CHARACTERISTICS OF RHIZOSPHERE AND BULK SOIL MICROBIAL COMMUNITIES IN RUBBER PLANTATIONS IN HAINAN ISLAND, CHINA

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GUO HC, WANG WB, LUO XH & WU XP. 2015. Characteristics of rhizosphere and bulk soil microbial communities in rubber plantations in Hainan Island, China. Phospholipid fatty acid analysis was used to investigate spatial variations in microbial communities of rhizosphere and bulk soil in rubber plantations in Hainan Island. Rhizosphere and bulk soil were collected from immature and mature rubber trees in areas with four different soil parent material types. For each site, total microbial biomass and biomass of bacteria, fungi, actinomycetes and ratio of fungi to bacteria in rhizosphere were significantly higher than those in bulk soil. The rhizosphere/bulk soil ratio for fungi in soil derived from basalt ranged from 10.44 to 12.33, which were significantly higher than those in soil derived from granitic gneiss, shallow marine deposits and granite (2.22–6.00). Total microbial biomass and bacterial biomass were positively correlated with soil organic carbon and total N in both rhizosphere and bulk soil. Total microbial biomass and biomass of bacteria, fungi and actinomycetes were correlated with soil total P in the rhizosphere. Rhizosphere total microbial biomass decreased in soil derived from basalt and increased in soil derived from shallow marine deposits with increasing age of rubber trees. The main factor affecting the composition of microbial communities in bulk and rhizosphere soil was soil parent material.

Keywords: Phospholipid fatty acid, soil parent material, basalt

INTRODUCTION

Rubber tree (*Hevea brasiliensis*) was first introduced to Hainan Island from Malaysia in 1906 (He & Huang 1987). This tree produces latex and timber, making it an economically important species. Since the 1950s, there has been a rapid expansion of rubber tree plantations in Hainan Island. At the end of 2010, rubber tree plantations covered 4.9×10^5 ha, accounting for 15.4% of the total land area of Hainan Island; these plantations made up the largest artificial ecosystem in the island (Statistical Bureau of Hainan Province 2011). Rubber tree plantations in Hainan Island are generally distributed between the central mountainous area and coastal area (Cheng et al. 2007). The main parent materials of soil in rubber plantations are granite, basalt, shallow marine deposits and granitic gneiss (He & Huang 1987, Zhang et al. 2007).

Soil microbes participate in almost every chemical transformation in the soil. They also play a vital role in soil fertility because they are involved in C, N and P cycles (Ushio et al. 2008), which provide nutrients for plant growth. Thus, soil microbes play an important role in nutrient conservation in tropical forests (Singh et al. 1989). With increasing rubber plantations, more studies have focused on potential ecological and environmental consequences of rubber tree cultivation. Such studies have evaluated effects of rubber tree cultivation on soil fertility, soil organic carbon and soil microbial biomass (Yang et al. 2004, Zhang et al. 2007). Some studies suggested that rubber tree cultivation resulted in significant decreases in soil organic carbon and microbial biomass (Yang et al. 2004, Zhang et al. 2007), while other studies showed that rubber plantations could potentially enhance soil carbon sequestration (Cheng et al. 2007). Rubber plantations are essentially a special type of secondary forest in tropical regions that are

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associated with human activities such as rubber tapping, land clearance and fertiliser application. Most studies have focused on microbial biomass and activity in surface or subsurface soil of rubber plantations (Cheng et al. 2007, Zhang et al. 2007). However, there is little information about effects of rubber cultivation on composition of microbial community in the rhizosphere and bulk soil of rubber trees.

Rhizosphere is the zone surrounding roots of plants. In this zone, there are complex interactions between plants, soil microorganisms and other components of the soil (nutrients and minerals). Plant species and soil type (its parent material) substantially affect the structure and function of microbial populations associated with the rhizosphere (Wagai et al. 2011, Kuramae et al. 2012, Prescott & Grayston 2013). Interactions among plant roots, soil minerals and soil microbial communities may be particularly important in the deeply weathered soil of rubber plantations. However, little is known about these interactions.

The main purpose of this study was to investigate the spatial variations in the microbial communities of rhizosphere and bulk soil in rubber plantations in Hainan Island. Soil samples from plantations of different ages and from plantations with soil derived from different parent materials were collected. The microbial communities in rhizosphere and bulk soil were analysed by phospholipid fatty acid analysis. Knowledge about factors affecting microbial communities may help assess the potential ecological consequences of rubber cultivation and to clarify effects of soil type on soil microbiology.

MATERIALS AND METHODS

Site description and selection

Hainan Island (latitude 18° 10' to 20° 09' N, longitude 108° 38' to 111° 02' E) is located in Hainan Province and lies off the southern coast of mainland China. The island has tropical monsoon climate with mean annual temperature of 23–26 °C and annual precipitation of 1500–2000 mm. Topography of the island is low on four sides and high in the centre. Approximately 20% of the island is mountainous, 15% hilly and 65% plains and tableland. Rubber plantations cover 15.4% of the total land area of the island and therefore make up the largest artificial ecosystem in the island. It usually takes 6 to 8 years before rubber trees are large enough to be tapped. Lateritic soil supports rubber plantations. To ensure that samples were representative of the variation in the island, sample sites were chosen according to soil parent materials before rhizosphere and bulk soil sampling was conducted. Rubber plantations in Hainan Island were divided into four areas according to the soil parent material types, i.e. basalt, granite, granitic gneiss and shallow marine deposits. The type of parent material and how the soil is formed will greatly influence the properties of the soil.

A total of 20 soil samples from 10 sites located in the four areas were collected. The rubber plantations were classified as immature (before tapping) and mature (after tapping) rubber fields. Of the 20 soil samples, 8 samples were collected from immature rubber fields and 12 from mature ones. The parent material of sites 1 and 2 was granitic gneiss, that of sites 3 to 5 was shallow marine deposits, that of sites 6 to 8 was basalt, and that of sites 9 and 10 was granite. The soils developed from granitic gneiss, shallow marine deposits, basalt, and granite are classified as Hiweatheri-Udic Ferrosols, Alliti-Udic Ferrosols, Typic Rhodi-Udic Ferralosols and Alliti-Udic Ferrosols respectively according to the Chinese Soil Taxonomy (Gong et al. 2000).

Soil sampling and chemical analysis

Surface (0 to 20 cm) rubber tree roots were carefully dug from the soil and shaken vigorously. The soil adhering to roots was treated as rhizosphere soil and the soil shaken from roots was treated as bulk soil. Each sample was a composite of seven individual rhizosphere soil samples, which were taken randomly from an area of approximately 1 ha. Unusual sites such as eroded sections, fence lines, fertiliser bands and old roads were avoided during sampling. Samples were taken to the laboratory in cool boxes with ice bags; roots and stones were removed. Each sample was divided into two parts—one part was air dried and ground to pass

through a 2-mm mesh size sieve to analyse soil properties, and the other part was freeze dried at -50 °C and then stored at -70 °C for microbial community analysis.

Soil was analysed using standard soil test methods described by Lu (1999). Soil pH was measured in a 1:1 soil:water mixture. Soil organic carbon was determined using the dichromate redox titration method. Soil total N was determined using the micro-Kjeldahl digestion followed by steam distillation. Total P and total K were digested with 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. Site location, landuse history at the site, and selected physical and chemical characteristics of the soil are shown in Table 1.

Phospholipid fatty acid analysis

Root material was removed from sieved soil samples before lipid extraction. Phospholipid fatty acids were extracted, fractionated and methylated according to the method of Wu et al. (2009). Each soil sample (3 g dry weight) was extracted with chloroform–methanol–citrate buffer mixture and the phospholipids were separated from other lipids on silica-bonded phase column. The phospholipid fraction

Table 1 Location, landuse history and properties of soil used in the study

Soil code	Location	Age of stand (years)	pH	SOC (%)	Total N (g kg^{-1})	Total P (g kg^{-1})	Total K (g kg^{-1})	Available P (mg kg^{-1})	Available K (mg kg^{-1})	C/N (%)
Soil derived from granitic gneiss										
1-1	19° 29' N, 109° 29' E	6	4.43	0.90	0.27	0.29	4.39	3.33	20.03	32.89
1-2	19° 29' N, 109° 29' E	6	4.34	0.88	0.26	0.32	4.36	3.30	13.58	33.64
2-1	19° 29' N, 109° 29' E	19	4.60	0.97	0.30	0.21	8.50	1.28	44.83	32.38
2-2	19° 29' N, 109° 29' E	19	4.43	0.74	0.27	0.21	9.72	0.63	28.46	26.85
Soil derived from shallow marine deposits										
3-1	19° 41' N, 109° 39' E	6	4.54	0.48	0.14	0.07	1.00	1.22	15.07	33.10
3-2	19° 41' N, 109°39' E	6	4.48	0.34	0.09	0.07	0.95	0.69	6.64	37.67
4-1	19° 42' N, 109° 39' E	10	4.66	0.77	0.25	0.17	0.50	4.73	14.57	31.25
4-2	19° 42' N, 109° 39' E	10	4.79	0.53	0.14	0.14	0.74	3.12	8.12	37.24
5-1	19° 42' N, 109° 39' E	29	4.48	0.78	0.22	0.10	0.81	1.61	11.60	35.41
5-2	19° 42' N, 109° 39' E	29	4.34	0.66	0.20	0.10	0.63	0.91	8.62	33.81
Soil derived from basalt										
6-1	19° 45' N, 109° 42' E	6	4.43	1.75	0.59	0.59	1.43	1.65	23.50	29.91
6-2	19° 45' N, 109° 42' E	6	4.31	1.21	0.35	0.49	1.34	1.50	9.61	34.61
7-1	19° 45' N, 109° 42' E	18	4.47	1.90	0.53	0.62	1.25	0.78	32.92	35.69
7-2	19° 45' N, 109° 42' E	18	4.35	1.24	0.38	0.59	1.22	1.38	11.60	32.78
8-1	19° 45' N, 109° 42' E	28	4.60	1.95	0.59	0.66	1.28	0.96	31.93	33.35
8-2	19° 45' N, 109° 42' E	28	4.54	1.08	0.35	0.62	1.25	0.92	9.61	30.66
Soil derived from granite										
9-1	18° 46' N, 109° 27' E	6	4.60	1.08	0.33	0.14	18.16	6.32	117.74	33.28
9-2	18° 46' N, 109° 27' E	6	4.55	0.86	0.33	0.12	18.94	3.82	92.94	26.41
10-1	18° 46' N, 109° 28' E	16	4.63	1.31	0.40	0.16	29.24	1.17	73.10	32.60
10-2	18° 46' N, 109° 28' E	16	4.67	1.13	0.35	0.16	28.58	0.83	65.66	32.08

Values represent means of three replicates; -1 = rhizosphere soil, -2 = bulk soil; SOC = soil organic carbon

was subjected to mild alkaline hydrolysis to produce fatty acid methyl esters before analysis. The internal standard was 19:0. Fatty acids were identified and quantified using gas chromatograph with flame ionisation detector, and the MIDI Sherlock microbial identification system (Version 4.5)

The following fatty acid nomenclature was used—total number of C atoms:number of double bonds, followed by the position of the double bond (ω) from the methyl end of the molecule. *Cis* geometry is indicated by the suffix c. The prefixes a and i refer to anteiso- and iso-branching, 10Me indicates a methyl group on the 10th C atom from the carboxyl end of the molecule and cy indicates cyclopropane fatty acids (Bossio et al. 2005). Values shown are means of two replicates.

Bacterial phospholipid fatty acids were represented by i15:0, a15:0, 15:0, i16:0, 16:1ω7c, i17:0, a17:0, cy17:0, 17:0, 18:1ω7c, and cy19:0ω8c (Bossio et al. 1998); fungal phospholipid fatty acids were represented by 18:2ω6,9c and 18:3ω6c (6,9,12) (Vestal & White 1989, Myers et al. 2001); and actinomycetic phospholipid fatty acids were represented by 16:0 (10Me), 17:0 (10Me), and 18:0 (10Me) (Turpeinen et al. 2004). The branched, saturated phospholipid fatty acids i14:0, i15:0, i16:0, i17:0, a15:0 and a17:0 were chosen to represent Gram positive bacteria (G^+) (O'Leary & Wilkinson 1988, Zogg et al. 1997). The monoenoic and cyclopropane unsaturated phospholipid fatty acids 16:1ω5c, 16:1ω7c, 16:1ω9c, 17:1ω8c, 18:1ω5c, 18:1ω7c, 18:1ω9c, cy17:0 and cy19:0 were chosen to represent Gram negative bacteria (G^-) (Ratledge & Wilkinson 1988, Mutabaruka et al. 2007).

Statistical analysis

Analysis of variance (ANOVA) was performed using Tukey's test to compare means. Correlations between microbial parameters and soil properties were tested by calculating Pearson's correlation coefficients. ANOVA and correlation analyses were carried out using SPSS Version 13. Multivariate techniques were used to analyse the data for soil characteristics and phospholipid fatty acid (as nmol phospholipid fatty acid g $soil^{-1}$). Principal coordinate analysis (PCA) and cluster analysis were performed using GenStat 12.1.

RESULTS

Chemical properties of rhizosphere and bulk soil

The 10 sample sites were located in areas with four different soil parent material types. Soil organic carbon and available K were higher in the rhizosphere soil than in the bulk soil at each site (Table 1). Rhizosphere soil pH was higher than that of bulk soil at all sites except for sites 4 and 10. Soil parent materials varied between the four sites, and greatly affected formation and properties of the soil at these sites. Total P was significantly higher in soil with basalt parent material than in soil with granitic gneiss, shallow marine deposit and granite parent materials. Total K and available K were significantly higher in soil with granite parent material than those in soil with granitic gneiss, shallow marine deposit and basalt parent materials.

Microbial communities of rhizosphere and bulk soil

Total microbial biomass and biomass of bacteria, fungi, actinomycetes, G^+ and G^- were higher in rhizosphere soil than in bulk soil at each site (Table 2). Ranges of rhizosphere:bulk soil ratios for the various components of the microbial biomass were as follows: 1.72–4.66 for total microbial biomass, 1.68–4.46 for bacteria biomass, 2.22–12.33 for fungi biomass, 1.31–5.32 for actinomycetes biomass, 1.41–3.95 for G^+ biomass and 1.92–4.15 for G^- biomass. The fungal to bacterial ratio was higher in the rhizosphere soil than in the bulk soil for all sites except for sample site 9 (Table 2).

Soil total microbial biomass varied with increasing years of rubber tree cultivation (Tables 1 and 2). There were no significant differences in total microbial biomass between the two bulk soil derived from granitic gneiss and between the two bulk soil derived from granite (Table 2). Similarly, there were no significant differences in total microbial biomass between the two rhizosphere soil derived from granitic gneiss and between the two rhizosphere soil derived from granite. In soil derived from shallow marine deposits, there were no significant differences in total microbial biomass between bulk soil from 10 and 29 years of rubber tree

Table 2 Total phospholipid fatty acid concentrations and signature groups in studied soil

Soil code	Total PLFAs (nmol g^{-1})	Bacteria (nmol g^{-1})	Fungi (nmol g^{-1})	Actinomycetes (nmol g^{-1})	G^+ (nmol g^{-1})	G^- (nmol g^{-1})	F/B	G^+/G^-	Total PLFAs	Bacteria	Fungi	Actinomycetes
									R/S ratio			
Soil with granitic gneiss												
1-1	15.66 a	6.93 a	0.66 b	1.35 a	3.54 a	2.75 a	0.095 b	1.28 a	2.22	2.07	6.00	1.80
1-2	7.04 b	3.35 b	0.11 c	0.75 b	1.78 b	1.39 b	0.033 d	1.28 a				
2-1	17.76 a	7.97 a	0.90 a	1.49 a	3.86 a	2.96 a	0.113 a	1.30 a	1.87	1.99	3.46	1.80
2-2	9.49 b	4.01 b	0.26 c	0.83 b	2.09 b	1.52 b	0.064 c	1.38 a				
Soil with shallow marine deposit												
3-1	9.49 c	4.27 c	0.34 b	0.71 c	2.15 c	1.42 c	0.080 a	1.51 b	2.09	2.03	3.09	1.78
3-2	4.53 d	2.10 c	0.11 c	0.40 d	1.19 d	0.74 d	0.053 b	1.61 ab				
4-1	16.12 b	7.69 b	0.40 b	1.41 b	3.92 b	2.71 b	0.052 b	1.45 b	2.17	2.32	3.08	1.86
4-2	7.42 c	3.32 c	0.13 c	0.76 c	1.96 c	1.15 c	0.040 c	1.70 a				
5-1	22.99 a	10.99 a	0.52 a	2.10 a	5.55 a	4.08 a	0.047 bc	1.36 b	3.34	3.40	4.73	2.84
5-2	6.89 c	3.23 c	0.11 c	0.74 c	1.70 d	1.24 c	0.033 c	1.37 b				
Soil with basalt												
6-1	47.61 a	20.15 a	1.80 a	4.36 a	11.02 a	6.48 a	0.090 b	1.70 a	4.66	4.46	10.59	5.32
6-2	10.21 d	4.52 c	0.17 c	0.82 c	2.79 c	1.56 b	0.038 c	1.79 a				
7-1	41.08 b	18.87 ab	1.48 b	3.96 a	10.54 a	6.64 a	0.079 b	1.59 a	2.94	2.60	12.33	3.54
7-2	13.99 d	7.25 c	0.12 c	1.12 c	4.36 c	2.57 b	0.016 d	1.70 a				
8-1	34.31 c	15.41 b	1.67 ab	3.01 b	8.56 b	5.29 a	0.108 a	1.62 a	3.16	2.86	10.44	3.63
8-2	10.87 d	5.38 c	0.16 c	0.83 c	3.31 c	1.76 b	0.030 c	1.88 a				
Soil with granite												
9-1	26.01 a	12.62 a	0.71 a	2.50 a	8.24 a	3.39 a	0.056 a	2.43 a	2.10	2.50	2.22	2.08
9-2	12.41 b	5.04 b	0.32 b	1.20 b	3.07 c	1.47 b	0.064 a	2.09 a				
10-1	25.18 a	11.54 a	0.75 a	2.30 a	5.86 b	3.96 a	0.065 a	1.48 b	1.72	1.68	4.17	1.31
10-2	14.64 b	6.88 b	0.18 c	1.76 b	4.17 bc	2.01 b	0.027 c	2.07 a				

Values represent means of two replicates; means within a column for each soil parent material followed by different letters are significantly different at the 0.05 level; -1 = rhizosphere soil, -2 = bulk soil; total PLFAs = total phospholipid fatty acids, G^+ = Gram positive bacteria, G^- = Gram negative bacteria, F/B = ratio of fungal to bacterial phospholipid fatty acids, R/S ratio = ratio of rhizosphere to bulk soil phospholipid fatty acids

cultivation. However, total microbial biomass in the rhizosphere soil significantly increased with increasing years of rubber tree cultivation. In soil derived from basalt, there were no significant differences in total microbial biomass between the three bulk soil. However, the total rhizosphere microbial biomass significantly decreased with increasing years of rubber tree cultivation.

Correlations between phospholipid fatty acid groups and soil properties

In the rhizosphere soil, total microbial biomass and the biomass of bacteria, fungi, actinomycetes, G^+ and G^- were positively correlated with soil organic carbon, total N and total P (at the 0.01 level) (Table 3). The G^+ to G^- ratio was positively correlated with soil available K (at the 0.01 level, Table 3). In the bulk soil, total microbial biomass and biomass of bacteria, G^+ and G^- were significantly positively correlated with soil organic carbon and total N. Fungal biomass was significantly positively correlated with available K but negatively correlated with the C/N ratio. Actinomycetes biomass was significantly positively correlated with total N, total K and available K. Biomass of G^- was significantly positively correlated with total P. The G^+/G^- ratio was positively correlated with available K.

Microbial community structure

Cluster analysis of the phospholipid fatty acid data showed that the microbial community structure in the 20 soil samples could be classified into three large groups (Figure 1a): (1) 1-1, 2-1, 3-1, 4-1, 5-1, 10-1, 10-2, 1-2, 4-2, 3-2, 2-2, 9-2 and 5-2; (2) 9-1, 6-2, 7-2 and 8-2 and (3) 6-1, 7-1 and 8-1. The first group (Group 1) was further subdivided into two groups: 1-1, 2-1, 3-1, 4-1, 5-1, 10-1 and 10-2; and 1-2, 4-2, 3-2, 2-2, 9-2 and 5-2. PCA of the phospholipid fatty acid data showed that the soil was clearly discriminated by their

Table 3 Pearson's correlation coefficients between soil properties and total phospholipid fatty acids/ signature groups in rhizosphere and bulk soil

Parameter	Total PLFAs	Bacteria	Fungi	Actinomycetes	G^+	G^-	F/B	G^+/G^-
				Rhizosphere soil				
pH	-0.36	-0.32	-0.32	-0.36	-0.29	-0.38	-0.18	0.18
SOC	0.91**	0.90**	0.94**	0.89**	0.88**	0.91**	0.38	0.22
Total N	0.92**	0.91**	0.96**	0.90**	0.88**	0.90**	0.39	0.24
Total P	0.83**	0.80**	0.95**	0.80**	0.77**	0.84**	0.53	0.05
Total K	-0.07	-0.05	-0.18	-0.05	-0.01	-0.13	-0.21	0.32
Available P	-0.26	-0.22	-0.41	-0.23	-0.10	-0.36	-0.47	0.59
Available K	0.10	0.15	-0.01	0.13	0.26	-0.01	-0.18	0.77**
C/N	-0.04	0.03	-0.12	0.00	0.04	0.10	-0.22	-0.03
				Bulk soil				
pH	0.13	0.08	0.18	0.32	0.12	-0.14	0.12	0.53
SOC	0.82**	0.85**	0.13	0.62	0.85**	0.87**	-0.55	0.41
Total N	0.88**	0.87**	0.38	0.67*	0.86**	0.86**	-0.35	0.47
Total P	0.42	0.53	-0.18	0.04	0.55	0.66*	-0.56	0.11
Total K	0.61	0.48	0.61	0.85**	0.46	0.27	0.19	0.57
Available P	-0.02	-0.11	0.25	0.04	-0.09	-0.14	0.19	0.12
Available K	0.58	0.40	0.80**	0.72*	0.41	0.18	0.39	0.64*
C/N	-0.54	-0.41	-0.85**	-0.43	-0.37	-0.37	-0.40	-0.23

Total PLFAs = total phospholipid fatty acids, G^+ = Gram positive bacteria, G^- = Gram negative bacteria, F/B = ratio of fungal to bacterial phospholipid fatty acids; SOC = soil organic carbon; ** = correlation is significant at the 0.01 level, * = correlation is significant at the 0.05 level

phospholipid fatty acid profiles (Figure 1b). The first and second dimensions explained 34.81 and 20.18% of variation in the data respectively. PCA results showed that the microbial community of the rhizosphere soil was distinct from that of the bulk soil at each site. Comparison of the rhizosphere microbial community structure between sites showed that 6-1, 7-1 and 8-1 clustered together closely, and were separated from 9-1 along the second dimension axis, and from 1-1, 2-1, 3-1, 4-1, 5-1 and 10-1 along the first dimension axis. Comparisons of the bulk soil microbial community structure between sites showed that 6-2, 7-2 and 8-2 clustered together closely and were separated from the seven bulk soil sites that clustered closely along the first dimension axis.

DISCUSSION

Phospholipid fatty acids are widely accepted as biomarkers of viable components of the soil microbial biomass. Consequently, phospholipid fatty acid analysis provides more detailed information than that obtained from culture-based analyses on active soil microbial community (Vestal & White 1989). Compared with bulk soil, rhizosphere soil often has greater microbial biomass and different microbial community compositions (Zak et al. 1996, Sørensen 1997). In this study, total microbial biomass and the biomass of bacteria, fungi, actinomycetes, G^+, G^- and the fungi:bacteria ratio were all significantly higher in rhizosphere soil than in bulk soil at each site. This may be due to of effects of plants on rhizosphere soil. Exudates and deposits from plant roots supply organic nutrients for microorganisms, and greatly affect their abundance and activity (Bardgett & McAlister 1999).

The rhizosphere:bulk soil ratio for components of the microbial population is widely used to evaluate the degree of rhizosphere effect (Morgan et al. 2005). The rhizosphere:bulk soil ratios for fungi in soil derived from basalt ranged from 10.44 to 12.33. These ratios were significantly higher than those in soil derived from granitic gneiss, shallow marine deposits or granite (ranging from 2.22 to 6.00). The magnitude of the rhizosphere effect depends on the nature and amount of root exudates, which are related to plant species and plant age, on edaphic factors such as soil type and fertility, and on climatic factors such as soil

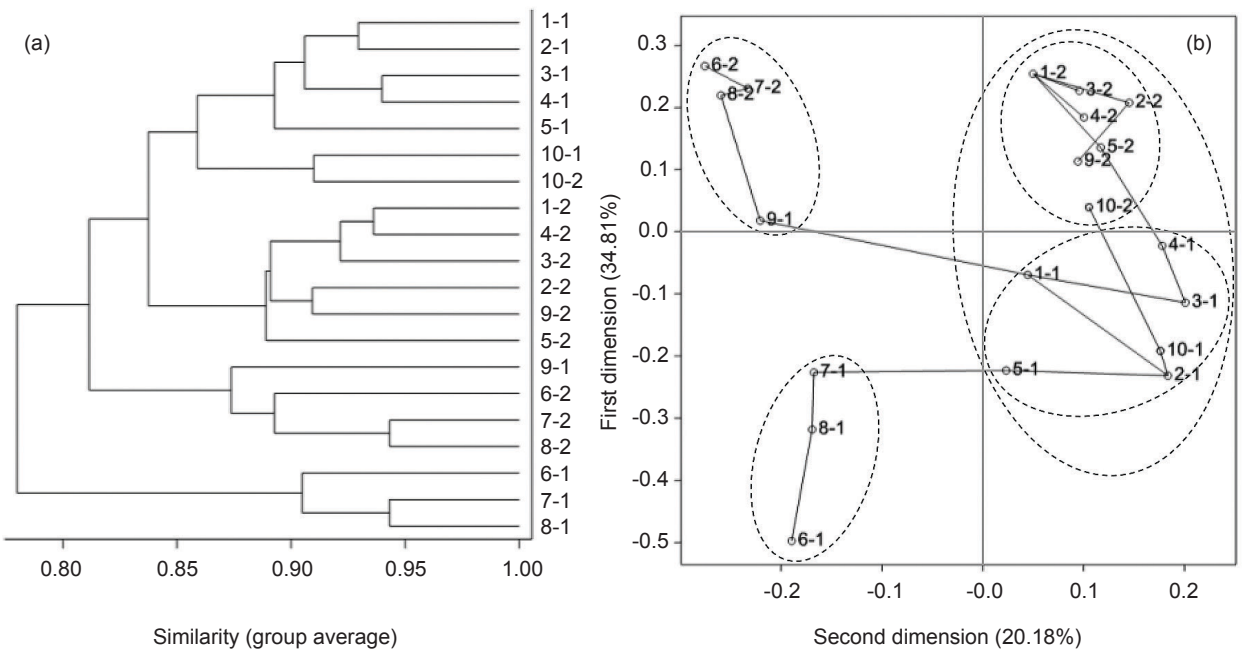

Figure 1 (a) Cluster analysis generated using group average method for soil microbial communities based on phospholipids fatty acid analysis and (b) minimum spanning tree constructed using principal coordinates analysis; -1 = rhizosphere soil, -2 = bulk soil

temperature and moisture availability (Pandey & Palni 2007). Fungi were more abundant in rhizosphere soil derived from basalt than in rhizosphere soil derived from other parent materials. In the present study, the rhizosphere effect on fungi community was more influenced by soil parent materials than by rubber tree age. There was also stronger rhizosphere effect on fungal communities than on bacterial and actinomycete communities in soil derived from basalt. This is inconsistent with results of other studies. It was reported that the difference between fungal populations in rhizosphere and bulk soil was generally smaller than the difference between bacterial populations in rhizosphere and bulk soil (Curl & Truelove 1986, Buyer et al. 2002). The stronger rhizosphere stimulatory effect on the fungal biomass in soil derived from basalt compared with that in soil derived from other parent materials may be related to edaphic properties of the soil. That is, root-induced processes may serve as a starter, but edaphic factors may be the main determinants of the enhanced fungal population in rhizosphere soil derived from basalt.

The rhizosphere total microbial biomass decreased in soil derived from basalt but increased in soil derived from shallow marine deposits with increasing years of rubber tree cultivation. Total microbial biomass was also not significantly different between bulk soil derived from basalt or between bulk soil derived from shallow marine deposits. In soil derived from shallow marine deposits, the three rhizosphere soil showed progressive increase in total microbial biomass with increasing age of the rubber tree plantation. Similar findings were also found on oil palm plantation soil derived from granite (Haron et al. 1998) and *Eucalyptus* plantation soil derived from arenaceous shale (Chen et al. 2013). This might result from the increase in soil organic carbon content (Table 1) that would favour microbial growth under the stimulation of plant roots (Gray & Williams 1971). Soil C concentration in the top soil was reported to decrease in the first 5 years after planting rubber trees, and then increase to reach a steady state after approximately 20 years because of increased inputs of leaf litter and root residues (de Blécourt et al. 2013). Our results showed that the rhizosphere microbial activity decreased dramatically with increasing age of rubber trees in soil derived from basalt. This might be partly explained by decreased soil fertility (for example, soil total K and soil available P) caused by loss of nutrients from the ecosystem via latex harvesting and partly by increased disturbance from agricultural practices and surface compaction after tillage (Zhang & Zhang 2005).

In general, microbial biomass is positively correlated with soil organic carbon and total N (Smith & Paul 1990). Our findings were consistent with this. Total phospholipid fatty acids were significantly correlated with soil organic carbon and total N. Total microbial biomass and biomass of bacteria, fungi and actinomycetes were also correlated with soil total P only in rhizosphere soil. This showed that the microbial activity in bulk soil was not affected by total P. Phosphorus is widely considered to constrain primary productivity in tropical forests on strongly weathered soils, in which P is mainly present in occluded inorganic forms. Phosphate is bound into the structure of secondary minerals and organic P compounds (Turner & Engelbrecht 2011). Trees have evolved various mechanisms to acquire P in soil with low P availability. Root-induced chemical changes in the forest rhizosphere may increase the solubility of inorganic P, e.g. exudation of organic acids decreases pH and increases P solubility, and excretion of phosphatases increases P availability by converting organic P into inorganic P (Fox et al. 2011). Greater P availability in rhizosphere soil than in bulk soil may explain the greater microbial biomass in rhizosphere soil detected in the present study.

Differences in microbial community structure have been attributed to differences in resource availability, particularly differences in types and amounts of root exudates, quantitative and qualitative changes in the inputs of organic substrates, differences in soil nutrient availability, and different plant species (Yeates et al. 1997, Ushio et al. 2008). Since all sample sites were in the same climatic region, the main factors responsible for differences in soil microbial communities in this study were soil type and plant age. There is no general decision about the key player: both factors can dominate depending on the biotic and abiotic conditions (Berg & Smalla 2009). Results from cluster analysis

and PCA showed that the main differences in composition between the 10 rhizosphere microbial communities and the 10 bulk soil microbial communities were attributed to soil parent materials. The soil derived from basalt showed significantly different microbial communities from those in soil derived from other parent materials. Different from findings from citrus orchard (Yao et al. 2000) and *Eucalyptus* plantation (Chen et al. 2013), i.e. plantation age had significant effect on total phospholipid fatty acid composition of soil microbial communities, rubber plantation age had weak effect on rhizosphere microbial community structure in this study. Soil parent materials and weathering degree control many soil characteristics, particularly soil texture, types of clay minerals present and the availability of soil nutrients to biota (Ulrich & Becker 2006). Unlike soil formed from granitic gneiss, granite and shallow marine deposits, those formed from basalt are relatively free of quartz and mica, which is regarded as one of the most weatherable rocks. Tropical soil derived from basalt has clay texture, is strongly structured and contains 2:1 lattice clay minerals. Most is highly fertile and unlike most tropical soil, is able to sustain continuous dry cropping (Young 1976). In this study, average soil organic carbon, total N and total P were higher in soil derived from basalt than their respective levels in soil derived from other parent materials. Soil developed from the same parent material is assumed to harbour the same specific microbial communities, as shown in a continental-scale study of soil bacterial communities (Fierer & Jackson 2006). In addition, the parent material may have stronger effect on soil microbial community at earlier stages than at later stages of ecosystem development (Wagai et al. 2011).

CONCLUSIONS

The total microbial biomass and biomass of most signature groups were significantly higher in rhizosphere soil than in bulk soil at all tested sites. Total microbial biomass and the biomass of bacteria, fungi and actinomycetes were also affected by soil total P in the rhizosphere environment. The rhizosphere stimulatory effect on fungal biomass and the difference in microbial community structure within rhizosphere and bulk soil were greater in soil derived from basalt than those derived from other parent materials. The microbial community structures in soil of rubber plantations in Hainan Island were strongly affected by soil parent material. To develop the best nutrient management practices for rubber tree plantations according to soil type (or soil derived from different parent materials), further studies should evaluate effects of organic manure and chemical fertiliser on microbial communities in the rhizosphere of rubber trees. This would help to optimise rubber tree growth and latex yield and minimise adverse environmental impacts on soil microbial populations.

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