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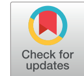
Land-use changes alter soil bacterial composition and diversity in tropical forest soil in China



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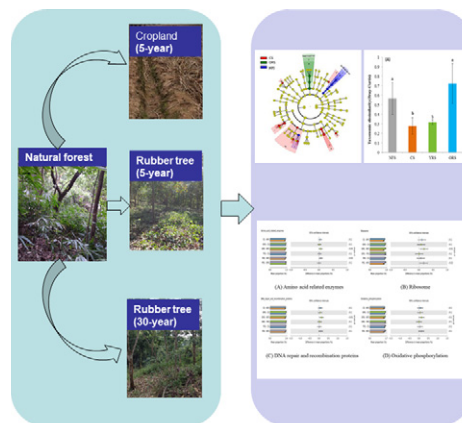
HIGHLIGHTS

- Land-use change remarkably alters soil bacterial community structure and function
- Significant effects of planting age on soil bacterial beta diversity
- Crucial roles of vegetation type in altering soil functional traits

GRAPHICAL ABSTRACT



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ABSTRACT

Tropical forests, under pressure from human activities, are important reservoirs of biodiversity and regulators of global biogeochemical cycles. Land-use and management are influential drivers of environmental change and ecosystem sustainability. However, only limited studies have analysed the impacts of planting age and vegetation type under land-use change on soil microbial community in tropical forests simultaneously. Here, we assessed soil bacterial community composition and diversity under different land-use in Hainan Province, China, using high-throughput sequencing combined with PICRUSt analysis. Land-use included natural forest, 5-year-old cropland, young (5-year-old) rubber tree plantation, and old (30-year-old) rubber tree plantation. Land-use changes altered the soil bacterial community composition but had a non-significant influence on alpha diversity ($P > .05$). We found that bacterial beta-diversity significantly decreased in young rubber tree plantation soils and cropland soils compared to natural forest as a control. In contrast, soil bacterial beta-diversity increased in old rubber tree plantation soils, indicating the effects of time since planting. There was no difference in microbial beta-diversity between soils from cropland and young rubber tree plantation. Soil bulk density and moisture, not pH, were the

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Vegetation type

main environmental factors explaining the variability in microbial diversity. PICRUSt analysis of soil bacterial predicted gene abundances within metabolic pathways and indicated that land-use change altered soil functional traits, e.g., amino acid-related enzymes, ribosomes, DNA repair/recombination proteins and oxidative phosphorylation. Also, vegetation type, not planting age, had significant impacts on soil functional traits. Overall, planting age had the greatest influence on soil bacterial beta-diversity, while vegetation type was more crucial for soil functional traits ($P < .05$).

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1. Introduction

Land-use change is one of the most crucial environmental pressures due to the influence of anthropogenic activities on natural landscapes, ecosystem functions and climate change (Abulizi et al., 2017; Foley et al., 2005; Muñoz-Rojas et al., 2015; Newbold et al., 2015; Papanastasis et al., 2017; Yu et al., 2013). Such effects of land-use change on global biology have attracted the interests of many ecologists and geographers (Lawler et al., 2014; Newbold et al., 2015; Song et al., 2015). Most studies have found that land-use change reduces biodiversity (Butler et al., 2010a; Butler et al., 2010b; Dormann et al., 2008; Newbold et al., 2015; Ussiri and Lal, 2013), but the main focus is on animals and plants (Albaladejo et al., 2013; de Assis et al., 2010; Ferreira et al., 2016; Fracetto et al., 2012; Stockmann et al., 2013); few studies have examined the effects on soil microbial diversity and functions under land-use change, particularly in tropical forests of China.

Soil microbial communities, including bacteria, fungi and archaea, are key players in ecosystems. They are closely related to nutrient and energy cycling, which support the ecosystem structure and functional stability (van der Heijden et al., 2008; Zak et al., 2003). Although soil microbial composition and diversity are reported to be influenced by different soil characteristics and vegetation types under land-use change, such as soil carbon, nutrient depletion, and reduced water holding capacity (Bossio et al., 2005; Kuczynski et al., 2010), the lack of comprehensive understanding on the effects on soil microbes of different land-use change requires more investigation.

Many previous studies have explored the impacts of forest land-use change on soil microbes with conflicting results and conclusions. Some studies found that changes in forest land-use type can alter soil microbial community composition (Kerfahi et al., 2014; Lupatini et al., 2013), increase soil microbial alpha-diversity, decrease beta-diversity, and lead to spatiotemporal homogenisation (Kerfahi et al., 2014; Purahong et al., 2014; Rodrigues et al., 2013; Vitali et al., 2016). However, others did not observe significant effects of land-use change on soil microbial alpha-diversity (Jesus et al., 2009; Tripathi et al., 2012). Besides, Lee-Cruz et al. and de Carvalho et al. found that the conversion of forest increased soil microbial beta diversity due to the increased environmental heterogeneity (de Carvalho et al., 2016; Lee-Cruz et al., 2013). The difference in original forest type and the land-use change afterwards may be the principal explanations for these controversial results (Lee-Cruz et al., 2013). Accordingly, studies on typical forest regions with a variety of land-use types under land-use change, such as different classic vegetation types and planting ages, can provide new clues and more reliable evidence to understand the effects of forest land-use change on soil microbes and practical suggestions for the effective management of forest conversion.

A comprehensive understanding of the biotic and abiotic factors that drive soil microbial community diversity could help in predicting changes in land use that affect ecosystems and global climate change since soil microbes are among organisms influencing biogeochemical processes and ecosystem stability. Studies have found that environmental factors such as pH and soil organic matters drive differences in soil microbial community dynamics under land-use change (Hartman et al., 2008; Vitali et al., 2016; Wakelin et al., 2008). Additionally, the temporal change in soil microbial community without land-use change has been intensively studied, suggesting that planting age of vegetation could significantly alter the composition and structure of microbial community (DeBruyn et al., 2011; Lipson, 2007; Zhang et al., 2011). Several studies found that the microbial community composition and alpha-diversity varied significantly over time, whereas the change in beta-diversity was relatively small (Fierer and Jackson, 2006; Wallenstein et al., 2007). However, the temporal change of soil microbial diversity under land-use change is unknown, especially in tropical rainforests of China.

Vegetation types are reported by some studies as the key driving forces affecting the structural and functional diversity of soil microbial communities to different extents (Berg and Smalla, 2009; Scherwinski et al., 2008; Wang et al., 2013; Wubet et al., 2012). For instance, Bossio et al. found significant changes in soil bacterial community composition after land-use change, with vegetation cover type as the dominant factor (Bossio et al., 2005). However, another study documented that soil traits played a relatively more important role in driving the variances of soil microbes compared with vegetation types (Fierer and Jackson, 2006). Till now, no conclusive result is widely accepted and more work about individual natural forest to various land-use types with different vegetation types can help in better understanding of the effects of land-use change on soil microbes.

In summary, land-use change would affect ecosystem functions and climate change by altering soil bacterial community composition and diversity. However, there is a considerable knowledge gap as regarding the effects of land-use change on soil microbial composition, diversity and functions when natural tropical forests are converted for agriculture. In this study, we examined the conversion of a tropical rainforest in Hainan Province (China) for rubber and rice production. It is a typical forest region with land-use change to cropland (5 years) and a rubber tree plantation (5 and 30 years). This study aimed to evaluate the effects of land-use change (from natural forest into cropland and artificial forests of different ages) on soil bacterial community and the predicted gene abundances within metabolic pathways using high-throughput sequencing and Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt) analysis. The objectives of this study are to (1) assess the response of soil bacterial community composition and diversity to land-use change; (2) identify the environmental factors that drive bacterial variability under different land-use types; (3) explore the changes in soil predicted gene abundances in metabolic pathways; and (4) determine whether different ages of artificial forests influence soil bacteria.

2. Materials and methods

2.1. Study area

The study was conducted in Hainan Province, China (19°26'54.96"N, 109°38'23.64"E, 244 m) (Fig. 1). The mean annual rainfall and air temperature are 651.4 mm and 30.5 °C, respectively. The soils are classified as Ultisol by the Food and Agriculture Organization soil taxonomy system, and their chemical and physical characteristics are listed in Table 1.

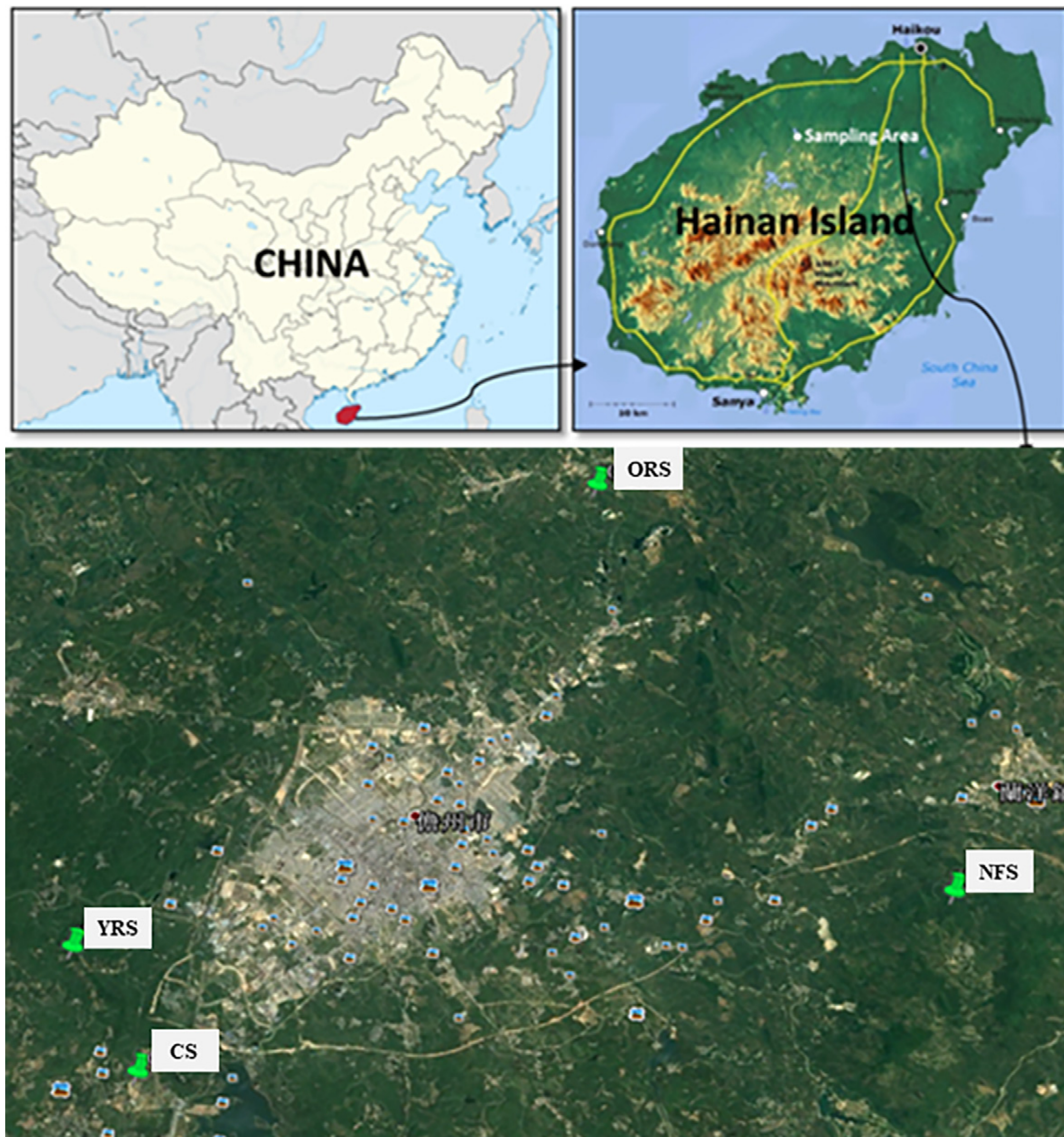

Fig. 1. Study sites. NFS (natural forest soils); CS (cropland soils); YRS (soils planted with young rubber tree of 5-year); ORS (soils planted with old rubber tree of 30-year).

2.2. Soil sampling

Soil sampling was conducted in 2012. Four land-use types were selected: natural forest soils (NFS), 5-year-old cropland soils (CS), young (5-year-old) rubber tree soils (YRS), and old (30-year-old) rubber tree soils (ORS). Cropland mainly grows rice and vegetables. Natural forests have no human management, rubber plantations receive NPK fertilizer (Table S2), and rice receives fertilizer and are annually waterlogged for 4–5 months.

The plots in each land-use type were subdivided into six subplots within 10 m acting as spatial replicates. In each subplot, three subsamples of topsoil (0–10 cm depth) within 5 m were collected using a spade and pooled to form a composite sample. Besides, volumetric metal rings (inner diameter, 70 ± 0.16 mm; height, 52 ± 0.16 mm) were used to collect intact soil cores to determine the soil bulk density.

Portions of the samples were used for measuring soil chemical and physical characteristics. They were passed through a 2-mm sieve, and a 300-g aliquot of each sample was separated, placed in a plastic bag,

Table 1
Physical and chemical characteristics of soils from study sites. Different letters ("a" and "b") indicate significant differences between treatments ($P < 0.05$) and the same letters indicate no significant($P > 0.05$).

Sample ID	Latitude	Longitude	Distance(m)	Soil density	Moisture (%)	pH	TC (%)	TOC (%)	TN (%)
NFS	19.4486	109.6399	0	1.25 ± 0.01a	16.38 ± 1.51b	4.51 ± 0.17a	1.13 ± 0.10b	0.91 ± 0.18b	0.12 ± 0.01a
CS	19.511	109.5062	15,656	1.13 ± 0.00a	30.20 ± 4.45a	4.47 ± 0.18a	2.17 ± 0.59ab	2.05 ± 0.82a	0.18 ± 0.03a
YRS	19.5375	109.5141	16,498	1.20 ± 0.08a	12.29 ± 2.80b	4.51 ± 0.26a	1.67 ± 0.10b	1.51 ± 0.10ab	0.17 ± 0.03a
ORS	19.5521	109.6522	11,593	1.27 ± 0.00a	13.67 ± 2.42b	4.57 ± 0.11a	2.83 ± 0.23a	2.43 ± 0.26a	0.27 ± 0.02a

and stored at 4 °C to measure the microbial biomass and activities within 2 days. The remaining soil samples were air-dried and stored at room temperature before chemical analysis.

2.2.1. Soil property analysis

Soil moisture, pH, and bulk density were measured using the Chinese Standard Protocol LYT-1999. Soil samples with metal rings were weighed and then dried at 105 °C to measure the soil bulk density. Total organic carbon (TOC) and total nitrogen (TN) were determined as described previously with modifications (Hedges and Stern, 1984). Briefly, 2 g of freeze-dried soil was blended and treated twice with 25 mL of HCl (1 M), followed by washing with ultrapure water to a final pH of 6–7. Then, TOC and TN were analysed using an elemental analyser (Vario EL cube; Elementar Analysensysteme GmbH, Langenselbold, Germany).

DNA extraction and polymerase chain reaction (PCR).

Soil total DNA was extracted in triplicates from each sample using the PowerSoil DNA extraction kit (MoBio, Carlsbad, CA, USA) following the manufacturer's instructions. Extracted DNA from the same sample was pooled and quantified with a NanoDrop 2000 spectrophotometer (Thermo Scientific, Waltham, MA, USA). The DNA was stored at −80 °C for further analysis. The primer pair (515F: 5′-GTGCCAGC MGCCGCGGTAA-3′, 806R: 5′-CCGGACTACVSGGGTATCTAAT-3′) targeting the V3 hypervariable region of the 16S rRNA gene was used for PCR (Sul et al., 2011). The 806R primer was labelled with a unique 12 bp barcode to distinguish among amplification products. PCR was performed in a 50-μL volumes containing 20 ng of DNA template, 25 μL of Taq premix buffer (TaKaRa, Shiga, Japan), 10 μM of each primer, and 22 μL of H_2O. Then, 16S rRNA genes were amplified in triplicates using the following process: 94 °C for 5 min, 28 cycles at 94 °C for 30 s, 55 °C for 30 s, 72 °C for 90 s, and a final extension at 72 °C for 5 min. The triplicated products were combined and tested using agarose gel (1.5%) and purified with the MicroElute Cycle-Pure Kit (Omega Bio-Tek, Norcross, GA, USA) following the manufacturer's instructions. Products from the same sample were combined and quantified as described above and sent to Beijing Genomics Institute (Shenzhen, China) for sequencing using the MiSeq 200 system (Illumina, San Diego, CA, USA). All the high-throughput sequencing data were submitted to NCBI (PRJAN561291).

2.3. Sequence analysis

For DNA amplicon sequencing data, we removed the low-quality reads (quality score < 20, length < 250) using Perl shell (reads_with_trimm_low_quality.pl, see SI). The 16S rRNA raw data were assembled using mothur v1.39.5 (Schloss et al., 2009). The sequence data were normalized according to the minimum number of samples to ensure the homogeneity of the sequence. The sequence data were then qualified, filtered, and clustered following the QIIME 1 manual (Caporaso et al., 2010). Operational taxonomic units (OTUs) with 97% similarity were selected, and the representative sequence set was chosen. Then, chimeric sequences were discarded (Edgar et al., 2011). OTUs were assigned to taxonomic groups using the Greengenes 13_5 database with the “assign_taxonomy.py” function.

2.4. Diversity analysis

Alpha-diversity (effective number of species, Chao1, Simpson and Shannon indices) and beta-diversity (weighted UniFrac) were calculated using the QIIME script. UniFrac distance, a web application that allows researchers to address many of these broader questions about the composition and evolution of bacterial communities, uses phylogenetic information to determine whether communities were significantly different and reveal broad patterns relating many environmental samples (Hamady et al., 2010; Lozupone et al., 2006; Lozupone et al., 2011). It has been widely used to analyze large data sets from next-generation sequencing technologies and in QIIME and Mothur analysis pipelines (Caporaso et al., 2010; Schloss et al., 2009). Thus, we used the weighted UniFrac distance to measure phylogenetic community similarity in our present study (Lozupone et al., 2011).

2.5. PICRUSt analysis

To predict the microbial functional responses to land-use change, we used PICRUSt 1.1.1 (http://picrust.github.com) to generate a functional profile using high-throughput sequencing results (Langille et al., 2013). PICRUSt has the accuracy of averagely 0.8 and up to 0.9 to predict microbial functions by testing a broad range of data sets from soils. Particularly when closely related reference genomes are available, PICRUSt is accurate and accepted by many researchers to study soil microbial functions (Langille et al., 2013; Ling et al., 2016; Zarraonaindia et al., 2015). We followed the suggested methods for OTU selection with Greengenes 13_5 using Galaxy (http://huttenhower.sph.harvard.edu/galaxy/). The predicted gene family abundances were analysed using Kyoto Encyclopedia of Genes and Genomes orthology group count level 3 and Storey FDR in STAMP software was used to avoid inflation of Type-I error (Parks et al., 2014).

2.6. Statistical analysis

Redundancy analysis (RDA) was performed to determine the relationships between soil microbial communities and selected soil properties by the “Bioenv” function in the “vegan” package (vegan v2.4-4) of R software (R Development Core Team, Vienna, Austria), using UniFrac weighting and the normalized distance matrix with default parameters. Before RDA, we firstly eliminated the spatial autocorrelation by removing two samples from total 26 samples. The physicochemical data comprised soil pH, TOC, TN, moisture and bulk density. The significance of each variable was determined using the permutation test with analysis of variance (ANOVA) in ‘vegan’. The linear discriminant analysis (LDA) effect size (LEfSe) can be used for high-dimensional biomarker discovery and identification of genomic features (e.g., genes, pathways, or taxa), to characterise the differences between two or more biological conditions (Segata et al., 2011). First, we used the non-parametric factorial Kruskal–Wallis rank sum test to detect features with significantly different abundances with respect to the class of interest. Next, biological significance was examined by pairwise testing of subclasses using the (unpaired) Wilcoxon rank-sum test (Segata et al., 2011). Finally, LDA was applied to estimate the effect size of each differentially abundant feature and performed dimension reduction and the “p.adjust” function (method = “Bonferroni”) was used to avoid inflation of Type-I error. Additional statistical analysis was conducted using SPSS for Windows (ver. 20.0; SPSS Inc., Chicago, IL, USA). One-way ANOVA and per multivariate analysis of variance (PERMANOVA) were used to analyze the significance of differences and variability in soil bacterial alpha-diversity and composition at the 95% confidence level ($P < .05$), respectively. The variation of soil bacterial beta-diversity was measured by the “adonis” function in the “vegan” package (vegan v2.4-4) of R software.

3. Results

3.1. Soil bacterial community composition

Soil bacterial community composition showed a clear distribution pattern at the phylum level (Fig. 2). Relatively abundant bacterial phyla in soil included *Proteobacteria*, *Planctomycetes*, *Nitrospirae*, *Verrucomicrobia*, *Bacteroidetes*, *Chloroflexi*, and *Actinobacteria* (total abundance > 90%). However, there was no significant change in soil bacterial community composition at the phylum level across different land-use changes ($P = .35$, Table S1). The relative abundance of *Actinobacteria* significantly decreased in ORS comparing with YRS, but

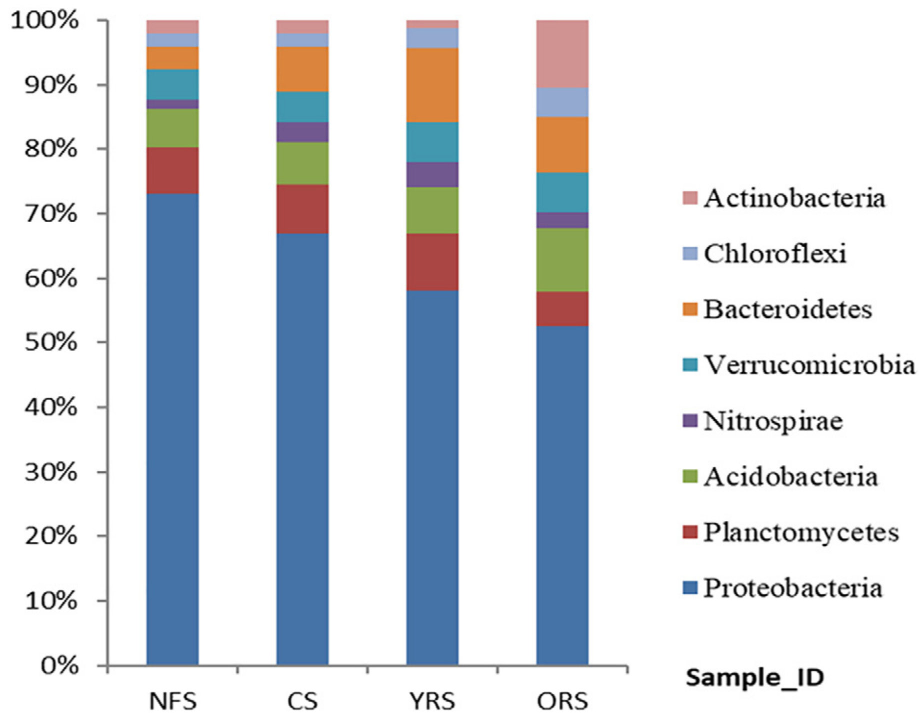

Fig. 2. Relative abundances (%) of soil dominant bacterial lineages at the phylum level across different land-use changes. NFS (natural forest soils); CS (cropland soils); YRS (soils planted with young rubber tree of 5-year); ORS (soils planted with old rubber tree of 30-year).

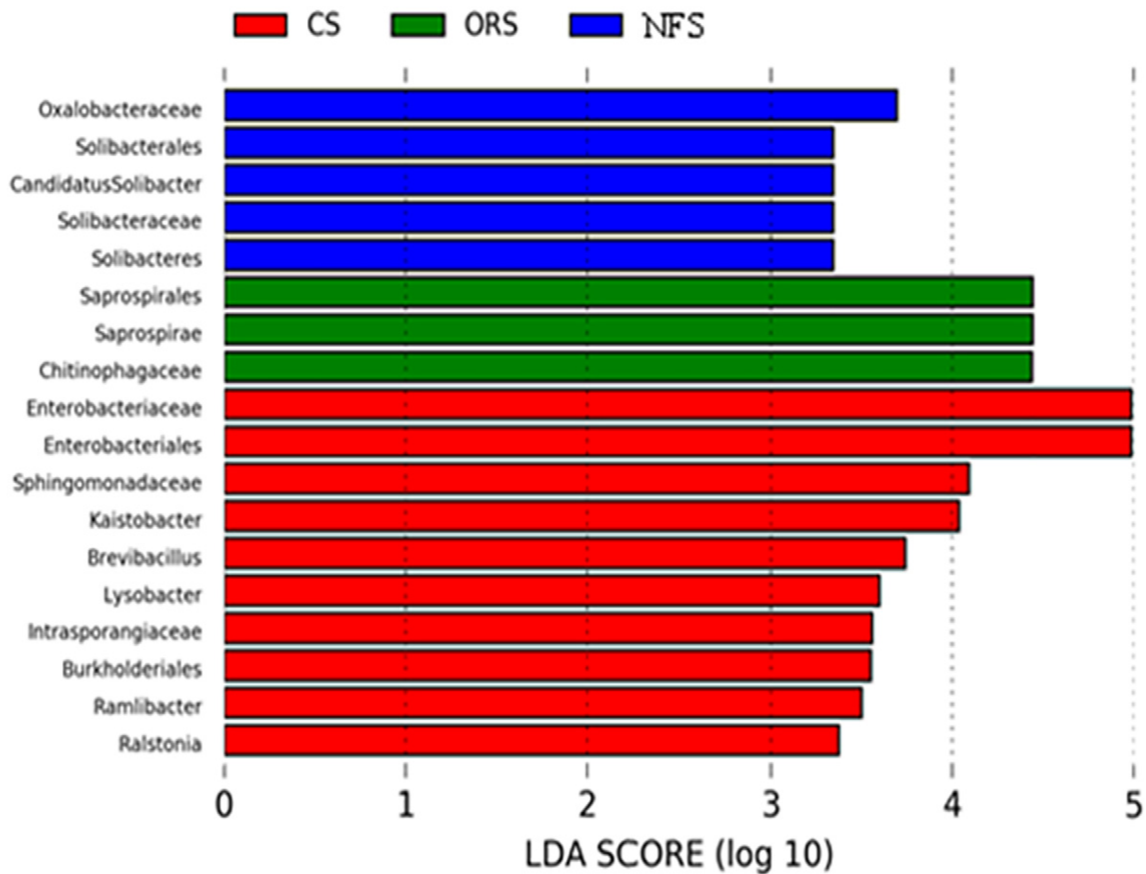

Fig. 3. Horizontal bar plot of the LDA scores calculated for features differentially abundant among study sites. Alpha value for the factorial Kruskal-Wallis test among classes and for the pairwise Wilcoxon test between subclasses uses the default one. Threshold on the logarithmic LDA score for discriminative features is 3.0. NFS (natural forest soils); CS (cropland soils); ORS (soils planted with old rubber tree of 30-year). LAD score represents the influence degree of significantly different species between different groups.

there was no significant change comparing ORS with other land-use types (Fig. S2). It is worth mentioning that *Proteobacteria* and *Proteobacteria/Acidobacteria* decreased with TOC, whereas *Acidobacteria* increased (Fig. S2, Table 1).

We further analysed the bacterial composition at the genus level and found significant variations across the land-use types, as revealed by the LEfSe. In total, eighteen bacterial genera with significant difference were detected (Fig. 3, $P < .05$). They were *Oxalobacteraceae*, *Solibacterales*, *Candidatus*, and *Solibacteres* in NFS, whereas *Chitinophagaceae* and *Saprospirales* for ORS (Fig. 3, $P < .05$). Moreover, the most differentially abundant bacterial taxa in CS were *Enterobacteriaceae*, *Enterobacteriales*, *Sphingomonadaceae*, *Kaistobacter*, *Brevibacillus*, *Lysobacter*, *Intrasporangiaceae*, *Burkholderiales*, *Ramlibacter*, and *Ralstonia* (Fig. 3, $P < .05$). No abundant bacterial taxa were significantly different in YRS comparing with other land-use types.

3.2. Soil bacterial diversity

3.2.1. Alpha-diversity

Alpha-diversity describes the species diversity within a single sample, including the indices of effective number of species, Chao1, Shannon and Simpson. Both effective number of species and Chao1 reflect the species richness in a sample, regardless of the abundance of each species in the community, whereas the Shannon and Simpson indices represent the species richness and species evenness of the community. Effective number of species increased slightly but non-significantly ($P > .05$) with land-use change compared with NFS, following the order of CS > ORS > YRS > NFS (Fig. 4). Consistent results were obtained for Simpson, Shannon and Chao1 indices which increased slightly but non-significantly ($P > .05$) with land-use change compared with NFS, following the order of CS > ORS > YRS > NFS (Fig. 4).

3.2.2. Beta-diversity

To evaluate the variability in community composition among soil samples across different land-use types, beta-diversity was assessed by Bray–Curtis and weighted UniFrac dissimilarity values. In contrast with alpha diversity, beta-diversity varied significantly in soils from cropland and rubber tree plantation compared with NFS ($P < .05$) (Fig. 5). The Bray–Curtis dissimilarity value of ORS was higher than NFS but non-significantly ($P > .05$), both significantly higher than those of CS and YRS ($P < .05$, Fig. 5A). The weighted UniFrac values illustrated similar results with Bray-Curtis (Fig. 5B).

3.3. Factors driving variability in the bacterial community

Based on the soil bacterial community composition and diversity indices, the main environmental factors driving bacterial variability were analysed using RDA. Environmental factors could explain 31.54% of the species variability, where RDA1 and RDA2 accounted for 27.80% and 3.7% of the total variance, respectively (Fig. 6). In addition, soil bulk density ($r^2 = 0.4857$, $P = .001$) and moisture ($r^2 = 0.2073$, $P = .015$) were the main environmental factors driving spatial variation. As pH varied non-significantly across land-use types (Table 1), it did not show significant effects on soil bacterial distribution ($r^2 = 0.0575$, $P = .551$).

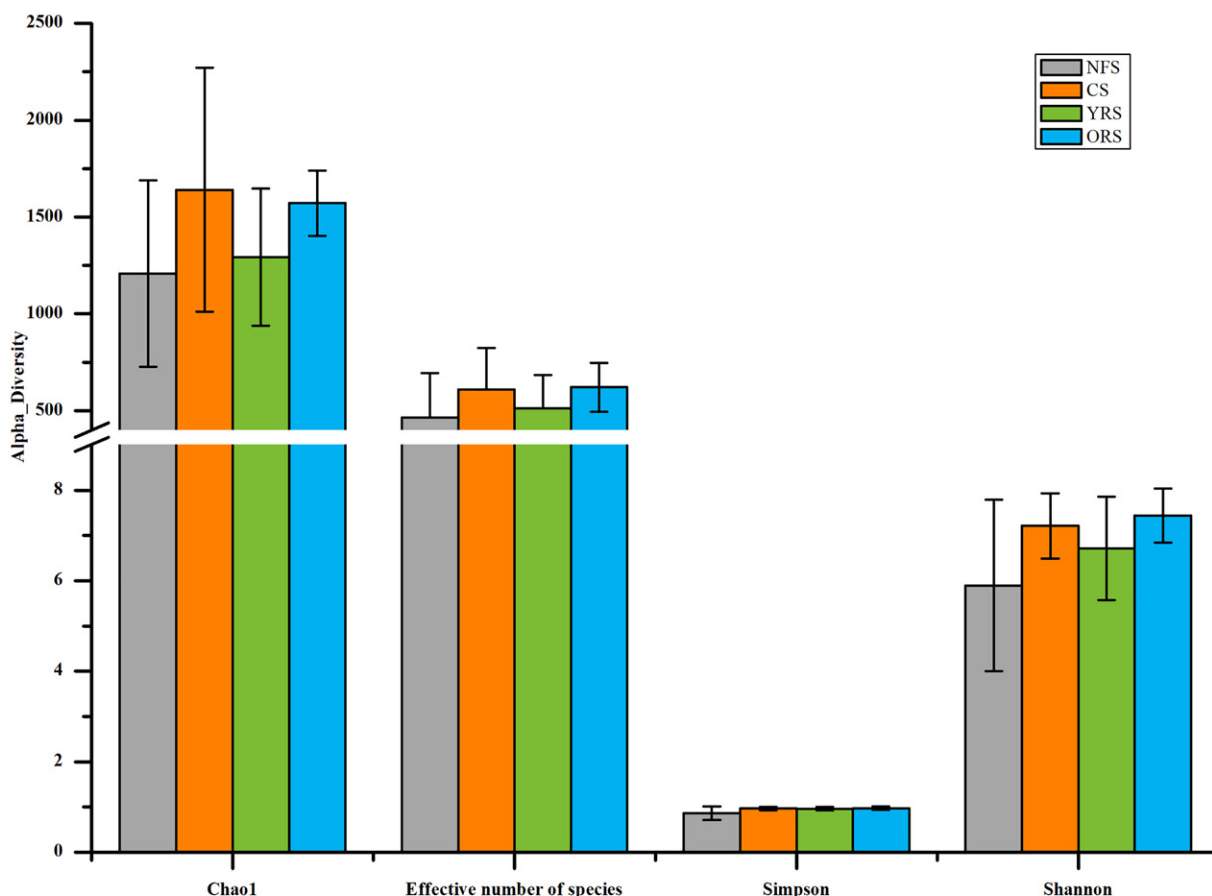

Fig. 4. Soil bacterial alpha diversity indices across different land-use changes ($n = 6$, ±95% CI). NFS (natural forest soils); CS (cropland soils); YRS (soils planted with young rubber tree of 5-year); ORS (soils planted with old rubber tree of 30-year).

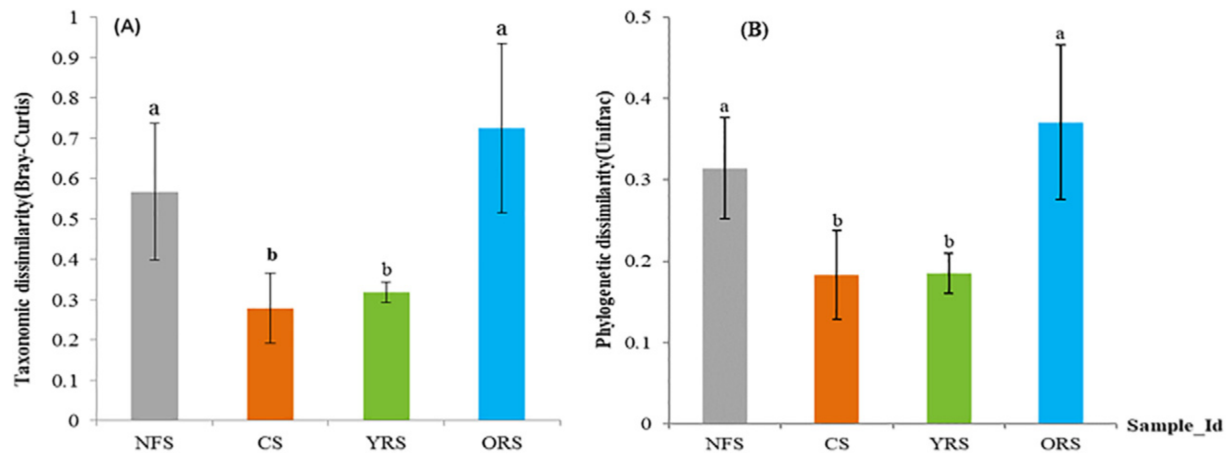

Fig. 5. Response of community similarity to ecosystem conversion. (A) Average taxonomic dissimilarity (Bray–Curtis). (B) Average phylogenetic dissimilarity (Weighted UniFrac, n = 6, ± 95% CI). NFS (natural forest soils); CS (cropland soils); YRS (soils planted with young rubber tree of 5-year); ORS (soils planted with old rubber tree of 30-year).

3.4. Effects of land-use change on predicted gene abundances within metabolic pathways

We used the PICRUSt analysis to assess the effects of land-use change on soil ecological functions by predicting gene abundances within metabolic pathways. Changes in the predicted gene abundances within metabolic pathways differed significantly across land-use changes in terms of amino acid-related enzymes, ribosomes, DNA repair and recombination proteins and oxidative phosphorylation ($P < .1$, Fig. 7). Besides, nitrogen metabolism related to nitrogen cycle showed little difference under land-use change with different vegetation types ($P > .1$). Compared with NFS, YRS and ORS exhibited significant differences in predicted gene abundances within metabolic pathways, whereas no notable difference was observed for CS ($P < .1$).

4. Discussion

4.1. Composition of soil bacterial community by land-use type

Our results suggested that land-use change significantly altered soil bacterial community composition at the genus level (Fig. 3), in agreement with previous studies (Jesus et al., 2009; Lee-Cruz et al., 2013; Rodrigues et al., 2013; Vitali et al., 2016). *Proteobacteria*/*Acidobacteria* ratio usually prefers labile organic C pools organic C quality, and it decreased with TOC in the present study. This result is inconsistent with most previous studies that *Proteobacteria*/*Acidobacteria* ratio normally increases with available C (Fierer et al., 2007; Thomson et al., 2010; Thomson et al., 2013). It may be explained by the lower pH in our study (Table 1), which is more suitable for *Acidobacteria*. Moreover, vegetation types, planting age and other undetected factors may have a

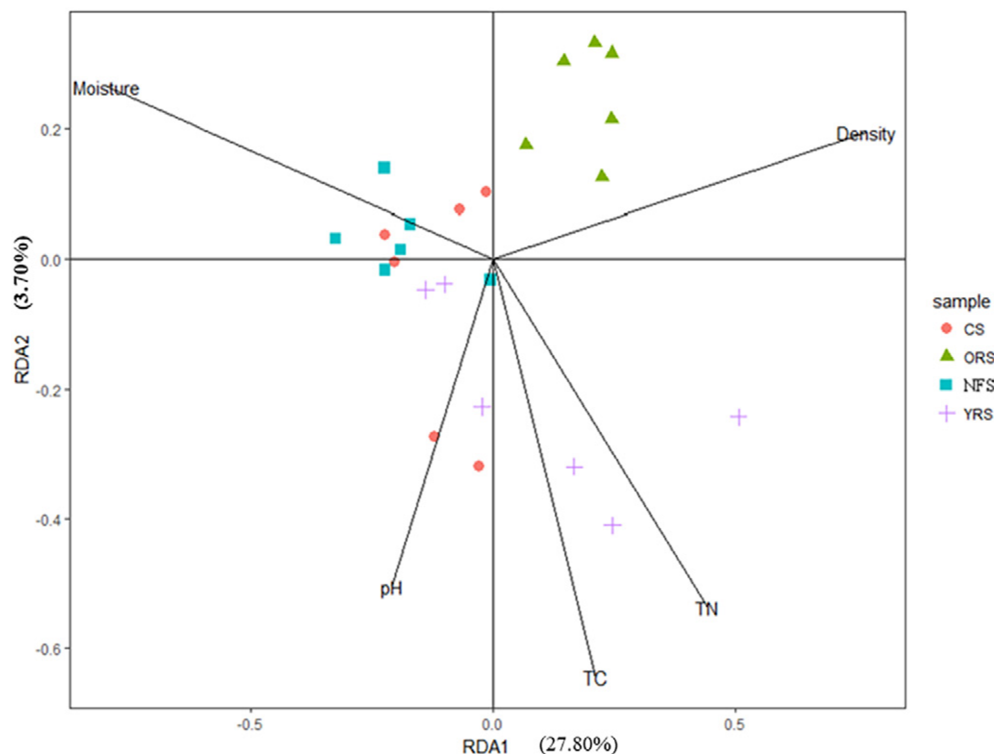

Fig. 6. Redundancy analysis (RDA) for the relationship between soil properties and soil microbial communities across different land-use changes. NFS (natural forest soils); CS (cropland soils); YRS (soils planted with young rubber tree of 5-year); ORS (soils planted with old rubber tree of 30-year).

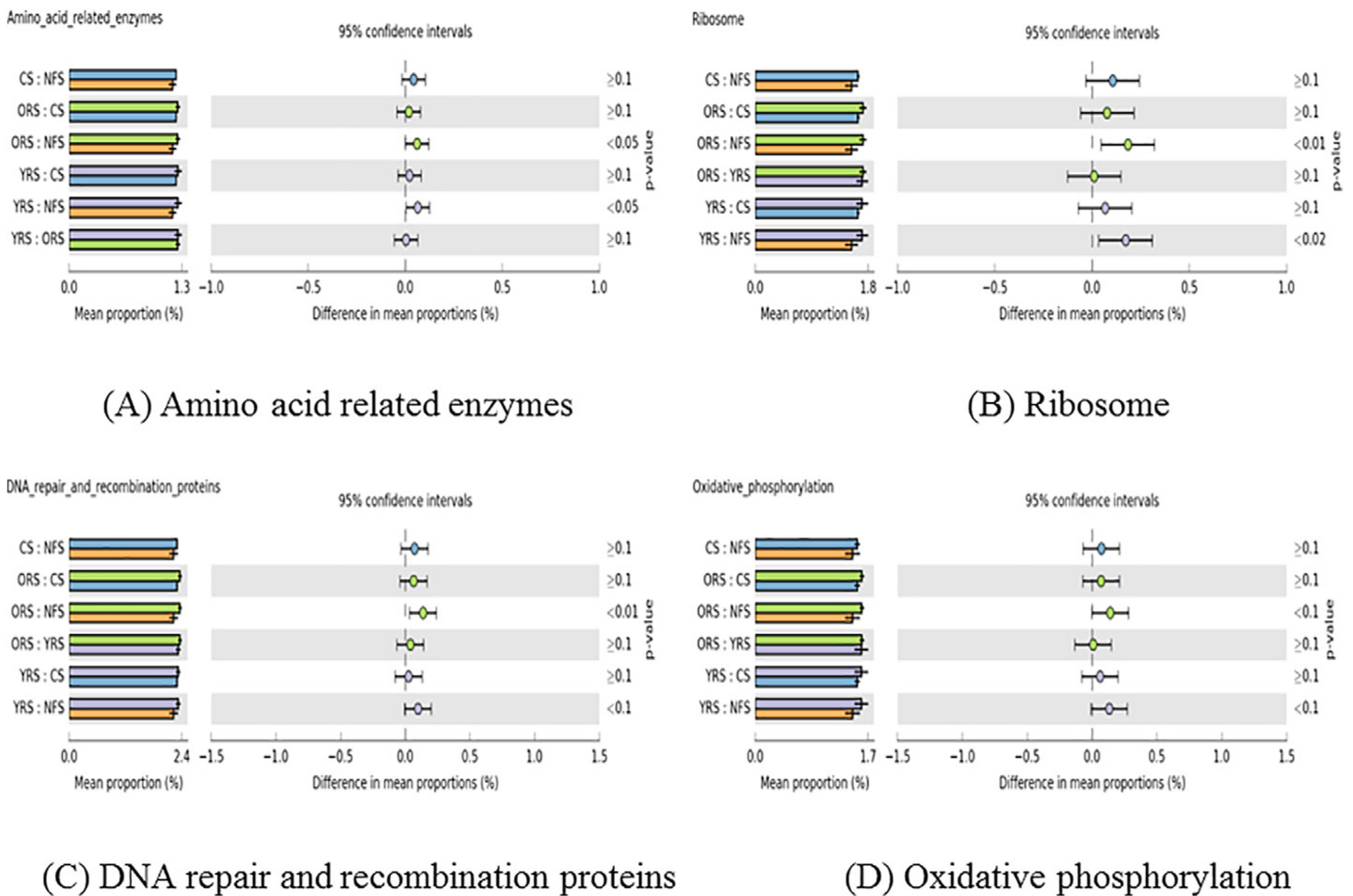

Fig. 7. Metabolic pathway of soil bacterial communities across different land-use changes predicted by PICRUSt. (A) Amino acid related enzymes; (B) Ribosome; (C) DNA repair and recombination proteins; (D) Oxidative phosphorylation. NFS (natural forest soils); CS (cropland soils); YRS (soils planted with young rubber tree of 5-year); ORS (soils planted with old rubber tree of 30-year). The labels on the left axes represent the differences in metabolic pathways between each two land-use types.

more significant influence on *Proteobacteria* and *Acidobacteria* than available C. Besides, the abundance of *Euryarchaeota* (*Archaea*) was significantly higher in NFS than other land-use types, possibly owing to the effects of cropland and management practices in rubber tree areas, such as fertilisation, crop rotation, and irrigation.

4.2. Bacterial diversity by land-use type

Some studies document that land-use conversion can alter soil microbial community composition and increase or decrease bacterial alpha-diversity (de Carvalho et al., 2016; Montecchia et al., 2015; Rodrigues et al., 2013). However, our results suggested no significant alpha-diversity change in different land-use changes, consistent with some other studies (de Carvalho et al., 2016; Rodrigues et al., 2013). It is possibly explained by soil management practices and microbial restoration in the study area, as the resistance of soil microbes to land-use change which might lead to slight alterations in bacterial diversity across different land-use types (Alele et al., 2014; Griffiths and Philippot, 2013). Another possible explanation is no-significant variation of pH which is suggested as a strong driving force on soil microbes by previous studies (Hartman et al., 2008; Vitali et al., 2016; Wakelin et al., 2008)(Hartman et al., 2008; Vitali et al., 2016; Wakelin et al., 2008).

In the present study, soil microbial beta-diversity differed significantly by land-use change with different planting ages. The land-use types with 5-year plantation (CS and YRS) had significantly lower beta-diversities than NFS, evidencing the homogenisation of soil bacterial communities. It might be explained by the loss of less-resilient members of the bacterial community, especially in the short planting age (Allison and Martiny, 2008). In contrast, the beta-diversity of ORS was similar with NFS, implying a recovery of soil bacterial communities and agreeing with some previous studies (de Carvalho et al., 2016; Lee-Cruz et al., 2013). This might be caused by the long-term soil management practices such as fertilisation (Table S2, Supporting Information) and an increasing number of existing species from the regional pool (Jesus et al., 2009), as illustrated in the Venn diagram of the shared and unique OTUs (Fig. S3, Supporting Information). Change in the relative abundance of dominant bacteria (*Proteobacteria*, *Planctomycetes*, *Nitrospirae*, *Verrucomicrobia*, *Bacteroidetes*, *Chloroflexi*, and *Actinobacteria*) and the decreasing unique OTUs in ORS further evidenced the recovery of existing species from the regional pool. Moreover, the significant difference in bacterial beta-diversity between ORS and YRS may be explained by the loss of some bacterial communities sensitive to disturbance through land-use change with a short planting age (Allison and Martiny, 2008), and subsequent recovery in a long planting age (Gregory et al., 2009). Besides, the differences in bacterial beta-diversity may be a consequence of habitat heterogeneity (Hanson et al., 2012; Kallimanis et al., 2008) as verified by RDA (Fig. 6). Soil bulk density and moisture were the main environmental factors driving the spatial variability, but pH had a small effect on soil bacteria. This is different with the findings of most studies showing that pH is the main environmental factor driving spatial and temporal bacterial distribution (Lauber et al., 2009; Rousk et al., 2010; Tripathi et al., 2012). This discrepancy may be attributed to the slight variation of pH with land-use change in our study (Table 1).

There was no significant difference in beta-diversity between CS and YRS, indicating that vegetation type has only considerable influence on soil bacterial beta-diversity, especially within short planting age.

Agricultural management practices, such as fertilisation and irrigation, result in the relatively similar of soil properties and lead to a homogenisation of soil bacterial communities in short term (Figuerola et al., 2015). This indicated that soil management plays a more important role than vegetation type on soil bacterial beta-diversity in short term, and sustainable soil management would be helpful to minimize the damage of land-use change to soil bacteria.

4.3. Predicted gene abundances within metabolic pathways

Because dominant bacteria have an important role in maintaining soil ecological functions, we used PICRUSt analysis for the predicted gene abundance within metabolic pathways based on high-throughput sequencing data. The predicted gene abundances within metabolic pathways in CS differed little comparing with those in NFS, whereas they exhibited significant differences between NFS and YRS or ORS (Fig. 7). The results indicated that the alterations in soil functional traits after land-use change were influenced by vegetation type, which potentially caused considerable shifts in soil properties (Roberts et al., 2009) and aggravated the changes in soil functional traits (Feng et al., 2018). Additionally, differences in vegetation litters and root exudates could change soil bacterial composition and diversity, exerting specific effects on soil functional microbes (Somers et al., 2004; Wardle et al., 2004). However, there was no notable difference in the predicted gene abundances within metabolic pathways between YRS and ORS, showing that planting age had a small influence on soil functional traits after land-use change (Fig. 7). It may be attributed to the stronger influence of vegetation type on functional traits than planting age. It is worth noting that no significant difference in the nitrogen metabolism was observed under land-use change with different vegetation types, possibly explained by the low abundance of functional bacterial taxa related to nitrogen cycle as revealed by high-throughput sequencing, and further work is suggested to target the functional genes associated with nitrogen cycle via metagenomics or GeoChip (Wardle et al., 2004).

5. Conclusions

In this study, we investigated the responses of soil bacterial community composition and diversity to land-use change from tropical natural forest to cropland and rubber plantation in the Hainan region of China. Land-use change altered the soil bacterial community composition and significantly influenced bacterial beta-diversity, but had only considerable influence on bacterial alpha-diversity. The key environmental factors shaping the bacterial community were soil bulk density and moisture, not pH. The significant effects of planting age suggested that soil bacterial beta-diversity could recover over time. Compared with planting age, vegetation type had a small influence on soil bacterial beta-diversity after land-use change. Moreover, the analysis of bacterial predicted gene abundances within metabolic pathways revealed that soil functional traits were significantly influenced by land-use changes and vegetation types, but not planting age. Our findings offer evidence for a strong effect of land-use change and management on soil bacterial communities, and a basis for further research on their structural and functional stability in converted tropical forest soils.

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Author contributions

The work presented here was carried out in collaboration between all authors. C.L and Y.S conceived the work and drafted the manuscript; Y.S, L.J and M.S. performed the experiments and analysed the data; D.Z, J.L, Y.L, N.O and G. Z participated in the acquisition, analysis, interpretation of data or provided constructive discussions.

Declaration of competing interest

The authors declare no competing interests.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2020.136526.

References

Abulizi, A., Yang, Y., Mamat, Z., Luo, J., Abdulslam, D., Xu, Z., et al., 2017. Land-use change and its effects in Charchan Oasis, Xinjiang, China. Land Degrad. Dev. 28, 106–115.
Albaladejo, J., Ortiz, R., Garcia-Franco, N., Navarro, A.R., Almagro, M., Pintado, J.G., et al., 2013. Land use and climate change impacts on soil organic carbon stocks in semi-arid Spain. J. Soils Sediments 13, 265–277.
Alele, P.O., Sheil, D., Surget-Groba, Y., Shi, L.L., Cannon, C.H., 2014. How does conversion of natural tropical rainforest ecosystems affect soil bacterial and fungal communities in the Nile River watershed of Uganda? PLoS One 9.
Allison, S.D., Martiny, J.B., 2008. Resistance, resilience, and redundancy in microbial communities. Proc. Natl. Acad. Sci. 105, 11512–11519.
de Assis, C.P., de Oliveira, T.S., Dantas, J.D.D., Mendonca, E.D., 2010. Organic matter and phosphorus fractions in irrigated agroecosystems in a semi-arid region of Northeastern Brazil. Agric. Ecosyst. Environ. 138, 74–82.
Berg, G., Smalla, K., 2009. Plant species and soil type cooperatively shape the structure and function of microbial communities in the rhizosphere. FEMS Microbiol. Ecol. 68, 1–13.
Bossio, D.A., Girvan, M.S., Verchot, L., Bullimore, J., Borelli, T., Albrecht, A., et al., 2005. Soil microbial community response to land use change in an agricultural landscape of Western Kenya. Microb. Ecol. 49, 50–62.
Butler, S.J., Boccaccio, L., Gregory, R., Vorisek, P., Norris, K.J.A., 2010a. Ecosystems, Environment. Quantifying the Impact of Land-use Change to European Farmland Bird Populations. 137 pp. 348–357.
Butler, S.J., Boccaccio, L., Gregory, R.D., Vorisek, P., Norris, K., 2010b. Quantifying the impact of land-use change to European farmland bird populations. Agric. Ecosyst. Environ. 137, 348–357.
Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., et al., 2010. QIIME allows analysis of high-throughput community sequencing data. Nat. Methods 7, 335–336.
de Carvalho, T.S., da Conceição Jesus, E., Barlow, J., Gardner, T.A., Soares, I.C., Tiedje, J.M., et al., 2016. Land use intensification in the humid tropics increased both alpha and beta diversity of soil bacteria. Ecology 97, 2760–2771.
DeBruyn, J.M., Nixon, L.T., Fawaz, M.N., Johnson, A.M., Radosevich, M., 2011. Global biogeography and quantitative seasonal dynamics of gemmatimonadetes in soil. Appl. Environ. Microbiol. 77, 6295–6300.
Dormann, C.F., Schweiger, O., Arens, P., Augenstein, I., Aviron, S., Bailey, D., et al., 2008. Prediction uncertainty of environmental change effects on temperate European biodiversity. Ecol. Lett. 11, 235–244.
Edgar, R.C., Haas, B.J., Clemente, J.C., Quince, C., Knight, R., 2011. UCHIME improves sensitivity and speed of chimera detection. Bioinformatics 27, 2194–2200.
Feng, Y., Wang, J., Yuan, K., Zong, W., Guo, D., 2018. Vegetation affects pool size and composition of amino acids in Tibetan alpine meadow soils. Geoderma 310, 44–52.
Ferreira, A.C.C., Leite, L.F.C., de Araujo, A.S.F., Eisenhauer, N., 2016. Land-use type effects on soil organic carbon and microbial properties in a semi-arid region of Northeast Brazil. Land Degrad. Dev. 27, 171–178.
Fierer, N., Jackson, R.B., 2006. The diversity and biogeography of soil bacterial communities. Proc. Natl. Acad. Sci. U. S. A. 103, 626–631.
Fierer, N., Bradford, M.A., Jackson, R.B.J.E., 2007. Toward an ecological classification of soil bacteria. 88, 1354–1364.
Figuerola, E.L.M., Guerrero, L.D., Turkowsky, D., Wall, L.G., Erijman, L., 2015. Crop monoculture rather than agriculture reduces the spatial turnover of soil bacterial communities at a regional scale. Environ. Microbiol. 17, 678–688.
Foley, J.A., DeFries, R., Asner, G.P., Barford, C., Bonan, G., Carpenter, S.R., et al., 2005. Global consequences of land use. Science 309, 570–574.
Fracetto, F.J.C., Fracetto, G.G.M., Cerri, C.C., Feigl, B.J., Neto, M.S., 2012. Carbon and nitrogen stocks in soil under castor bean in the semi-arid Caatinga of Brazil. Revista Brasileira De Ciencia Do Solo 36, 1545–1552.
Gregory, A.S., Watts, C.W., Griffiths, B.S., Hallett, P.D., Kuan, H.L., Whitmore, A.P., 2009. The effect of long-term soil management on the physical and biological resilience of a range of arable and grassland soils in England. Geoderma 153, 172–185.
Griffiths, B.S., Philippot, L., 2013. Insights into the resistance and resilience of the soil microbial community. FEMS Microbiol. Rev. 37, 112–129.

Hamady, M., Lozupone, C., Knight, R., 2010. Fast UniFrac: facilitating high-throughput phylogenetic analyses of microbial communities including analysis of pyrosequencing and PhyloChip data. ISME J 4, 17–27.

Hanson, C.A., Fuhrman, J.A., Horner-Devine, M.C., Martiny, J.B., 2012. Beyond biogeographic patterns: processes shaping the microbial landscape. Nat. Rev. Microbiol. 10, 497.

Hartman, W.H., Richardson, C.J., Vilgalys, R., Bruland, G.L., 2008. Environmental and anthropogenic controls over bacterial communities in wetland soils. Proc. Natl. Acad. Sci. 105, 17842–17847.

Hedges, J.I., Stern, J.H., 1984. Carbon and nitrogen determinations of carbonate-containing solids1. Limnol. Oceanogr. 29, 657–663.

van der Heijden, M.G.A., Bardgett, R.D., van Straalen, N.M., 2008. The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. Ecol. Lett. 11, 296–310.

Jesus, E.D., Marsh, T.L., Tiedje, J.M., Moreira, F.M.D., 2009. Changes in land use alter the structure of bacterial communities in Western Amazon soils. ISME J. 3, 1004–1011.

Kallimanis, A.S., Mazaris, A.D., Tzanopoulos, J., Halley, J.M., Pantis, J.D., Sgardelis, S.P., 2008. How does habitat diversity affect the species–area relationship? Glob. Ecol. Biogeogr. 17, 532–538.

Kerfahi, D., Tripathi, B.M., Lee, J., Edwards, D.P., Adams, J.M., 2014. The impact of selective-logging and forest clearance for oil palm on fungal communities in Borneo. PLoS One 9.

Kuczynski, J., Liu, Z.Z., Lozupone, C., McDonald, D., Fierer, N., Knight, R., 2010. Microbial community resemblance methods differ in their ability to detect biologically relevant patterns. Nat. Methods 7, 813–U67.

Langille, M.G.I., Zaneveld, J., Caporaso, J.G., McDonald, D., Knights, D., Reyes, J.A., et al., 2013. Predictive functional profiling of microbial communities using 16S rRNA marker gene sequences. Nat. Biotechnol. 31 (814-+).

Lauber, C.L., Hamady, M., Knight, R., Fierer, N., 2009. Pyrosequencing-based assessment of soil pH as a predictor of soil bacterial community structure at the continental scale. Appl. Environ. Microbiol. 75, 5111–5120.

Lawler, J.J., Lewis, D.J., Nelson, E., Plantinga, A.J., Polasky, S., Withey, J.C., et al., 2014. Projected land-use change impacts on ecosystem services in the United States. Proc. Natl. Acad. Sci. U. S. A. 111, 7492–7497.

Lee-Cruz, L., Edwards, D.P., Tripathi, B.M., Adams, J.M., 2013. Impact of logging and forest conversion to oil palm plantations on soil bacterial communities in Borneo. Appl. Environ. Microbiol. 79, 7290–7297.

Ling, N., Zhu, C., Xue, C., Chen, H., Duan, Y., Peng, C., et al., 2016. Insight into how organic amendments can shape the soil microbiome in long-term field experiments as revealed by network analysis. Soil Biol. Biochem. 99, 137–149.

Lipson, D.A., 2007. Relationships between temperature responses and bacterial community structure along seasonal and altitudinal gradients. FEMS Microbiol. Ecol. 59, 418–427.

Lozupone, C., Hamady, M., Knight, R., 2006. UniFrac – An online tool for comparing microbial community diversity in a phylogenetic context. BMC Bioinformatics 7, 371.

Lozupone, C., Lladser, M.E., Knights, D., Stombaugh, J., Knight, R., 2011. UniFrac: an effective distance metric for microbial community comparison. ISME J. 5, 169–172.

Lupatini, M., Jacques, R.J.S., Antoniolli, Z.I., Suleiman, A.K.A., Fulthorpe, R.R., Roesch, L.F.W., 2013. Land-use change and soil type are drivers of fungal and archaeal communities in the Pampa biome. World Journal of Microbiology & Biotechnology 29, 223–233.

Montecchia, M.S., Tosi, M., Soria, M.A., Vogrig, J.A., Sydorenko, O., Correa, O.S., 2015. Pyrosequencing reveals changes in soil bacterial communities after conversion of Yungas forests to agriculture. PLoS One 10.

Muñoz-Rojas, M., Jordán, A., Zavala, L., De la Rosa, D., Abd-Elmabod, S., Anaya-Romero, M., 2015. Impact of land use and land cover changes on organic carbon stocks in Mediterranean soils (1956–2007). Land Degrad. Dev. 26, 168–179.

Newbold, T., Hudson, L.N., Hill, S.L., Contu, S., Lysenko, I., Senior, R.A., et al., 2015. Global effects of land use on local terrestrial biodiversity. Nature 520, 45–50.

Papanastasis, V.P., Bautista, S., Chouvardas, D., Mantzanas, K., Papadimitriou, M., Mayor, A.G., et al., 2017. Comparative assessment of goods and services provided by grazing regulation and reforestation in degraded Mediterranean rangelands. Land Degrad. Dev. 28, 1178–1187.

Parks, D.H., Tyson, G.W., Hugenholtz, P., Beiko, R.G., 2014. STAMP: statistical analysis of taxonomic and functional profiles. Bioinformatics 30, 3123–3124.

Purahong, W., Hoppe, B., Kahl, T., Schloter, M., Schulze, E.D., Bauhus, J., et al., 2014. Changes within a single land-use category alter microbial diversity and community structure: molecular evidence from wood-inhabiting fungi in forest ecosystems. J. Environ. Manag. 139, 109–119.

Roberts, P., Newsham, K.K., Bardgett, R.D., Farrar, J.F., Jones, D.L., 2009. Vegetation cover regulates the quantity, quality and temporal dynamics of dissolved organic carbon and nitrogen in Antarctic soils. Polar Biol. 32, 999–1008.

Rodrigues, J.L.M., Pellizari, V.H., Mueller, R., Baek, K., Jesus, E.D., Paula, F.S., et al., 2013. Conversion of the Amazon rainforest to agriculture results in biotic homogenization of soil bacterial communities. Proc. Natl. Acad. Sci. U. S. A. 110, 988–993.

Rousk, J., Baath, E., Brookes, P.C., Lauber, C.L., Lozupone, C., Caporaso, J.G., et al., 2010. Soil bacterial and fungal communities across a pH gradient in an arable soil. ISME J. 4, 1340–1351.

Scherwinski, K., Grosch, R., Berg, G., 2008. Effect of bacterial antagonists on lettuce: active biocontrol of Rhizoctonia solani and negligible, short-term effects on nontarget microorganisms. FEMS Microbiol. Ecol. 64, 106–116.

Schloss, P.D., Westcott, S.L., Ryabin, T., Hall, J.R., Hartmann, M., Hollister, E.B., et al., 2009. Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. Appl. Environ. Microbiol. 75, 7537–7541.

Segata, N., Izard, J., Waldron, L., Gevers, D., Miropolsky, L., Garrett, W.S., et al., 2011. Metagenomic biomarker discovery and explanation. Genome Biol. 12, R60.

Somers, E., Vanderleyden, J., Srinivasan, M., 2004. Rhizosphere bacterial signalling: a love parade beneath our feet. Crit. Rev. Microbiol. 30, 205–240.

Song, W., Deng, X.Z., Yuan, Y.W., Wang, Z., Li, Z.H., 2015. Impacts of land-use change on valued ecosystem service in rapidly urbanized North China Plain. Ecol. Model. 318, 245–253.

Stockmann, U., Adams, M.A., Crawford, J.W., Field, D.J., Henakaarchchi, N., Jenkins, M., et al., 2013. The knowns, known unknowns and unknowns of sequestration of soil organic carbon. Agric. Ecosyst. Environ. 164, 80–99.

Sul, W.J., Cole, J.R., Jesus, E.D., Wang, Q., Farris, R.J., Fish, J.A., et al., 2011. Bacterial community comparisons by taxonomy-supervised analysis independent of sequence alignment and clustering. Proc. Natl. Acad. Sci. U. S. A. 108, 14637–14642.

Thomson, B.C., Ostle, N., McNamara, N., Bailey, M.J., Whiteley, A.S., RIJMe, G., 2010. Vegetation Affects the Relative Abundances of Dominant Soil Bacterial Taxa and Soil Respiration Rates in an Upland Grassland Soil. 59 pp. 335–343.

Thomson, B.C., Ostle, N.J., McNamara, N.P., Oakley, S., Whiteley, A.S., Bailey, M.J., et al., 2013. Plant soil interactions alter carbon cycling in an upland grassland soil. 4, 253.

Tripathi, B.M., Kim, M., Singh, D., Lee-Cruz, L., Lai-Hoe, A., Ainuddin, A.N., et al., 2012. Tropical soil bacterial communities in Malaysia: pH dominates in the equatorial tropics too. Microb. Ecol. 64, 474–484.

Ussiri, D.A., Lal, R., 2013. Land management effects on carbon sequestration and soil properties in reclaimed farmland of Eastern Ohio, USA. Open Journal of Soil Science 3, 46.

Vitali, F., Mastromei, G., Senatore, G., Caroppo, C., Casalone, E., 2016. Long lasting effects of the conversion from natural forest to poplar plantation on soil microbial communities. Microbiol. Res. 182, 89–98.

Wakelin, S.A., Macdonald, L.M., Rogers, S.L., Gregg, A.L., Bolger, T.P., Baldock, J.A., 2008. Habitat selective factors influencing the structural composition and functional capacity of microbial communities in agricultural soils. Soil Biol. Biochem. 40, 803–813.

Wallenstein, M.D., McMahon, S., Schimel, J., 2007. Bacterial and fungal community structure in Arctic tundra tussock and shrub soils. FEMS Microbiol. Ecol. 59, 428–435.

Wang, W., Zeng, W.J., Chen, W.L., Zeng, H., Fang, J.Y., 2013. Soil respiration and organic carbon dynamics with grassland conversions to woodlands in temperate China. PLoS One 8.

Wardle, D.A., Bardgett, R.D., Klironomos, J.N., Setälä, H., Van Der Putten, W.H., Wall, D.H., 2004. Ecological linkages between aboveground and belowground biota. Science 304, 1629–1633.

Wubet, T., Christ, S., Schoning, I., Boch, S., Gawlich, M., Schnabel, B., et al., 2012. Differences in soil fungal communities between European beech (Fagus sylvatica L.) dominated forests are related to soil and understory vegetation. PLoS One 7.

Yu, G., Yu, Q., Hu, L., Zhang, S., Fu, T., Zhou, X., et al., 2013. Ecosystem health assessment based on analysis of a land use database. Appl. Geogr. 44, 154–164.

Zak, D.R., Holmes, W.E., White, D.C., Peacock, A.D., Tilman, D., 2003. Plant diversity, soil microbial communities, and ecosystem function: are there any links? Ecology 84, 2042–2050.

Zarraonaindia, I., Owens, S.M., Weisenhorn, P., West, K., Hampton-Marcell, J., Lax, S., et al., 2015. The soil microbiome influences grapevine-associated microbiota. MBio 6, e02527-14.

Zhang, N.L., Xia, J.Y., Yu, X.J., Ma, K.P., Wan, S.Q., 2011. Soil microbial community changes and their linkages with ecosystem carbon exchange under asymmetrically diurnal warming. Soil Biol. Biochem. 43, 2053–2059.