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Change in Soil Microbial Community Compositions and Diversity Following the Conversion of Tropical Forest to Rubber Plantations in Xishuangbanan, Southwest China

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Abstract

The ecological consequences of converting tropical forests to rubber plantations on the soil microbial compositions and diversity remain unknown. By using an Illumina MiSeq sequencing analysis, we assessed the compositions and diversity of bacterial and fungal community in soils of rubber plantation (or rubber forest, RF), secondary tropical forest (STF), and tropical seasonal rainforest (TSR) in Xishuangbanna, southwest China. Our findings revealed that (a) for bacterial composition, *Bacillaceae* was the most dominant family (13.60%) in RF soil, while it only accounted for 4.13% in STF and 6.92% in TSR. For fungal composition, the largest family in soils of RF was *Basidiomycota_unclassified*. However, the largest family in STF and TSR was *Russulaceae*. (b) Number of operational taxonomic units, Chao index, and Shannon index of bacterial community in soil of RF were significantly higher than those of TSR and STF. However, these diversity indices of fungal community in RF were significantly lower than those of TSR and STF. (c) Soil pH and total phosphorus were very important drivers for bacterial community, whereas soil organic matter and total nitrogen were the most important factors for fungal community. (d) The microbial biomass carbon in RF was relative lower than those in STF and TSR, which suggested that the total microbial biomass decreased after forest conversion. To protect the total diversity of this region, the individual farmers should use herbicides as little as possible to reserve ground vegetation. And the government could outline a land-use policy that prohibits the cultivation in areas of natural vegetation.

Keywords

bacterial compositions, bacterial diversity, rubber plantation, secondary tropical forest, tropical seasonal rainforest

Introduction

Due to intensifying human disturbance, over half of the world's tropical forests are reforested or afforested secondary forests or plantations (Wang et al., 2017). Xishuangbanna, one of the richest regions in China, belongs to the Indo-Burma biodiversity hotspot (Myers, Mittermeier, Mittermeier, Da Fonseca, & Kent, 2000). However, the region's biodiversity is threatened by the dramatic land use or land cover changes in past 40 years, especially the decrease in natural forest cover from about 70% in the 1970s to 50% in the 2000s due to the expansion of rubber plantations (Li, Aide, Ma, Liu, & Cao, 2007; Li, Ma, Aide, & Liu, 2008; Li, Ma,

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Liu, & Liu, 2009). The area of rubber monoculture plantations was 4.5% of the total area of Xishuangbanna in 1988, 9.9% in 2002, and 22.2% in 2010, and rubber monoculture expanded to higher elevations and onto steeper slopes between 1988 and 2010 (Chen et al., 2016). Rubber cultivation has significantly improved local livelihoods (Bierregaard, Gascon, Lovejoy, & Mesquita, 2001), for example, if the rubber farmer has 1 ha of tapped rubber plantation, he will gain an increase of about US$1,470 per year, accounting about 50% to 60% of his total income (Liu, 2015). However, the conversion of nature tropical forests to rubber plantations has posed great challenges for local ecosystem, such as changes of soil carbon stock, soil biomass, and biodiversity (Bierregaard et al., 2001; Soares-Filho et al., 2009). The expansion of rubber plantation and its impacts on the environment in Xishuangbanna have attracted much attention by local government and ecologists. However, till now there are very limited understanding of how does forest conversion affect soil microbial composition and diversity in Xishuangbanan.

Compared with primary tropical forest, rubber plantations with simpler structure have less litter (Deng et al., 2003). Moreover, rubber plantations lacking understory vegetation cover can cause higher soil erosion (Li, Ma, Liu, & Liu, 2012). Application of N fertilizer, straw return after harvest, and cultivation could markedly influence the relative contribution of bacteria and fungi to specific soil nitrification processes and cycling of carbon (Wang, Liu, Zhang, & Cai, 2015). Previous studies have shown that conversion of rain forests to managed systems results in more pronounced changes in soil microbial community compositions that total phospholipid fatty acids especially that of gram-negative bacteria decreased (Krashevska, Klarner, Widyastuti, Maraun, & Scheu, 2015). Lan, Li, Wu, & Xie (2017) also found soil bacterial community compositions have changed after tropical secondary forest conversion to rubber plantation in Hainan Island of China. The compositions and diversity of soil microbial communities are largely believed to directly influence a wide range of ecosystem processes (Schimel, 1995). However, how does microbial community (including bacteria and fungi) response to forest conversion, and the mechanisms responsible for shifts in the soil microbial community remain largely unknown (De Vriesa, Hoffl, Van Eekerenc, Brussaard, & Bloem, 2006).

In this study, we focused on the effects of forest conversion to rubber plantations on the soil bacterial and fungal community compositions and diversity. We investigated the bacterial and fungal community of rubber plantations (rubber forest [RF]), secondary tropical forest (STF), and tropical seasonal rainforest (TSR) using a high-throughput Illumina MiSeq sequencing analysis. In light of the existing work, the objectives of the current study were (a) to clarify whether there are significant differences in soil bacterial and fungal compositions and diversity among RF, STF, and TSR communities and (b) to understand how conversion of tropical forest to rubber plantations affects both bacterial and fungal communities.

Methods

Study Site

The study site is located in the Xishuangbanna National Nature Reserve in Southwestern China (101°34′E, 21°36′N). It has a mountainous topography, which borders Myanmar in the southwest and Laos in the southeast. The region is dominated by a typical monsoon climate with an alternation between a dry season and a rainy season. The mean annual temperature is 21.0°C, and the mean annual precipitation is 1532 mm, of which approximately 80% occurs between May and October (the rainy season). The dry season is from November to April (Zhang & Cao, 1995). The vegetation of Xishuangbanna is classified into four main vegetation types: tropical rain forest, tropical seasonal moist forest, tropical montane evergreen broad-leaved forest, and tropical monsoon forest (Cao & Zhang, 1997; Zhu, 2006; Zhu, Cao, & Hu, 2006).

Sample Collection

We selected a total of nine 20×20 m plots within the rubber plantations (RF), STF, and TSR with each forest three plots. The three plots of TSR were distributed in Xishuangbanna nature reserve (Figure 1). The elevation of RF, STF, and TSR was 580 m, 630 m, and 750 m, respectively. TSR were mainly composed of *Mezzettiopsis creaghii*, *Ardisia neriifolia*, *Saprosma ternatum*, *Gironniera subaequalis*, *Aidia pycnantha*, *Drypetes indica*, and *Mussaenda pubescens*. STF were mainly composed by *Litsea mollis*, *Litsea umbellate*, *Litsea elongate*, *Schefflera bodinieri*, *Phoebe lanceolate*, *Ficus hirta*, *Goniothalamus griffithii*, and *Pratia nummularia*. The plot of RF was located in individual farms and was mainly composed by herbaceous species, such as *Cyrtococcum patens*, *Commelina communis*, and *Cyclosorus interruptus*. All soils of the three forests are Latosols. The RF was converted from STF about 20 years ago. The age of STF is about 20 to 30 years. Fertilizers and herbicides were applied in the RF but not applied in the STF and the TSR. Soil samples of 0 to 20 cm in depth were collected in September, 2015. Five soil samples were randomly collected within each plot using a 5-cm diameter stainless steel cylinder. The soil samples collected from each plot were mixed and homogenized for a total of three composite samples

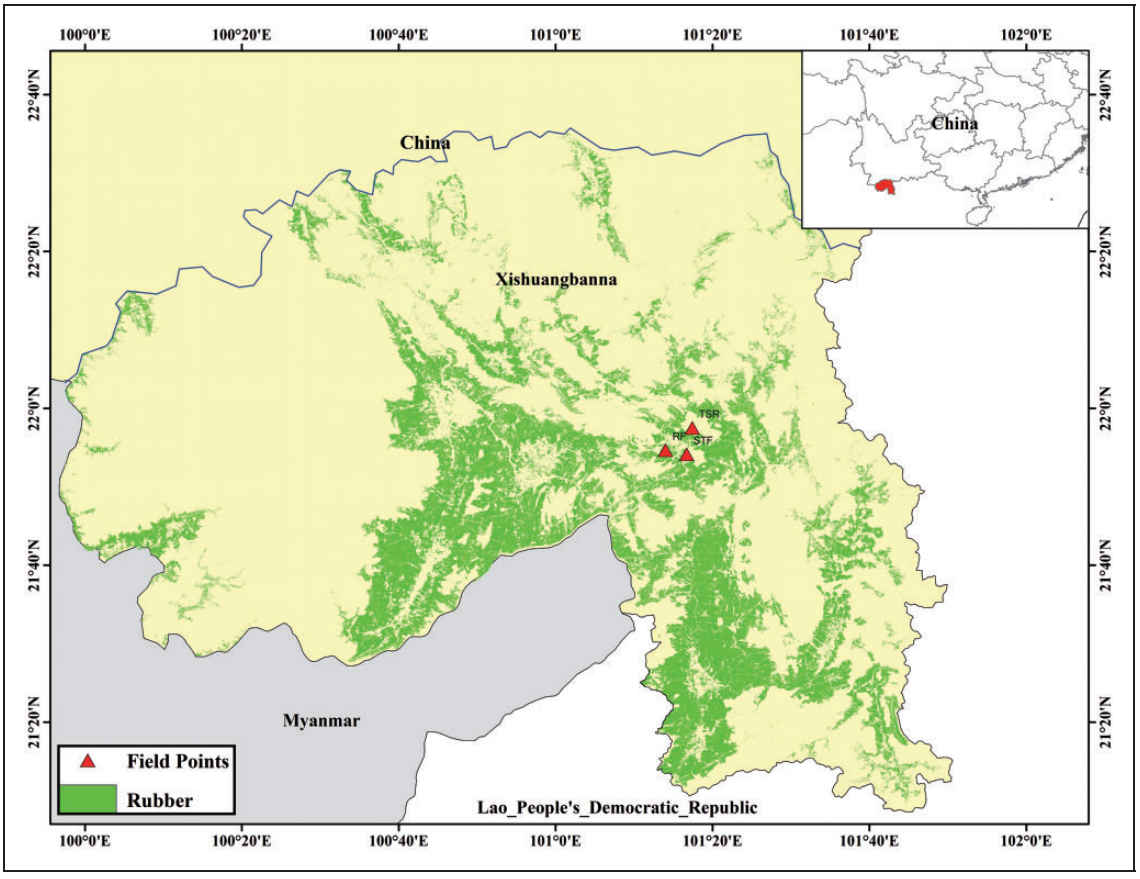

Figure 1. The location of study site.
RF = rubber forest; STF = secondary tropical forest; TSR = tropical seasonal rainforest.

per forest type and a grand total of nine soil samples. The composite soil samples were then divided into three parts: One was sieved through a 2-mm mesh immediately and stored at 4°C until analysis of microbial biomass carbon (MBC), the other was air-dried and passed through a 0.25-mm sieve for soil organic matter (SOM), soil pH, and total nitrogen (TN), and the third was stored at −80°C for future analyses (DNA extraction). Soil was analyzed using standard soil test methods described by Lu (1999). Soil pH was measured in a 1:1 soil: water mixture. Soil moisture was measured gravimetrically. Soil TN was determined using the micro-Kjeldahl digestion followed by steam distillation. Total P and total K were digested with NaOH. MBC analyzed by the chloroform fumigation and extraction method and calculated using the correction factors 0.35 (Shen et al., 2014). Forests and soil properties are shown in Table 1.

DNA Extraction, Polymerase Chain Reaction Amplification, and Illumina MiSeq Sequencing

Microbial DNA was extracted from 5.0 g of soil using the E.Z.N.A.®Soil DNA Kit (Omega Bio-tek, Norcross, GA, USA) according to manufacturer's protocols. For bacteria, the V3-V4 hypervariable regions of the bacteria 16S rRNA gene were amplified with primers 338F (5′- ACTCCTACGGGAGGCAGCAG-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′) by thermocycler polymerase chain reaction system (Xu, Tan, Wang, & Gai, 2016; GeneAmp 9700, ABI, USA). Fungal Internal Transcribed Spacer (ITS) Region 1 was amplified using ITS1F (5′-CTTGGTCATTTAGAGG AAGTAA- 3′) and ITS2 (5′- GCTGCGTTCTTCATCG ATGC-3′) primer pair (Bokulich & Mills, 2013; Kerfahi, Tripathi, Dong, Go, & Adams, 2016). Polymerase chain reactions were performed in triplicate, and samples analyzed in a 20 μL mixture containing 4 μL of 5 × FastPfu Buffer, 2 μL of 2.5 m MdNTPs, 0.8 μL of each primer (5 μM), 0.4 μL of FastPfu Polymerase, and 10 ng of template DNA. Amplicons were extracted from 2% agarose gels and purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) according to the manufacturer's instructions and quantified using QuantiFluor™ -ST (Promega, USA). Purified amplicons were pooled in equimolar and paired-end sequenced (2 × 250) on an Illumina MiSeq platform according to the standard protocols.

Table 1. Soil Properties of the Three Forest Types in Xishuangbanna, Southwest China.

Forest types	pH	WC	MBC (mg/kg)	SOM (g/kg)	TK (g/kg)	TP (g/kg)	TN (g/kg)
RF	4.85 ± 0.04a	36.14 ± 3.90a	145.01 ± 15.86a	21.94 ± 1.05a	10.32 ± 1.09a	0.42 ± 0.01a	2.53 ± 0.15a
STF	4.09 ± 0.05b	29.73 ± 10.40a	231.89 ± 46.69b	28.64 ± 1.75b	8.83 ± 2.50b	0.34 ± 0.00b	2.29 ± 0.10b
TSR	4.31 ± 0.03c	26.02 ± 11.36a	219.12 + 29.98b	27.84 ± 1.81b	6.87 ± 1.78b	0.31 ± 0.05c	1.95 ± 0.18c

Note. Values shown are means and standard error ($n = 3$). RF = rubber plantation; STF = secondary tropical forest; TSR = tropical seasonal rainforest; MBC = microbial biomass carbon; SOM = soil organic matter; TN = total nitrogen; TP = total phosphorus; TK = total potassium; WC = water content; pH = soil pH.
Letters "a" to "c" denote a statistically significant difference at $p < .05$.

Statistical and Bioinformatics Analysis

Raw FASTQ files were demultiplexed and quality filtered using QIIME (Version 1.17). The aligned sequences were clustered into operational taxonomic units (OTUs) defined by 97% similarity (Stackebrandt & Goebel, 1994) using CD-HIT-OTU program (Wu, Zhu, Fu, Niu, & Li, 2011). The phylogenetic affiliation of each 16S and 18S rRNA gene sequence was analyzed by RDP Classifier against the silva (SSU117/119)16S and 18S rRNA database using a confidence threshold of 70% (Amato et al., 2013). We calculated the coverage percentage using Good's (1953) method, and Shannon's diversity index and nonparametric measures of species richness (Chao1) were calculated for each sample using the Mothur (Schloss et al., 2009). Principal coordinate analysis (PCoA), based on Bray–Curtis distances of family composition data, was performed to interpret the relative similarity of the microbial communities. Analysis of similarities was used to test statistically whether there is a significant difference among forest types. Seven environmental variables were forest types (in the data set, 1 means RF; 2 means STF; 3 means TSR), SOM, TN, total phosphorus (TP), total potassium (TK), water content (WC), and soil pH, respectively. To reveal the correlations between the microbial communities and environmental factors, a redundancy analysis was performed based on seven variables and taxon composition (family level) data in the nine communities, using the "Vegan" package within R (http://cran.rproject.org/web/packages/vegan). Statistical significance was assessed using the Monte Carlo permutation's method, based on 999 permutations. The statistical analyses were conducted with SPSS 16.0. A multiple comparison based on Duncan's method was used to test the significant difference on compositions and diversity between dry season and rainy season.

Results

Taxonomic Composition

All sequences were classified to from phylum to OTU according to the program Mothur using the default setting (97% sequence identity). At family level, 253 bacterial families and 118 fungal families were identified from the nine soil samples (3 RF, 3 STF, and 3 TSR). Dominant family of both bacteria and fungi were obviously different between forest types. RF was different from STF and TSR in both bacterial and fungal community composition, but the latter two forests (STF and TSR) were very similar. For bacterial composition, *Bacillaceae* was the most dominant family (13.60%) in RF soil, while it only accounted for 4.13% in STF and 6.92% in TSR (Figure 2). However, the largest family in STF and TSR was *Subgroup_2_norank*. The results also revealed that the relative abundance of *Acidobacteriaceae_Subgroup_1* decreased after forest conversion, while *Bacillaceae* increased. For fungal composition, the largest family in soils of RF was *Basidiomycota_unclassified*. However, the largest family in STF and TSR was *Russulaceae* (Figure 3). In addition, after forest conversion, the relative abundance of *Basidiomycota_unclassified* increased and companied with a decrease in *Russulaceae*.

Hierarchically clustered heatmaps analysis based on the bacterial (Appendix A) and fungal (Appendix B) community profiles at the family level showed that STF and TSR communities grouped together. To sum up, heatmaps further confirmed that both bacterial and fungal compositions in soils of RF were significantly different from STF and TSR.

Bacterial Diversity

Number of OTUs and diversity estimations of the16S and 18S rRNA gene libraries of the three communities from the Illumina MiSeq sequencing analysis are shown in Figure 4. First, the number of OTUs, Chao diversity, and Shannon diversity of bacterial communities in soils of RF were significantly higher than those in STF and TSR, whereas there were no significant differences among the Simpson diversities of the three communities. For fungal communities, the number of OTUs and Chao diversity of RF were significantly lower than that in soils of STF and TSR, whereas there were no significant differences among the Shannon and Simpson diversities of the three forest types.

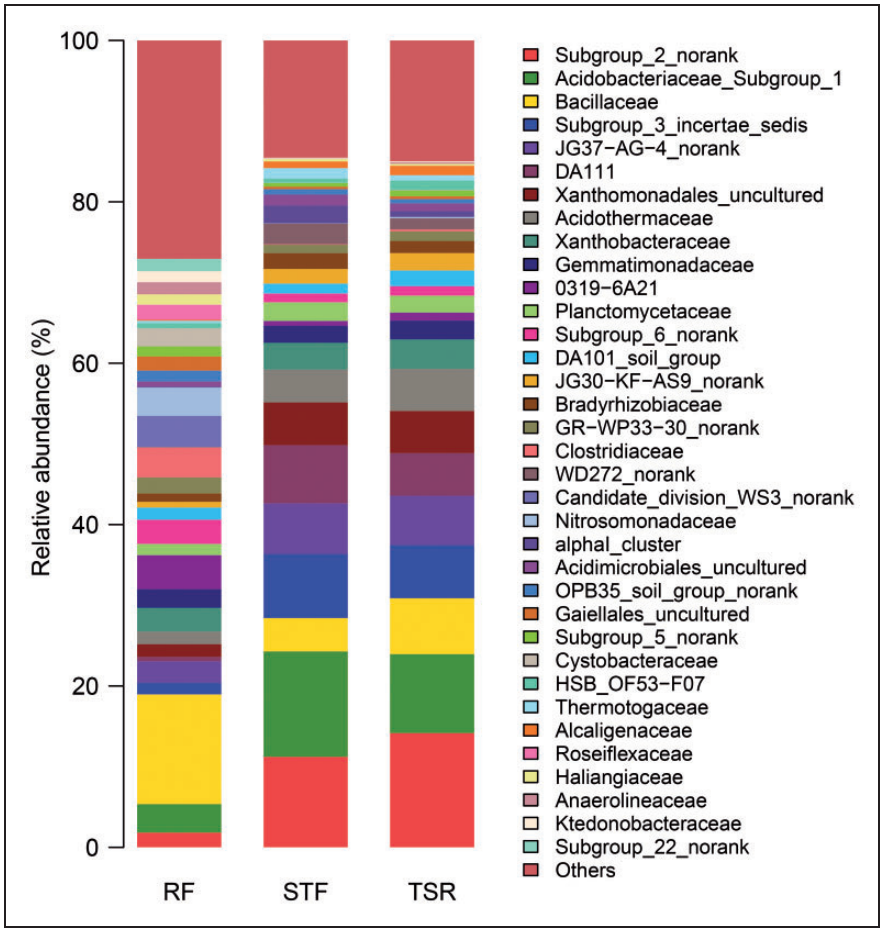

Figure 2. Family compositions of soil bacteria in the three forest types.
RF = rubber forest; STF = secondary tropical forest; TSR = tropical seasonal rainforest.

PCoA of the bacterial and fungal communities of the three types of forest revealed considerable spatial heterogeneity of bacterial and fungal taxonomic compositions among soils of RF, STF, and TSR (Figure 5). For bacteria, PCoA 1 and 2 explained 92.59% and 2.81% of the total variance, respectively (Figure 5(a)). For fungi, PCoA 1 and 2 explained 33.10% and 17.66% of the total variance, respectively (Figure 5(b)). For both bacteria and fungi, points of soil samples in STF and TSR were closer together on the ordination showed the two types of forest are more similar in taxonomic compositions. The results were confirmed by analysis of similarities results ($p < .001$).

Influencing Factors

Seven variables and abundance data from dominant phyla of bacteria and fungi in soil of the nine communities were used for redundancy analysis (Figure 6). For bacteria, the first axis of ordination was strongly positively correlated with soil pH, TP, and TK but negatively correlated with forest types, TN, and TK, which explained 74.93% of the total variance ($p = .001$; Monte Carlo permutation test with 1,000 permutations). The second axis of ordination was positively correlated with SOM, TN, and WC, which explained 12.53% of the total variance. This combination of variables explained 90.09% of the total variance of phylum abundance. Soil pH, TP, forest types, and TK explained 70.85%, 63.55%, 41.85%, and 35.88% of the total variance, respectively. Other factors had little effect on bacterial community compositions. TN, SOM, and WC explained 8.69%, 6.87%, and 5.08%, respectively.

For fungi, the first axis of ordination was strongly positively correlated with soil pH, TP, TK, and SOM but negatively correlated with forest types, which explained 40.47% of the total variance ($p = .001$; Monte Carlo permutation test with 1,000 permutations). The second axis of ordination was positively correlated with soil pH but negatively associated with WC, which explained 34.30% of the total variance. This combination of variables explained 78.70% of the total variance of phylum abundance. TN, SOM, forest types, and TK

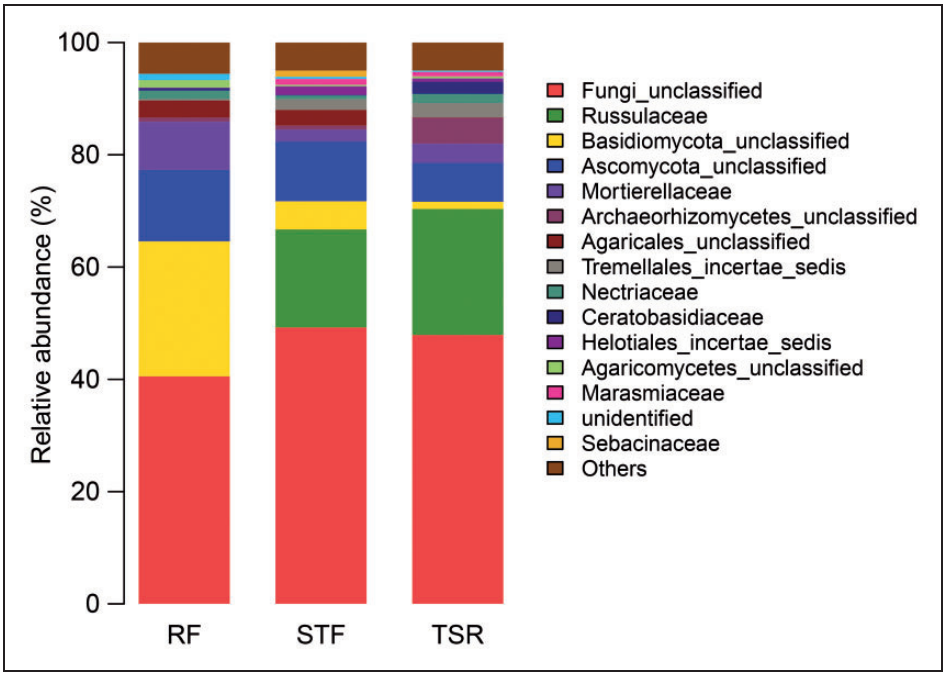

Figure 3. Family compositions of soil fungi in the three forest types.
RF = rubber forest; STF = secondary tropical forest; TSR = tropical seasonal rainforest.

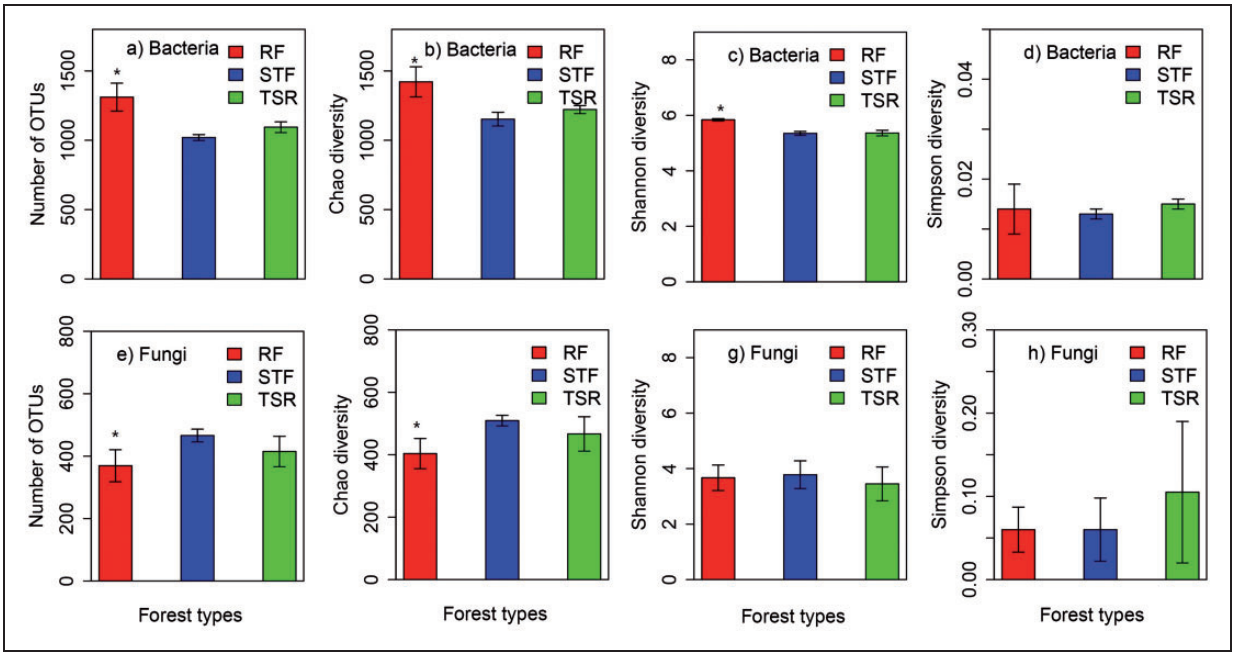

Figure 4. Number of OTUs, Chao, Shannon, and Simpson index of soil bacterial (a to d) and fungal (e to h) communities in the three forest types. Values shown are means, and vertical bars are standard error ($n = 3$). Asterisk represents families for which their abundance was significantly different among RF, STF, and TSR ($p < .05$).
RF = rubber forest; STF = secondary tropical forest; TSR = tropical seasonal rainforest.

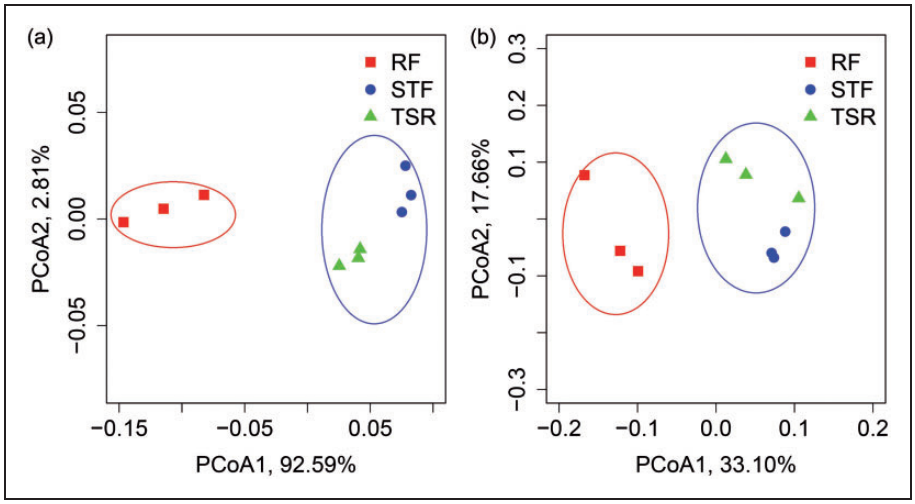

Figure 5. Principal coordinates analysis (PCoA) of the bacterial (a) and fungal (b) communities of the three forest types for bacteria, PCoA 1 and 2 explained 92.59% and 2.81% of the variance, respectively. For fungi, PCoA 1 and 2 explained 33.10% and 17.66% of the variance, respectively. Points those are closer together on the ordination show communities that are more similar.
RF = rubber forest; STF = secondary tropical forest; TSR = tropical seasonal rainforest.

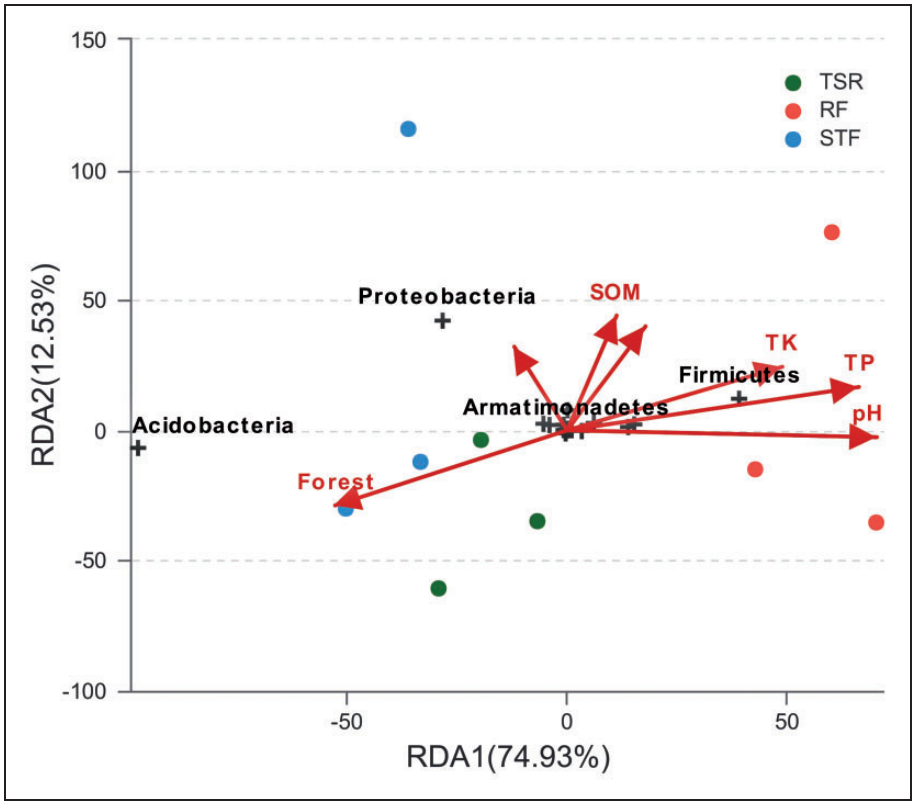

Figure 6. Redundancy analysis ordination of the study plots and phylum compositions of bacteria across three forest types. RDA 1 and 2 explained 74.93% and 12.53% of the variance, respectively.
RF = rubber forest; STF = secondary tropical forest; TSR = tropical seasonal rainforest; SOM = soil organic matter; TN = total nitrogen; TP = total phosphorus; TK = total potassium; WC = water content; pH = soil pH.

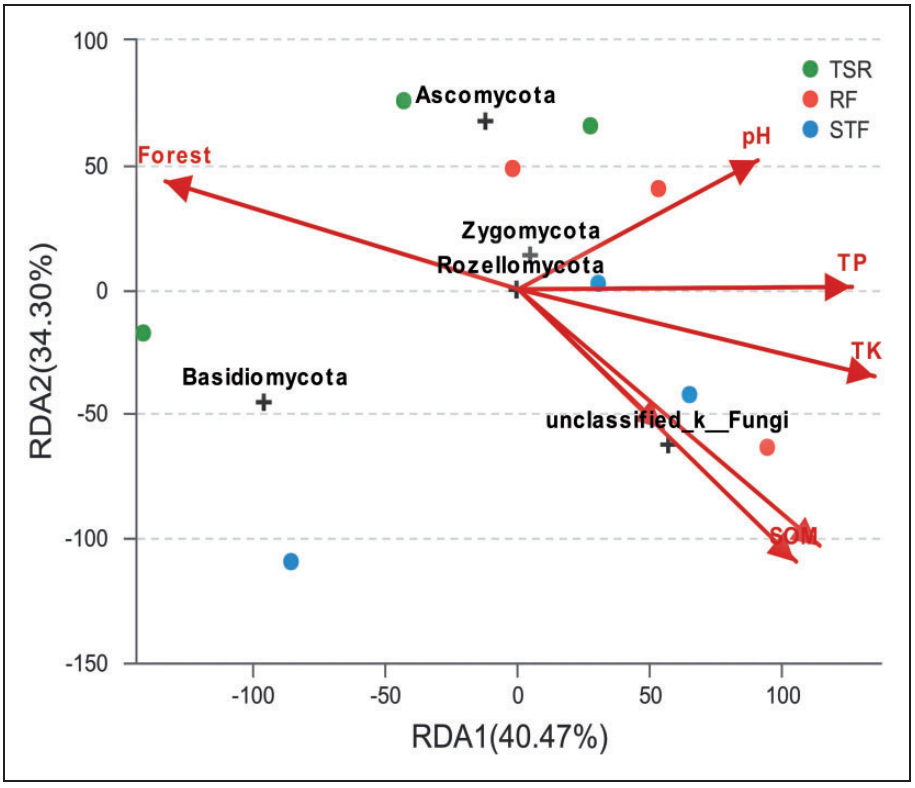

Figure 7. Redundancy analysis ordination of the study plots and phylum compositions of bacteria across three forest types. RDA 1 and 2 explained 40.47% and 34.30% of the variance, respectively.
RF = rubber forest; STF = secondary tropical forest; TSR = tropical seasonal rainforest; SOM = soil organic matter; TN = total nitrogen; TP = total phosphorus; TK = total potassium; WC = water content; pH = soil pH.

explained 25.10%, 23.98%, 22.43%, and 22.26% of the total variance, respectively. TP, soil pH, and WC explained 18.31%, 12.06%, and 6.57%, respectively (Figure 7).

Ratio of Fungal to Bacterial Diversity

To clarify the effects of forest conversion on the relation of growth and decline of bacterial and fungal communities, we calculated the ratios of fungal to bacterial OTUs, abundance-based coverage estimator (ACE), and Chao diversity (Figure 8). The mean ratio of fungal to bacterial OTUs of soil communities in RF was 0.28, which was significantly lower than in STF (0.46) and TSR (0.38). The mean ratio of fungal to bacterial diversity indices (both ACE and Chao) of RF was also significantly lower than STF and TSR.

Discussion

Taxonomic Compositions

The replacement of natural forests to managed system directly affects the bacterial taxonomic compositions (Figuerola et al., 2012). Our findings revealed that there were significant differences among the bacterial and fungal compositions at family level in the soils of RF, STF, and TSR; however, STF and TSR had similar taxonomic compositions. This indicated that the conversion of STF or TSR to RF resulted in the changes in the soil microbial compositions of the soils in Xishuangbanna. Our results were also agree with previous studies conducted in Amazon region (Neill et al., 1997; Neill, Piccolo, Melillo, Steudler, & Cerri, 1999) and Hainan Island (south of China; Lan, Li, et al., 2017) that bacterial compositions of the soil significantly changed after forest conversion. Soil properties, including pH, soil moisture, SOM, TN, and the carbon-to-nitrogen ratio, are all correlated with bacterial community structures and diversity (Lee, Barbier, Bottos, Mcdonald, & Cary, 2012; Smith, Barrett, Tusnady, Rejto, & Cary, 2010; Van Horn et al., 2013; Zeglin et al., 2011).The changes of soil properties such as nutrient levels and abiotic conditions occurred with the conversion from forest to managed system (agricultural practices and plantation; Alele, Sheil, Surget-Groba, & Shi, 2014). Bacterial communities exhibited significant responses to the N addition (Mueller, Belnap, & Kuske, 2015). The conversion of

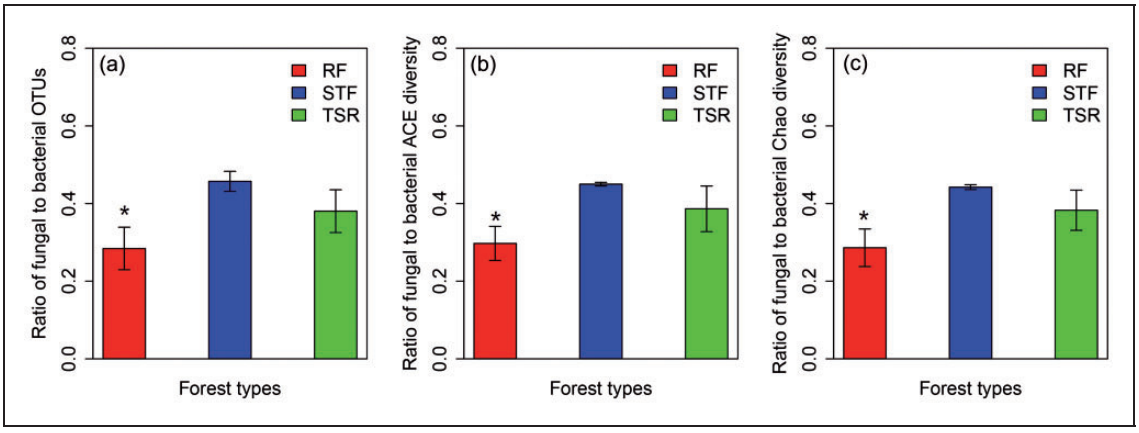

Figure 8. Ratio of fungal to bacterial OTUs (a), ACE diversity (b), and Chao diversity (c) of the three forest types. Asterisk represents significantly different among RF, STF, and TSR ($p < .05$).
RF = rubber forest; STF = secondary tropical forest; TSR = tropical seasonal rainforest.

the tropical forest to rubber plantation in Xishuangbanna would increase NH4-N concentrations and decrease SOM concentration (Li et al., 2012). In our study, soil nutrients and soil pH increased after forest conversion (Table1). Beside this, Lan, Li, et al. (2017) have examined that soil nutrition (TN, TP, and TK), which explained 43.05% of the total variance of bacterial taxonomic composition, was one of the most important factors affecting the soil bacterial community structures in Hainan Island, south of China. In this study, the TN, TK, and total TP of soils in RF were significantly higher than in TSF and TSR (Table 1). TP and TK explained 63.55% and 35.88% of the total variance of phylum composition. In fact, the properties of the soil have changed after forest conversion in this region (Table 1). In addition, soil pH of RF was significantly higher than in STF and TSR. Our research also revealed that the most important factor affecting bacterial community was soil pH, which explained 70.85% of the total variance. However, Krashevska et al. (2015) also found that pH was the most significant driver of soil microbial community composition in rainforests and rubber plantations. Therefore, we deduced that forest conversion results in the changes in soil nutrition and soil pH, which affected the microbial compositions of soils in Xishuangbanna.

Microbial Diversity

The relationship between microbial diversity and ecosystem function (Langenheder, Bulling, Solan, & Prosser, 2010), or plant or animal diversity (Rodrigues et al., 2013), has not been definitively established. Consequently, Zak, Holmes, White, Peacock, and Tilman (2003) reported that plant species richness is an important factor in this biotic interaction; however, this interaction has been contested for tropical ecosystems (Fierer & Jackson, 2006). In our study, the number of OTUs, Chao diversity index, and Shannon diversity index of bacterial community of the RF were significantly higher than TSR and STF, which demonstrated that forest conversion resulted in an increase in the diversity of bacteria. This has been attributed to a direct contact between bacteria and substrate under agriculture practice, which facilitating bacterial growth (Beare, Hus, Coleman, & Hendrix, 1997). Microbial community biomass and fungal abundance significantly increased with greater plant diversity (Zak et al., 2003). Our results also implied that high richness of plant species above ground does not result in a high diversity of bacteria below aground. However, the number of OTUs and Chao diversity index of fungal communities in the RF were significantly lower than those in the TSR and STF communities, which indicated forest conversion to rubber plantations would reduce fungal diversity. Microorganisms, like all other organisms, have habitat preferences that are affected by land-use changes (Da Jesus, Marsh, Tiedje, & Des Moreira, 2009; Martiny et al., 2006). Furthermore, Rodrigues et al. (2013) have revealed that local taxonomic and phylogenetic diversity of soil bacteria increases after conversion, but the communities become more similar across space. The main reason for high diversity of bacterial communities in RP maybe the results of agricultural management, such as the application of fertilizers, which would result in high diversity of soil bacteria (Kerfahi et al., 2016; Lan, Li, et al., 2017).

It is worth noting that the MBC in RF was relative lower than those in STF and TSR (Table 1), which suggested that the total microbial biomass decreased after forest conversion. Similar results were also found in Hainan (Lan, Li, et al., 2017). While, close-to-nature management, an approach in maintaining plant diversity

in rubber plantations (Lan, Wu, Chen, & Xie, 2017), could be useful to increase the total microbial biomass.

Ratio of Fungal to Bacterial Diversity

Previous studies have shown that as ecosystems mature, there is a switch in dominance from bacterial to fungal biomass (Harris, 2009). Consequently, De Vriesa et al. (2006) have demonstrated that the fungal or bacterial biomass ratio quickly responded to changes in management. In our study, ratios of fungal to bacterial OTUs and Chao diversity of soils in RF community were significantly lower than in STF and TSR. The RF, which are frequently fertilized, had significantly higher soil nutrients compared with STF and TSR. Our results may imply that bacteria dominate under conventional tillage, whereas fungi dominate under notillage (De Vriesa et al., 2006). Our work also suggested that the microbial community *follows* and is dependent on what is going on in the above-ground community and can indicate the impact of management practices (Harris, 2009). Given the distinct roles of soil bacteria and fungi in major nutrient cycles, the resilience of fungi and sensitivity of bacteria to N amendments suggests that increased N input predicted for many ecosystems could shift nutrient cycling toward pathways driven primarily by fungal communities (Mueller et al., 2015).

Implications for Conservation

Due to the high biodiversity and endemism, the tropical rainforests in Xishuangbanna are among the world's most important for their conservation values. But these areas are under pressure of rubber tree cultivation. We are concerned about that whether if soil microbial diversity will decline dramatically after forest conversion. Our results revealed soil bacterial and fungal compositions had changed significantly after STF or TSR had converted to RF. Another important finding was that after forest conversion in this region, the increase in bacterial diversity was always accompanied by a decrease in fungal diversity. Therefore, we deduced that management practices, such as the application of fertilizers in RF, would increase the soil nutrition and soil pH, which would result in the changes in soil microbial composition and diversity in this region. Although the diversity of bacteria in soil may increase after the conversion of STF (or TSR) to RF, we could not ignore the fact that there was a decline in total microbial biomass to some extent. Thus, not only soil bacterial diversity but also total microbial biomass are important factors to be monitored when assessing agricultural impact on tropical forests. To increase the community diversity of rubber plantation, it is necessary as far as possible to minimize the intensity of human management. For individual farmers, close-to-nature management, which tries to increase the stability, species and structural diversity, and health of forests while maintaining production (Bieling, 2004), maybe a potentially effective management. In this way, the total microbial biomass and total diversity would increase to some extent. However, to protect the total diversity of this region, the government could outline a land-use policy that prohibits the cultivation in areas of natural vegetation.

Appendix A

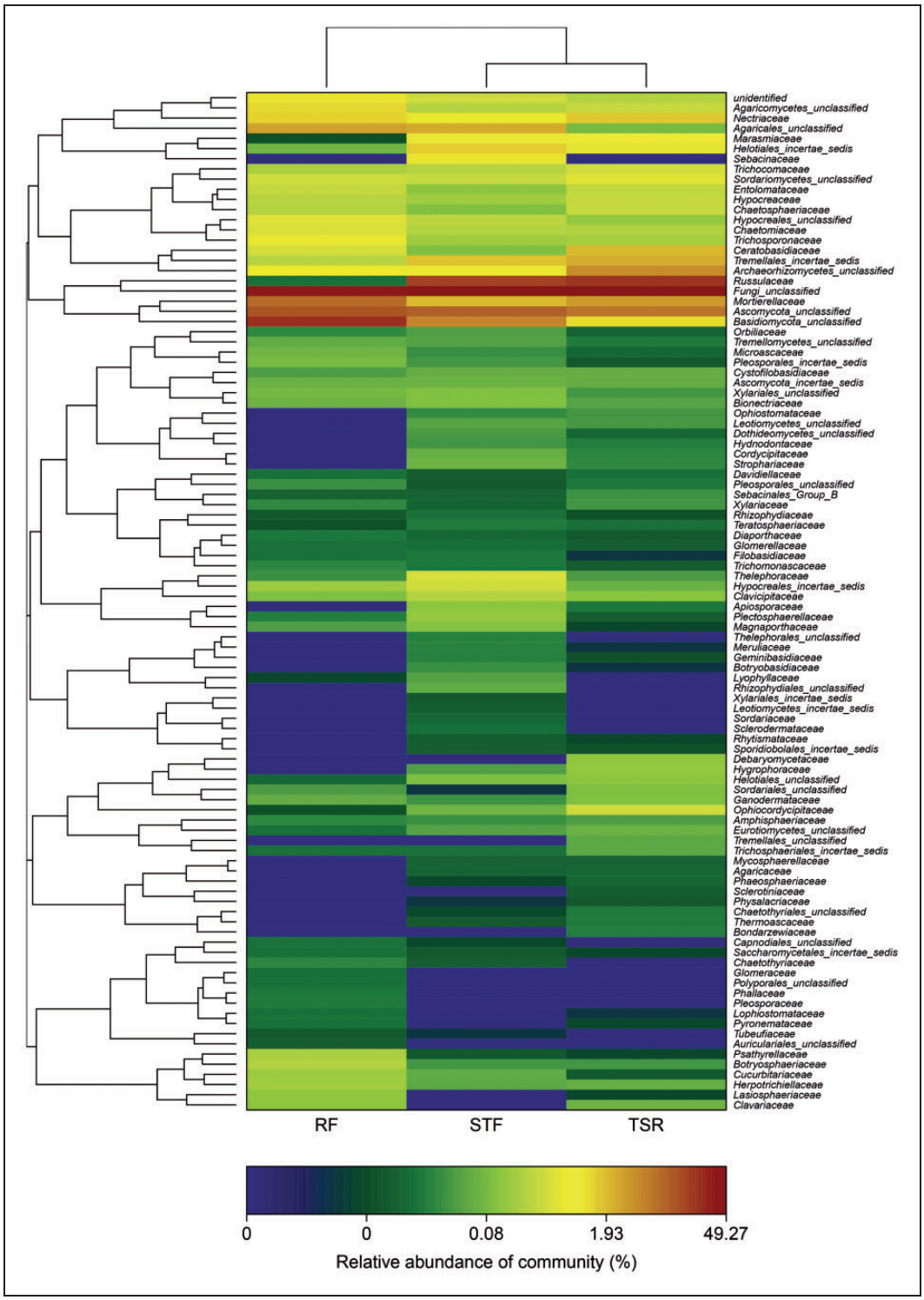

Figure A1. Double hierarchical dendrogram showing the bacterial distribution among the three forest types. The bacterial phylogenetic tree was calculated using the neighbor-joining method, and the relationship among samples was determined by Bray–Curtis distance and the complete clustering method. The heatmap plot depicts the relative percentage of each bacterial family (variables clustering on the *Y*-axis) within each communities (*X*-axis clustering). The relative values for bacterial family are depicted by color intensity with the legend indicated at the bottom of the figure.
RF = rubber forest; STF = secondary tropical forest; TSR = tropical seasonal rainforest.

Appendix B

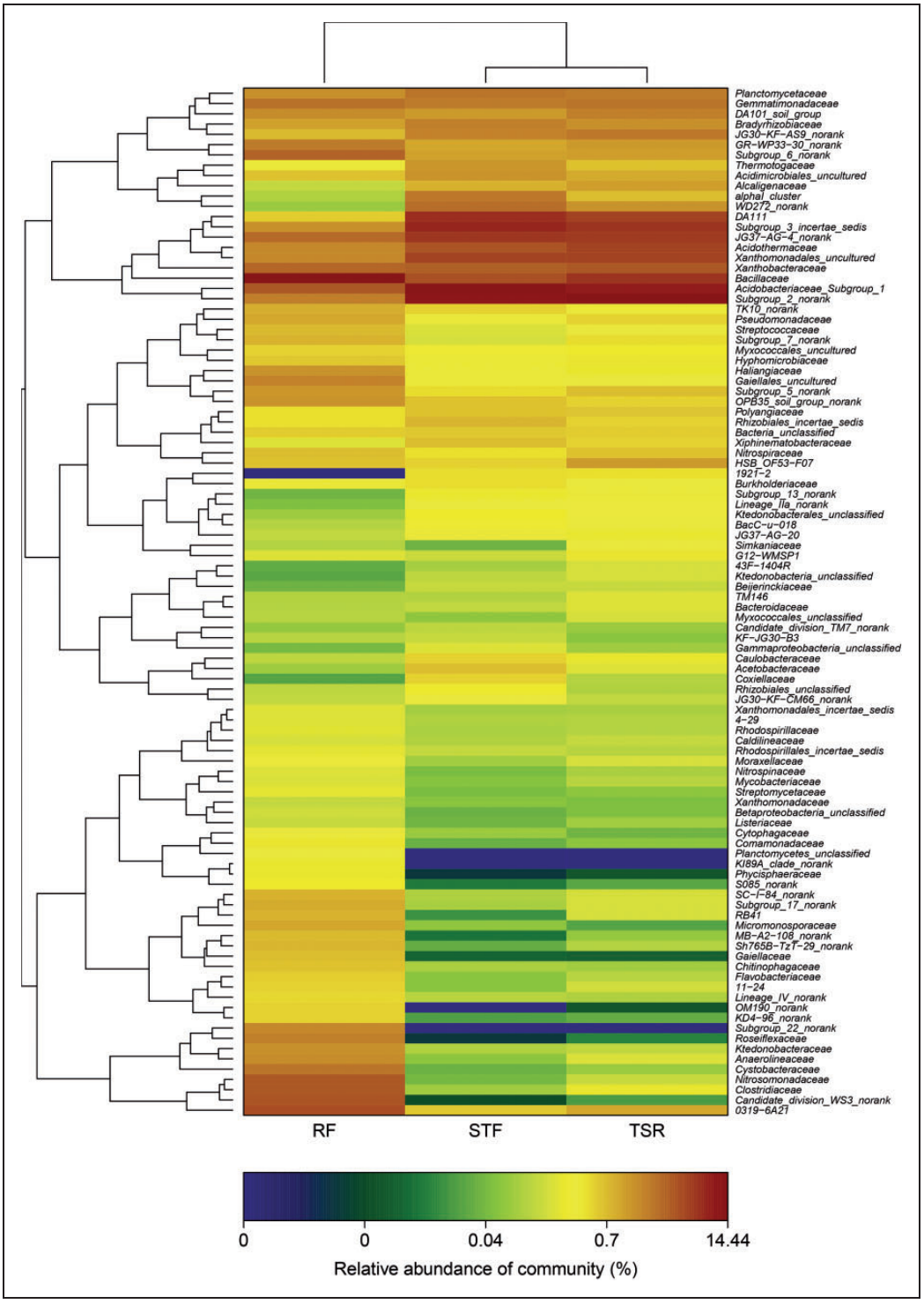

Figure B1. Double hierarchical dendrogram showing the fungal distribution among the three forest types. The fungal phylogenetic tree was calculated using the neighbor-joining method, and the relationship among samples was determined by Bray distance and the complete clustering method. The heatmap plot depicts the relative percentage of each bacterial family (variables clustering on the *Y*-axis) within each communities (*X*-axis clustering). The relative values for fungal family are depicted by color intensity with the legend indicated at the bottom of the figure.
RF = rubber forest; STF = secondary tropical forest; TSR = tropical seasonal rainforest.

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Author Contributions

G. L., Y. L., and M. T. J. contributed equally to this work.

Declaration of Conflicting Interests

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