Soil bacterial communities are influenced more by forest type than soil depth or slope position

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Abstract

Soil depth, slope position and different plantations can influence bacterial community composition in *Camellia oleifera* forests. However, prior studies have focused on the impacts of different depths, slope positions, and forest types on bacterial diversity independently, without comparing their combined impacts. This study aimed to assess variation in soil bacterial community structures according to soil depth and slope position and different forest types in the same plot. The composition of soil bacterial communities was evaluated using high-throughput sequencing of the 16S rRNA gene. Results indicate that the soil organic carbon, humus, and total organic content increased, and the bacterial composition and structure were significantly altered in response to the *G. jasminoides + C. oleifera* low-yielding forest in comparison to the other three forest types. The highest soil bacteria numbers, Alpha and beta diversity, which improved the soil microecological environment, were associated with the *G. jasminoides + C. oleifera* forests and not the depth or slope position treatments. The slope position did not have a significant influence on the soil physicochemical and bacterial properties. Structural equation modeling suggested that *G. jasminoides + C. oleifera* significantly affected the soil bacterial community diversity by mediating the soil pH and NH_4–N. The effects of forest type on soil bacterial diversity were more important than soil depth and slope position. This specific intercropping system was found to be an effective strategy to improve soil health.

Soil bacterial communities are influenced more by forest type than soil depth or slope position

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Abstract

Soil depth, slope position and different plantations can influence bacterial community composition in *Camellia oleifera* forests. However, prior studies have focused on the impacts of different depths, slope positions, and forest types on bacterial diversity independently, without comparing their combined impacts. This study aimed to assess variation in soil bacterial community structures according to soil depth and slope position and different forest types in the same plot. The composition of soil bacterial communities was evaluated using high-throughput sequencing of the 16S rRNA gene. Results indicate that the soil organic carbon, humus, and total organic content increased, and the bacterial composition and structure were significantly altered in response to the *G. jasminoides + C. oleifera* low-yielding forest in comparison to the other three forest types. The highest soil bacteria numbers, Alpha and beta diversity, which improved the soil microecological environment, were associated with the *G. jasminoides + C. oleifera* forests and not the depth or slope position treatments. The slope position did not have a significant influence on the soil physicochemical and bacterial properties. Structural equation modeling suggested that *G. jasminoides + C. oleifera*significantly affected the soil bacterial community diversity by mediating the soil pH and NH_4–N. The effects of forest type on soil bacterial diversity were more important than soil depth and slope position. This specific intercropping system was found to be an effective strategy to improve soil health.

Keywords : *Camellia oleifera* , bacterial communities, soil physicochemical properties, forest type, depth, slope position

1 | INTRODUCTION

Camellia oleifera is an evergreen tree belonging to the Theaceae family, which originates in China, and is a well-known woody oil tree species used to produce Camellia seed oil (Zhang et al., 2020b). Hunan Province is a key region for *C. oleifera* production in China. The centralization and continuous distribution patterns of the large-scale*C. oleifera* forests in this region have laid the foundation for their future industrialization (Han, 2020).

Soil is one of the most important environmental factors that impact plant physiology and affects the size and stability of *C. oleifera* yields. Soil nutrient content and microbial communities are crucial components of every ecosystem and are important drivers of global biogeochemistry. Soil bacterial community composition can be used to determine the type of land use and differentiate sites grouped according to key physicochemical properties (Hermans et al., 2020).

As the main source of plant nutrients, the soil nutritional status is a key factor that directly influences plant growth and development (Xiao and He, 2019). Soil microorganisms are used as an index for soil fertility and play a prominent role in soil productivity and fertility, organic matter decomposition, nutrient cycling, and soil aggregate formation (Six et al., 2004; Zhalnina et al., 2015; Müller et al., 2016; Nacke et al., 2016; Ding et al., 2017). Plant diversity, nutrient concentration, soil moisture, and soil type are also correlated with variation in bacterial communities (Hermans et al., 2017).

Land use can have long-term effects on the soil microbiota structure and diversity (Goss-Souza et al., 2017). Soil microbial characteristics are controlled by changes in moisture and temperature and driven by seasonal fluctuations and are closely associated with soil chemical characteristics (Jiao et al., 2018), forest habitat, and other ecosystem processes such as growth and development. Moderate growth of soil microorganisms may promote transformation and storage of soil nutrients and cause hormone disruption in the rhizosphere,

as well as affecting soil physicochemical properties. Metabolites produced by soil microorganisms can be used as a source of nutrients for plant growth, thereby affecting plant growth and development, succession, and community diversity (Dunn et al., 2006; Van der Heijden et al., 2006). The differences in the soil physico-structural characteristics under different vegetation management measures in different forest types are likely to affect soil bacterial diversity, community structures, and the evolution of soil physico-structural properties and their ecological function. Hermans et al. (2017) showed that key bacterial taxonomic groups can reflect the impacts of specific anthropogenic activities and provide strong evidence that microorganisms could be used as indicators for soil condition.

The main areas for growing *C. oleifera* in the southern region of China have predominantly acidic red and yellow soils. Red soil has poor breathability, low organic matter content, and is relatively nutrient-poor, which negatively affects plant growth. Compound, *C. oleifera* special, and bioorganic fertilizers promote improved soil nitrogen content, bacterial community abundance, biological activity, and plant yield. The use of biological agents instead of chemical fertilizers reduces environmental pollution and increases the yield of*C. oleifera* (Wu et al., 2019).

In the red soil hilly region of southern China, *C. oleifera* helps enhance and maintain soil fertility and the ecological quality of the tree species planted (Tu et al., 2019). However, considering the long growth cycle and low level of gains during the early growth stages of economic forests, identifying a novel scientific approach for forestry production is vital. Agroforestry management is an emerging land use and management method that could address this problem that has attracted considerable global attention. Intercropping *C. oleifera* with peanuts has been found to improve soil porosity and conductivity, as well as rhizosphere bacterial and fungal populations when compared with*C. oleifera* monocultures (Kroon et al., 2019; Lu et al., 2019; Liu et al., 2020). Previous studies on plant–soil interactions have highlighted many advantages associated with intercropping, such as improved yield, accretion, decomposition of organic matter, and enhanced iron and phosphorus availability. In mixed forest stands, bacterial community diversity increased, inhibiting soil erosion, improving the microclimate under the forest floor, increasing crop yields, and contributing to the sustainable development of agriculture and forestry (Dollinger and Jose, 2018; Mosquera-Losada et al., 2018). Intercropping also improves forest productivity, provides important non-economic benefits including social and environmental benefits. It can also increase farm yields and agricultural income, improving the livelihood of farmers (Li et al., 2019; Quandt et al., 2019).

Studies on soil bacterial communities in*C. oleifera* forests have suggested that different climates, plantations, and morphological site variation, such as soil depth can influence the bacterial community composition of *C. oleifera* forests (Tobias-Hünefeldt et al., 2019). However, the structural composition of the bacterial microbiome in the*C. oleifera* intercropping system still requires further exploration. Previous studies have focused on the impacts of different depths, slope positions, and forest types on bacterial diversity independently, without comparing their combined impacts (Lee et al., 2015; Jiang et al., 2021).

Studying different tea-oil forests will help to deepen our understanding of soil fertility and development in different forest types. It will also help to explain the role of microorganisms in the growth and development of tea-oil forest vegetation. It will also provide a baseline for developing effective afforestation and forestry techniques to improve the structure of existing tea-oil forests, promoting their sustainable development.

In this study, we analyzed the effects of different soil depths, slope positions, and different *C. oleifera* forest types in the same plot in Tangjiapu, Dingcheng District, China, to examine the variation in the soil bacterial structures. We also analyzed the correlation between soil physicochemical properties and soil microorganisms to determine whether depth, slope and forest type influenced the soil bacteria for *C. oleifera* in the same plot. The study provides reference data for informing effective and sustainable management of*C. oleifera* in low-yielding forest plantations.

2 | MATERIALS AND METHODS

2.1 | Site description

The experimental site was at a forest farm in Tangjiapu (111.844347°E, 29.188179°N), Dingcheng District, Changde, Hunan Province, China. The regional climate is subtropical monsoon, with a mean annual total rainfall of 1,200–1,900 mm, mean annual temperature of 16.7 °C, and the soil at the experimental site is quaternary red clay with a pH of 5.

2.2 | Experimental design

For the study, 36 soil samples were collected at random from different forest types (NA, *C. oleifera* new afforestation; GNA,*Gardenia jasminoides* + *C. oleifera* new afforestation; GLF,*G. jasminoides* + *C. oleifera* low-yielding forest; and LF,*C. oleifera* low-yielding forest) in April 2019, with nine soil samples collected from each forest type. In each block, a south to north topographic profile was selected, and the sampling plots were set in three slope positions at the base of the slope, on the midslope and the top of the slope. The angles of each of these were the same for each forest type. Stratified sampling was performed at different soil depths (0–20 cm, 20–40 cm, and 40– 60 cm) for each slope position, and three replicate plots were selected. Following collection, the samples were immediately frozen in liquid nitrogen, placed in a sterile plastic bag, labeled, transferred to the laboratory in a portable refrigerator on dry ice, and stored at –80 °C prior to bacterial community analysis.

2.3 | Analysis of soil physicochemical properties

The soil pH was measured using a pH meter in a soil: water suspension (1:5 w/v), after being shaken for 30 min. The soil organic carbon (SOC) and soil organic matter (SOM) were determined using the $K_2Cr_2O_7$-H_2SO_4oxidation–reduction colorimetric method (Schulz, 2002). The soil total nitrogen (TN) was measured using the Kjeldahl digestion method (Krishnamoorthy et al., 1982). The total organic carbon (TOC) was measured using a TOC analyzer (Elementar GmbH, Langenselbold, Germany). The humus content (HC), TN, total carbon (TC), nitrate nitrogen (NO_3–N), nitrite nitrogen (NO_2–N), and ammonium nitrogen (NH_4–N) levels were determined using colorimetry (Wang et al., 2011). Measurements of available phosphorus (AP) and available potassium (AK) were performed using the methods described by Mitchell et al. (2010). Soil available nitrogen (AN) was determined using a diffusion method (Khan et al., 1997).

2.4 | DNA extraction

Bacterial DNA was extracted from the soil samples using a soil microbe DNA kit (QIAGEN, USA) following the manufacturer's protocols. The concentration and purification of the DNA samples were determined using a NanoDrop 2000 UV-vis spectrophotometer (Thermo Scientific, USA), and quality checked using 1% agarose gel electrophoresis.

2.5 | PCR amplification and MiSeq high-throughput sequencing

Bacterial 16S rRNA was amplified using the 338F primer set (5'-ACTCCTACGGGAGGCAGCA-3') and 806 R (5'-GGACTACHVGGGTWTCTAAT-3')(Chen et al., 2018). The reaction mixtures (20 μL) contained 5 × FastPfu Buffer (4 μL), 2.5 mM dNTPs (2 μL), 5 μM forward primer (0.8 μL), 5 μM reverse primer (0.8 μL), FastPfu polymerase (0.4 μL), and template DNA (10 ng). The reaction volume was made up to 20 μL with ddH2O. The thermocycler settings were as follows: 2 min at 95 , followed by 27 cycles for 30 s at 95 °C, 2 min at 45 °C, 3 min at 72 °C, and finally 10 min at 72 °C. Amplified products were visualized using 2% agarose gel and purified followed the manufacturer's protocols for the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, USA) and quantified using QuantiFluor-ST (Promega, USA). Sequencing was performed using the Illumina MiSeq platform (Illumina, San Diego, CA, USA) in Majorbio Bio-Pharm Technology Co. Ltd. (Shanghai, China).

2.6 | Data processing

The similarities and differences between the samples were based on operational taxonomic unit (OTU) clustering, and representative sequences in the OTU clusters were obtained. The most abundant sequences were selected as representative OTUs and used in various OTU analyses. The soil bacteria alpha diversity was calculated after randomly subsampling sequences to an equal number. Four metrics were used for alpha

diversity analysis, namely the observed species richness (Chao) index, abundance-based coverage estimator (ACE) index, Shannon index, and Simpson's index (Prober et al., 2015; Gao et al., 2018). To analyze the species turnover, we calculated the Bray–Curtis dissimilarity for the same subplot with different forest types, slope positions, and depths. Principal coordinate analysis (PCoA) of the β-diversity was calculated based on the Bray–Curtis algorithm. The structure equation model (SEM) was used to assess the direct effects of planting *G. jasminoides in C. oleifera* on soil physicochemical properties and soil bacterial characteristics (He et al., 2020).The data were analyzed using the free online Majorbio Cloud Platform (www.majorbio.com). Statistical differences were tested by ANOVA.

2.7 | Statistical analysis

Paired-end reads of the 16S rRNA gene were assembled using Flash (v1.2.11) (https://ccb.jhu.edu/software/FLASH/index.shtml) to obtain the raw tags. The relative abundance taxonomic summaries, beta diversity, and rarefactions were examined with QIIME (v1.9.1) (http://qiime.org/install/index.html). The OTU clustering of sequences was performed using UPARSE (v7.0.1090) (http://www.drive5.com/uparse/). Taxonomic classification was conducted using the ribosomal database project classifier (v2.11) (https://sourceforge.net/projects/rdp-classifier/). Concatenated sequences were detected using USEARCH (v7.0) (http://www.drive5.com/usearch/). Alpha and beta diversity estimates were calculated using MOTHUR (v1.30.2) (https://www.mothur.org/wiki/Download_mothur). Linear discriminant analysis (LDA) effect size (LEfSe) was performed to evaluate the different taxa among the soil depth, slope position, and forest types (http://huttenhower.sph.harvard.edu/galaxy/root?tool_id=lefse_upload). SEM was used to assess the effects of the *G. jasminoides*intercropping.

3 | RESULTS

3.1 | *Physicochemical analysis of the soil samples*

Soil physicochemical properties that could be directly influenced by the soil depth and forest type are shown in Figure 1 and Tables 1 and 2. There were no significant differences in the five physical indicators evaluated, namely soil moisture content [SMC], pH, electric conductivity [EC], total dissolved solids [TDS], and specific soil weight [GS] in the soil samples from different depths ($P > 0.05$; Figure 1). In contrast, the slope position and forest type did influence the physical indicators (Figure 1). The EC decreased from the -base to the top at different slope positions, while intercropping caused fluctuations in the soil EC and the TDS.

Preliminary analysis results for the soil chemical parameters are presented in Table 1. The TOC, SOM, TN, TC, NH_4–N, and AP content were highest in the 0–20 cm depth layer. The increase in the soil chemical parameter values of the surface soil was likely due to the decomposition and transformation of forest tree litter and biological residues, which are major sources of organic matter accumulation. The slope position did not significantly affect the soil physical and chemical indicators. The TOC, HC, SOM, TN, TC NH_4–N, AN, and AP contents in the GLF were significantly higher than for the other stands, indicating that *G. jasminoides* plays an important role in modulating the fertility of the soil in the *C. oleifera*low-yielding forest.

A comparison of the soil nutrient content at different soil depths determined that the NH_4–N and AN levels were the lowest in the 40–60 cm depth layer and that the AP levels were highest in the 0–20 cm depth layer (Table 2). The NO_3–N and AK levels did not significantly vary by soil layer. No significant differences in NO_2–N levels were recorded among the different plantations, but the highest NO_3–N levels were observed in LF. Comparing the soil nutrient content from the different forest types revealed that the NH_4–N level for the intercropping was significantly lower than that of the monoculture($p < 0.05$). It could be due to the*G. jasminoides* and newly planted *C. oleifera* substantially increasing plant uptake of tNH_4–N. Therefore, the nitrogen supplying capacity of the forest soil became insufficient.

3.2 | Microbial diversity and richness in the soil samples

Alpha diversity reflects the richness and diversity of the bacterial community indices. The Chao index is used to estimate the species richness, and the ACE index is used to indicate the number of OTUs in the

soil community. Therefore, the higher the value of these indices, the higher the richness. The Shannon and Simpson's indices are used to characterize species diversity within a community. A high Shannon index indicates high microbial diversity. The results from the diversity (Shannon), richness (Chao), ACE, and Simpson's indices for the bacterial communities are shown in Figure 2. The species richness and number of OTUs in the soil bacterial community in GLF were significantly higher than those in the LF (Figure 2). There were no significant differences in the determined characteristics between the soil layer depth and slope positions. These results indicated that forest type has a more pronounced effect on the soil bacterial community than the depth and the slope position.

3.3 | Distribution of bacteria in soil samples

At the phylum level, Proteobacteria, Chloroflexi, Acidobacteria, and Actinobacteria were the dominant bacteria in all the samples, accounting for 88.23% of the total sequence data. Other abundant phyla were GAL15, WPS-2, Verrucomicrobia, Bacteroidetes, Gemmatimonadetes, and Planctomycetes (Figure 3a). The phyla with comparatively high relative abundance, namely, Chloroflexi, Acidobacteria, Actinobacteria, and WPS-2, were all significantly lower at the 40–60 cm soil depth than for the other depths, except for Proteobacteria (Figure 3b). At different slope positions, the relative abundance of Proteobacteria was the lowest at the top of the slope, while that of Acidobacteria was the highest. The relative abundance of Actinobacteria was lowest at the midslope (Figure 3c). In the different forest types, the abundance of Proteobacteria was the highest in LF. The abundance of Chloroflexi and Acidobacteria were highest in NA and lowest in LF, while the abundance of Actinobacteria was highest at LF and lowest in GNA. There were no significant differences in the abundance of Chloroflexi between the forest types (Figure 3d).

Plantation altered the relative abundance of the dominant phyla (Figure 4) in different forest types, with Acidobacteria, WPS-2, and Rokubacteria showing highly significant differences($p< 0.01$.Significant differences in the abundance of Actinobacteria, unclassified_k_norank_d_Bacteria, and Gemmatimonadetes were recorded, while the relative abundance of Proteobacteria did not differ significantly. There were no significant differences in the bacterial communities at different depths or slopes.

3.4 | Beta diversity analysis of the soil samples

Distance was used to identify the variations that influence soil microbial communities in different soil samples (Figure 5). The samples were divided into different groups based on forest type, slope position, and differences in soil depth. When all the samples were divided into three groups according to the depth, no significant differences in the microbial communities were recorded (Figure 5a). When samples were divided into top, midslope, and base of the slope groups, the groups did not form distinct clusters based on the Bray–Curtis distance of PCoA analysis (Figure 5b). Four groups from different forest types were not distinct, indicating no difference in the bacterial community structures between the groups that were tested (Figure 5c).

3.5 | Linear discriminant analysis effect size

LEfSe was used to evaluate the different taxa among the different soil depths, slope positions, and forest types (LDA $>$ 3). The results of the LEfSe analysis suggest that there was no significant difference in the taxa across the different soil depth layers and slope positions (Figure 6). However, several significant microbial biomarker taxa were present across the different forest type groups. Furthermore, 117 species were differentially abundant among the forest type groups. There were 32 biomarkers in the NA, of which the Acidobacteria contributed the most, followed by Chloroflexi at the phylum level of the bacterial communities. There were 49 and 17 biomarkers in GLF and LF, respectively, with the Proteobacteria contributing the most at both sites. There were 19 biomarkers in GNA, and the dominant taxon was Gemmatimonadetes. These results indicate that the rhizosphere soil exhibited a higher number of the dominant genera from the bacterial or fungal communities than the endophytic environments.

3.6 | Relationships between soil microbial communities and soil physicochemical properties

To explore the influence of soil environmental factors on soil bacterial communities, the relationships between microbial preponderant phyla and the main soil properties were analyzed using Spearman's correlation

heatmap. At the phylum level, environmental factors and the 20 most abundant bacterial communities were correlated with the depth, slope position, and forest types (Figure 7). The results of the depth and slope position were the same. Approximately seven bacterial phyla were significantly correlated, five were negatively correlated, and two were positively correlated with AK. Five bacterial phyla were significantly correlated, one was negatively correlated, and four were positively correlated with pH. Five phyla were significantly correlated with the concentration of AN. The concentrations of AP, NH_4–N, and SMC were significantly correlated with four phyla. No bacterial phyla were significantly correlated with NO_2–N in the different forest types. Forest type has been found to have a potential impact on the bacterial microbial community and has led to differences in the microbial abundance.

3.7 | Correlation analysis of soil properties and bacterial communities

We constructed SEMs to further investigate the impact of the depth, slope position, and forest type on the bacterial communities. SEM explained 83.2% and 72.1% of the variation under new afforestation and low-yield forest, respectively, (new afforestation: $\chi 2 = 22.586$, P-value = 0.832, AIC = 118.586, BIC = 161.324; low-yielding forest: $\chi 2 = 25.086$, P-value = 0.721, AIC = 121.086, BIC = 163.824) (Figure 8). *G. jasminoides* in a low-yielding *C. oleifera* forest had significant direct effects on the Shannon diversity index, while *G. jasminoides* in the *C. oleifera* new afforestation had no significant effect on the Shannon diversity index. Planting *G. jasminoides* in the low-yielding *C. oleifera* forest led to a change in the EC values, which influenced the pH values. As a direct factor, pH influenced the ACE richness index. The NH_4–N to NO_3–N transition was enhanced (Figure 8b). Our results suggest that planting *G. jasminoides*could effectively promote the conversion of NH_4–N to NO_3–N in low-yielding *C. oleifera*forests.

4 | DISCUSSION

The highest TOC, SOM, TN, TC, NH_4–N, and AP content levels were identified in the soil samples at the 0–20 cm depth, indicating that the chemical characteristics of the soil were in balance (Wang et al., 2017). The low-yielding *G. jasminoides* + *C. oleifera* forest had significantly increased TOC, HC, SOM, TN, TC NH_4–N, AN, and AP content levels, and the availability of soil nutrients subsequently increased. This could be because agroforestry trees provide nutrients that fulfill agricultural demands. Another reason may be the increased abundance of related bacteria, including rhizobia, phosphate-solubilizing bacteria, and potassium-solubilizing bacteria, in soil nutrient cycling. However,*G. jasminoides* intercropping in a new afforestation area had marginal effects on these indicators. This supports the hypothesis that anthropogenic activities could considerably alter the original distribution of nutrients in low-yield forests by changing the planting patterns and altering the growth conditions to modify the soil nutrient status. This investigation found that slope position had few effects on the soil physicochemical properties.

Understanding the impacts of forestry practices on the soil microbiota will aid in the development of sustainable forestry practices. Our results support the hypothesis that intercropping alters the below-ground microbial community and composition. *G. jasminoides* supplementation in a new area of*C. oleifera* afforestation increased the soil bacterial abundance and improved the bacterial alpha diversity. The soil depth and the slope position had no significant effect on the alpha diversity of *C. oleifera.* These findings were in contrast with previous studies, suggesting that bacterial diversity was significantly greater at the lower slope position compared with the upper slope position (Sun et al., 2018). Different bacterial taxa showed variable abundance with varying soil depths (Eilers et al., 2012). This suggests that forest types had more pronounced effects on the bacterial communities than the depth and the slope position in the same plot.

Based on high-throughput sequencing, Proteobacteria, Chloroflexi, and Acidobacteria were identified as the main bacterial phyla in the soil communities, and this was consistent with the findings of previous studies (Li et al., 2018, 2019). Proteobacteria and Acidobacteria are the primary soil bacteria taxa associated with SOM decomposition. Changes in the SOM content are highly correlated with the abundance of Proteobacteria (Fierer et al., 2007; Banerjee et al., 2016). The Proteobacteria abundance is highest in disturbed forest soils (Noble et al., 2020), implying a prominent role in carbon turnover. Proteobacteria were also found to be more abundant in the organic layer of deciduous forests (Eilers et al., 2012;, Mundra et al., 2021). This could

be because higher levels of soil compaction in the deeper layers reduces O_2 availability, limiting the growth and activity of many microorganisms (Hartmann et al., 2014). However, the reason behind the increase in the abundance of Proteobacteria in the 40–60 cm soil layer requires further investigation. Acidobacteria is the common bacterial phyla in soil and can degrade the cellulose and lignin of plant residues and reduce nitrate, nitrite, and potentially nitric oxide, which play a major role in the carbon cycle and nitrogen circuits (Ward et al., 2009; Kalam et al., 2020). Previous studies have demonstrated that Acidobacteria diversity is inversely related to soil depth. The abundance of Acidobacteria is highest in soils in unmanaged forests (Kuske et al., 2002; Sheng et al., 2019), which was largely confirmed in this study. This could be attributed to the Acidobacteria oligotrophic nature or the ecological K-strategy (Ward et al., 2009; Kielak et al., 2016. Chloroflexi have been identified in many environments through marine and freshwater sediments, and they exhibit biological activity in extreme soil environments (Neilson et al., 2012). Chloroflexi efficiently degrades chlorides (Zhang et al., 2020a) and can metabolize additional complex carbon sources (McGonigle et al., 2020). It is likely that Chloroflexi species play a role in the material circulation of the soil bio-chemical layers.

In our research, the dominant soil microbes significantly differed in their relative abundance among the different forest types. Our results confirmed that the *G. jasminoides* plantation altered the relative abundance of the dominant phyla and that there were no significant differences in the bacterial communities in relation to the depth and slope positions. These observations suggested that different forest substructures affected the soil ecological environment. A study on the effects of leguminous supplementation on the resilience of soil bacterial communities and nutrient content in Chinese fir plantations showed that functional plant supplementation significantly increases the diversity and richness of soil microbes, accelerates the transformation and absorption of soil nutrients, and promotes the growth of Chinese fir (Zhang et al., 2020c). Complex bacterial community structures increase soil resistance to adverse environmental factors and are important for protection of the soil ecosystem. As key components of the soil ecosystem, soil physicochemical properties and microorganisms have an irreplaceable role. These interspecific interactions can help maintain the stability and ecological functions of microbial communities and re-construct stable core microbial communities. Based on the LEfSe analysis, the variable distribution of these biomarkers across different forest types indicate that the influence of the soil bacterial community structure on *C. oleifera* may be highly important.

SEM analysis showed that the *G. jasminoides* plantation significantly influenced soil bacterial communities by changing NO_3–N and secondarily affecting pH and EC. However, these implications in the new *C. oleifera* afforestation were less pronounced than in the low-yielding *C. oleifera* forest treatment and, may not have had as strong a stimulatory effect on the bacterial activity as expected. This indicates that the effects of the *G. jasminoides* plantation in *C. oleifera* forest may be more important than the depth and slope position when considering bacterial function in the ecosystem.

5 CONCLUSIONS

Soil depth has been shown to have important effects on soil microbial community structures (Brewer et al., 2019). Cultivating *G. jasminoides* with *C. oleifera* is a promising method to promote plant growth. It increases the soil fertility and microbial diversity and promotes the network structure and growth of key microbial organisms, improving potential ecosystem functioning in *C. oleifera* plantations. These beneficial effects were more pronounced when different forest types were examined and were less apparent when the soil depth and slope position were analyzed. When *G. jasminoides* was introduced into the *C. oleifera* stand, it significantly affected microbial communities and the soil physicochemical properties. The SOC, HC, and TOC content levels were higher in the *G. jasminoides* with the low-yielding *C. oleifera* forest than in the other stands. As revealed by the SEM, pH, and NH_4–N were the principal drivers shaping the soil microbial structure, and microbial composition was significantly influenced by land use change rather than by depth and slope position. The litter from multiple tree species improves soil structure and promotes the build-up of diverse microbial communities when compared with homogenous litter. Replacing monocultures with mixed-species stands is a promising approach for the maintenance of soil biodiversity and stability and the improvement of soil nutrient content.

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CONFLICT OF INTEREST

All authors declare that there are no competing interests.

AUTHOR CONTRIBUTIONS

The main structure of the article was developed after several discussions between the co-authors. Yun Wang and Peng Xie contributed equally to this work. Yun Wang and Peng Xie collected samples, conducted the laboratory assays, processed sequencing data, conducted statistical analysis, and wrote the manuscript. Jiyun She contributed to experimental concepts and design and trained and supervised Yun Wang and Peng Xie in designing experiments, analyzing data, and writing the manuscript. Yun Wang, Peng Xie, Aihua Deng, and Kerui Huang performed the experiments, and Shaogang Fan provided informative suggestions during the experimental design and revision of the article. All authors have read and approved the final manuscript.

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Figure Captions

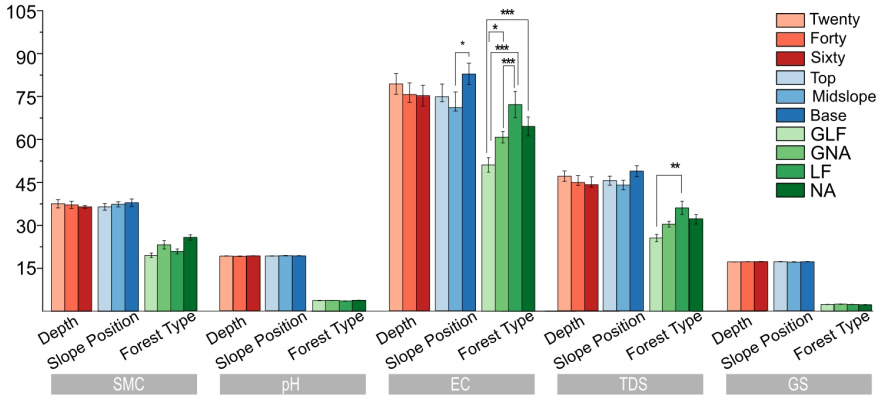

Figure 1 . Physical properties of the soil. The soil was sampled at different depths (a), at different slope positions (b), and in different forest types (c). SMC (%), soil moisture content; EC ($\mu s \cdot cm^{-1}$), electric conductivity; TDS ($\mu g \cdot L^{-1}$), total dissolved solid; GS ($g^{*}cm^{-3}$), specific soil weight. Forest types: NA,*Camellia oleifera* new afforestation; GLF, *Gardenia jasminoides* in a low-yielding *C. oleifera* forest; LF, low-yielding *C. oleifera* forest; GNA, *G. jasminoides* in*C. oleifera* new afforestation. Twenty, soil layer at 0–20 cm depth; Forty, soil layer at 20–40 cm depth; Sixty, soil layer at 40–60 cm depth. The data are shown as the mean +- SD. (depth and slope positon n = 9forest type n = 12; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$). Statistical analysis was performed using one-way analysis of variance (ANOVA), and Tukey's multiple comparison test was used to correct for multiple comparisons.

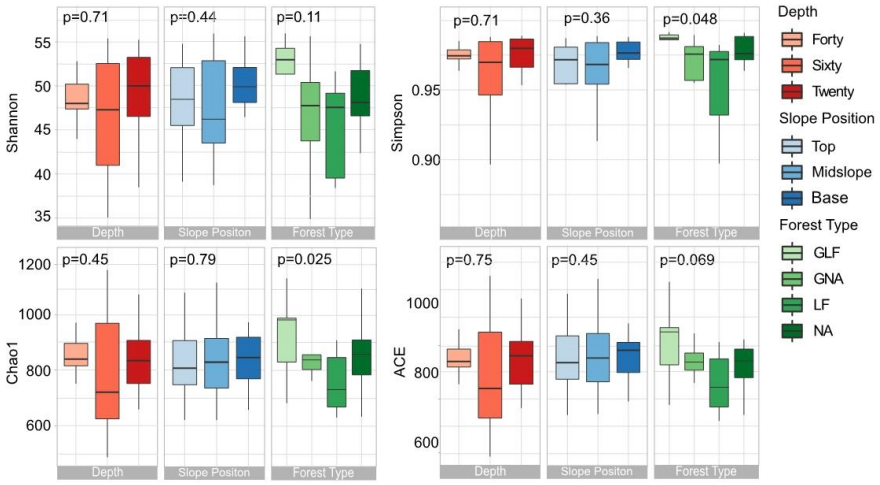

Figure 2 . General bacterial alpha diversity patterns. Boxplot of alpha diversity indices, including community richness (Ace and Chao) and diversity (Shannon and Simpson). Microbial alpha diversity in the soil samples from different forest types (a), different depths (b), and different slope positions (c). NA, *C. oleifera* new afforestation; GLF, *G. jasminoides* in a low-yielding *C. oleifera* forest; LF, low-yielding *C. oleifera* forest; GNA, *G. jasminoides*in *C. oleifera* new afforestation. Twenty, soil layer at 0–20 cm depth; Forty, soil layer at 20–40 cm depth; Sixty, soil layer at 40–60 cm depth. OUT, operational taxonomic unit; ACE, abundance-based coverage estimator. The data are represented as a box-and-whisker plot representing median values with interquartile ranges. Different colors indicate different sampling campaigns and sampling locations (depth and slope positon n = 9forest type n = 12; $^{*}P < 0.05$, $^{**}P < 0.01$, [Student's t -test for estimator]).

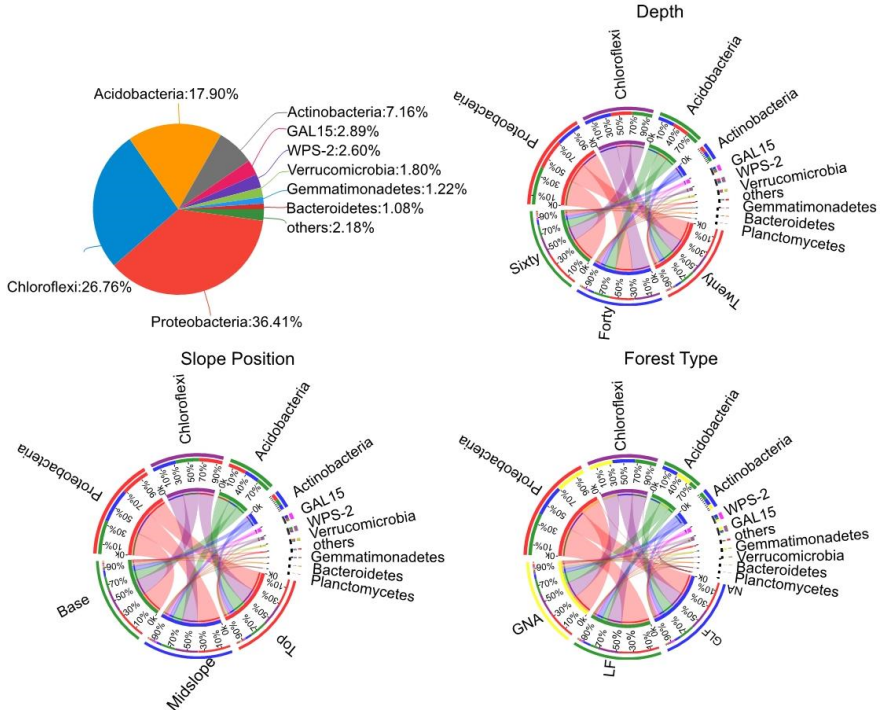

Figure 3 . Relative abundance of different soil bacteria at the phylum level. Pie chart showing the percentage of species as designated by color (a). In the circos plot, the colors in the left semi-circle represent the species composition, and the outermost circles represent the grouping information. The colors of the inner circles represent species, and the length represents the relative abundance of the species. The right semi-circle indicates the distribution proportion of species in different samples at the taxonomic level for that cluster, the colors of the outermost circles represent species, the colors of the inner circles represent grouping information, and the length represents the fraction of that species in that cluster (b–d). The soil was sampled from different depths (b), slope positions (c), and forest types (d) depth and slope positon (n = 9), forest type (n = 12)). m1 (NA),*C. oleifera* new afforestation; m2 (GLF), *G. jasminoides* in a low-yielding *C. oleifera* forest; m3 (LF), low-yielding *C. oleifera* forest; m4 (GNA), *G. jasminoides* in *C. oleifera*new afforestation. Twenty, soil layer at 0–20 cm depth; Forty, soil layer at 20–40 cm depth; Sixty, soil layer at 40–60 cm depth. Here, "others" indicates taxa with a maximum abundance of < 0.5% in any sample.

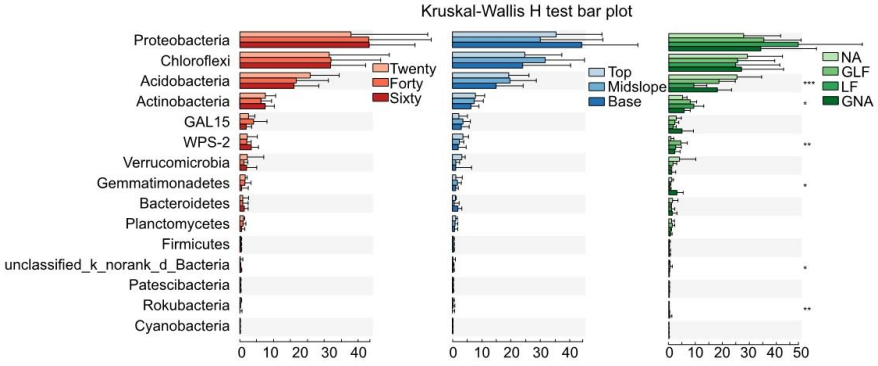

Figure 4 . Bacterial community diversity assessments based on forest type (a), depth (b), and slope position (c) using the Kruskal–Wallis H test bar plot. The image represents the difference between proportions in 95% confidence intervals. "Unclassified" includes all unclassified species obtained directly from the database through sequence alignment. The X-axis represents the average relative abundance of different species, The Y-axis represents the species, and different colors indicate the different groups. NA, *C. oleifera* new afforestation; GLF,*G. jasminoides* in a low-yielding *C. oleifera* forest; LF, low-yielding *C. oleifera* forest; GNA, *G. jasminoides* in*C. oleifera* new afforestation. Twenty, soil layer at 0–20 cm depth; Forty, soil layer at 20–40 cm depth; Sixty, soil layer at 40–60 cm depth. Depth and slope position (n = 9), forest type (n = 12); $^{*}P < 0.05$, $^{**}P < 0.01$

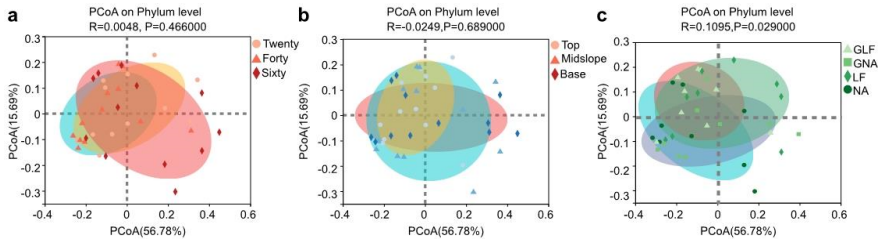

Figure 5 . Principal coordinate analysis (PCoA) of the soil bacterial community composition as affected by forest type (a), depth (b), and slope position (c), and based on the Bray–Curtis distance. Points of the same color belong to the same group, and soil group points that are the same are identified using ellipses. Abscissa represents the first principal component, ordinate represents the second principal component, and the percentage represents the contribution of the principal component to the sample difference. Box plots represent the discrete distribution of the different groups of samples along the PCoA1 axis and different colors represent the different sample groups. Values of R^2 and P were calculated using analysis of similarities. NA,

Camellia oleifera new afforestation; GLF,*Gardenia jasminoides* in a low-yielding *C. oleifera* forest; LF, low-yielding *C. oleifera* forest; GNA, *G. jasminoides*in *C. oleifera* new afforestation. Twenty, soil layer at 0–20 cm depth; Forty, soil layer at 20–40 cm depth; Sixty, soil layer at 40–60 cm depth.

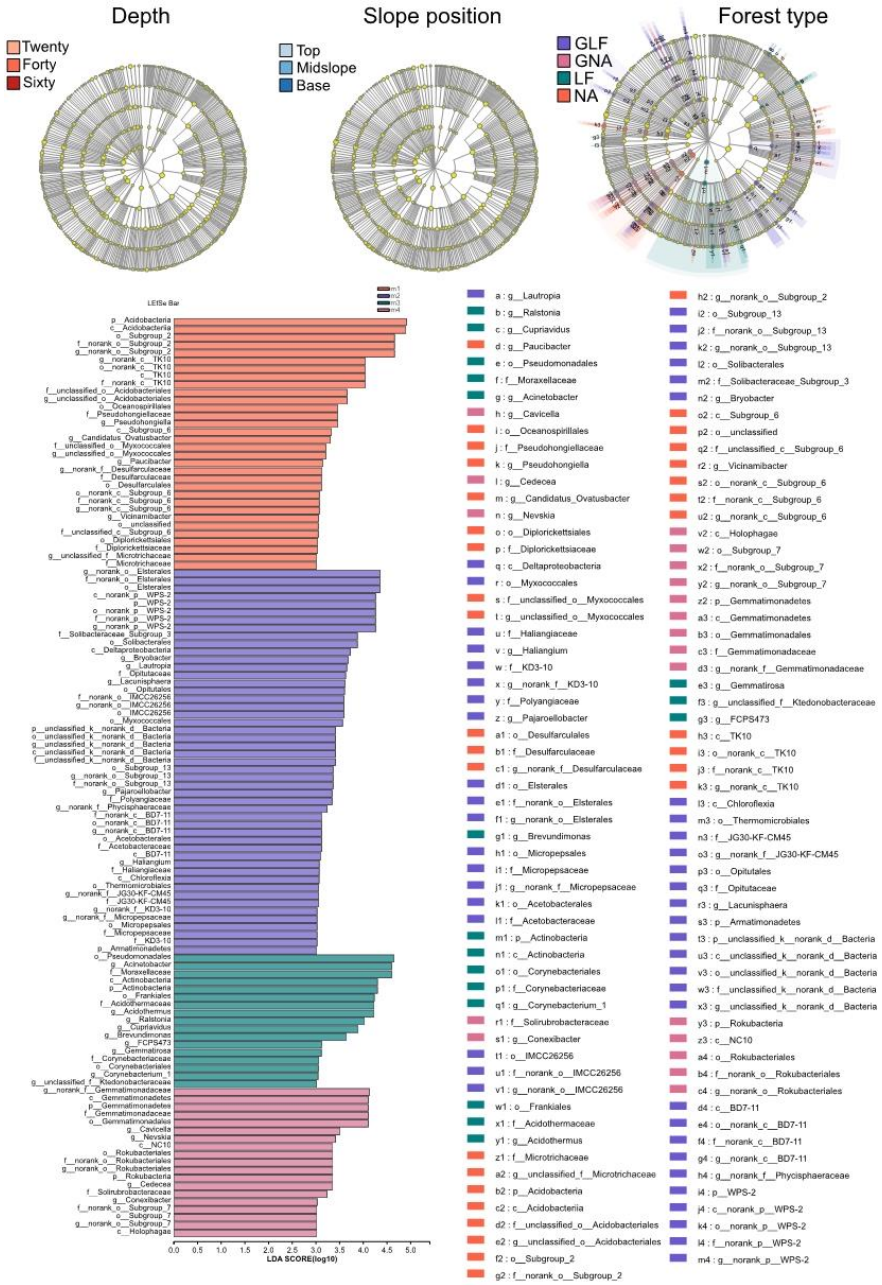

Figure 6 . LEfSe (LDA effect size) analysis for forest type (a), depth (b), and slope position (c). The colored nodes from the inner to the outer circles represent taxa from the phylum to genus level. The node color denotes different taxa with a more significant role. Taxa without significant differences are labeled in yellow, while significantly different taxa are labeled using the color for each group. A cut-off value of [?] 3.0 was used for the linear discriminant analysis (LDA). NA, *C. oleifera* new afforestation; GLF, *G. jasminoides* in a low-yielding *C. oleifera* forest; LF, low-yielding *C. oleifera* forest; GNA, *G. jasminoides* in*C. oleifera*

new afforestation. Twenty, soil layer at 0–20 cm depth; Forty, soil layer at 20–40 cm depth; Sixty, soil layer at 40–60 cm depth.

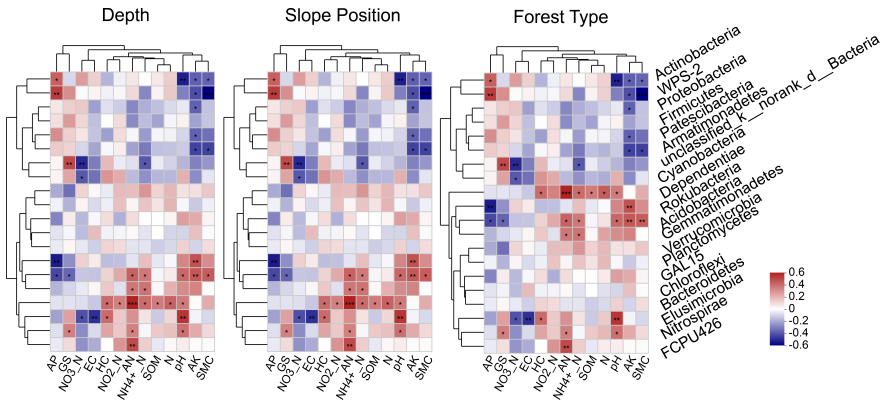

Figure 7 . Correlation between environmental factors and the top 20 most abundant bacterial communities at the phylum level. The X and Y axes represent horizontal and vertical angular environmental factors (species composition), respectively. HC, humus content; GS, specific gravity of solid particles; AP, available phosphorus; TOC, total organic carbon; SOM, soil organic matter; N, soil total nitrogen; C, soil total carbon; NO_3–N, nitrate-nitrogen; EC, soil electric conductivity; TDS, total dissolved solid; AK, available potassium; SMC, soil moisture capacity; AN, available nitrogen; NO_2–N, nitrite nitrogen; NH_4–N, ammonium nitrogen. The red shades represent positive correlation, and the blue shades represent negative correlation. Darker shades represent stronger correlation. (Depth and slope positon (n = 9), forest type (n = 12); $^*P< 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.)

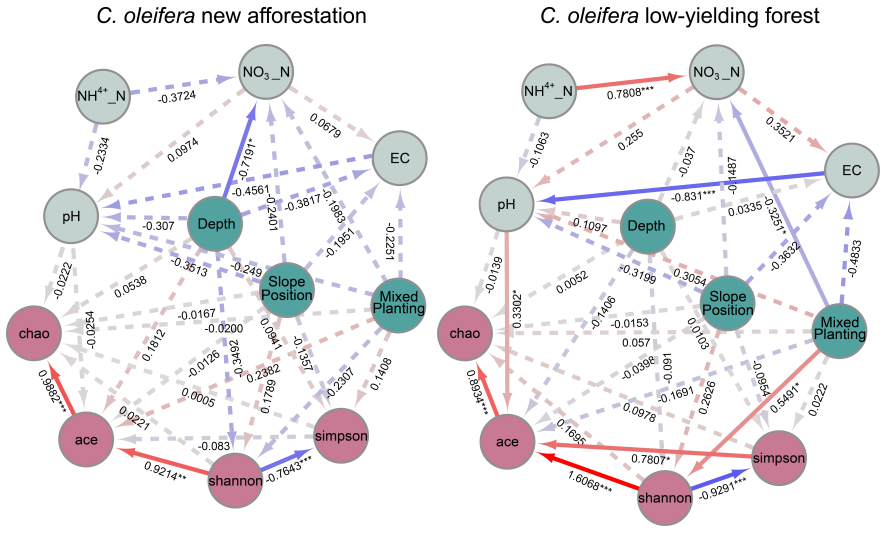

Figure 8 . Effects of new afforestation under monoculture and intercropping with *G. jasminoides* (a) and low-yield forests under monoculture and intercropping with *G. jasminoides* (b) on bacterial functional communities, as determined using structural equation models. Solid lines indicate that the effects were at a positive level, dashed lines indicate that the effects were at a negative level. The red shades represent positive regulations, and the blue shades represent negative regulations. Darker shades of red represent high regulation strength, and light shades represent low regulation strength. Asterisks indicate the significance level: $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P< 0.001$.)

Tables

Table 1 . Effects of depth, slope position, and forest type on the soil nutrients (1)

Sampling condition		TOC	SOM	HC	TN	TC
		($g \cdot kg^{-1}$)	(%)	($mg \cdot kg^{-1}$)	(%)	(%)
Depth (cm)	0-20	12.19 ± 1.13^{Aa}	0.02 ± 0.00^{Aa}	5.36 ± 0.87^{Aa}	0.18 ± 0.01^{Aa}	1.51 ± 0.15^{Aa}
	20-40	9.50 ± 0.72^{ABb}	0.02 ± 0.00^{Ab}	4.58 ± 0.62^{Aa}	0.15 ± 0.01^{AB}b	1.06 ± 0.08^{Bb}
	40-60	8.52 ± 0.85^{Bb}	0.01 ± 0.00^{Ab}	4.99 ± 0.62^{Aa}	0.14 ± 0.01^{Bb}	1.01 ± 0.11^{Bb}
Slope position	Top	10.76 ± 1.04^{Aa}	0.01 ± 0.00^{Aa}	4.76 ± 1.19^{Aa}	0.16 ± 0.01^{Aa}	1.12 ± 0.11^{Aa}
	Midslope	10.29 ± 1.14^{Aa}	0.00 ± 0.00^{Aa}	5.54 ± 0.83^{Aa}	0.15 ± 0.01^{Aa}	1.25 ± 0.11^{Aa}
	Base	9.38 ± 0.67^{Aa}	0.01 ± 0.00^{Aa}	4.76 ± 0.78^{Aa}	0.15 ± 0.01^{Aa}	1.20 ± 0.17^{Aa}
Forest Type	NA	9.76 ± 1.61^{ABb}	0.02 ± 0.00^{ABb}	4.17 ± 0.96^{Bb}	0.15 ± 0.01^{Bb}	1.10 ± 0.18^{Bb}
	GNA	8.26 ± 0.42^{Bb}	0.01 ± 0.00^{Bb}	4.05 ± 0.33^{Bb}	0.14 ± 0.00^{Bb}	0.95 ± 0.05^{Bb}
	LF	9.923 ± 0.84^{ABb}	0.02 ± 0.00^{ABb}	3.77 ± 0.25^{Bb}	0.15 ± 0.01^{ABb}	1.10 ± 0.09^{Bb}
	GLF	13.01 ± 1.05^{Aa}	0.02 ± 0.00^{ABb}	7.80 ± 0.69^{Aa}	0.19 ± 0.01^{Aa}	1.62 ± 0.16^{Aa}

Data are presented as mean ± SD (depth and slope positon n = 9, forest type n = 12). Different lowercase letters in the same column represent significant differences at P [?] 0.05, and different uppercase letters represent significant differences at P [?] 0.01. One-way ANOVA was used for the statistical test. NA, *Camellia oleifera*new afforestation; GLF, *Gardenia jasminoides* in a low-yielding*C. oleifera* forest; LF, low-yielding *C. oleifera* forest; GNA, *G. jasminoides* in *C. oleifera* new afforestation. TOC, total organic content; HC, humus content; SOM, soil organic matter; TN, soil total nitrogen; TC, soil total carbon.

Table 2 . Effects of depth, slope position, and forest type on soil nutrient content (2)

Sampling condition		NH_4^+-N	NO_3-N	NO_2-N	AN	AK	A
		($mg \cdot kg^{-1}$)	($mg \cdot kg^{-1}$)	($g \cdot kg^{-1}$)	($mg \cdot kg^{-1}$)	($mg \cdot kg^{-1}$)	(
Depth (cm)	0-20	7.18 ± 1.38^{Aa}	4.27 ± 1.35^{Aa}	1.26 ± 0.12^{Aa}	99.25 ± 9.58^{Aa}	5.04 ± 0.89^{Aa}	1
	20-40	$4.76 \pm 1.1A^{Bab}$	2.62 ± 0.96^{Aa}	1.13 ± 0.07^{Aab}	81.20 ± 5.31^{ABab}	4.62 ± 0.98^{Aa}	0
	40-60	3.124 ± 0.32Bb	3.08 ± 0.49^{Aa}	0.95 ± 0.07^{Ab}	74.39 ± 4.41^{Bb}	4.11 ± 0.44^{Aa}	0
Slope position	Top	6.51 ± 1.20^{Aa}	3.07 ± 0.54^{Aa}	1.20 ± 0.11^{Aa}	89.88 ± 7.26^{Aa}	4.69 ± 0.66^{Aa}	0
	Midslope	3.99 ± 0.83^{Aa}	4.79 ± 1.39^{Aa}	1.19 ± 0.11^{Aa}	84.99 ± 4.58^{Aa}	4.87 ± 0.93^{Aa}	1
	Base	4.08 ± 0.82^{Aa}	2.96 ± 0.36^{Aa}	0.99 ± 0.09^{Aa}	77.24 ± 7.72^{Aa}	3.95 ± 0.60^{Aa}	1
Forest type	NA	6.46 ± 1.75^{Aab}	2.76 ± 0.39^{Bb}	1.27 ± 0.13^{Aa}	90.33 ± 10.50^{Aa}	7.53 ± 0.93^{Aa}	0
	GNA	2.23 ± 0.33^{Bc}	1.82 ± 0.32^{Bb}	1.25 ± 0.19^{Aa}	84.46 ± 5.26^{Aab}	2.72 ± 0.35^{Bb}	0
	LF	3.76 ± 0.82^{ABbc}	7.39 ± 1.52^{Aa}	0.95 ± 0.08^{Aa}	65.59 ± 3.88^{Ab}	3.51 ± 0.23^{Bb}	1
	GLF	6.98 ± 0.74^{Aa}	2.41 ± 0.27^{Bb}	1.18 ± 0.12^{Aa}	92.45 ± 7.17^{Aa}	4.06 ± 0.38^{Bb}	1

Data are presented as mean ± SD (depth and slope positon n = 9, forest type n = 12). Different lowercase letters in the same column represent significant differences at P [?] 0.05, and different uppercase letters represent significant differences at P [?] 0.01. One-way ANOVA was used for the statistical test. NA, *Camellia oleifera*new afforestation; GLF, *Gardenia jasminoides* in a low-yielding*C. oleifera* forest; LF, low-yielding *C. oleifera* forest; GNA, *G. jasminoides* in *C. oleifera* new afforestation. NH_4–N, soil ammonia nitrogen; NO_3–N, soil nitrate nitrogen; NO_2–N, soil nitrate nitrogen; AN, alkaline hydrolysable nitrogen; AK, available potassium; AP, available phosphorus.

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soil-bacterial-communities-are-influenced-more-by-forest-type-than-soil-depth-or-slope-position