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The carbon and nitrogen stoichiometry in litter-soil-microbe continuum rather than plant diversity primarily shapes the changes in bacterial communities along a tropical forest restoration chronosequence

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ABSTRACT

Soil bacteria play core roles in mediating the functional linkage between above- and belowground components during forest restoration. However, the pattern and mechanism through which plants and soils influence bacterial communities remain unclear. This study aimed to quantify the contributions of plant community and soil characteristics to shifts in bacterial composition and diversity along a tropical forest restoration chronosequence in the Xishuangbanna. We found a negative effect of forest restoration on bacterial composition and a positive impact on the diversity. Forest restoration changed bacterial communities from being oligotrophic *Acidobacteria* and *Actinobacteria* dominated to copiotrophic *Proteobacteria* and *Firmicutes* dominated. Forest restoration also induced a 1.5–1.6 fold increase in OTU richness and diversity of bacterial communities. Soil variables, including plant litter, contributed 46.3–58.1% to the variations in bacterial composition and diversity, while the contribution of plant community was 6.4–13.8%. Furthermore, the increase in plant richness and diversity had minor contribution to variations in bacterial diversity and composition during forest restoration. Soil bacterial diversity was primarily explained by the elevated levels of carbon and nitrogen stoichiometry in the litter-soil-microbe continuum, but litter was explained by the increased plant diversity. In contrast, soil bacterial composition was negatively correlated with biomass, nitrogen, and carbon:nitrogen of the litter, as well as the level of soil carbon and nitrogen pools. Our data suggested that the carbon and nitrogen stoichiometry in litter-soil-microbe continuum rather than plant diversity primarily shaped the changes in bacterial composition and diversity along tropical forest restoration.

1. Introduction

The tropical forests are suffering from a serious human destruction, resulting in biodiversity loss and land degradation (Abson et al., 2012). There is therefore an urgent need to restore the tropical forests in order to return native biodiversity and ecosystem services. The development of tropical forests is essentially a feedback process between above- and belowground components that would shape the composition and successional changes in plants and microbes (Delgado-Baquerizo et al., 2017; Yang et al., 2020). During tropical forest restoration, the increased plant community complexity and litter productivity may improve soil fertility, thereby increasing microbial biomass, abundance and diversity (Yao et al., 2018). In turn, soil microorganisms can promote plant development through stirring nutrient release from decomposition and mineralization, and thus regulate carbon (C) and nitrogen (N) cycling within ecosystem (Lu et al., 2019a; Wang et al., 2020). As dominant soil groups, bacteria have crucial roles in mediating functional connection between above- and belowground components (Liang et al., 2017). Therefore, it may be pivotal to identify how the changes in bacterial communities can be regulated by the feedback between plant community and soil properties along tropical forest restoration.

The shifts in bacterial communities are primarily regulated by plant communities as well as C and N stoichiometry in litter-soil-microbe continuum (Stefanowicz et al., 2016; Pascual et al., 2017; Yao et al., 2018). During forest restoration, the increased plant composition and diversity not only improve micro-environment through increase of

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Table 1
The alterations in vegetation community characteristics during tropical forest restoration in the Xishuangbanna.

Restoration stage	11-yr	41-yr	52-yr
Elevation (m)	594	551	610
Dominant trees	*Mallotus paniculatus*, *Alchornea tiliifolia*	*Millettia leptobotrya*, *A. tiliifolia*, *Canthium horridum*	*Syzygium oblatum*, *M. leptobotrya*, *A. tiliifolia*, *Ficus semicordata*, *Castanopsis indica*, *Engelhardia spicata*
Canopy coverage (%)	56	85	91
Average height (m)	8.62 ± 1.37^{c}	10.73 ± 2.26^{b}	18.84 ± 4.13^{a}
Average DBH (cm)	7.07 ± 0.43^{c}	10.26 ± 1.27^{b}	21.76 ± 6.68^{a}
Litter thickness (cm)	1–2	2–4	4–6
Litter biomass (t ha^{-1} a^{-1})	2.57 ± 0.74^{c}	5.71 ± 1.15^{b}	7.18 ± 1.27^{a}
Tree species richness	0.229 ± 0.025^{c}	0.455 ± 0.034^{b}	1.353 ± 0.347^{a}
Understory species richness	0.474 ± 0.045^{c}	0.672 ± 0.085^{b}	1.616 ± 0.174^{a}
Tree shannon diversity	0.935 ± 0.195^{c}	1.117 ± 0.247^{b}	1.335 ± 0.335^{a}
Understory shannon diversity	1.139 ± 0.116^{b}	1.353 ± 0.154^{b}	1.766 ± 0.285^{a}

Values of average height, DBH (diameter at breast height), litter biomass, hierarchical species richness and diversity are mean ± SE. Different lowercase letters in the same line were significantly different ($P < 0.05$) among the variables.

vegetation coverage, but also elevate the level of nutrient stoichiometry through the input of plant litter and root exudation, thus promoting the development of soil bacteria (Zhang et al., 2016; Ren et al., 2018). In contrast, soil characteristics have direct functional roles in regulating bacterial communities, as different patterns of C and N combinations and allocations in soil-microbe continuum can diversify bacterial communities (Zhang et al., 2020). Furthermore, the C and N stoichiometry of litter-soil continuum can exert separated or combined effects on bacterial communities, as different functional groups have differentiated requirements for nutrient type and dose (Ren et al., 2017; Lu et al., 2019a). For example, the taxa of *Acidobacteria* and *Bacteroidetes* are often associated with C cycle, and thus had a high abundance in C-rich soil habitat (Zhang et al., 2020). On the other hand, the functional groups such as *Proteobacteria*, *Firmicutes*, *Chloroflexi,* and *Actinobacteria* mainly participate in N cycle, consequently having a high assemblage in N-rich soil environments (Pfister et al., 2010). Moreover, soil bacteria can be divided into oligotrophs and copiotrophs according to their different requirements of C and N levels. The high abundances of oligotrophic *Acidobacteria* are often observed in early restoration stages where there were low levels of C and N in soils, whereas, the late stage can harbour a high abundance of copiotrophic bacteria (e.g., most of *Proteobacteria*) due to high levels of C and N pools (Zhang et al., 2020). However, there is no consistent conclusion about the linkages of bacterial community with plant diversity and nutrient stoichiometry in litter-soil-microbe continuum (Chen et al., 2016). As a consequence, it remains unclear about the relative contributions of plant diversity and nutrient stoichiometry to shifts in bacterial communities during forest restoration (Zhang et al., 2016, 2020).

In Xishuangbanna, Yunnan, Southwest of China, a large area of primary tropical rainforests was severely destroyed by slash-burn agricultural practices. The ceasing of slash-burn farming was followed by a sequence of restoration stages of tropical forests. It is still unclear about the associations of plant diversity and nutrient stoichiometry with the shifts in bacteria community along secondary tropical forest development. Our study aimed to (i) probe the response of bacteria community change to tropical forest restoration, (ii) determine whether plant diversity and nutrient stoichiometry (e.g., C, N and C:N stoichiometry) of litter-soil-microbe continuum have differentiable effects on bacterial composition and diversity, and (iii) identify the major environmental variables in determining shifts in soil bacterial community during tropical forest restoration. We hypothesized that (i) tropical forest restoration can have a positive effect on bacteria composition and diversity, as plant diversity and nutrient stoichiometry of litters and soils would increase following forest development, (ii) nutrient stoichiometry in litter-soil continuum might contribute more to bacterial development compared with plant diversity, given that nutrient availability can directly provide the substrates for microbes during tropical forest restoration in the Xishuangbanna.

Table 2
The carbon and nitrogen stoichiometry in litter-soil-microbe continuum and other measured edaphic properties along restoration chronosequence in the tropical Xishuangbanna. Significant levels: $^{**}P < 0.01$, $^{*}P < 0.05$.

Restoration stage	11-yr	41-yr	52-yr	F value
Litter C (g kg^{-1})	279 ± 22.6^{c}	335 ± 35.4^{b}	428 ± 41.9^{a}	52.7**
Litter N (g kg^{-1})	10.9 ± 1.16^{c}	17.3 ± 1.77^{b}	38.3 ± 2.04^{a}	28.4*
Litter C:N	25.5 ± 1.07^{a}	15.4 ± 1.07^{b}	11.2 ± 1.07^{c}	14.8*
MBC (g kg^{-1})	0.94 ± 0.06^{c}	1.45 ± 0.07^{b}	2.05 ± 0.08^{a}	66.5**
MBN (g kg^{-1})	0.05 ± 0.01^{c}	0.09 ± 0.01^{b}	0.13 ± 0.02^{a}	48.2**
Microbial C:N	18.8 ± 1.35^{a}	16.1 ± 1.14^{b}	15.8 ± 1.05^{b}	7.17
Soil C:N	33.2 ± 4.12^{a}	30.2 ± 3.68^{b}	28.4 ± 2.75^{c}	16.9*
SOC (g kg^{-1})	21.6 ± 1.47^{c}	27.8 ± 1.35^{b}	32.9 ± 1.25^{a}	33.6**
ROC (g kg^{-1})	9.43 ± 0.99^{c}	12.2 ± 1.06^{b}	19.4 ± 1.17^{a}	16.8*
DOC (g kg^{-1})	1.08 ± 0.06^{c}	1.43 ± 0.09^{b}	1.95 ± 0.11^{a}	12.9*
TN (g kg^{-1})	0.65 ± 0.01^{c}	0.92 ± 0.02^{b}	1.16 ± 0.04^{a}	11.2*
DON (mg kg^{-1})	95 ± 8.01^{c}	127 ± 8.82^{b}	188 ± 11.7^{a}	21.6*
NH_4-N (mg kg^{-1})	82.4 ± 5.24^{b}	$86. \pm 6.37^{b}$	90.6 ± 10.5^{a}	5.25
NO_3-N (mg kg^{-1})	46.6 ± 2.33^{c}	70.4 ± 4.48^{b}	89.6 ± 6.27^{a}	13.8*
SW (%)	28.7 ± 1.58^{a}	29.6 ± 1.69^{a}	30.3 ± 1.78^{a}	9.26
BD (g cm^{-3})	1.37 ± 0.06^{a}	1.28 ± 0.05^{b}	1.15 ± 0.04^{c}	11.8*
Clay content (<0.002 mm, %)	21.5 ± 1.07^{c}	26.3 ± 1.79^{b}	30.5 ± 2.06^{a}	22.6*
pH	5.25 ± 0.16^{a}	4.56 ± 0.11^{a}	4.24 ± 0.10^{a}	2.94

The data are mean ± SE. The different lowercase letters in the same row indicate significant differences among three sites ($p < 0.05$). SW: soil water content; BD: bulk density; MBC: microbial biomass carbon; MBN: microbial biomass nitrogen; Litter C: litter carbon; Litter N: litter nitrogen; SOC: soil organic carbon; ROC: readily oxidizable carbon; DOC: dissolved organic carbon; TN: total nitrogen; DON: dissolved organic nitrogen.

2. Methods

2.1. Experiment designs

The experiment was established in the Xishuangbanna Tropical Botanical Garden (21°55′ N, 101°15′ E), Chinese Academy of Sciences.

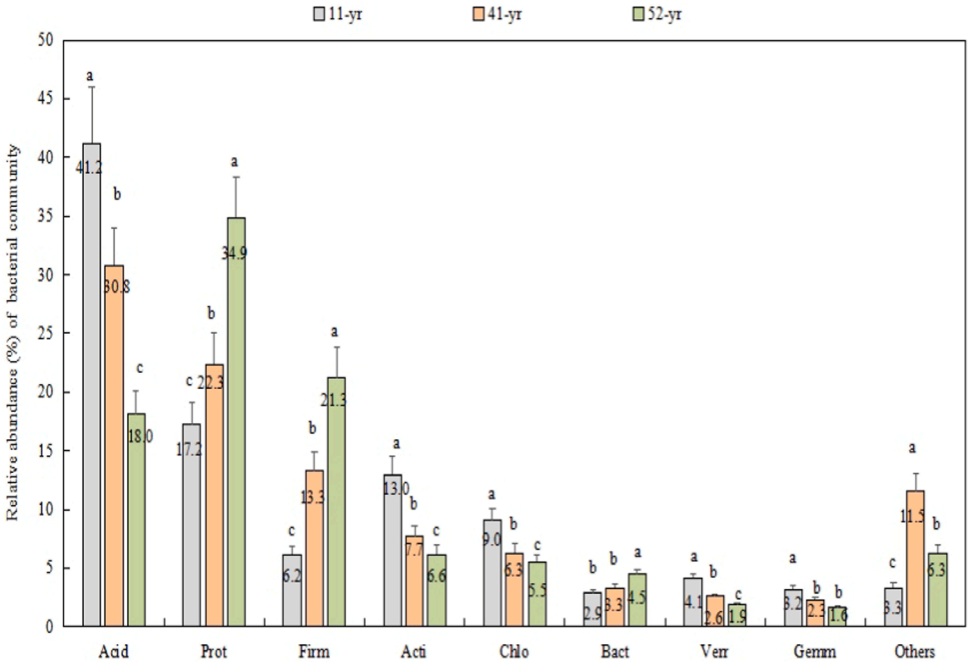

Fig. 1. The shift in taxonomic composition of soil bacterial communities along tropical forest restoration chronosequence in the Xishuangbanna. Columns are mean ± SE (standard error). Different letters show the significant difference ($P < 0.05$) among three restoration phases (ANOVA with Tukey's Honest Significant Difference (HSD)). Acid: *Acidobacteria*; Prot: *Proteobacteria*; Firm: *Firmicutes*; Acti: *Actinobacteria*; Chlo: *Chloroflexi*; Bact: *Bacteroidetes;* Verr: *Verrucomicrobia;* Gemm: *Gemmatimonadetes.*

Average rainfall amounts to ≃1557 mm, while mean air temperature is approximately 21.5 °C. The ≃87% of total rainfall is distributed in wet seasons (from May to October). The thick fog prevails from night to daytime and keeps airs and soils at a high moisture, flourishing tropical forests in this region. The soils are originated from cretaceous sandstone and their taxonomies belong to Oxisols.

The slash-burn farming was a common practice that destructed primary tropical rainforests in the Xishuangbanna. A protection program was developed to cease the destruction of tropical rainforest in the 1960s, which promoted forest restoration. Consequently, a series of phases were developed from secondary forest restoration. A chronosequence of restoration stages, i.e., 11-yr-old *Mallotus paniculatus* community, 41-yr-old *Mellettia leptobotrya* community, and 52-yr-old *Syzygium oblatum* community, was chosen to distinguish the effects of plant community and nutrient stoichiometry on shifts in bacteria communities during tropical forest restoration in 2017 (Table 1).

The three restoration stages (200–1000 m apart), i.e., 11-, 41-, and 52-year-old sites, were established from slash-and-burn agriculture lands abandoned in 2006, 1976, and 1965, respectively. At each restoration stage, we randomly set up three reduplicative sites (50 × 40 m) in 2017 and they are ~200 m apart. At each site, three plots (15 × 10 m, 10 m apart) were randomly repeated at each stage. The 9-plots in total were established at every restoration stage. The sites had the similarity of classification of soil texture and type, micro-topography, management history, and burning severity of slash-and-burn. Therefore, similar conditions were guaranteed in these restoration stages.

2.2. Soil physicochemical analysis

At each site, we sampled litters and soils in above reduplicative plots in June 2017. Three litter samples in the traps (15 × 15 cm; ~4 m apart) were collected at each plot. The litter samples were fully mixed into a composite sample at each site and then measured litter quality and biomass after a dried procedure at 70 °C. At per plot, three replicated soils (4 m apart) were sampled at 0–10 cm depth with a method of soil core (Ø 5 cm × 10 cm deep). Each sample was sieved with a 2 mm sifter, removing all of visible substances. The fully mixed samples were divided into three parts. One part was air-dried for analyses of carbon (C) and nitrogen (N) concentrations and the pH. The other two parts were used for measurements of microbes, soil water content, and dissolved organic carbon (DOC) (stored at −80 °C), as well as microbial C and N concentrations (stored at 4 °C). Soil water content (SW) was calculated as [(wet weight of soils-dry weight of soils) × 100]/dry weight of soils. The dry weight was determined following oven drying at 105 °C for 24 h. A core method was utilized to measure bulk density (BD), and soil clay content (<0.002 mm; %) was determined by a hydrometer method. The glass electrode was used to determine soil pH in a solution (g m L^{-1}) of 1:2.5 soil:water, while microbial biomass carbon and nitrogen (MBC and MBN) were measured using chloroform fumigation extraction (Beck et al, 1997). Litter C, soil organic carbon (SOC), total nitrogen (TN), and litter N were determined according to Wang et al. (2020). Readily oxidizable carbon (ROC) and DOC were measured by the methods described by Xu et al. (2010). Dissolved organic nitrogen (DON) was measured by TOC-VCPH analyzer (Shimadzu Scientific Instruments, Columbia), and the NO_3^-–N and NH_4^+–N concentrations were determined by UV–VIS spectrophotometer (UV mini 1240, Shimadzu, Japan).

2.3. DNA extraction, amplification, and sequencing data processing

DNA extraction and sequencing analyses were described in Lu et al. (2019b) and Zhao et al. (2016). Each of 3 × 1.0 g fresh soil sample was used to extract DNA using MoBio powersoil DNA extraction kits (MoBio Laboratories, Catalog No. 12888–50, USA) based on the instructions of manufacturer. The PCR was used to amplify bacterial 16S rDNA through 341F and 805R primers (Lu et al., 2019b). High-throughput sequencing (300 bp PE) was then conducted on the Illumina MiSEQ platform (San Diego, CA, USA).

The bioinformatic classifications for the sequences were produced from the Ribosomal Database Project (RDP) with less than 3% dissimilarity according to recommended standards (Lu et al., 2019b). The operational taxonomic units (OTUs) at 97% identity were clustered through the Mothur program. OTU richness was determined using Chao1 and ACE indices, while bacterial diversity was estimated by Shannon-Wiener index (Zhao et al., 2016).

2.4. Statistic analysis

The plant community diversity was measured using the indices of

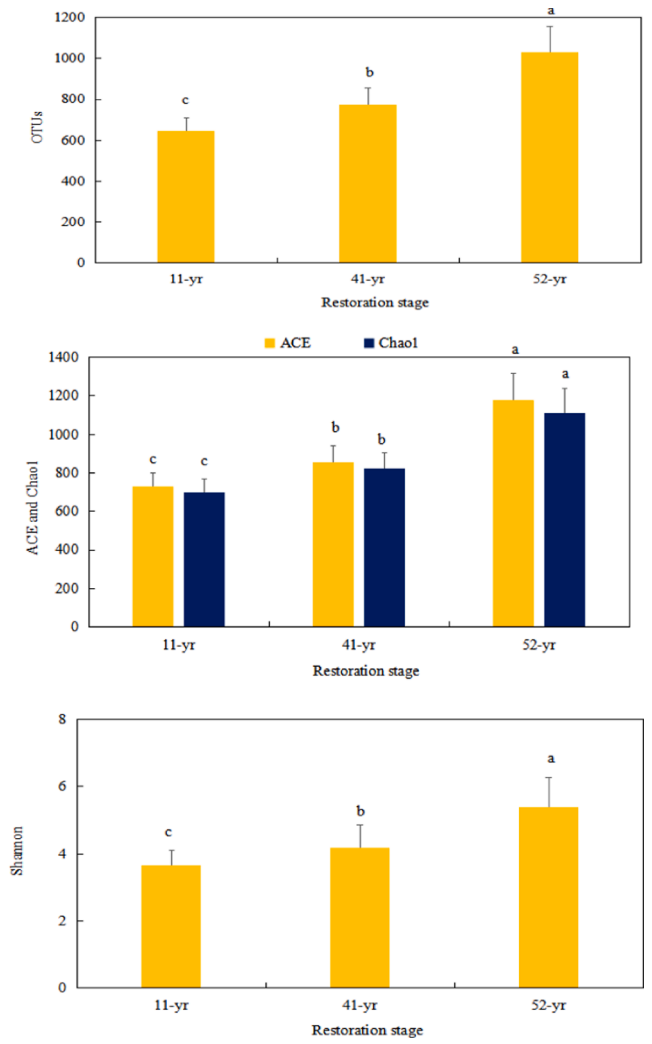

Fig. 2. The changes in the bacterial diversity along tropical forest restoration chronosequence in the Xishuangbanna. Columns are mean ± SE (standard error). Different lowercase litters are significantly different ($P < 0.05$) among three restoration phases (ANOVA with Tukey's Honest Significant Difference (HSD)).

Margalef richness (*MR*) and Shannon-Wiener (H'). The *MR* and H' were determined by $MR = (S - 1) / \ln n$ and $H' = -\sum(p_i/\ln p_i)$, where S, n and p_i are the species number, the sum of all species, and the density proportion of i species, respectively (Lu et al., 2019b). The data were normalized and homogenized for variance prior to analysis. A Duncan test was used for multiple comparisons, while Tukey' Honest Significant Difference (HSD) was employed for the mean comparison ($P < 0.05$). A one-way analysis of variance (ANOVA) was utilized to test the variables among the three restoration stages.

The Non-metric multidimensional scaling (NMDS) was utilized to illustrate the impacts of soil variables (using the envfit function) on shifts in bacterial composition and diversity along forest restoration. Redundancy analysis (RDA) was applied to explore the associations of bacterial composition and diversity with plant and soil characteristics. Structural equation model (SEM) was utilized to identify the direct and indirect links between forest restoration chronosequence, hierarchical vegetation diversity, litter stoichiometry, soil properties, bacterial diversity and composition using lavaan package (Rosseel, 2012). Variation partition analysis (VPA) was used to explain how plant community and soil characteristics contributed to the variations in bacterial composition and diversity using vegan package. R statistical software version 3.2.2 was used in the statistical analyses (R Core 466 Team, 2015).

3. Results

3.1. Shifts in plant and soil variables along tropical forest restoration

The complexity and diversity of the tropical plant communities varied significantly along forest restoration chronosequence (Table 1). During the forest development from 11-yr to 52-yr-old site, tree height and DBH and litter biomass increased 1.2–3.1 fold, while the hierarchical species richness and shannon diversity increased 1.4–7.8 times for the trees and shrubs (Table 1; $P < 0.05$).

The stoichiometric characteristics of most nutrients in litter-soil-microbe continuum varied significantly across the three restoration stages (Table 2). The carbon and nitrogen concentrations in the litters increased by 1.5–3.5 fold in 52-yr-old stage compared with 11-yr restoration stage, while litter C:N ratio decreased by more than 2.3 fold. Compared with 11-yr-old site, the C and N concentrations in microbial biomass increased by 2.2 fold in 41-yr-old site and 2.6 fold in 52-yr-old site, respectively; however, there was no difference in microbial C:N.

The values of soil carbon pool (i.e., TOC, ROC, and DOC) increased 1.7–2.1 fold following forest restoration. The concentrations of TN, DON, and NO_3-N increased 1.8–2.0 fold, whereas those of NH_4-N were not significantly affected by forest restoration. Soil C:N ratio decreased significantly along forest restoration. Furthermore, soil texture (i.e., clay content) increased, and soil BD decreased followed forest development (Table 2; $P < 0.05$).

3.2. Changes in bacterial composition and diversity along tropical forest restoration

Soil bacterial OTUs were sorted into two taxonomic taxa (Bacteria and Archaea), 42 phyla, 94 classes, and 216 orders. The dominant phyla of bacterial communities were *Acidobacteria* (30.0%), *proteobacteria* (24.8%), *Firmicutes* (13.5%) in three restoration stages (Fig. 1). Other dominated bacterial taxa were *Actinobacteria* (9.0%), *Chloroflexi* (6.9%), *Bacteroidetes* (3.5%), *Verrucomicrobia* (2.9%) and *Gemmatimonadetes* (2.4%). Thus, the eight preponderant bacterial groups accounted for 93.0% of the total bacterial communities among the three restoration stages.

The relative abundances of soil bacteria varied with the restoration gradient (Fig. 1; $P < 0.05$). The abundances of copiotrophs (i.e., *Proteobacteria*, *Firmicutes,* and *Bacteroidetes*) increased by 56.5–244.4% across the three restoration stages, while those of oligotrophs (i.e., *Acidobacteria*, *Actinobacteria*, *Chloroflexi*, *Verrucomicrobia*, and *Gemmatimonadetes*) decreased by 39.9–56.1% (Fig. 1; $P < 0.05$). Furthermore, soil bacterial communities were dominated by *Acidobacteria* in the 11-yr and 41-yr sites, whereas *proteobacteria* were the dominant taxa in the 52-yr site.

The secondary succession of tropical forests also had a significant effect on bacterial diversity ($P < 0.001$; Fig. 2). The OTU richness of soil bacteria increased from 19.7% to 58.9% during the forest restoration, while the diversity indices (i.e., ACE, Chao, and Shannon) of bacterial communities in 52-yr-old site increased by 47.6–62.2% compared with 11-yr-old site (Fig. 2; $P < 0.01$).

3.3. Linking bacterial community changes to plant and soil characteristics

The results from the SEM explained 96.6% and 88.5% of the variations in bacterial composition and diversity, respectively (Fig. 3A and 3B). The tropical forest restoration directly and indirectly affected soil bacterial composition and diversity ($P < 0.05$ or 0.01). The restoration chronosequence had a negative direct effect on bacterial composition and a positive direct impact on the diversity. In contrast, the indirect effects of forest restoration on bacterial composition and diversity were both positive and negative. The indirect effects of forest restoration gradient on bacterial composition and diversity were primarily through

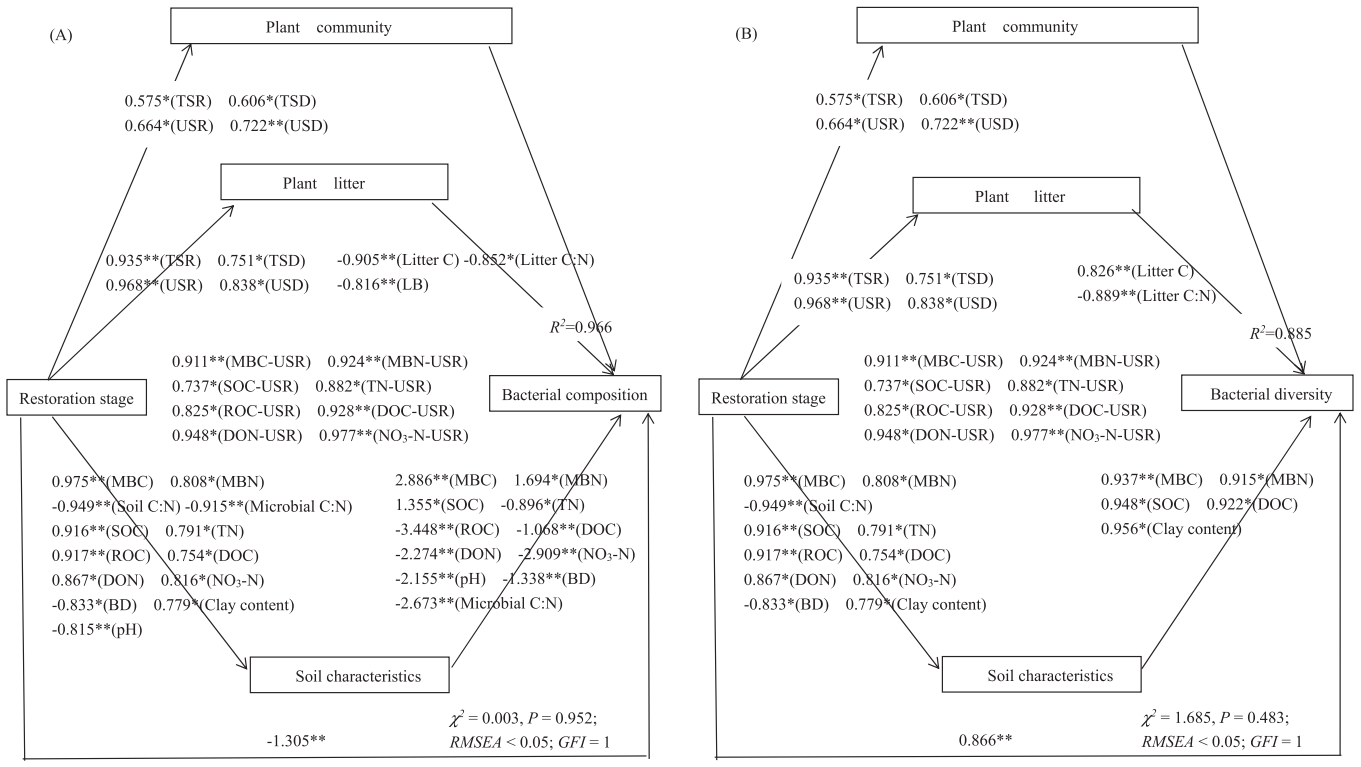

Fig. 3. Structural equation models (SEM) determining the impacts of tropical forest restoration, vegetation diversity, and edaphic characteristics on bacterial community composition (a) and diversity (b). The values of R^2 show explained variance proportion in the model. Solid lines show significant paths (**: $P < 0.01$, *: $P < 0.05$). TSR: tree species richness; USR: understory species richness; TSD: tree shannon diversity; USD: understory shannon diversity; LB: litter biomass; Litter C: litter carbon; Litter N: litter nitrogen; BD: bulk density; MBC: microbial biomass carbon; MBN: microbial biomass nitrogen; SOC: soil organic carbon; ROC: readily oxidizable carbon; DOC: dissolved organic carbon; TN: total nitrogen; DON: dissolved organic nitrogen.

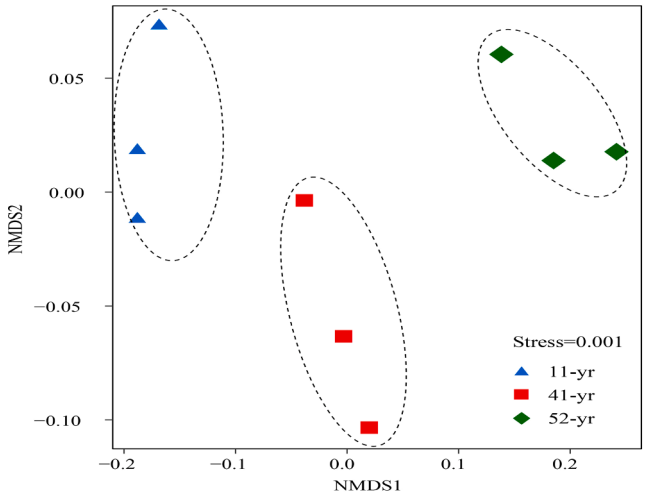

Fig. 4. Non-metric multidimensional scaling (NMDS) showing the impacts of soil variables on the shifts in bacterial composition and diversity along forest restoration chronosequence in the Xishuangbanna.

the C and N stoichiometry in litter-soil-microbe continuum and not via plant species richness (i.e., TSR and USR) and diversity (TSD and USD). The changes in MBC, MBN, and SOC had positive indirect effects on bacterial composition, while litter C, LB, DOC, ROC, NO_3-N, DON, pH and the C:N ratio in litters and microbes had negative direct effects (Fig. 3A; $P < 0.05$ or 0.01). Furthermore, we observed a positive indirect effect of litter C, MBC and MBN, SOC, DOC, and clay content as well as a negative effect of litter C:N ratio on bacterial diversity (Fig. 3B; $P < 0.05$ or 0.01).

The NMDS demonstrated a high-degree separation of sampling restoration stages based on bacterial composition and diversity (Fig. 4). The results showed a distinct impact of soil variables on microbial composition and diversity along forest restoration chronosequence (Fig. 4). The data from VPA showed that soil characteristics (including plant litter) explained 46.3% of the total variations in bacterial composition, whereas plant diversity explained 13.8% (Fig. 5A). Two parameters co-explained 35.6% of the total variations in bacterial composition (Fig. 5A). For the bacterial diversity, soil characteristics contributed 58.1% to the explanation of the total variations, but plant diversity contributed 6.4% (Fig. 5B). Two parameters co-contributed 28.2% to the total variations in bacterial diversity (Fig. 5B).

The relative abundances of dominant bacterial taxa were closely associated with the C and N stoichiometry in litter-soil-microbe continuum (Fig. 6A), but they had a little linkage with hierarchical species richness (TSD and USD) and diversity (TSR and USR) (Fig. 6A). The nutrient stoichiometry had different effects on special bacterial groups along forest secondary succession (Fig. 6A). In 52-yr site, the abundances of *Proteobacteria* and *Firmicutes* were primarily explained by the concentrations of MBC, MBN, NO3-N, DON, ROC, and LB. The *Bacteroidetes* were mainly contributed by the values of SW, SOC, LC, and DOC. In 11-yr site, the litter and microbial C:N ratio contributed mostly to the changes in abundances of oligotrophs (i.e., *Acidobacteria, Actinobacteria, Actinobacteria, Actinobacteria*, *Gemmatimonadetes*, and *Verrucomicrobia*), followed by pH, soil C:N, and BD. However, the abundances of copiotrophic functional groups (i.e., *proteobacteria*, *Firmicutes*, *Bacteroidetes*) were negatively correlated with soil BD, pH, and the C:N ratio in litter-microbe-soil stoichiometry. In contrast, the oligotrophic taxa were negatively associated with the C an N stoichiometry in litter-soil-microbe continuum, as well as the plant richness and diversity.

The results from RDA also indicated that the bacterial diversity was

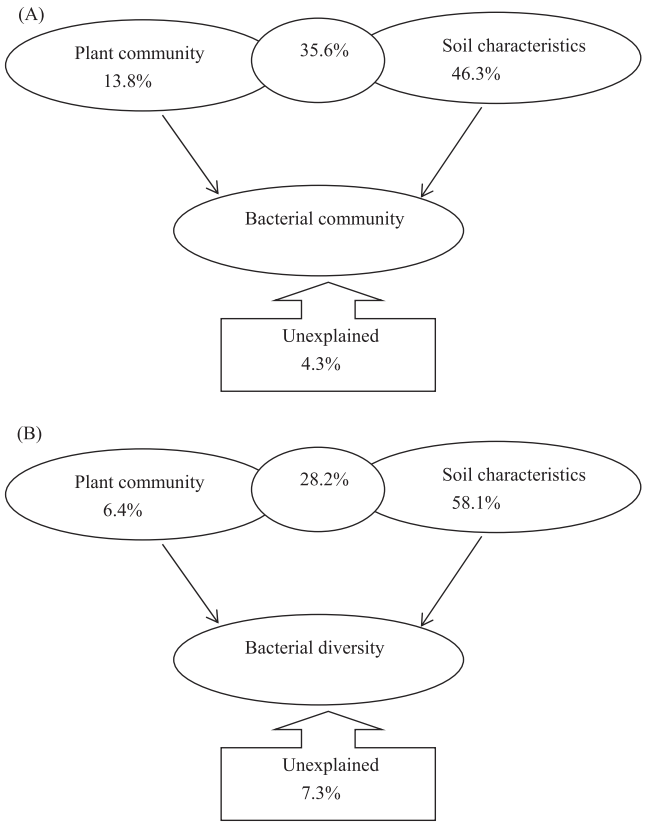

Fig. 5. Variation partition analysis (VPA) for the contributions of plant community and soil characteristics (including plant litter) to the variations in soil bacterial composition and diversity along a tropical forest restoration chronosequence.

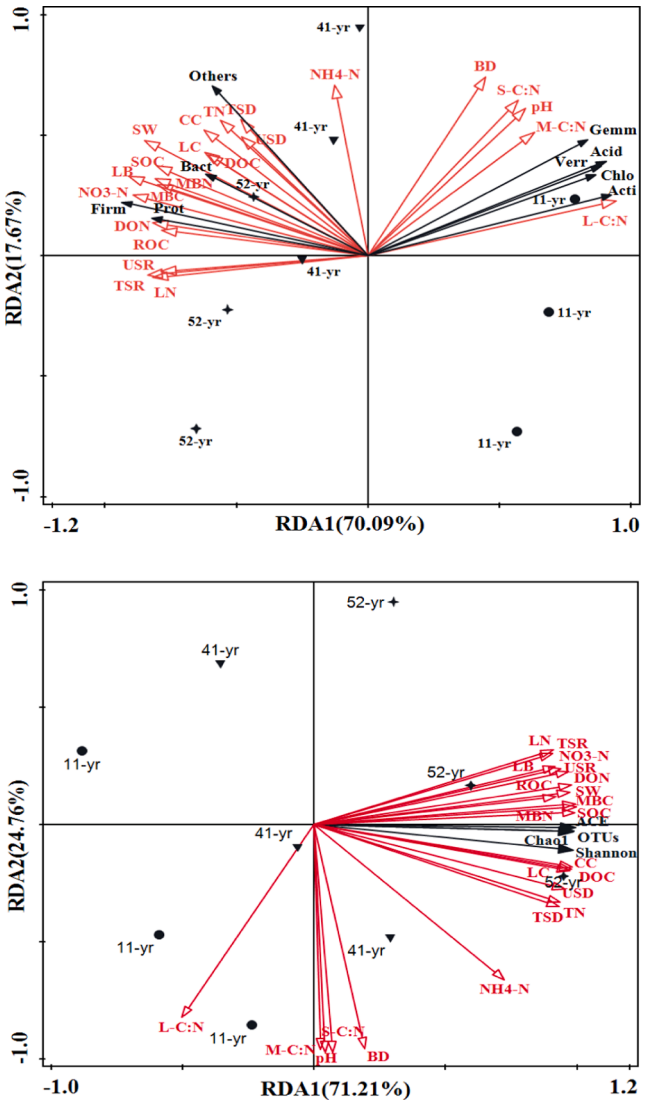

Fig. 6. Redundancy analysis (RDA) for the associations of abundance composition and diversity of bacterial communities with plant and soil characteristics. TSR: tree species richness; USR: understory species richness; TSD: tree shannon diversity; USD: understory shannon diversity; LB: litter biomass; LC: litter carbon; LN: litter nitrogen; L-C:N: litter C:N; SW: soil water content; BD: bulk density; CC: clay content; MBC: microbial biomass carbon; MBN: microbial biomass nitrogen; M-C:N: microbial C:N; S-C:N: soil C:N; SOC: soil organic carbon; ROC: readily oxidizable carbon; DOC: dissolved organic carbon; TN: total nitrogen; DON: dissolved organic nitrogen; NH4-N:NH_4-N; NO3-N: NO_3-N. Acid: *Acidobacteria*; Prot: *Proteobacteria*; Firm: *Firmicutes*; Acti: *Actinobacteria*; Chlo: *Chloroflexi*; Bact: *Bacteroidetes;* Verr: *Verrucomicrobia;* Gemm: *Gemmatimonadetes.*

mainly determined by the elevated levels of C and N stoichiometry in litter-soil-microbe continuum, but not by the increased plant diversity (Fig. 6B). In 52-yr site, the OUTs and diversity indices (i.e., Shannon, Chao1, and ACE) of bacterial communities were primarily explained by the changes in values of CC, LC, DOC, SOC, MBC, and MBN, followed by the parameters of SW, ROC, TN, DON, USD, and TSD (Fig. 6B). In 11-yr and 41-yr sites, however, the carbon and nitrogen stoichiometry had little effect on bacterial diversity.

4. Discussion

This study observed a pronounced increase in the level of C and N stoichiometry in the plant-soil-microbe continuum along tropical forest restoration. A possible reason for this may be that the rapid growth of leguminous plant species (*M. leptobotrya*) induced a significant increase in input of high-protein leaf litter (Wang et al., 2021). The changes in C and N concentrations in soil-litter continuum can exert crucial impact on the microbiome through meeting the nutritional needs (Delgado-Baquerizo et al., 2018; Wang et al., 2020). In view of the roles that plant composition, plant diversity, and nutrient stoichiometry play in regulating bacteria in soils, it is important to identify the relative contributions of plant community and soil characteristics to the shifts in bacterial communities following tropical forest development.

We also found a positive effect of tropical forest restoration on bacterial diversity. Soil bacterial diversity increased primarily due to the increased soil clay content and the level of C and N stoichiometry in litter-soil-microbe continuum following forest development. This may be attributed to the fact that increased litter and soil nutrients can meet the bacterial metabolism demands for building their own biomass and for maintaining a high diversity (Chen et al., 2016; Zhao et al., 2019). Furthermore, the increase in soil C and N concentrations favored the development of copiotrophic functional groups such as *proteobacteria*, *Firmicutes*, and *Bacteroidetes*, which may be another important reason to explain the high bacterial diversity in late restoration stage (Wu et al., 2015; Högberg et al., 2007). In consequence, the increased level of C and N stoichiometry had a direct functional effect on bacterial diversity (Pérez-Ramos et al., 2012). In contrast, hierarchical plant richness and diversity increased during the forest restoration, but they contributed little to the increase in bacterial diversity. This finding was discrepant with the results reported by Nakayama et al. (2019) who observed a significant impact of plant diversity on bacterial community. This contradiction might be linked with the deficient indirect effects of the microhabitats on bacteria adaptability under plant coverage change during forest restoration (Zhang et al., 2016). Plant diversity can

promote the diversification of soil microorganism, probably through increasing the diversified soil environments (Li et al., 2020). However, there was no significant alterations of soil water and the pH as well as no effect of bulk density on bacteria during forest restoration, which may be an important reason why plant diversity had litter association with bacterial diversity.

This study observed a negative impact of tropical forest restoration on bacterial composition, which may be due to the direct functional role of nutrient stoichiometry of litter-soil-microbe continuum in regulating bacterial communities. For this study, the C and N concentrations in litters and soils had a negative effect on bacterial composition. This may be attributed to the fact that low-resource habitat in early restoration stage can result in more unique niches especially for oligotrophic groups, but a high resource in late stage was not prone to produce differentiated niches for bacterial developments (Chao et al., 2020). Accordingly, the nutrient stoichiometry in litter-soil continuum may have crucial roles in determining niche-driven bacterial communities during tropical forest restoration (Zhang et al., 2018). Furthermore, the direct effect of restoration chronosequence on bacterial composition was negative. The negative direct effect may be due to the decreased abundance of oligotrophic functional groups along forest restoration, as the bacterial composition was dominated by the oligotrophs (Bledsoe et al., 2020). Moreover, we observed a minor contribution of plant diversity to the changes in bacterial community composition, probably due to the deficiency of indirect microhabitat control and direct symbiosis impact on bacteria (Ren et al., 2018). Likewise, aboveground vegetation affected belowground bacteria possibly indirectly through the effects of dominant plant species and litter production on soil nutrient stoichiometry.

The characteristics of bacterial diversity substantially depend on the composition change along forest restoration (Zhang et al., 2016). Soil bacterial composition was dominated by the abundances of *Acidobacteria*, *Proteobacteria*, *Firmicutes*, and *Actinobacteria*. During forest restoration, the relative abundances of k-strategist (e.g., *Proteobacteria* and *Firmicutes*) increased, while those of r-strategist (i.e., *Acidobacteria* and *Actinobacteria*) decreased. The increased abundances of k-strategist were closely correlated with the increased supplies of C and N substances from litter-soil continuum (Zhang et al., 2020; García-Carmona et al., 2021). In contrast, the linkage of r-strategist abundance with C and N stoichiometry was negative, which may be due to the adaptation result of bacterial taxa to resource-limited environments (Zhang et al., 2020). Therefore, the changes in bacterial composition and diversity along tropical forest restoration were mainly determined by the interplay between nutrient stoichiometry and ecological adaptation of dominant bacterial taxa.

The abundance proportion of copiotrophic and oligotrophic groups in bacterial composition varied with restoration stages, which was probably associated with the separated or combined effects of C and N stoichiometry in the litter-soil continuum (Ren et al., 2017; Lu et al., 2019a; Sun and Badgley, 2019; Zhang et al., 2020). A low level of C and N combinations in the litter and the soil at early restoration stage favored the development of oligotrophic taxa (García-Carmona et al., 2021). In contrast, a high level of C and N combinations in the litter-soil continuum at late restoration stage increased the abundances of copiotrophic taxa in bacterial communities (Mise et al., 2018; Liang et al., 2021). Furthermore, the C and N stoichiometry in the litter-soil continuum had a separated effect on the developments of oligotrophic and copiotrophic groups along forest restoration. In early restoration stage, the low litter and soil quality (high C:N ratio) had a main contribution to the increase in the abundances of oligotrophic *Acidobacteria*, *Actinobacteria*, and *Chloroflexis* associated with C cycle (Zhang et al., 2020). In later stage, the high N level in the litter-soil continuum primarily contributed to the increased abundances of *Proteobacteria* and *Firmicutes* linked with N cycle (Pfister et al., 2010). Consequently, the C and N stoichiometry can exert a separated role in determining the development of bacterial trophic types (Mcgee et al., 2018). Therefore, our results indicated that the shifts in bacterial community composition were primarily regulated by the interactions between bacterial trophic types and nutrient stoichiometry of litter-soil-microbe continuum along forest restoration gradient.

5. Conclusion

This study observed a negative impact of tropical forest restoration on bacterial composition and a positive influence on the diversity. The restoration chronosequence resulted in a 1.48–1.62 fold increase in bacterial diversity and changed bacterial communities from being oligotrophic *Acidobacteria* and *Actinobacteria* dominated to copiotrophic *Proteobacteria* and *Firmicutes* dominated. The increased bacterial diversity was closely associated with the elevated carbon (C) and nitrogen (N) concentrations in litter-soil-microbe continuum. In contrast, a negative correlation of bacterial composition was observed with the changes in biomass, C, and C:N of the litter, as well as the level of soil C and N pools. Furthermore, plant diversity had minor contribution to bacterial composition and diversity in contrast to soil characteristics. Therefore, the results suggest that the shifts in bacterial composition and diversity following forest restoration are primarily determined by C and N stoichiometry in litter-soil-microbe continuum not via plant diversity.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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