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Variations in Rhizosphere Microbial Communities of Rubber Plantations in Hainan Island, China

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The aim of this study was to describe rhizosphere soil microbial community characteristics of rubber plantations varying in soil parent material and years of plantation in Hainan Island, southern China. Soils were taken from rhizosphere of grass field, immature rubber plantation (before tapping) and mature rubber plantation (after tapping) at each of three soil type areas in Hainan Island. Microbial biomass was estimated from the total extractable phospholipid fatty acids (PLFAs) and PLFA profiles were analysed to determine the microbial community structure. Total microbial biomass of soils with shallow marine deposit significantly increased with increasing years of rubber cultivation and declined significantly with increasing years of rubber cultivation of soils with basalt. The ratio of fungi to bacteria (F/B) was significantly higher in rubber plantations than in grass field on soils with the same parent material. Soil organic carbon (C), total nitrogen (N) and total phosphorus (P) were significantly positively correlated with total microbial biomass, bacteria biomass, fungi biomass and actinomycete biomass. The primary difference in the microbial community structure of the 11 soils was between soils with basalt versus soils with granite and shallow marine deposit. Soil total N, total organic C and total P may be important factors influencing the structure of the soil microbial community in this study.

Keywords: Microbial community; phospholipid fatty acid; rhizosphere; rubber plantation

Rubber tree is a rubber producing arbor, which was firstly introduced from Malaya to Hainan Island in 1906[1]. It is of major economic importance because of its latex and timber. Since the 1950s, rubber plantations have developed rapidly in Hainan Island. At the end of 2010, the rubber plantation covered 4.9×10^5 ha, accounting for 15.4% of the total land area of Hainan Island, which is the largest artificial ecosystem in Hainan Island[2]. Rubber plantations in Hainan Island are generally distributed in the region between the central mountainous area and the coastal area[3].The main soil parent materials of rubber plantations in Hainan Island are granite, basalt and shallow marine deposit[1,4].

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With the continuous increase in rubber plantations, the need for studies on the potential of ecological and environmental consequences of rubber cultivation significantly increased. Many studies have been conducted on the effect of rubber cultivation on soil fertility, soil organic carbon and soil microbial biomass[4–8]. Most results from previous researches showed that rubber cultivation led to significant decline in soil organic carbon and microbial biomass[4,6]. In contrast, other studies suggested that rubber plantations could potentially enhance soil carbon sequestration[3,9]. Rubber plantation is basically a special type of secondary forest in the tropical region with human activities, such as rubber tapping and land clearing as well as fertiliser application. Fertiliser is usually applied in rectangular or square patches in between rows. Long-term high fertilisation always results in spatial variability of soil nutrients in rubber plantations. The different selection of sampling sites may finally result in different conclusions. Most researches in the past mainly focused on microbial biomass and activity in surface or subsurface soil of rubber plantations[3,4]. However, top soil properties cannot represent rhizosphere soil. There was little information on effects of rubber cultivation on the rhizosphere microbial community. It has long been recognised that soil directly adjacent to roots has a greater microbial biomass and a different composition from the bulk soil[10,11].

Accordingly, the main purpose of the present study was to investigate spatial variation in rhizosphere soil microbial community under rubber forest ecosystems with different soil parent material and years of rubber cultivation by using phospholipid fatty acid analysis. Knowledge on this may help us to assess potential ecological consequences of rubber cultivation on soil microbiology.

EXPERIMENTAL

Site Description and Selection

Hainan Island has a tropical monsoon climate with a mean annual temperature of 23 – 26ºC and annual precipitation of 1500 – 2000 mm. It usually takes six to eight years before the rubber plants are matured enough to be tapped. The type of soil that supports rubber plantations is lateritic soil. In order to make the samples representative, sample sites were chosen according to soil types before rhizosphere soil sampling. The rubber plantations in Hainan Island were divided into three soil type areas according to parent materials including granite, basalt and shallow marine deposit. In September 2010, eleven soil samples were collected from the three soil type areas (*Figure 1*). Soil samples from adjacent grass (mainly *Eleusine indica*) fields (GR) with less human disturbance in each area were also obtained. Grass fields were used as controls for com-parison with immature (R1, before tapping)and mature (R2, after tapping) rubber fields. Of 11 soil samples, three samples were taken from different grass fields, three samples from immature rubber fields and five samples from mature rubber fields. Parent materials of soils 1 to 3, soils 4 to 7 and soils 8 to 11 are granite, shallow marine deposit and basalt, respectively. The soils developed from granite, shallow marine deposit and basalt are classified as Hiweatheri-Udic Ferrosols, Alliti-Udic Ferrosols and Typic Rhodi-Udic Ferralosols according to the Chinese Soil Taxonomy, respectively[12], or Acrisols, Acrisols and Ferralsols in the World Reference Base for Soil Resources, respectively[13].

Soil Sampling and Chemical Analysis

Surface (from 0 to 200 mm) plant roots, including rubber tree and grass were carefully dug out of the soil, vigorously shaken and

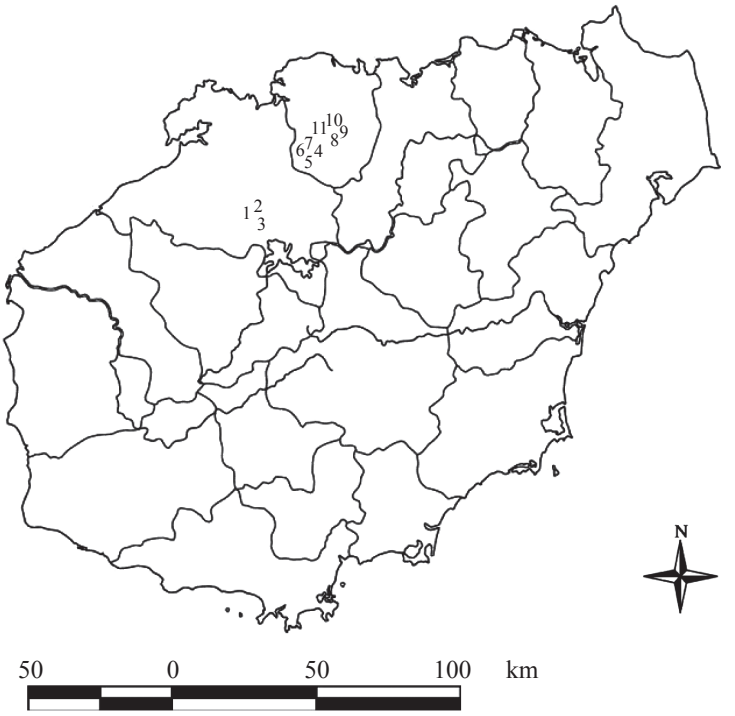

Figure 1. Location of the studied site.

the remaining soil attached to the roots were treated as the rhizosphere. Each sample was a composite of seven individual rhizosphere soil samples which were taken randomly at nearly one hectare of land. Unusual sites such as eroded sections, fence lines, fertiliser bands and old road beds were avoided during sampling.

Samples were taken to the laboratory in cool boxes with ice bags. Roots and stones were removed subsequently. One portion of the soil sample was air dried and ground to pass through a 2 mm sieve for soil property analyses and another portion of the soil sample was freeze-dried at –50ºC and stored at –70ºC for microbial community analysis. The soils were analysed using standard soil test methods as described by Lu[14]. Soil pH was measured in a 1:1 soil: water ratio. Soil organic carbon (SOC) was determined using the dichromate oxidation method. Soil total nitrogen (N) was determined using the semi-micro Kjeldahl method. Total phosphorus (P) and total potassium (K) were digested with Sodium Hydroxide (NaOH). Soil available P (Bray 1) was extracted with NH_4F-HCl solution. Soil available K was extracted with 1 mol L^{-1} NH_4OAc. The land use history, location and selected physical and chemical characteristics of these soils are described in *Table 1*.

Phospholipid Fatty Acid Analysis (PLFA)

Root material was removed from sieved soil samples before lipid extraction. The PLFAs were extracted, fractionated and

TABLE 1. LAND USE HISTORY, LOCATION, AND PROPERTIES USED IN THE STUDY

Soil no.	Land use	Latitude/ longitude	Parent materials	pH	SOC	TN g kg^{-1}	TP	TK	Available P (mg kg^{-1})	Available K (mg kg^{-1})	C/N %
1	GR	N19º29', E109º29'	Granite	4.78 a	11.27 d	0.38 d	0.25 cd	12.48 a	1.16 cd	32.43 b	29.91
2	R1-5 yr	N19º29', E109º29'	Granite	4.43 f	9.02 e	0.27 f	0.29 c	4.39 c	3.33 b	20.03 de	32.89
3	R2-18 yr	N19º29', E109º29'	Granite	4.60 c	9.73 e	0.30 e	0.21 de	8.50 b	1.28 cd	44.83 a	32.38
4	GR	N19º41', E109º39'	Shallow marine deposit	4.64 b	7.53 f	0.22 h	0.10 f	1.43 d	1.06 cd	17.05 de	33.93
5	R1-5 yr	N19º41', E109º39'	Shallow marine deposit	4.54 d	4.75 g	0.14 i	0.07 f	1.00 d	1.22 cd	15.07 de	33.10
6	R2-9 yr	N19º42', E109º39'	Shallow marine deposit	4.66 b	7.73 f	0.25 h	0.17 e	0.81 d	4.73 a	14.57 e	31.25
7	R2-28 yr	N19º42', E109º39'	Shallow marine deposit	4.48 e	7.83 f	0.22 g	0.10 f	0.50 d	1.61 c	11.60 e	35.41
8	GR	N19º45', E109º42'	Basalt	4.52 d	13.67 c	0.43 c	0.58 b	1.18 d	1.08 cd	18.04 de	31.70
9	R1-5 yr	N19º45', E109º42'	Basalt	4.43 f	17.53 b	0.59 a	0.59 b	1.28 d	1.65 c	23.50 cd	29.91
10	R2-19 yr	N19º45', E109º42'	Basalt	4.47 e	19.00 a	0.53 b	0.62 ab	1.43 d	0.78 d	32.92 b	35.69
11	R2-27 yr E109º42'	N19º45',	Basalt	4.60 c	19.51 a	0.59 a	0.66 a	1.25 d	0.96 d	31.93 bc	33.35

Means within a vertical column not followed by the same letter are significantly different at the 0.05 level. GR: grass field, R1: rubber before tapping, R2: rubber after tapping, TN: total N, TP: total P, TK: total K.

methylated using a modified method by Wu *et al*.[15]. Three grams (dry equivalent weight) was extracted with a chloroform-methanol-citrate butter mixture and the phospholipids were separated from other lipids on a silica-bonded phase column (SPE-Si, Supelco, Poole, UK). The phospholipid fraction was subjected to mild alkaline hydrolysis for producing fatty acid methyl esters before analysis. *c*19:0 was used as the internal standard. Fatty acids were analysed by Agilent 6890 gas chromatography with a flame ionisation detector carried out by a MIDI Sherlocks microbial identification system (Version 4.5, MIDI, Newark, NJ, USA).

The fatty acid nomenclature was used as follows: total number of carbon atoms: number of double bonds, followed by the position of the double bond (ω) from the methyl at the end of the molecule. *cis* geometry is indicated by the suffix *c*. The prefixes *a* and *i* refer to ante iso and iso-branching, 10Me indicates a methyl group on the tenth carbon atom from the carboxyl end of the molecule and *cy* indicates cyclopropane fatty acids[16]. Each value represents the mean of two replicates.

Bacterial PLFAs were represented by *i*15:0, *a*15:0, 15:0, *i*16:0, 16:1ω7*c*, *i*17:0, *a*17:0, *cy*17:0, 17:0, 18:1ω7*c* and *cy*19:0ω8*c*17; fungal PLFAs were represented by18:2ω6, 9*c* and 18:3ω6*c* (6,9,12)18, 19; actinomycetic PLFAs were represented by 16:0 (10Me), 17:0 (10Me), 18:0 (10Me)[20]. The branched, saturated PLFAs *i*14:0, *i*15:0, *i*16:0, *i*17:0, *a*15:0, *a*17:0 were chosen to represent Gram-positive bacteria (G^+)[21,22]; the monoenoic and cyclopropane unsaturated PLFAs 16:1ω5*c*, 16:1ω7*c*, 16:1ω9*c*, 17:1ω8*c*, 18:1ω5*c*, 18:1ω7*c*, 18:1ω9*c*, *cy*17:0, *cy*19:0 were chosen to represent Gram-negative bacteria (G^-)[23,24]. The following ratios of fatty acid relative abundance used as indexes of environmental stress were also calculated in this study: cyclopropyl:precursor [(*cy:pre*), (*cy*17:0 + *cy*19:0)/(16:1ω7 + 18:1ω7)]; total saturated:total monounsaturated [(*sat:mono*), (14:0 + 15:0 + 16:0 + 17:0 + 18:0 + 20:0)/(16:1ω9c + 16:1ω7*c* + 16:1ω5*c* + 17:1ω8*c* + 18:1ω9*c* + 18:1ω7*c* + 18:1ω5*c* + 19:1ω11*c* + 20:1ω9*c*)][25,26,27].

Statistical Analysis

Analysis of variance (ANOVA) was performed by using Tukey's comparison of the means. Correlation between microbial parameters and soil properties was tested by the Pearson correlation coefficient. ANOVA and correlation analysis were carried out using SPSS Version 13.0. Multivariate techniques were used to analyse the data for soil characteristics and PLFA data (as nmol PLFA g $soil^{-1}$). Principal component (PCA) and cluster analyses were performed using GenStat 9.2. Redundancy analyses (RDA) were performed using CANOCO 4.5. RDA is an ordination technique based on PCA, in which ordination axes are constrained to be linear combinations of environmental variables, thus allowing direct assessment of the relationship between environmental variables and variation in the multivariate data. Environmental variables tested in this study were soil characteristics. In graphical outputs, the position of the sites along the axes is determined by the loading scores, which describe the relative importance of a variable along the ordination axis.

RESULTS

Soil Characteristics

The 11 soils were located in three soil type areas of Hainan Province, latitude and longitude ranged from N19º29' to N19º45', E109º29' to E109º42', respectively. Soil

parent materials of the three areas vary greatly affecting the formation and properties of these soils. Soil pH, organic C, total N, total P, total K, available P, available K and C/N of the 11 soils ranged from 4.43 to 4.78, 4.75 to 19.51 g kg^{-1}, 0.14 to 0.59 g kg^{-1}, 0.07 to 0.66 g kg^{-1}, 0.50 to 12.48 g kg^{-1}, 0.78 to 4.73 mg kg^{-1}, 11.60 to 44.83 mg kg^{-1}, 29.91 to 35.69%, respectively. Total P was significantly higher in the soils with basalt than in the soils with granite and shallow marine deposit, total K was significantly higher in the soils with granite than in the soils with shallow marine deposit and basalt (*Table 1*).

PFLAs

Thirty-nine PLFAs were detected for the 11 soils. Among these, PLFA i19:0, 19:1ω11*c* were only detected in soils (soils 8 to 11) developed from basalt. The total microbial biomass ranged widely from 9.49 to 46.99 nmol g^{-1} in the 11 soils, which was significantly higher in the soils with basalt than in the soils with granite and shallow marine deposit. Total microbial biomass of soils with shallow marine deposit significantly increased with increasing years of rubber cultivation and declined significantly with increasing years of rubber cultivation of soils with basalt. The ratio of fungi to bacteria (F/B) was significantly higher in rubber plantations than in grass field on soils with the same parent material. There was no clear similar trend of total microbial biomass and F/B from immature rubber plantation to mature rubber plantation in the three areas (*Table 2*). Soil organic C, total N, total P were significantly positively correlated with total microbial biomass and most of the signature groups except F/B, *cy/pre* and *sat/mono*. The ratio of G^+ to G^- was negatively correlated with soil total K (*Table 3*).

Microbial Community Structure

Cluster analysis of the PLFAs data showed that the microbial community structure of 11 soils can be classified into two large groups (*Figure 2a*): (1) soil 1 to soil 7, (2) soil 8 to 11. The first group (Group 1) was further subdivided into: soil 1 and soil 4; soil 2, soil 3, soil 5 and soil 6; soil 7, respectively. The second group (Group 2) was further subdivided into soil 8; soil 9; soil 10 and soil 11, respectively. Principal component analysis of the PLFA data showed that the soils were also clearly discriminated by their PLFA profiles (*Figure 2b*). The principal component PC 1 and PC 2 explained 72.4% and 10.2% of the variation in the data, respectively. Soil 8 to 11 *versus* soil 1 to 7 were separated into two groups along the PC1 axis. PCA results also showed that soil 10 and soil 11 were clustered together closely, which were distinct with soil 8 and soil 9 along the PC2 axis. Soil 1 and soil 4 were clustered together more closely than soil 2, soil 6, soil 5 and soil 3 while soil 1 to 6 were distinct with soil 7 along PC2 axis.

The Relationships between Soil Microbial Community Structure and Soil Properties

The RDA performed with the soil properties and PLFA data showed that 87.5% of total variation could be explained by the first two axes. Axis 1 and axis 2 explained 79.7% and 7.8% of the variation, respectively. Axis 1 separated soils with basalt from the other soils. Among all soil properties, soil total N exhibited the greatest influence on the soil microbial community structure followed by soil organic C and soil total P, all of which was distributed in a positive direction along axis 1 (*Figure 3*).

TABLE 2. TOTAL PLFAs CONCENTRATION AND SIGNATURE GROUPS IN SOIL STUDIED

	Bacteria	Fungi	Actinomycete	Total PLFAs	G^+	G^-	F/B	G^+/G^-	*cy/pre*	*sat/mono*
				nmol g^{-1}						
				Soils with granite						
1	12.40a	0.48c	2.36a	24.19a	6.06a	5.01a	0.039 c	1.21a	2.45a	1.30a
2	6.93b	0.66b	1.35b	15.66b	3.54b	2.75b	0.095 b	1.28a	2.73a	1.51a
3	7.97b	0.94a	1.49b	17.76b	3.86b	2.96b	0.118 a	1.30a	1.40b	1.11a
				Soils with shallow marine deposit						
4	9.19ab	0.30b	2.17a	19.75a	4.90ab	3.56a	0.033 c	1.38a	2.72a	1.68a
5	4.27c	0.34b	0.71c	9.49c	2.15c	1.42b	0.080 a	1.51a	1.19b	1.34ab
6	7.69b	0.40b	1.41b	15.99b	3.92bc	2.71ab	0.052 b	1.45a	1.51b	1.41ab
7	10.99a	0.56a	2.10a	22.99a	5.55a	4.08a	0.051 b	1.36a	1.57b	1.22b
				Soils with basalt						
8	15.72b	0.47c	2.34c	31.75c	8.70b	6.12a	0.030 c	1.42a	3.61a	1.70a
9	20.15a	1.91a	4.36a	46.99a	11.02a	6.48a	0.095 ab	1.70a	1.22b	1.24b
10	18.87a	1.55b	3.96a	40.96b	10.54a	6.64a	0.082 b	1.59a	1.85b	1.27b
11	15.41b	1.67b	3.01b	34.24c	8.56b	5.29a	0.108 a	1.62a	1.69b	1.14b

Means within a vertical column for each soil parent material not followed by the same letter are significantly different at the 0.05 level. F/B is the ratio of fungal to bacterial PLFAs.

TABLE 3. PEARSON'S CORRELATION COEFFICIENTS BETWEEN TOTAL PLFAS CONCENTRATION, SIGNATURE GROUPS AND SOIL PROPERTIES FROM ALL SITES

	Bacteria	Fungi	Actinomycete	Total PLFA	G^+	G^-	F/B	G^+/G^-	*cy/pre*	*sat/mono*
pH	-0.27	-0.41	-0.28	-0.34	-0.31	-0.19	-0.37	-0.44	0.09	0.00
SOC	0.91**	0.87**	0.88**	0.91**	0.92**	0.87**	0.34	0.61*	0.01	-0.31
TN	0.91**	0.87**	0.88**	0.92**	0.92**	0.88**	0.32	0.60*	0.01	-0.30
TP	0.86**	0.79**	0.78**	0.86**	0.88**	0.84**	0.29	0.61*	0.16	-0.13
TK	-0.15	-0.15	-0.16	-0.19	-0.21	-0.06	0.06	-0.67*	0.13	-0.24
Available P	-0.41	-0.27	-0.39	-0.39	-0.41	-0.44	-0.03	-0.16	-0.10	0.16
Available K	0.27	0.50	0.28	0.27	0.24	0.27	0.57	-0.05	-0.16	-0.56
C/N	-0.08	0.00	-0.02	-0.07	-0.04	-0.07	0.07	0.08	-0.06	-0.05

Eleven soil samples were used in this analysis.
** Correlation is significant at the 0.01 level.
* Correlation is significant at the 0.05 level.

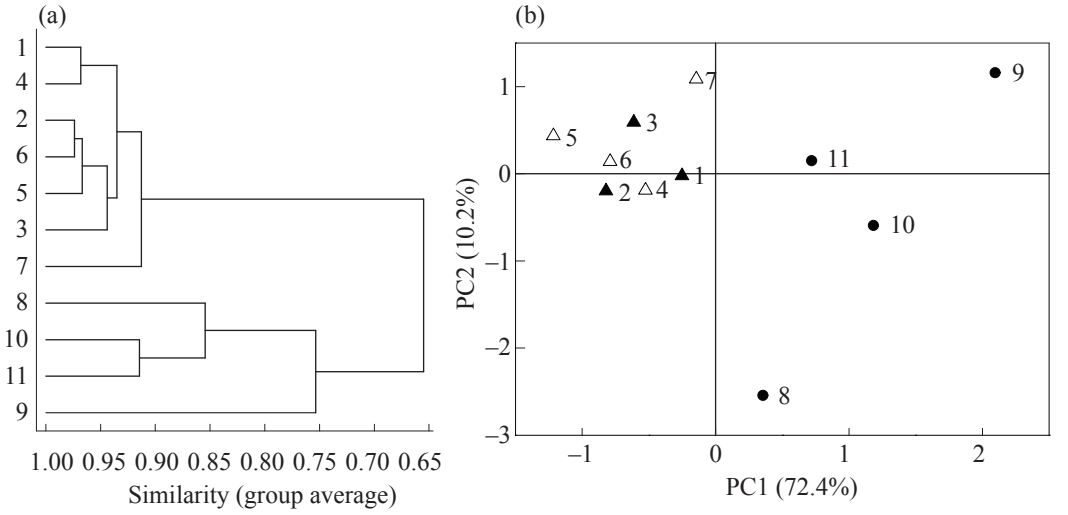

Figure 2. (a) Cluster analysis of lipids biomarker using group average method; and (b) the principal component analysis of lipids biomarker. ▲ Soils with granite, △ Soils with shallow marine deposit, ● Soils with basalt.

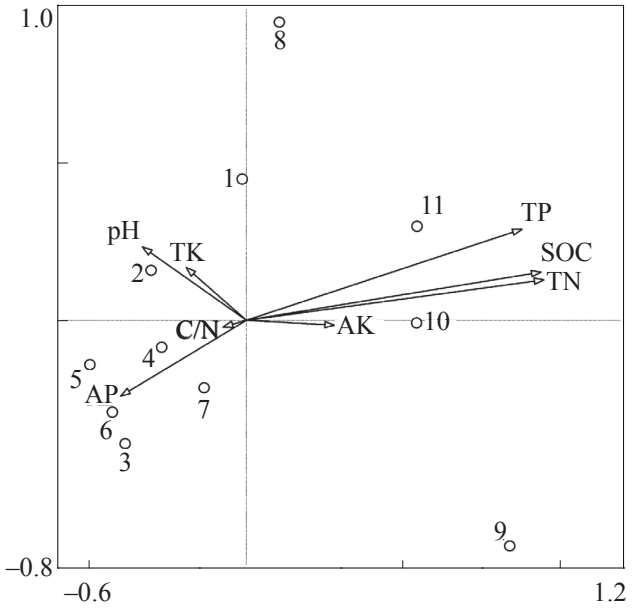

Figure 3. RDA ordination biplot of the 11 sites. Vectors represent the soil characteristics from 0 to 6 cm while centroids show the management factors that are significant by the Monte Carlo test ($p < 0.05$). Axes 1 and 2 represent 79.7% and 7.8% of the variation in the data, respectively.

DISCUSSION

Soil Microbial Biomass

The ratio of soil fungal biomass to bacterial biomass is an indicator of ecosystem self-regulation[28,29]. Higher F/B are suggested to be indicative for a more sustainable agroecosystem with lower impact on the environment, in which organic matter decomposition and N mineralisation dominate the provision of plant nutrients for crop growth[29,30]. It was found that the F/B of the rubber plantation was significantly higher than that of grass field on each soil type area in this study. This increase was mainly caused by an increase of fungal biomass. Environmental factors affecting fungal:bacterial dominance are: physical disturbance and tilling effects, direct and indirect nutrient effects, soil pH effects, soil moisture, temperature and elevated CO_2 effects[31,32]. Allison *et al*.[33] suggested that C inputs in the grassland mainly come from rapidly metabolised root exudates, which limit the development of fungi as this group uses more recalcitrant sources of C. As litter recalcitrance increases, the fungal role is expected to increase[34] and the most probable important reason for the greater importance of fungi in the decomposition of recalcitrant litter is related to their lignin degrading ability[35]. There is a general consensus in the literature that fungi are capable of degrading lignin whereas bacteria are not. This argument is valid for explaining our results, since the recalcitrant organic matter was suggested to be high in the topsoil of rubber plantations[8].

Microbial biomass is generally positively correlated with soil organic C and total N[36]. This is consistent with our findings that total PLFAs and most of the signature groups were significantly correlated with soil organic C and total N. We also found that microbial biomass was significantly positively correlated with soil total P and not soil available P. It was consistent with recent findings, that P can be a co-limiting nutrient for microbial growth along with C and N[37,38]. The quantities and forms of P in soil depend on soil-forming factors and may modify C and N accumulation during pedogenesis[39]. Thus, both soil P and C availability may concurrently limit the microbial activity in soils.

Microbial Community Structure

Rhizosphere is the zone surrounding the roots of plants in which complex relations exist among the plant, the soil microorganisms and the soil itself. Plant species, as well as the soil type, have a substantial influence on the structure and function of rhizosphere associated microbial populations[40–42]. As reviewed by Berg and Smalla[43], there is no general decision about the key player; both factors can dominate depending on biotic and abiotic conditions. In the present study, the results suggested that the primary differences in composition of 11 soil microbial communities are between soils with basalt *versus* soils with granite and shallow marine deposit and that the effect of plant species and plantation history were small. Different from soils with granite and shallow marine deposit, soils with basalt are relatively free of quartz and mica. Allitisation, ferritisation and biological enrichment of soil developed from basalt were intense as soils with basalt were weathered and tended to be finely textured and fertile[44]. It was also found that the average organic carbon, total nitrogen and total phosphorus of soils with basalt were greater than soils with granite and shallow marine deposit in this study. Different soil types or soils developed from the same parent material were assumed to harbour specific microbial communities, as shown in a continental-scale

study of soil bacterial communities[45]. Certain characteristics of soil environments can lead to overall similarities. Although spatial and temporal variations in soil properties may result in the formation of many discrete microhabitats on a much smaller scale, microorganisms have the ability to persist despite unfavourable conditions for growth, thereby permitting almost a universal presence of species[41,46].

Many studies have suggested that RDA is a suitable method for quantifying the relationship between environmental factors and changes in microbial community of soils[47,48]. As with RDA, it was found that soil PLFA profiles were strongly affected by soil total N, soil organic C and soil total P. Soil total N, total organic C and total P may be important factors influencing the composition of the soil microbial community in the present study. Previous studies had also reported that soil pH and C/N were related to soil microbial community structure[45,49,50], but it was not valid in this study possibly because all soils had similarly low pH and C/N.

Soil microorganisms participate in almost every chemical transformation taking place in soil and play a vital role in soil fertility as a result of their involvement in the cycle of carbon, nitrogen and phosphorus[51]. Studies showed that soil microbial biomass and microbial community could be sensitive as indicators of soil health and quality[29,52]. A healthy soil condition is a guarantee for normal growth of the rubber tree. In order to improve soil health of rubber plantations, more organic manure and nitrogen fertiliser should be applied on the soil with low microbial biomass.

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