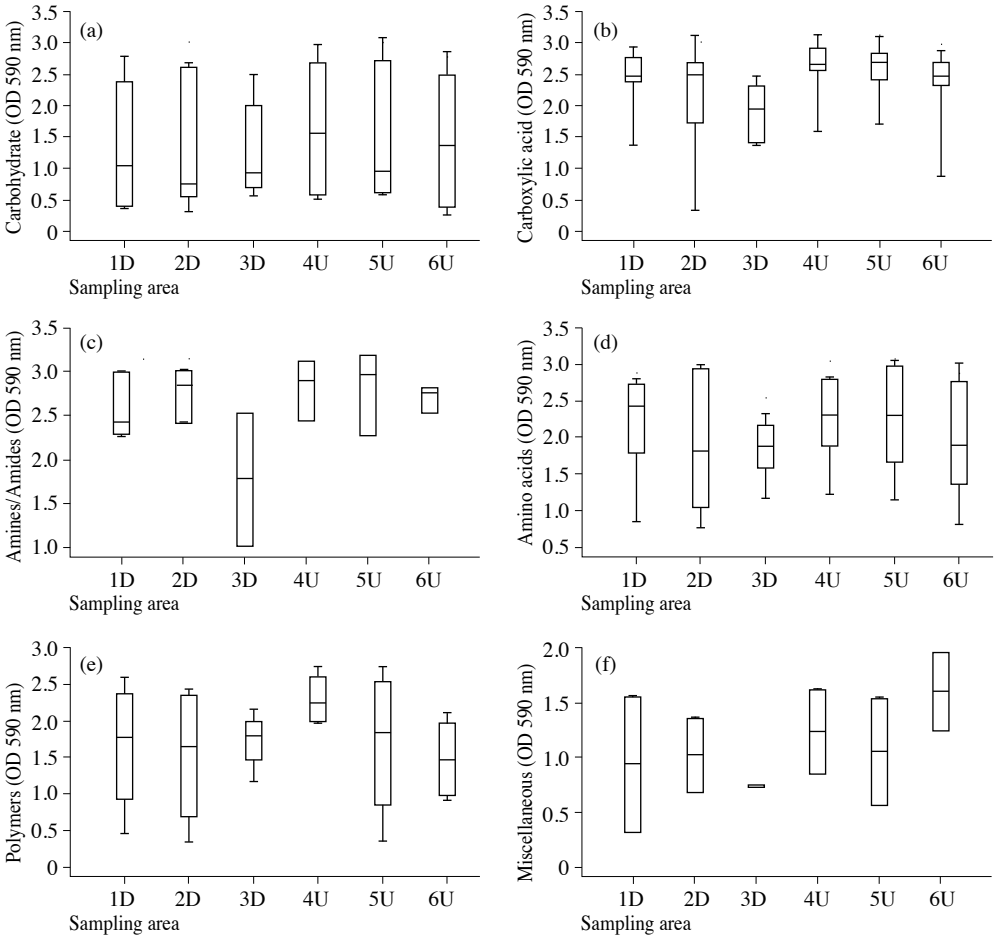


Figure 6. Carbon substrates groups utilized by all areas in 72 h using box-whisker diagrams. Carbon substrates were divided into 6 groups, i.e. carbohydrate (a), carboxylic acids (b), amines/amides (c), amino acids (d), polymers (e) and miscellaneous (f). The lower and upper boxes show the lower and upper quartiles respectively

Beattie and Lindow (1999) found that dominant Denaturing Gradient Gel Electrophoresis (DGGE) bands in the BIOLOG wells were from 8 major carbon sources (pyruvic acid methyl ester, D-mannitol, N-acetyl-D-glucosamine, glucose-1-phosphate, α-D-lactose, L-asparagine, D-galacturonic acid and D-galactonic acid). Optical densities less than 0.25 (threshold for positive response) were recorded for β-methyl-D-glucoside and α-D-lactose for undisturbed areas. β-methyl-D-glucoside is produced in the leaves of many plants and is broken down by *β-glucosidases* that break down a variety of carbohydrates (Aubert et. al 2004). Only a few *β-glucosidases* are able to cleave the side chain of β-methyl-D-glucoside due to its break down selectivity which could make it less favourable by the microbes.

Conclusion

It can be concluded that land clearing (disturbed areas) may influence the microbial metabolic diversity and carbon-mineralization, however, it does not change

the microbial function. Thus, suitable interim measures need to be planned towards microbial diversity conservation.

Acknowledgement
The authors would like to express gratitude to the Unit Perancang Ekonomi Negeri (UPEN) Terengganu for funding the project. Thanks are also due to Mr Azrizal Ahmad Rashdi, Ms Norzaimawati Aman Nejis and Ms Rosnah Hassan for their technical assistance. The authors would also like to thank MARDI, Lembaga Kemajuan Terengganu Tengah (KETENGAH) and all the staff involved in the Taman Tropika Kenyir project for their support and encouragement.

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Abstrak

Pulau Tekak Besar merupakan salah satu daripada 340 pulau yang terdapat di Tasik Kenyir iaitu tasik terbesar buatan manusia di Asia Tenggara. Kajian ini dijalankan untuk menilai kesan pembersihan tanah terhadap kepelbagaian fungsi bakteria tanah di Pulau Tekak Besar. Kajian telah dijalankan di enam kawasan pensampelan, 3 kawasan terganggu (pembersihan tanah) dan 3 kawasan tidak terganggu, dengan menggunakan sistem Biolog EcoPlate ™ yang mengandungi 31 sumber karbon berguna untuk analisis komuniti tanah yang memberikan tindak balas yang dipanggil sebagai cap jari metabolik. Kepelbagaian fungsi bakteria dinilai melalui aktiviti mikroorganisma {unit membentuk koloni (CFU) dan pembangunan warna purata (AWCD)} serta profil fisiologi komuniti (kekayaan dan Indeks Shannon Weaver) menggunakan cap jari metabolik yang dihasilkan di dalam masa 48 dan 72 jam. Secara umumnya, nilai AWCD bagi kawasan tidak terganggu lebih tinggi berbanding kawasan terganggu. Ini menunjukkan bahawa komuniti mikroorganisma tanah di kawasan tidak terganggu mempunyai kepelbagaian metabolik dan karbon-mineral yang lebih tinggi daripada kawasan terganggu. Kekayaan spesis bagi kedua-dua kawasan tidak signifikan dan ini menunjukkan bahawa mikroorganisma di kawasan tidak terganggu dan terganggu mempunyai bilangan sumber karbon teroksida yang sama. Ini menunjukkan bahawa kedua-dua kawasan mempunyai bilangan kumpulan mikroorganisma yang sama. Mikroorganisma di kawasan tidak terganggu menggunakan sumber-sumber karbon yang lebih tinggi berbanding dengan kawasan yang terganggu. Substrat seperti 'D-galacturonic acid', 'D-mannitol', 'D-galactonic acid γ-lactone' dan 'N-acetyl-D-glucosamine' telah dimetabolismakan dengan tinggi di kedua-dua kawasan. Melalui kajian ini, dapat disimpulkan bahawa pembersihan tanah (kawasan terganggu) mempengaruhi kepelbagaian metabolik mikroorganisma dan karbon-mineral. Namun, fungsi mikroorganisma dari kedua-dua kawasan tidak berubah.

Accepted for publication on 2 January 2013