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Soil Bacterial Diversity Impacted by Conversion of Secondary Forest to Rubber or Eucalyptus Plantations: A Case Study of Hainan Island, South China

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Rubber plantation (RP) is the most important economic forest of Hainan Island, South China. In addition, eucalyptus plantations (EP), used as shelter forests, were planted to protect the rubber plantations from typhoon hazards in the 1980s. To date, few studies have examined the effects on bacterial composition and diversity after secondary tropical forests (SF) have been converted into RP or EP. This study investigated the bacterial communities of RP, EP, and SF using an Illumina high-throughput sequencing analysis. Our findings revealed the following First, there were significant differences between RP, EP, and SF in bacterial compositions at both the phylum and family levels. Second, the Shannon indices of RP and EP were significantly higher than that of SF. The Simpson dominance index of SF was 0.012, which was significantly higher than those of RP and EP, indicating that the diversities of RP and EP were higher than that of SF. Abundance rank curves confirmed that the taxonomic compositions in EP and RP were relatively uniformly distributed compared with that in SF, which results in the higher diversities of RP and EP. Third, Soil nutrition (total nitrogen, total phosphorus, and total potassium), which explained 43.05% of the total variance of taxonomic composition, was the most important factor affecting the soil bacterial community structure in this region. In conclusion, soil nutrition has increased, mainly due to the application of fertilizers, after SF conversion to RP and EP, which, in turn, has resulted in significant changes in the bacterial community composition as well as a general increase in bacterial community diversity.

Keywords: rubber plantation, tropical secondary forest, eucalyptus plantation, bacterial community, bacterial diversity

Hainan Island has a long history of plantation forestry, starting with rubber (Zhai et al. 2012). Now rubber trees represent one of the most economically important plant species in tropical ecosystems. Rubber plantations (RP) occupy approximately one-quarter of the total flora of Hainan Island, South China (Wang et al. 2012), and rubber is one of the most important vegetation species in the region (Lan et al. 2013). Eucalyptus plantations (EP), as shelter forests, were planted in the 1980s to protect the RP from the typhoon hazards, and some of them have been conserved up to now for their protective effect.

The diversity and composition of soil bacterial communities are thought to have a direct influence on a wide range of ecosystem processes (Schimel 1995, Balser et al. 2002). In tropical forest sites converted to agriculture had a less diverse community of microorganisms capable of degrading the specific substrates than the unconverted forest sites (Griffiths and Philippot 2013). Land-use change and management practices are normally applied to manipulate environments to improve conditions that relate to production and remediation (Shange et al. 2012). Management practices, such as the application of nitrogen (N) fertilizer and lime, straw return after

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harvest, and cultivation could markedly influence the relative contribution of bacteria to specific soil nitrification processes (Wang et al. 2015). Microbial taxa associated with specific components of the soil N cycle (for example, nitrifiers) often change in relative abundance when soils are amended with N. The N additions may result in a shift from a more oligotrophic bacterial community to one that is more copiotrophic (Fierer et al. 2011). However, Guo et al. (2015) found that the main factor affecting the composition of microbial communities in bulk and rhizosphere soil was soil parent material. We know far less about how fertilizer inputs will influence the structure and associated functioning of the entire belowground microbial community (Fierer et al. 2011). Forest conversion always is accompanied by changes in management practices (for example, the application of fertilizers). The effects of secondary tropical forest (SF) conversion to RP or EP on the soil microbial community diversity are still unknown.

The development of high-throughput sequencing has led to a revolution in the characterization of complex microbial populations (McKenna et al. 2008, Costa et al. 2012, Su et al. 2013). This study investigates the bacterial community of SF, RP, and the shelter forest (i.e., EP) using an Illumina high-throughput sequencing analysis. We propose the hypotheses that the soil bacterial community structure will change and bacterial community diversity will increase after SF conversion to EP and RP due to the management practices (mainly the application of fertilizers) in RP and EP. Here we attempt to answer the question: What are the differences in soil bacterial composition and diversity between RP, EP, and SF? The results imply how the conversion of an SF into an RP or EP affects bacterial diversity.

Materials and Methods

Study Site

Hainan Island (18°10′–20°10′ N and 108°37′–111°03′ E) is the largest tropical island in China. With an area of 33,920 km^2 (Lopez et al. 2009), it is the largest island in the Indo-Burma biodiversity hotspot (Francisco-Ortega et al. 2010). The study area was located in the west central part of Hainan Island. This area has a tropical monsoon climate with a rainy season from May to October and a dry season from November to April (Luo 1985). The annual average temperature is 23.5° C. The hottest month, July, has an average temperature of 27.8° C. The coldest month, January, has an average temperature of 17.5° C. The full temperature range annually is approximately 3–33° C. The mean annual precipitation is 1,815 mm, and approximately 84% is accumulated between the months of May and October.

Sample Collection

We selected a total of 18 20- × 20-m plots including 6 plots in an RP, 6 in an EP, and 6 in a tropical SF. The parent materials of the three forests are all granitic gneiss. The RP was about 25 years old and is considered to be in a mature forest condition. The ages of the EP and SF were about 30 years. Fertilizers were applied in the RP and EP (earlier stage) but were not applied in the SF. Mineral soil samples at 0 to 30 cm depths were collected in March 2014. The litter layer was removed before mineral soil sampling. Five soil samples were randomly collected at each plot using a stainless steel cylinder with a 5-cm diameter. Samples collected from each plot were mixed and homogenized for a total of three composite samples per forest type, resulting in a 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 carbon, 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 total N was determined using micro-Kjeldahl digestion followed by steam distillation. Total phosphorus (TP) and total potassium (TK) were digested with NaOH. MBC was analyzed by the chloroform fumigation and extraction method and calculated using the correction factor 0.35 (Shen et al. 2014).

DNA Extraction and Polymerase Chain Reaction (PCR) Amplification

Microbial DNA was extracted from 5.0 g of soil using the E.Z.N.A. Soil DNA Kit (Omega Bio-tek, Norcross, GA) according to the manufacturer's protocols. The V4–V5 region of the bacterial 16S ribosomal RNA gene was amplified by PCR (95° C for 2 min, followed by 25 cycles at 95° C for 30 s, 55° C for 30 s, and 72° C for 30 s and a final extension at 72°C for 5 min) using the primers 515F (5′-barcode-GTGCCAGCMGCCGCGG-3′) and 907R (5′-CCGTCAATTCMTTTRAGTTT-3′), where the barcode is an eight-base sequence unique to each sample. PCRs were performed in triplicate in a 20-μl mixture containing 4 μl of 5× astPfu buffer, 2 μl of 2.5 mM deoxynucleoside triphosphates (dNTPs), 0.8 μl of each primer (5 μM), 0.4 μl of FastPfu polymerase, and 10 ng of template DNA.

Illumina MiSeq Sequencing

Amplicons were extracted from 2% agarose gels and purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA) according to the manufacturer's instructions and quantified using QuantiFluor-ST (Promega). Purified amplicons were pooled in equimolar and paired-end sequenced (2 × 250) on an Illumina MiSeq platform according to the standard protocols.

Statistical and Bioinformatics Analysis

Raw FASTQ files were demultiplexed and quality filtered using QIIME (version 1.17) with the following three criteria: the 250-bp reads were truncated at any site receiving an average quality score of <20 over a 10-bp sliding window, discarding the truncated reads that were shorter than 50 bp; exact barcode matching, 2-nucleotide mismatch in primer matching, and reads containing ambiguous characters were removed; and only sequences that overlap longer than 10 bp were assembled according to their overlap sequence. Reads that could not be assembled were discarded. The aligned sequences were clustered into operational taxonomic units (OTUs) defined by 97% similarity (Stackebrandt and Goebel 1994) using the CD-HIT-OTU program (Wu et al. 2011). The phylogenetic affiliation of each 16S rRNA gene sequence was analyzed by RDP Classifier[1] against the SILVA (SSU 117/119) 16S rRNA database using a confidence threshold of 70% (Amato et al. 2013). We also calculated the coverage percentage using Good's method (Good 1953) and the Shannon diversity and Simpson dominance indices using the mothur program (Schloss et al. 2009).

Shannon's diversity is defined as

$$H_{\text{Shannon}} = -\sum_{i=1}^{S_{\text{obs}}} \frac{n_i}{N} \ln \frac{n_i}{N} \quad (1)$$

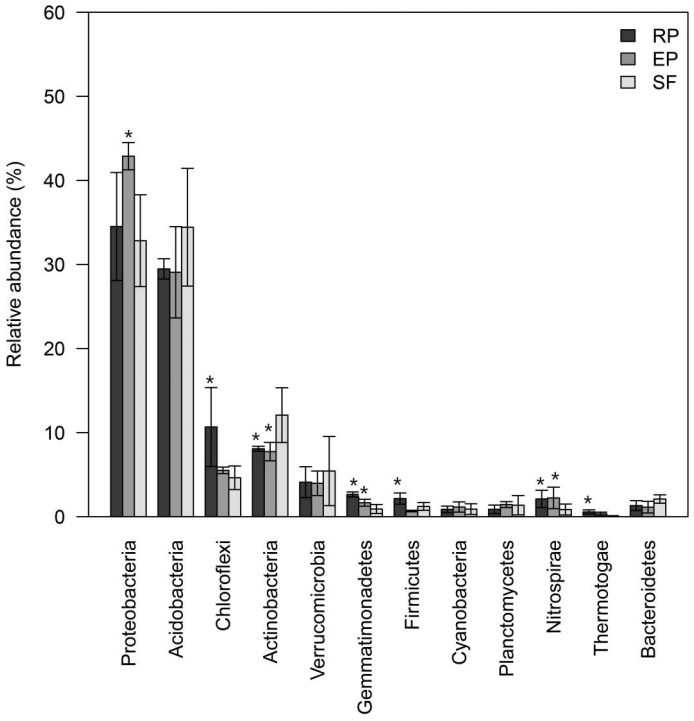

Figure 1. Phylum compositions of soil bacteria of the three forest types. Values shown in the figure are mean relative abundances ($n = 6$). *Significant differences ($P < 0.05$) between RP, EP, and SF.

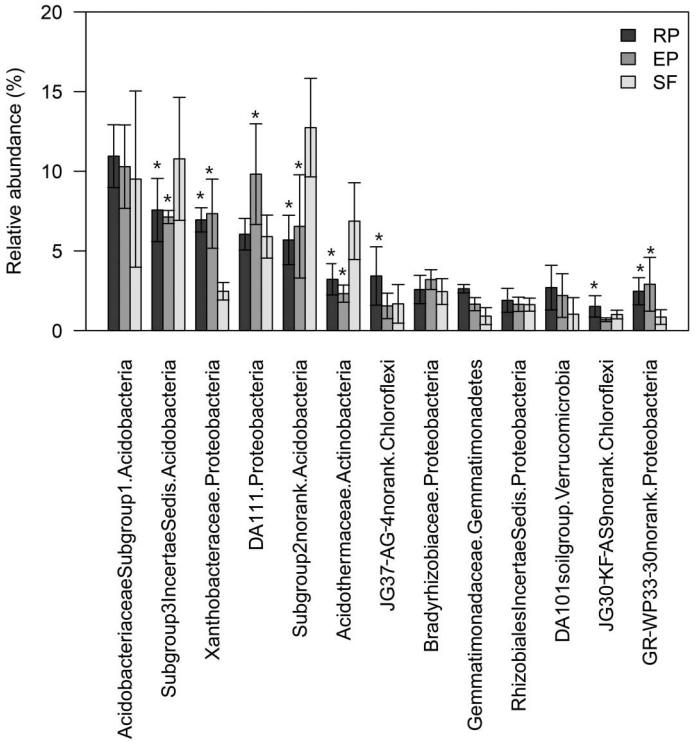

Figure 2. Family compositions of soil bacteria of the three forest types. Values shown in the figure are mean relative abundances ($n = 6$). *Significant differences ($P < 0.05$) between RP, EP, and SF. The *x* coordinate is a combination of family name and phylum name.

Simpson's dominance is defined as

$$D_{\text{Simpson}} = \frac{\sum_{i=1}^{S_{\text{obs}}} n_i\,(n_i - 1)}{N(N-1)} \qquad (2)$$

where S_{obs} is the observed number of OTUs, n_i is the number of OTUs containing i sequences, and N is the number of total sequences. The greater the H_{Shannon}, the higher the diversity of community is. However, the greater the D_{Simpson}, the lower the diversity of the community is.

Principal coordinate analysis (PCoA), based on the phylum and family composition data in the 18 communities, was performed to interpret the relative similarity of the microbial communities from each sample site. Analysis of similarities (ANOSIM) was used to test statistically whether there is a significant difference between the taxonomic compositions of RP, EP, and SF. To reveal the correlations between the microbial communities and environmental factors, a redundancy analysis (RDA) was performed based on seven soil variables and taxon composition (phylum level) data in the 18 communities, using the Vegan package within R.[2] The seven soil variables are MBC, soil organic matter (SOM), TN, TP, TK, water content (WC), and soil pH. Statistical significance was assessed using the Monte Carlo permutation 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 composition and diversity between RP, EP, and SF.

Results

Taxonomic Composition

We used the ILVA database for the classification. All sequences were classified to phylum and species according to the mothur program using the default setting. Thirty different phyla were identified from the 18 samples. Proteobacteria, Acidobacteria, Chloroflexi, Actinobacteria, and Verrucomicrobia were the most abundant groups in the three communities, and these phyla accounted for about 80% of the total reads (Figure 1). The relative abundance of Proteobacteria in EP was significantly higher than that in RP. However, the relative abundance of Chloroflexi in RP was significantly higher than those in EP and SF. In addition, the relative abundances of Acidobacteria and Actinobacteria in RP and EP were significantly lower than that in SF. At the family level, 260 different families were identified from the 18 samples. The most abundant families in the three communities were AcidobacteriaceaeSubgroup1, Subgroup2norank, Subgroup3IncertaeSedis, DA111, Xanthobacteraceae, Acidothermaceae, and JG37-AG-4norank. The relative abundances of families in the three communities were quite different (Figure 2). For example, the relative abundances of Subgroup3IncertaeSedis, Subgroup2norank, and Acidothermaceae in RP and EP were significantly lower than that in SF. However, the relative abundance of Xanthobacteraceae was higher in SF than that in other communities. In all, the three forests showed very different 16S rRNA profiles at both the phylum level and family level.

For the PCoA results of phylum composition (Figure 3A), PCoA 1 and 2 explained 41.34 and 21.00% of the total variance, respectively. Most points of SF were distributed on the left part of the figure, and most points of EP and RP were distributed on the middle-right of the figure. PCoA results of family composition (Figure 3B) revealed similar results; however, it was easy to divide these 18 samples into two parts: one was SF and the other was EP and RP,

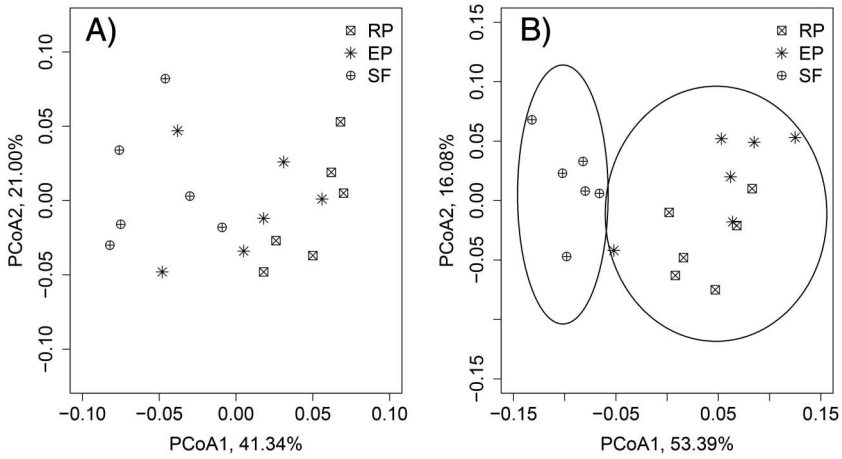

Figure 3. PCoA of the bacterial communities of the three forest types. A. PCoA results of sample scores based on phylum composition. PCoA 1 and 2 explained 41.34 and 21.00% of the variance, respectively. B. PCoA results of sample scores based on family composition, PCoA 1 and 2 explained 53.39 and 16.08% of the variance, respectively. Points closer together on the ordination show communities that are more similar.

which indicated that EP and RP were significantly different from SF in family composition. This was further confirmed by the ANOSIM results ($P < 0.05$).

Bacterial Diversity

Diversity estimations of the 16S rRNA gene libraries of the three communities from the pyrosequencing analysis are shown in Figure 4. Generally speaking, the Shannon indices of RP and EP were significantly higher than that of SF (Figure 4A). However, there was no significant difference between EP and RP. The Simpson dominance index of SF was 0.012, which was significantly higher than those of RP (0.0061) and EP (0.0068) (Figure 4B) indicating that the taxonomic compositions of RP and EP were more uniformly distributed. Rank abundance curves of the SF decayed faster than those of EP and RP (Figure 5). This further confirmed that the taxonomic compositions of EP and RP were more uniformly distributed than in SF.

RDA Analysis

Seven soil variables and abundance data from 30 phyla in the nine communities were used for RDA (Figure 6). The first axis of ordination was strongly negatively correlated with soil pH, WC, TN, and TP but positively correlated with MBC, which explained 36.77% of the total variance ($P = 0.001$; Monte Carlo permutation test with 1,000 permutations). The second axis of ordination was negatively correlated with SOM, but positively correlated with TK, which explained 19.88% of the total variance. This combination of variables explained 60.99% of the total variance in phylum abundances (Figure 6). TN, TP, and TK together explained 43.05% of the total variance, and SOM explained 14.76%. However, WC, soil pH, and MBC explained 7.37, 6.39, and 3.24%, respectively (Figure 7).

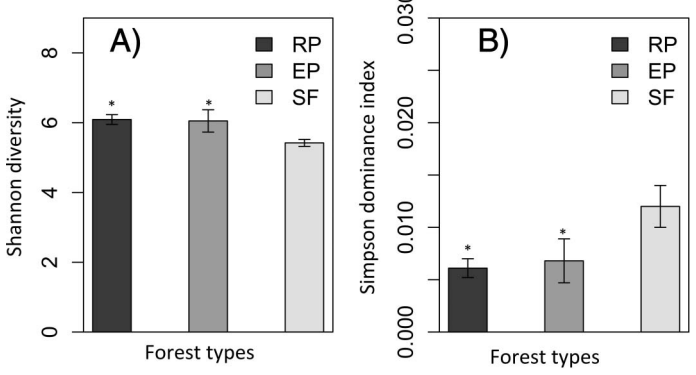

Figure 4. Shannon diversity and Simpson dominance of the three forest types. Values shown in figure are means of Shannon and Simpson indices ($n = 6$). Values shown in the figure are means, and vertical bars are SE ($n = 6$). *Significant differences ($P < 0.05$) between RP, EP, and SF.

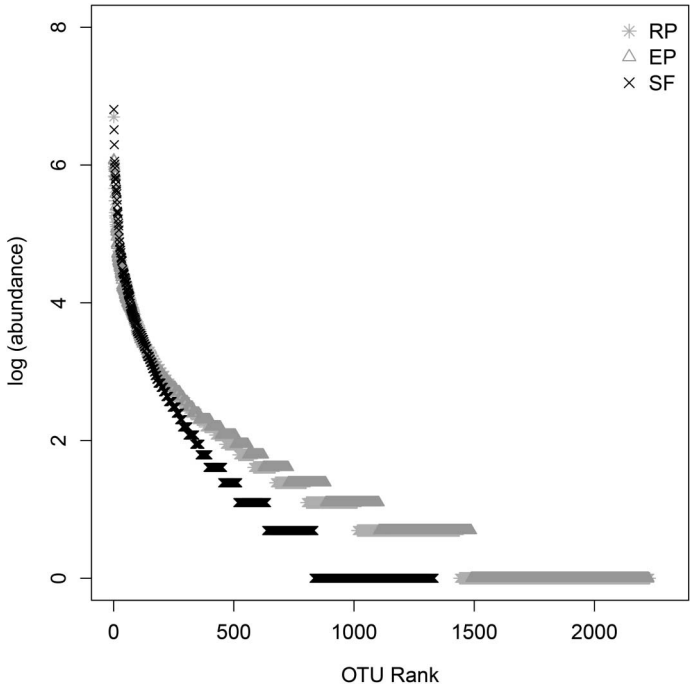

Figure 5. Rank-abundance curve (log scale) for the three forest types. OTUs are ranked in order from the most abundant to the least abundant for each soil bacterial community.

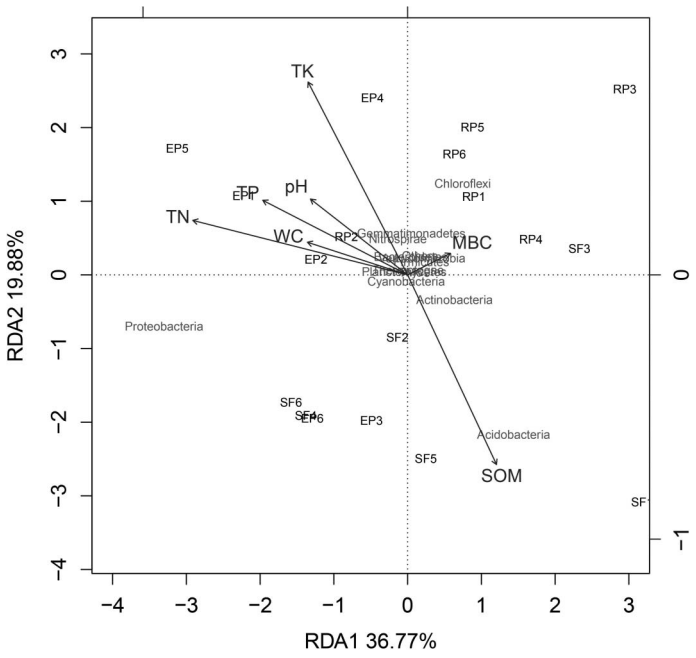

Figure 6. Redundancy analysis ordination of the study plots and phylum compositions across three forest types. RDA1 and RDA2 explained 36.77 and 19.88% of the variance, respectively.

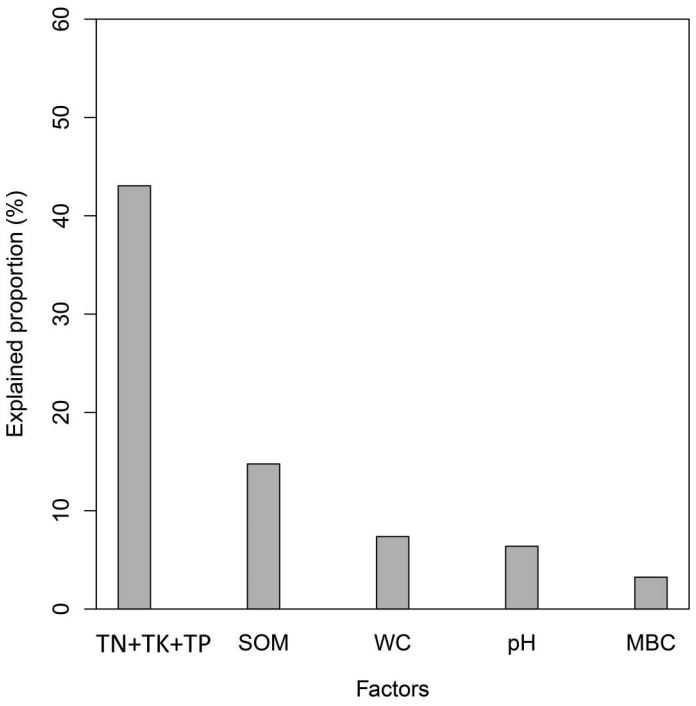

Figure 7. Explained proportion (%) of variance of phylum composition for the three forests by soil properties based on RDA.

We also found some linkages among forest types, phylum composition, and soil properties (Figure 6). First, the phylum composition of SF was positively correlated with SOM, RP was positively correlated with MBC, and EP was positively correlated with soil chemistry variables (TN, TP, and TK). In addition, Acidobacteria was positively correlated with SOM, and Proteobacteria was positively correlated with TN. Finally, different phyla were associated with (dominant in) different forest types. For example, Acidobacteria was associated (dominant in) with SF, Proteobacteria was associated with (dominant in) EP, and Chloroflexi was associated with RP.

Discussion

Taxonomic Composition

Up to now, studies examining the effects of SF conversion to RP or EP on the soil microbial community diversity have been relatively limited. In the present study, we examined and defined the bacterial communities under RP, EP, and SF. The dominant phyla in the three forests were Proteobacteria, followed by Acidobacteria, Chloroflexi, and Actinobacteria. Acidobacteria dominance was common in forest soils. For example, Acidobacteria accounted for 63% in the Brazilian Atlantic Forest soils and 35% in spruce-fir-beech forest soils (Hackl et al. 2004). In a meta-analysis of 16S rRNA gene sequences from distinct soils, Janssen (2006) determined that the most abundant bacterial phyla were Proteobacteria and Acidobacteria.

Our results also revealed that the relative abundances of the dominant phyla and families in RP and EP were quite significantly different from those in SF, thus supporting our hypothesis that the bacterial communities had altered when SF converted to EP or RP. In addition, this result supports earlier findings (Krashevska et al. 2015) that conversion of rainforests into production systems results in pronounced changes in microbial community composition. In contrast, a similar study conducted in the Nile River Watershed of Uganda revealed that soil microbial communities are relatively resilient to forest conversion and despite substantial and consistent change in the soil environment, the effects of conversion differed widely among sites (Alele et al. 2014).

Certain bacterial phyla can be differentiated into copiotrophic and oligotrophic categories that correspond to the *r*- and *k*-selected categories used to describe the ecological attributes of plants and animals (Fierer et al. 2007). In our study, soil nutrition (TN, TP, and TK) of RP and EP was relatively richer than that in SF. Acidobacteria were positively associated with SF (high between-plot variability, which was not significant) (Figure 6).The Acidobacteria are typified by small cells, slow growth, and the ability to use a wide variety of complex carbon substrates; thus, they could be considered oligotrophs (Fierer et al. 2007). The relative abundances of Firmicutes phyla in RP and EP were significantly higher than that in SF (with lower soil nutrition). The Firmicutes may exhibit rapid growth followed by abundant spore formation and are generally

Table 1. Soil properties of the three forest types in Hainan, South China.

Forest type	Soil pH	WC (%)	MBC (mg/kg)	SOM (%)	TK	TP	TN
					. . . . . .(g/kg)		
RP	4.72 ± 0.29a	15.81 ± 4.53a	59.64 ± 33.93a	1.99 ± 0.15a	45.71 ± 3.03a	1.29 ± 0.57a	1.07 ± 0.09a
EP	4.47 ± 0.31a	16.49 ± 3.20a	64.91 ± 20.07a	1.92 ± 0.22a	35.04 ± 1.74b	0.58 ± 0.12b	0.83 ± 0.10b
SF	4.05 ± 0.25b	14.84 ± 4.57a	94.49 ± 29.06b	2.53 ± 0.31b	30.22 ± 0.67c	0.39 ± 0.08c	0.76 ± 0.13b

Values are means ± SE ($n = 6$). a, b, and c indicate significant differences ($P < 0.05$).

considered copiotrophs (Mueller et al. 2015).This result indicates that management practices, such as application of fertilizers, may result in a shift from a more oligotrophic bacterial community to one that is more copiotrophic (Fierer et al. 2011), just as fertilizer often increases the abundance of *r*-selected "weedy" plant species over longer-lived, slower-growing *k*-selected plant species. In addition, Chloroflexi were positively associated with RP ($P < 0.5$) (with higher soil nutrition) (Figure 6); however, Mueller et al. (2015) found that the relative abundance of Chloroflexi decreased in soils due to increased soil N. Although we know that the composition of the microbial community present in a soil is strongly dependent on its physicochemical properties (Griffiths and Philippot 2013), it is very difficult to distinguish the importance of these properties for the dominance of Chloroflexi in RP.

Bacterial Diversity

Soil pH has been reported to be the major factor governing bacterial diversity in soils (Fierer and Jackson 2006). In our study, there were significant differences in the soil properties among the three forest types (Table 1). The mean soil pHs in RP and EP were 4.72 and 4.47, respectively, which were slightly higher than that in SF (4.05). However, soil pH explained only 6.39% of total variance of taxonomic compositions of the 18 communities, which suggests that soil pH may not be the most important factor affecting bacterial communities in this region. It has been shown that the physiochemical characteristics of forest soils can influence soil bacterial diversity (Faoro et al. 2010), although the concentrations of MBC and SOM in SF were significantly higher than those in RP and EP. MBC and SOM combined explained about 10% of the total variance. Because of the application of fertilizers, the concentrations of TN, TP, and TK in RP and EP were significantly higher than that in SF. TN, TP, and TK together explained 43.5% of the total variance in the taxonomic composition at the phylum level, indicating that soil nutrition was the most important factor affecting the bacterial composition diversity in this region.

Our results also showed that OTU richness of RP and EP was significantly higher than that of SF (Figure 5), which was consistent with the results of Rodrigues et al. (2013) in that the local taxonomic (OTU) richness of soil bacteria increased after tropical rain forests were converted to pasture. Rodrigues et al. (2013) also revealed that communities become more similar across space (local scale of <10 km). Previous studies also have shown that conversion of tropical rainforest ecosystems threatens the microbial diversity because microorganisms, like all other organisms, have habitat preferences and may be affected by land-use changes (Martiny et al. 2006, da C Jesus et al. 2009). In this study, the lower Shannon diversity and higher Simpson dominance indices of SF than of RP and EP indicate that the diversities of RP and EP were relatively higher than that of SF. The main reason for the high diversities of the bacterial communities of RP and EP may be management practices, such as the application of fertilizers, which would result in higher diversity of soil bacterial as discussed above.

Conclusion

Our findings revealed there were significant differences among RP, EP, and SF in bacterial community composition at both the family and phylum levels (particularly at the family level). The Shannon indices of RP and EP were significantly higher than that of SF. RP and EP were converted from tropical secondary forest; thus, we suspect this conversion caused significant changes in the composition of the soil bacterial communities. However, bacterial diversity was not reduced after this conversion. Management practices, such as the application of fertilizers in RP and EP would markedly influence soil fertility, which appears to result in changes in the soil bacterial composition and diversity of this region.

Endnotes

1. For more information, see rdp.cme.msu.edu/.
2. For more information, see cran.r-project.org/web/packages/vegan.

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