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Effects of Soil Properties and Plant Diversity on Soil Microbial Community Composition and Diversity during Secondary Succession

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Abstract: Soil microbial communities play an important role in maintaining the ecosystem during forest secondary succession. However, the underlying mechanisms that drive change in soil microbial community structures during secondary succession remain poorly defined in species-rich subtropical coniferous forests. In this study, Illumina high-throughput sequencing was used to analyze the variations in soil microbial community structures during forest secondary succession in subtropical coniferous forests in China. The role of soil properties and plant diversity in affecting soil bacterial and fungal communities was determined using random forest and structural equation models. Highly variable soil microbial diversity was observed in different stages of secondary succession. Bacterial community diversity rose from early to middle and late successional stages, whereas fungal community diversity increased from early to middle successional stages and then declined in the late stage. The relative abundance of *Acidobacteria*, *Gemmatimonadetes*, *Eremiobacterota (WPS-2)*, *Rokubacteria*, and Mortierellomycota increased during succession, whereas the relative abundance of Ascomycota and Mucoromycota decreased. The community composition and diversity of the soil microbial community were remarkably influenced by plant diversity and soil properties. Notably, tree species richness (TSR) displayed a significant and direct correlation to the composition and diversity of both bacterial and fungal communities. The carbon-to-nitrogen (C:N) ratio had a direct impact on the bacterial community composition and diversity, and pH had a marked impact on the fungal community composition and diversity. Furthermore, succession stage and plant diversity indirectly impacted the composition and diversity of soil bacterial and fungal communities via soil properties. Overall, it can be concluded that soil intrinsic properties and plant diversity might jointly drive the changes in soil microbial community composition and diversity during secondary succession of subtropical coniferous forests.

Keywords: *Pinus kesiya* forest; plant diversity; soil property; secondary succession; bacteria; fungi; structural equation models

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1. Introduction

Secondary succession is becoming one of the research focuses of terrestrial ecosystems with global land use changes [1]. Forest succession is driven by aboveground–belowground interactions [2,3]. Because soil microbes have essential roles in key ecosystem processes [4–6], such as plant productivity [7], nutrient cycling, water regulation, and litter decomposition [8], they are increasingly regarded as vital edaphic variables affecting secondary succession [9,10]. To clarify the roles of soil microbial communities in secondary succession, it is necessary to identify the factors that drive the changes in soil microbial community structures in the successional process [11]. Therefore, how the soil microbial community structure varies during long-term succession needs to be determined.

During ecological succession, soil microbial communities can vary dramatically [12–14]. For example, microbial communities change substantially in the transition from grassland to pioneer forest, but less so in the transition from pioneer stage to later stage [15]. Liu et al. [10] found linear increases in the diversity of bacteria and fungi along secondary succession, whereas the abundance increased in the early stages and then plateaued. There are major shifts in the composition of both bacterial and fungal communities during succession in a tropical forest [16], semiarid abandoned farmland [17], grassland [18,19], and a desert steppe [20]. However, it is difficult to identify consistent patterns for the changes in soil microbial communities during succession because of environmental heterogeneity. In addition, whether some microbial species can be regarded as biomarkers for secondary succession remains unclear [10]. Therefore, because of the large heterogeneity among subtropical forest types, gaps remain in the understanding of changes in soil microbial communities during secondary succession in those forests.

Soil microbial community composition and diversity are affected by multiple biotic and abiotic factors, such as forest succession, plant diversity, carbon:nitrogen (C:N) ratio, pH, nutrient availability, and land use type [21–24]. In one study, soil properties rather than the plant community played the decisive part in shaping the soil microbial community [25], whereas in others, the plant community was decisive [26,27]. However, there is accumulating evidence that soil abiotic properties and plant community characteristics are simultaneously important in regulating soil microbial communities [2,28,29]. The composition and diversity of tree species can directly affect the functions of soil microbial communities by altering the quantity and quality of resources [23,30,31]. It is thus necessary to find out the relations between the composition and diversity of soil bacterial and fungal communities, and plant diversity and soil properties, in order to predict the changes in soil microbial community structures during ecosystem development [8]. Accordingly, the relations between vegetation and soil biotic and abiotic properties during long-term succession need to be studied further [10].

In this study, to identify the key factors that drive changes in soil microbial communities in different stages of succession, we evaluated the effects of plant diversity and soil properties on the composition and diversity of soil bacterial and fungal communities during forest secondary succession in subtropical China. Three secondary successional stages were examined, including a *Pinus kesiya* forest, a mixed *P. kesiya* and evergreen broad-leaved forest, and an evergreen broad-leaved forest. Typically, a *P. kesiya* forest is the first stage in the restoration of evergreen broad-leaved forests after clear-cutting, and there is a predictable sequence of these forest types in succession [32,33]. The composition and diversity of soil bacterial and fungal communities were measured using high-throughput sequencing. The objectives were: (1) to investigate how the soil bacterial and fungal communities changed during secondary succession in a subtropical forest; (2) to identify the plant and soil factors that shaped the soil bacterial and fungal communities during secondary succession. We hypothesized that: (1) the composition and diversity of soil bacterial and fungal communities would clearly shift during succession, with greater changes occurring in fungal communities than in bacterial communities because of the changes in plant species with succession [27]; (2) because plant diversity and soil abiotic properties are the primary regulators of variations in multifunctionality [34], they would be the drivers of alterations in soil microbial communities during succession.

2. Materials and Methods

2.1. Study Sites

The study was conducted in Pu'er (22°34′ N–22°53′ N, 100°56′ E–101°9′ E) in Yunnan Province in Southwest China. The area has a typical monsoon climate, characterized by distinct dry and rainy seasons [35]. The average annual temperature in the region is 17.7 °C. The mean annual precipitation is 1490 mm, and the precipitation is mainly concentrated from May to October. The soil type is latosol [33]. The climax vegetation community in the area is monsoon evergreen broad-leaved forest [32]. Similar to other regions in subtropical

China [36,37], the study area has a distinct successional gradient composed of pine forest (early stage), pine and broad-leaved mixed forest (middle stage), and monsoon evergreen broad-leaved forest (late stage).

2.2. Field Sampling

On the basis of species composition, three secondary forest stands in different successional stages were selected. By evaluating tree rings and consulting historical documents, the age of the three secondary forest communities were approximately 40, 70, and 90 years old. Detailed descriptions of the stands are presented in Table 1 and Table S1. In May 2018, seven 30 m × 30 m plots were established in each successional stage, for a total of 21 plots. Within each plot, all living woody plants with a diameter at breast height ≥ 1 cm were recorded. The plants were identified by species and then numbered. Three 1 m × 1 m subplots were established in each plot to determine the species richness and abundance in the herb layer. During the vegetation survey, five soil samples were collected from the depth of 0–10 cm in each plot using a five-point sampling method and then mixed into one composite sample. All composite soil samples were sieved through a mesh 2 mm in size and then divided into two parts. One part was transported to the lab in a portable refrigerator at −20 °C, and then stored at −80 °C until the next step for molecular analysis. The other part was air dried for physicochemical analyses.

Table 1. Soil properties and stand attributes in different stages of secondary succession of a *P. kesiya* forest.

Properties	Stages of Succession		
	Early	Middle	Late
SOC ($g \cdot kg^{-1}$)	18.99 ± 1.83^{c}	28.80 ± 2.09^{b}	43.81 ± 2.39^{a}
TN ($g \cdot kg^{-1}$)	0.90 ± 0.07^{c}	1.65 ± 0.10^{b}	2.86 ± 0.13^{a}
TP ($g \cdot kg^{-1}$)	0.19 ± 0.02^{c}	0.27 ± 0.02^{b}	0.39 ± 0.03^{a}
TK ($g \cdot kg^{-1}$)	4.37 ± 0.79^{b}	6.90 ± 1.01^{ab}	8.59 ± 1.21^{a}
C:N ratio	21.07 ± 1.02^{a}	17.52 ± 0.80^{b}	15.29 ± 0.43^{b}
HN ($mg \cdot kg^{-1}$)	80.47 ± 6.08^{c}	139.99 ± 5.85^{b}	246.64 ± 24.35^{a}
AP ($mg \cdot kg^{-1}$)	7.35 ± 1.41^{a}	9.65 ± 1.09^{a}	9.21 ± 2.74^{a}
AK ($mg \cdot kg^{-1}$)	91.63 ± 14.51^{b}	108.19 ± 11.36^{ab}	140.87 ± 9.18^{a}
pH	4.28 ± 0.10^{a}	4.07 ± 0.04^{b}	4.10 ± 0.04^{ab}
SWHC (%)	32.80 ± 1.54^{c}	41.01 ± 1.25^{b}	52.22 ± 3.34^{a}
TSR	26 ± 1^{b}	34 ± 2^{a}	34 ± 2^{a}
HSR	13 ± 1^{a}	14 ± 1^{a}	14 ± 1^{a}

Different lowercase letters in the same row indicate significant differences at $p < 0.05$. Abbreviations: SOC, soil organic carbon; TN, total nitrogen; TP, total phosphorus; TK, total potassium; HN, hydrolyzable nitrogen; AP, available phosphorus; AK, available potassium; SWHC, soil water holding capacity; TSR, tree species richness; HSR, herb species richness.

2.3. Soil Physicochemical Properties

Standard methods were used to test soil physicochemical properties [38], including the maximum soil water holding capacity (SWHC), pH, soil organic carbon (SOC), total nitrogen (TN), total phosphorus (TP), total potassium (TK), hydrolyzable N (HN), available P (AP), and available K (AK). The C:N ratio was calculated as a mass ratio of SOC and TN.

2.4. DNA Extraction, Illumina Sequencing, and Data Processing

The TIANamp Soil DNA Kit (Tiangen BiotECH Co., Beijing, China) was used to extract the soil DNA from 0.25 g of fresh soil according to the guidelines. The composition and diversity of soil bacterial and fungal communities were measured using 16S rRNA and ITS genes high-throughput sequencing [39]. The 16S rRNA was amplified by the V3–V4 region with the universal primers 338F (5′-ACTCCTACGGGAGGCAGCA-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′). Targeting the fungal community, the broad-spectrum primers ITS1 (5′-CTTGGTCATTTAGAGGAAGTAA-3′) and ITS2 (5′-

GCTGCGTTCTTCATCGATGC-3′) were used. According to standard protocols, an Illumina HiSeq2500 platform (Biomarker Technologies, Beijing, China) was used to conduct amplicon sequencing. UCHIME software was used to identify and remove chimera sequences [2]. USEARCH was used to cluster tags at the 97% similarity level to obtain operational taxonomic units (OTUs) [40], and then taxonomic annotation of OTUs was carried out based on the SILVA (bacteria) and UNITE (fungi) databases [41,42]. The raw sequencing data are available at the NCBI Sequence Read Archive (SRA) under the accession number SRP315258: PRJNA722794.

2.5. Data Analyses

The alpha diversity of soil bacterial and fungal communities in the three succession stages was compared using one-way ANOVA, including the number of OTUs (richness) and the Chao1 and Shannon indexes [43,44]. Additionally, nonmetric multidimensional scaling (NMDS), based on the Bray–Curtis dissimilarity matrices of OTUs of bacterial and fungal communities (Hellinger transformed), was used to test for differences in beta diversity using the "vegan" packages [31,43]. The NMDS analysis had certain reliability when *Stress* < 0.2. The composition of soil bacterial and fungal communities in each plot was represented by the averaged values of the Bray–Curtis dissimilarity matrices [8]. In addition, analysis of similarities (ANOSIM) was used to determine the differences in soil microbial community composition [25,45]. The effects of plant diversity and soil properties on the composition and diversity of soil bacterial and fungal communities in the different secondary successional stages of the *P. kesiya* forest were analyzed using random forest and structural equation models (SEMs) in "randomForest" and "lavaan" packages, respectively [39]. First, random forest models measured the relative contributions of successional stage, plant diversity, and the different soil properties to the soil bacterial and fungal richness and community composition to determine the main response variables during the forest secondary succession. Second, an SEM measured the direct and indirect effects of successional stage, plant diversity, and soil properties on the soil bacterial and fungal richness and community composition based on the following theoretical framework: (1) successional stage, plant diversity, and soil properties directly affect the soil bacterial and fungal richness and community composition; (2) successional stage indirectly affects the soil bacterial and fungal richness and community composition by affecting plant diversity and soil properties; (3) plant diversity indirectly affects the soil bacterial and fungal richness and community composition by affecting soil properties. The Shipley test of D-separation based on the "piecewiseSEM" package was used to check the missing paths [46]. Finally, the best-fit SEM was determined using a chi-square test ($p > 0.05$), root mean square error of approximation ($RMSEA < 0.05$), and goodness-of-fit index ($GFI > 0.95$) [4,47]. All analyses were performed in R3.6.2 [48].

3. Results

3.1. Soil Properties and Vegetation Characteristics

Soil properties (Table 1) and the composition of plant community (Table S1) were different in the three stages of succession. The SOC, TN, TP, TK, HN, AK, and SWHC increased significantly during secondary succession ($p < 0.05$). However, the C:N ratio decreased remarkably by 28.18% from the early stage to the late stage ($p < 0.05$). The pH decreased significantly from the early to middle stage ($p < 0.05$), but then remained approximately the same in the late stage. Available P was not significantly different among the succession stages. According to the vegetation data, tree species richness (TSR) was markedly higher in the middle and late stages than in the early succession stage ($p < 0.05$). However, herb species richness (HSR) was similar among the three succession stages.

3.2. Soil Bacterial and Fungal Community Composition and Diversity

For bacteria, 1139 OTUs were obtained and, for fungi, 667 OTUs were obtained. ANOVA analysis of alpha diversity showed remarkable differences among the three suc-

cession stages, except for the Shannon index (Figure 1). The OTU and Chao1 index of the bacterial community increased significantly from the early stage to the middle and late stages of succession ($p < 0.05$, Figure 1a,b). The OTU and Chao1 index of the fungal community was also significantly higher in the middle and late stages than in the early stage, but the index was notably higher in the middle stage than in the late stage ($p < 0.05$, Figure 1c,d).

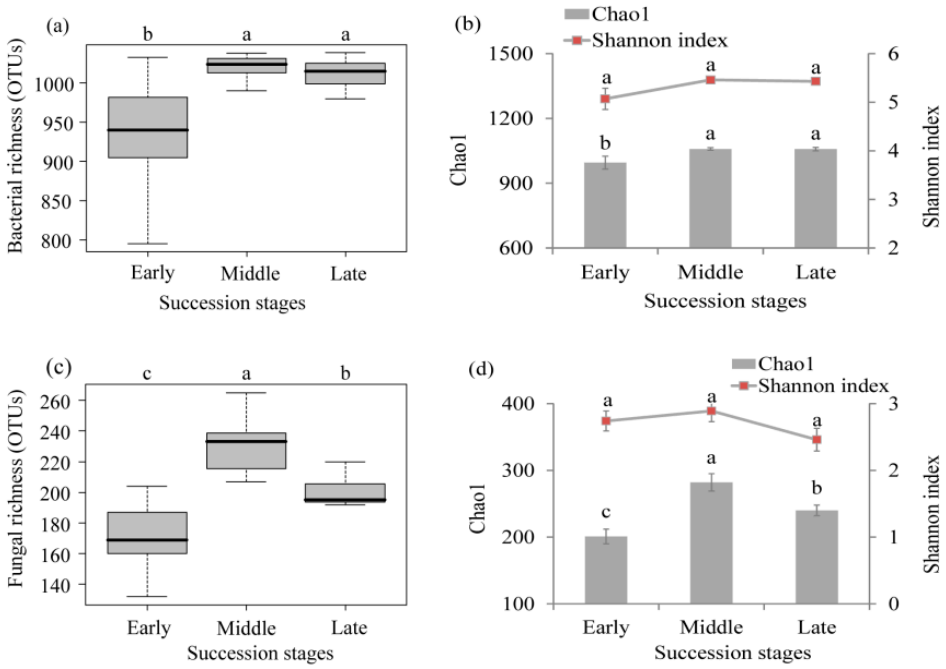

Figure 1. Alpha diversity of soil bacterial (**a**,**b**) and fungal (**c**,**d**) communities in different stages of secondary succession in a *P. kesiya* forest. Different letters above the box plots indicate significant differences ($p < 0.05$) between succession stages.

The NMDS analysis indicated that the bacterial and fungal communities were different in the three succession stages (*Stress* = 0.0956 and 0.1109, respectively) (Figure 2a,c). Similarly, according to the ANOSIM, the composition of both bacterial ($R = 0.448$, $p = 0.001$) and fungal ($R = 0.425$, $p = 0.001$) communities differed significantly among succession stages (Figure 2b,d).

Representative OTU sequences were aligned to the microbial reference database to obtain the classifications of the corresponding species. Twenty-two bacterial phyla and eight fungal phyla were identified. Generally, *Acidobacteria*, *Proteobacteria*, and *Actinobacteria* played a leading role in the bacterial communities, which accounted for more than 88% of the total bacterial sequences (Figure 3a). Notably, succession stage had no significant effect on the relative abundance of the top seven bacterial phyla (except *Acidobacteria*), but the relative abundance of the less-abundant phyla, including *Gemmatimonadetes*, *WPS-2*, and *Rokubacteria*, increased significantly from the early to late stage of succession ($p < 0.05$, Table S2). The dominant fungal phylum was Basidiomycota (58.33%), which was followed by Ascomycota (15.84%), Mortierellomycota (15.55%), and Mucoromycota (3.78%) (Figure 3b). The relative abundance of the dominant fungal phyla (except Basidiomycota) was distinctly influenced by successional stage ($p < 0.05$, Table S2). The relative abundance of Ascomycota and Mucoromycota decreased strongly and significantly from the early to late successional stages, whereas the abundance of Mortierellomycota increased significantly from the early and middle stages to the late stage (Table S2).

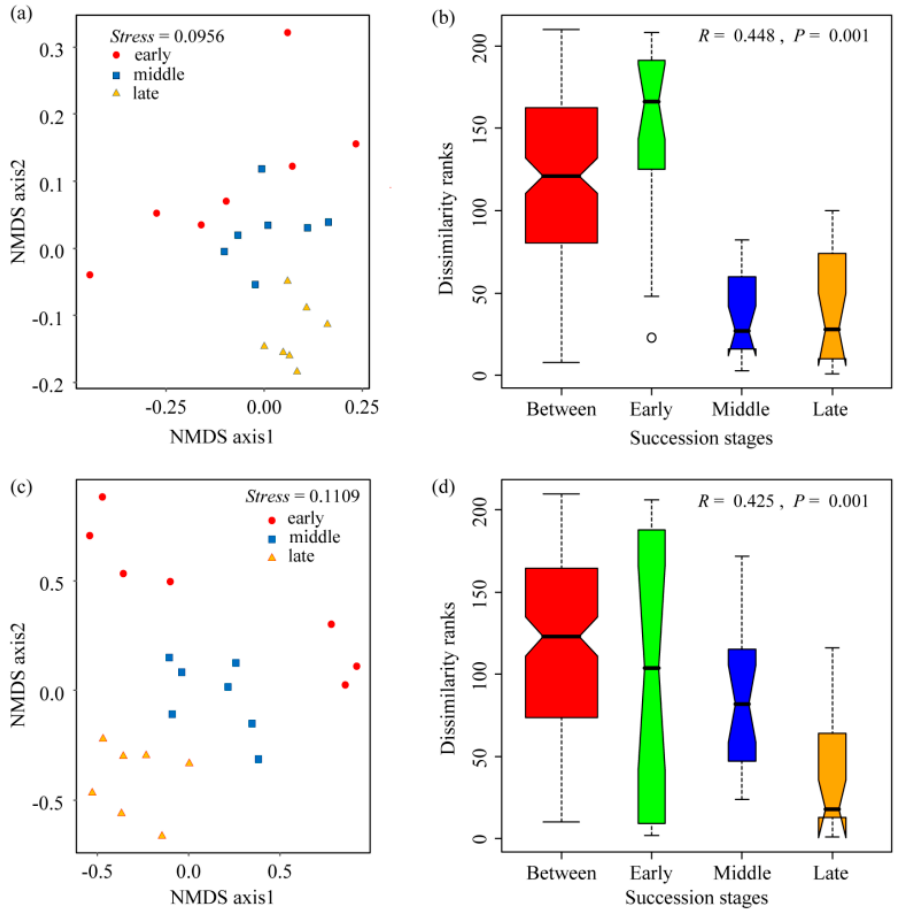

Figure 2. Nonmetric multidimensional scaling (NMDS) (**a**,**c**) and analysis of similarities (**b**,**d**) based on Bray–Curtis dissimilarities of soil bacterial (**a**,**b**) and fungal (**c**,**d**) communities during secondary succession in a *P. kesiya* forest.

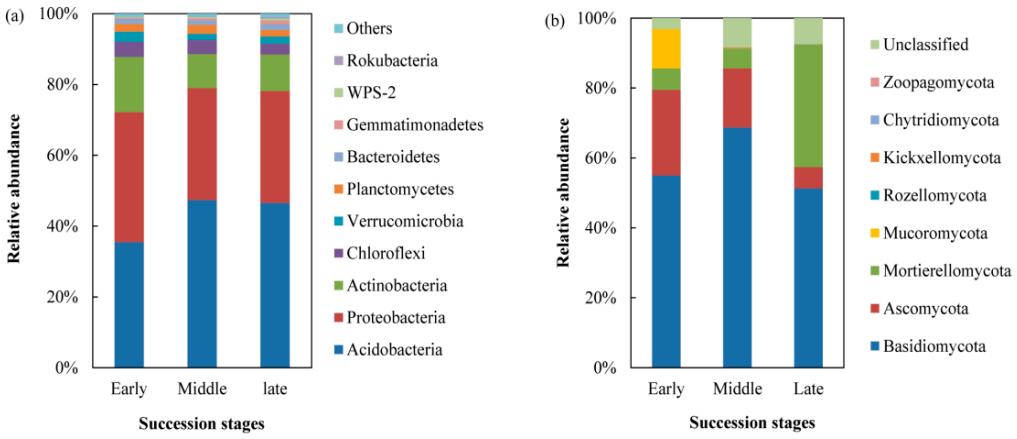

Figure 3. Relative abundance (%) of the phyla in soil bacterial (**a**) and fungal (**b**) communities in different stages of secondary succession in a *P. kesiya* forest.

3.3. Effects of Plant Diversity and Soil Properties on the Composition and Diversity of Soil Microbial Communities

Random forest analyses were used for indentifying the soil properties and vegetation characteristics that were important in predicting composition and diversity of the soil microbial communities (Figure 4). In the bacterial communities, random forest models explained 29.20% of the variance in composition and 47.45% in diversity. In the fungal communities, the models explained 12.36% of the variance in composition and 32.39% in

diversity. Tree species richness and the C:N ratio were the primary predictors of bacterial community diversity, whereas succession stage, TN, HN, C:N ratio, and TSR were the primary predictors of bacterial community composition. The primary predictors of fungal community diversity were succession stage, TN, HN, SWHC, and SOC, whereas SOC, TN, HN, and AP were the primary predictors of fungal community composition.

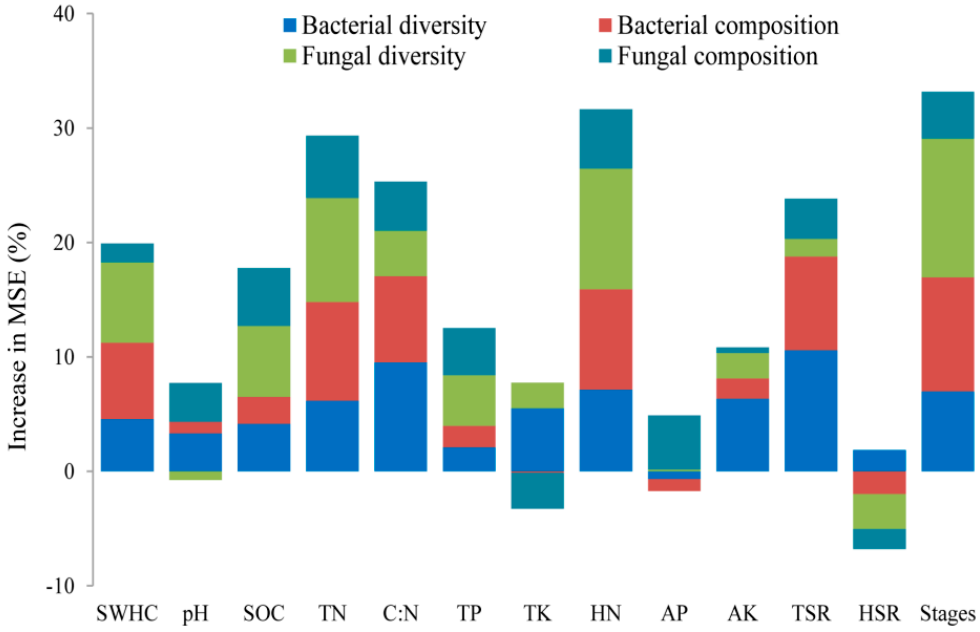

Figure 4. Main predictors of the composition and diversity of soil bacterial and fungal communities based on random forest models. Abbreviations: SWHC, soil water holding capacity; SOC, soil organic carbon; TN, total nitrogen; TP, total phosphorus; TK, total potassium; HN, hydrolyzable nitrogen; AP, available phosphorus; AK, available potassium; TSR, tree species richness; HSR, herb species richness.

The SEM was used to gain deeper insights into the effects of succession stage, soil properties, and vegetation characteristics on composition and diversity, and of soil microbial communities. The best-fit model was selected with $\chi^2 = 0.281$; $n = 1$; $p = 0.596$; *RMSEA* < 0.001; and *GFI* = 1. Multiple abiotic and biotic pathways regulated soil microbial community composition and diversity (Figure 5). For example, TSR had a notable direct influence on the composition and diversity of both bacterial and fungal communities. The C:N ratio had a significant negative direct effect on bacterial community diversity, but a significant positive direct effect on bacterial community composition (Figure 5a,b). Soil pH had a significant positive direct effect on bacterial community diversity, and AK had a significant positive direct effect on bacterial community composition. In fungal communities, soil pH and TP had striking negative direct effects on diversity, whereas TK and AP had significant positive direct effects (Figure 5c). Soil pH and TK had significant positive direct effects on fungal community composition (Figure 5d).

Indirect effects of succession stage, plant diversity, and soil properties on the composition and diversity of bacterial and fungal communities were also identified. For example, HSR indirectly affected the bacterial community composition by regulating AK (Figure 5b), and succession stage indirectly affected soil microbial communities through changes in TSR. In addition, succession stage indirectly affected the soil microbial community composition and diversity via soil pH, TP, TK, AK, and C:N ratio (Figure 5).

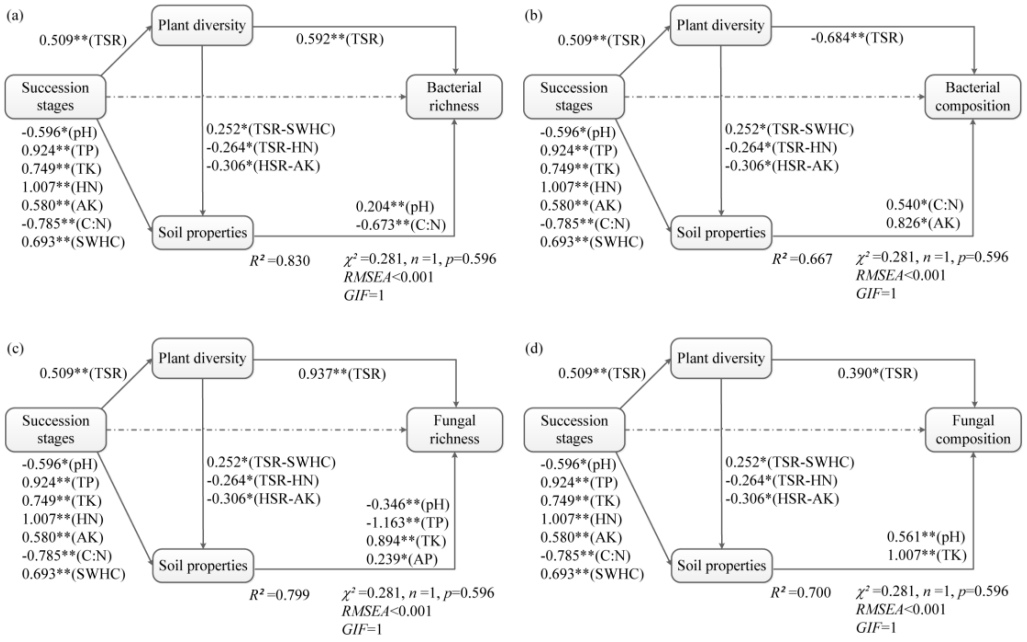

Figure 5. Effects of plant diversity, succession stage, and soil properties on the diversity (**a**,**c**) and composition (**b**,**d**) of soil bacterial (**a**,**b**) and fungal (**c**,**d**) communities based on a structural equation model. Solid lines represent significant paths (* $p < 0.05$; ** $p < 0.01$); dashed lines represent nonsignificant paths ($p \geq 0.05$). Abbreviations: SWHC, soil water holding capacity; SOC, soil organic carbon; TN, total nitrogen; TP, total phosphorus; TK, total potassium; HN, hydrolyzable nitrogen; AP, available phosphorus; AK, available potassium; TSR, tree species richness; HSR, herb species richness.

4. Discussion

Our results showed forest secondary succession altered the diversity of soil bacterial and fungal communities. This finding is in agreement with those of previous studies in temperate and subtropical forests [31,49]. In this study, the diversity of the soil bacterial community increased from the early to middle and late stages, while fungal community diversity was highest in the middle successional stage. This difference might be due to the different nutritional preferences of bacteria and fungi [10]. The direction of secondary succession was from a *Pinus* forest to an evergreen broad-leaved forest in the present study [50], and changes were observed in plant species, forest type, and soil fertility. Such changes could provide different environmental conditions for bacteria and fungi [23].

Generally, soil bacteria exhibit faster growth and turnover rates than fungi during early successional stages [51,52]. In *P. kesiya* forests in the early successional stage, biomass production is relatively high and soil fertility is relatively low [50]. Because these conditions lead to greater competition for available resources and lower plant diversity, the result can be lower soil bacterial and fungal community diversity in early succession, as observed in this study. In addition, the decomposition of conifer species litter is slower than that of evergreen broad-leaved species [53]. Thus, in early succession, the environment did not support high soil microbial diversity. However, because plants and soil fungi usually form symbioses, the increase in plant diversity with succession significantly positively affected soil fungal community diversity. These results provided further evidence that secondary succession could indirectly affect the soil microbial community structure [49].

The findings of this study suggested that TSR was the key driver shaping soil fungal community diversity, with the path coefficient from plant diversity to soil microbial diversity greater for soil fungi than for bacteria (Figure 5). Previous studies have demonstrated that plant communities significantly affect soil microbial communities via litter chemical properties and root exudates [22,23,28]. The diversity of soil bacterial and fungal communities was affected by different successional stages [21,52]. Our results showed that

the differences in fungal community diversity between successional stages were greater than those in soil bacterial community diversity, with the highest fungal community diversity in the middle successional stage (Figure 1). This might be attributed to the middle successional stage featuring more plant species with a particular soil fungal guild, such as the ectomycorrhizal fungi specifically associated with *P. kesiya*, *Alnus nepalensis*, and Fagaceae species [54,55]. Furthermore, the composition of soil microbial communities may be determined by the dominant plant species [27,56]. A previous study indicated that plant attributes (such as plant cover and functional traits) were also important factors affecting soil microbial communities [57]. In fact, the vegetation of middle successional stages contains more plant species because it includes both early and late successional stages [31]. With more plant species, more fungal species can coexist, which increases soil fungal community diversity. These fungi include the Basidiomycota and Ascomycota, which can easily metabolize the organic substrates in rhizodeposition [52]. Moreover, NMDS analysis showed that the distance in fungal community composition between successional stages was larger than that in bacterial community composition (Figure 2a,c), which suggested that fungi were more sensitive to environmental variations than the bacteria [17]. Taken together, these results confirm our hypothesis that the shift of fungi with succession was more obvious than bacteria.

Soil properties also significantly affected the composition and diversity of soil bacterial and fungal communities. In this study, soil pH and C:N ratio played critical roles in shaping soil microbial communities. Soil pH is a major factor that affects the soil bacterial community structure [8,56]. Extensive studies have shown that forest soil acidification on decadal scales is caused by trees [29]. The relative abundance of *Acidobacteria* increased during secondary succession. This phylum tends to be more abundant at lower pH levels [23,58] and, therefore, the increase in *Acidobacteria* could be explained by the decrease in soil pH in the middle and late successional stages. Soil C:N ratio and P concentration are usually considered limiting factors for soil microbial diversity [8,59]. In addition, secondary succession affects soil C and N cycles [56]. The soil C:N ratio significantly affected bacterial community diversity and composition, which suggests that the determinants of the soil bacterial community could be the variables related to soil carbon and nitrogen transformation. Soil fungal community structure is also influenced by soil pH [11,23,60]; similar findings were observed in the present research (Figure 5c,d). TK was found to be a strong factor influencing the fungal community structure, which may be attributed to the easy leaching of potassium from soil [44]. Furthermore, the solubility and availability of potassium could be partially affected by soil fungi [61]. Total P limited soil fungal community diversity, whereas the effect of AP was positive and significant. This may be attributed to Mortierellomycota being able to dissolve phosphorus [62]. We found a significant decrease in the abundance of Ascomycota with the accumulation of soil nutrients, which was in line with the works by Liu et al. [10]. The phenomenon might be explained by the fact that Ascomycota was able to grow in low-nitrogen conditions and had negative correlations with nitrate and nitrite [63]. Taken together, these findings demonstrate that indices of soil quality can be predictors of changes in soil microbial community diversity during pedogenesis [8].

5. Conclusions

In summary, the structure of soil bacterial and fungal communities showed significant differences at the three stages in the secondary succession of a *P. kesiya* forest, and the changes in plant diversity and those of most soil properties during succession could explain the differences in the microbial communities. In addition, plant diversity was a better predictor of fungal community diversity than bacterial community diversity. The relative abundance of dominant soil microbial phyla could also predict the direction of forest secondary succession, indicating that some phyla could be an important reference for future afforestation management in most subtropical zones in China. Future research should focus on the effects of specific plant species and plant attributes on functional groups

of soil microbial communities to provide more precise reference for the management of forest secondary succession.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/f12060805/s1, Table S1: dominant plant species in different stages of secondary succession of a *P. kesiya* forest, Table S2: relative abundance of phyla in bacterial and fungal communities in different stages of secondary succession of a *P. kesiya* forest.

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