Response of bacterial community structure and diversity to different fertilization regimes in the rhizosphere soil of Camellia oleifera

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Abstract

Camellia oleifera is a high quality woody oilseed crop that produces a low yield in the central subtropics of China. Although fertilization can efficiently increase *C. oleifera* yield in these areas, the influences of fertilization continuously on soil microbiota and soil fertility remain poorly understood. The purpose of this study was to determine the influences of the type and amount of fertilizer on the soil properties of *C. oleifera*. Here, we compared the effects of organic fertilizer, organic–inorganic compost, no fertilizer control, and low (F1), medium (F2), and high (F3) amounts of continuously applied organic–inorganic compost at the sapling stage All chemical indicators and copiotropic bacteria measured were significantly lower in organic fertilizer and without fertilizer. Successive fertilization over two years with organic-inorganic compost significantly altered the relative abundance of the dominant bacterial groups at the phylum levels. the abundance of these phyla was the same in F2 and F3 treatments which was higher than their abundance in the other treatments. The relative abundance of kopiotrophic bacteria, especially *proteobacteria* and *gemmatimonadetes*, increased significantly and similarly with F2 and F3 treatments. With successive fertilizations, the levels of total nitrogen (TN), total phosphorus (TP) and organic matter (OM) were the vital factors affecting bacterial communities, which was confirmed by structural equation models, redundant analyzes and random forest models. These results suggest that the continuous application of moderate amounts of organic-inorganic compost is the main driver for the improvement of soil bacterial communities, and this was mainly achieved by altering the levels of OM, TN and TP, thus affecting the copiotropic bacterial abundance. This study provides a scientific basis for optimal fertilization of C. oleifera forest.

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Camellia oleifera is a high quality woody oilseed crop that produces a low yield in the central subtropics of China. Although fertilization can efficiently increase *C. oleifera* yield in these areas, the influences of fertilization continuously on soil microbiota and soil fertility remain poorly understood. The purpose of this study was to determine the influences of the type and amount of fertilizer on the soil properties of *C. oleifera* . Here, we compared the effects of organic fertilizer, organic–inorganic compost, no fertilizer control, and low (F1), medium (F2), and high (F3) amounts of continuously applied organic–inorganic compost at the sapling stage All chemical indicators and copiotropic bacteria measured were significantly lower in organic fertilizer and without fertilizer. Successive fertilization over two years with organic-inorganic compost significantly altered the relative abundance of the dominant bacterial groups at the phylum levels. the abundance of these phyla was the same in F2 and F3 treatments which was higher than their abundance in the other treatments. The relative abundance of kopiotrophic bacteria, especially *proteobacteria* and *gemmatimonadetes* , increased significantly and similarly with F2 and F3 treatments. With successive fertilizations, the levels of total nitrogen (TN), total phosphorus (TP) and organic matter (OM) were the vital factors affecting bacterial communities, which was confirmed by structural equation models, redundant analyzes and random forest models. These results suggest that the continuous application of moderate amounts of organic-inorganic compost is the main driver for the improvement of soil bacterial communities, and this was mainly achieved by altering the levels of OM, TN and TP, thus affecting the copiotropic bacterial abundance. This study provides a scientific basis for optimal fertilization of C. oleifera forest.

Keywords:*Camellia oleifera* plantation, Organic–inorganic compost, Successive fertilization, Bacterial communities, Structural equation modeling

1. Introduction

Camellia oleifera is a unique plant known for producing edible oil and is widespread in subtropical and warm temperate mountainous regions (Zeng et al., 2013).*C. oleifera* forests cover an area of 4.7 million hectares in China, and are widely cultivated in tropical, subtropical, and even southern temperate countries, such as Vietnam and Burma (Y. Wang et al., 2019). Thus, *C. oleifera* are called "oriental olive oil" and is considered a healthy edible oil by the Food and Agriculture Organization of the United Nations (FAO). and could potentially be planted in similar places worldwide (Hu et al., 2005). Planting*C. oleifera* in hilly and mountainous areas may solve the problem of edible oil shortage worldwide (C. Liu et al., 2021). However, the *C. oleifera* forest area in China is more than 75%, while their oil yield is approximately 150 kg per hectare, which is far less than that of olive oil (approximately 2,700 kg per hectare) (Shi et al., 2011). Traditional *C. oleifera* forests require almost no fertilization or only a small amount of organic fertilizer in the winter due to low economic benefits (Tu et al., 2016). How to fertilize? How can soil quality be improved? How to increase the output of *C. oleifera* ? Because organic and inorganic fertilizers promote yield, carrying out appropriate fertilizer management measures for improving soil bacterial communities and soil properties have been considered as one of the strategies for improving the oil yield of *C. oleifera* (Dezhi, 2016).

Soil bacterial communities are the most important part of the soil ecosystem and playing the vital role in yield regulation (Knelman & Nemergut, 2014). Therefore, the soil microbial structure is particularly important for *C. oleifera* planting. Previous studies showed that increasing the pH of *C. oleifera* rhizosphere in different seasons improved the utilization of soil nutrients by plants and rhizosphere microbes (Jun-Jie et al., 2008). Moreover, appropriate trace element contents enhanced the increase of bacterial communities in the soil (Yong et al., 2019). Soil nitrogen, potassium and phosphorus levels are the main factors affecting the composition of bacterial communities (Archer, 2016). Moreover, organic matter and the ratios of total soil carbon to nitrogen also effect bacterial communities (Rabbi et al., 2018). Although the effects of fertilization patterns on soil microorganisms and aggregates have been widely studied, how to improve the soil environment? How can copiotrophic bacterial abundance be improved? The analysis of various fertilizers and fertilizer amounts on soil bacterial communities and soil properties should be addressed.

Soil properties and parent rock material strongly influence the structure and diversity of the soil bacterial community and *C. oleifera* yield (J. Li et al., 2019). The high clay content, nutrient content, and cation exchange capacity of loamy clay soil are associated with high bacterial microbial diversity and activity (Shrestha et al., 2013). The Hunan Province of China is the main production area of tea oil, and its hilly areas are rich in quaternary red clay (Deng et al., 2021). Although clay particles are acidic, sticky, and compact, resulting in poor soil quality (J. Liu et al., 2018). Organic–inorganic compost and organic fertilizer are more effective than inorganic fertilizers in improving soil N content, microbial community number, biological intensity, and *C. oleifera*yield (H. Wang et al., 2014). Therefore, the application of organic-inorganic compost and organic fertilizers should be increased according to the nutritional status of the soil (Sarango et al., 2021; Watson et al., 2021). However, what the type of fertilizer and how much fertilizer is suitable for *C. oleifera* soil is necessary to be studied.

In this study, we used random forest models, redundancy analysis (RDA) and structural equation modeling (SEM) to analyze the relationship between soil bacterial communities and soil properties. This analysis was exploratory, because, to date, few studies have investigated the influences of sequential fertilization on the structure and diversity of soil bacterial community in *C. oleifera* plantations. We hypothesized that: (1) continuous fertilization will improve the structure and diversity of soil bacterial community in *C. oleifera* plantations; and (2) certain chemical properties have important implications for the structure and diversity of the soil bacterial community. To test these hypotheses, we (1) explored the influences of six fertilization treatments on the structure and diversity of soil bacterial community, and (2) identified chemical factors influencing the structure and diversity of soil bacterial community to provide guidelines for soil nutrient management and fertilizer application for *C. oleifera*plantations.

2. Materials and methods

2.1 Study area

This research was carried out in Youxian County, Hunan Province, China (113°24' E and 27°03' N), which is a major development and production areas of *C. oleifera* and is known as the "Hometown of*Camellia oleifera* in China". This region forms a part of the evergreen broadleaved forest belt, and features four distinct seasons, subtropical monsoon climate and abundant rainfall. The average annual temperature and annual rainfall was approximately 17.6 and 1,410 mm.

The *C. oleifera* forest area is located in a hilly area with an average inclination of < 15deg and the altitude is less than 300 m above sea level. According to Chinese Soil Taxonomy, the soil type at the study site is Allitic-Udic Ferrosols, derived from Quaternary laterite (CRGCST, 2001). The main variety of *C. oleifera* in this region is Xianglin No. 1, with fruit maturation from October 22 to 28. Xianglin No. 1 was planted in 2012 with a planting density of about 1667 trees per hectare and a row spacing of 2m x 3m.

2.2 Experimental design

In the center of the *C. oleifera* forestland mentioned above, two areas with the same slope and growth conditions were selected. Three randomized blocks (20 m x 30 m) were set up in each area, and each block

was divided into six plots as six treatments. The organic–inorganic compost in this research was micro-balanced organic fertilizer produced by Run Feng Da in China. Six different fertilizer treatments, namely, organic fertilizer (OF), organic–inorganic compost (SF; the total N, P, and K content of SF was the same as that of OF), no fertilizer control (CK), and three different amounts of organic–inorganic compost (low [F1], medium [F2], and high [F3]) applied at 1:3:5 ratio (Table 1).

To apply the fertilizer, a trench (15–25 cm deep and 30 cm wide) was built. The fertilizer was distributed evenly in the trench. After fertilizer application, the trench was filled with soil, and the soil level was recorded. The commercially available organic fertilizer used in this study contained 8% NPK (N: P_2O_5: K_2O = 1:1:1), > 45% organic matter, and no trace elements. Organic–inorganic compost contained 11% NPK (N: P_2O_5: K_2O = 5:2:4) and different amounts of trace elements (0.2% calcium amino acid chelate Ca, 0.1% copper oxychloride, 0.1% magnesium chloride, 0.05% ferric glycine complexes, 0.1% glycine zinc, 0.1% EDTA–$MnNa_2$, and 0.15% borax decahydrate).

2.3 Collection of soil samples

Each sampling point was located 30 cm away from the plant. The taproot of each *C. oleifera* sapling was dug into the soil layer 0-20 cm after removing the topsoil. Together with the taproot soil we could find fibrous soil. Soil samples from the rhizosphere were collected by vigorously shaking the fibrous root to remove loose soil (loose soil). Fine-grained soil (<4 mm) was considered rhizosphere soil. Eight soil samples were taken from each block using the "S-path" method, and pooled. Additionally, one sample from each block was obtained by the quartering method (Su & Han, 2000). Three repeats were performed for each treatment. Eighteen soil samples were collected on September 28, 2017, and divide each rhizosphere soil sample into two. A portion (5–10 g of fresh soil weight) was transferred to a sterilized centrifuge tube, and immediately stored it in liquid nitrogen and returned it to the laboratory for bacterial diversity analysis. When the remaining sample was air-dried, it was passed through 2-mm sieves for chemical property determination.

2.4 Analysis of soil physicochemical properties

Soil texture was determined with the Bouyoucos hydrometer (Klute et al., 1986), and soil pH with a pH meter (PHS3E; soil: water = 1:2.5) (B. Li et al., 2011). Soil organic matter (OM) content was measured with a visible spectrophotometer using the dichromate wet combustion method (Rayment & Higginson, 1992). An automated batch chemical analyzer was used to determine the soil total phosphorus (TP) content after digestion (WestCo Scientific Instruments, Brookfield, CT, USA). Flame photometry was accustomed to measure the soil total potassium (TK) content after digestion (Yanu & Jakmunee, 2015). Soil total nitrogen (TN) was determined using the Kjeldahl method (Tsiknia et al., 2014). Soil available phosphorus (AP) and available potassium (AK) were extracted with Mehlich 3 (Ojekanmi & Chang, 2014), and then quantified using an automatic discontinuous chemical analyzer and flame photometer, respectively. Soil available calcium (ACa), available iron (AFe), available magnesium (AMg), available manganese (AMn), available zinc (AZn), and available copper (ACu) were measured using the Smartchem 200 Discrete Chemistry analyzer and the Mehlich 3 (Ryan et al., 2006).

2.5 Bacterial DNA

Extraction of genomic DNA from samples by CTAB/SDS method. DNA concentration and purity were checked on 1% agarose gels. DNA samples were diluted to a concentration of 1 ng/μL with sterile water. 16S rRNA genes were amplified using V3+V4:341F (CCTAYGGGRBGCASCAG) and 806R (GGACTAC-NNGGGTATCTAAT) primers. PCR reaction at 30 μ L reaction volume, containing 15 μL Phusion® High-Fidelity PCR Master Mix (New England Biolabs), 0.2 μM forward and reverse primers and about 10 ng DNA matrix. Thermocycling provides for a first denaturation at 98°C for 1 minute; Denature at 98°C for 30 cycles for 10 seconds, anneal at 50°C for 30 seconds and elongate at 72°C for 30 seconds; Finally, it is extended to 72 for 5 minutes. Equal volumes of 1x loading buffer (containing SYB Green) and PCR products were mixed and electrophoresed on a 2% agarose gel for detection. Use the NEB Next Ultra DNA Library Prep Kit from Illumina (NEB, USA) to create a sequencing library and add indexing code according to the manufacturer's recommendations. Sequencing the library on the Illumina HiSeq platform (Caporaso et al., 2012), thereby

resulting in 250-bp paired-end reads. The sequence reads were quality checked and then aligned. Operational Taxonomic Units (OTUs) have been bundled withUPARSE v7.0.1001 (http://drive5.com/uparse/), with 97% of the marks on clusters (Edgar 2013). The ribosomal database entries were used for classification. Beta diversity was determined by calculating the weighted UniFrac distance, which is a quantitative calculation of microbial ecology (QIIME2) (Chang Q, 2011).

2.6. Structural Equation Modeling

Structural equation modeling (SEM) is a method for creating, estimating, and testing causal models (Bollen & Kenneth, 1989). The model includes observable variables and latent variables that cannot be observed directly. An observable variable used to indirectly measure these potential variables. Traditional statistical methods cannot effectively resolve these potential variables, while SEM can resolve the potential variables and their indicators simultaneously. Potential variables are represented by ellipses In SEM while rectangles represent observable variables. The number on the potential variable arrow pointing to the observed variable represents the weight of the indicator, and number on remaining arrows represents the path coefficient. Determine the direct contribution of environmental factors and bacterial community structure diversity through the application of SEM in IBM SPSS Amos 24.0. In order to conform to the model, the meaningless path has been eliminated. Verification of model applicability with the chi-square test (CMIN/DF <5, model suitability reduction rates GFI and PL > 0.5, and RMSEA < 0.08) and standardized path coefficients ($p < 0.05$) (Wetzels et al., 2009).

2.7 Statistics analyses

One-way analysis of variance (ANOVA) with Tukey's tests for significant differences was used to estimate differences in soil properties, bacterial diversity, most bacterial abundant phyla and OTUs among the six different treatments. Principal coordinate analysis (PCoA), multivariate analysis of variance of permutation, RDA and Mantel test were performed using R's (version 3.6.1) "vegan" package (Dixon, 2003). These analyzes aimed to determine the relationship between soil properties and soil bacterial community composition. Random forest modeling was used to quantitatively evaluate the key predictors of bacterial diversity on soil properties, analysis using random forest package (Liaw & Wiener, 2002). The A3 and rfPermute packages are used to determine the model and predict the significance level, respectively (Archer, 2016; Fortmannroe, 2015).

3. Results

3.1 Changes in rhizosphere soil physicochemical properties

Significant differences in soil OM, TN, TP, TK, AK, and AFe contents were revealed among six fertilization regimes (p <0.05), but sand, silt, clay, pH, ACa, AMg, AMn, ACu, and AZn contents (Table 2) had no significant difference ($p > 0.05$),. The gradient experiment (F1, F2, and F3) increased the contents of soil OM, major elements, and trace elements. The application of organic fertilizer and organic–inorganic compost increased soil pH and the percentage of silt fraction, and reduced the percentage of sand fraction, with F2 exhibiting the highest silt content (33.2%) among all treatments tested. Soil pH ranged from 4.57 to 4.78 under different fertilizer treatments; soil pH was lowest with CK and highest with F3 treatment. Organic–inorganic compost increased the contents of Ca, Mg, and trace elements; the contents of these elements showed the following order among gradient and comparison experiments, respectively: F3 > F2 > F1 and SF > OF > CK.

3.2 Richness and diversity of bacterial community in rhizosphere soil

The 16S rDNA sequencing results revealed that there were 25,775 OTUs in total, with each sample ranging from 1,189 to 1,792, and the average similarity of 1,432 OTUs was 97% (Fig. 1). The average number of OTUs in the gradient experiment were significantly higher than those in the comparison experiment. The sequence coverage rate of all samples was 98% (based on a good coverage rate and no data displayed), which indicated that the probability of a sample gene sequence being detected was very high, reflecting the

real-time status of soil bacterial communities. The quantity of sequencing data in the dilution curve was reasonable, and more data would produce only a few new OTUs.

In general, the gradient fertilizer treatment ($p < 0.05$) significantly increased the richness and diversity of soil bacterial communities, and F2 showed the highest bacterial community richness among all treatments. The richness and diversity of bacterial community of the F1 and F2 treatments was higher than that of the F3 treatment. The Sobs, Chao1, and Shannon indices of bacterial communities were significantly higher in the gradient experiments than in the comparison experiment, although coverage did not show significant differences among different treatments, except CK. The richness index of OF and SF treatments was higher than that of CK. Similarly, the richness index of F1 and F2 was higher than that of F3. Diversity index did not show significant difference between the comparison and gradient experiments (Table 3).

3.3 Distribution and composition of rhizosphere bacterial communities

The bacterial community composition of organic fertilizer and gradient fertilizer treatment was significantly different from that of the control. Gradient fertilizer application significantly increased the relative abundance of *Acidobacteria* , *GAL15* , and *Verrucomicrobia* , but significantly reduced the relative abundance of *Firmicutes* (Fig. 2; $p < 0.05$). The predominant microbial taxa within different treatments were *Chloroflexi* , *Proteobacteria* , *Actinobacteria* , and *Acidobacteria* (Fig. S1); the sequence reads of these taxa accounted for 43.22–60.85% (average 53.34%), 11.84–17.97% (average 13.65%), 9.61–18.09% (average 14.31%), and 5.22–19.89% (average 9.91%) of the total bacterial sequence data, respectively. A clustered heatmap grouped by bacterial abundance has been established. The results showed that the gradient experiments are enriched in *Verrucomicrobia* , *Nitrospirae* , *Gemmatimonadetes* , and *GAL 15* (Fig. 2 and Fig. S2).

Clustering analysis divided the six treatments into two groups, depending on the soil microbial community characteristics. F1, F2, and F3 treatments were clustered together in one group, while the CK, OF, and SF treatments formed the second group. Continued analysis divided the different treatments into four groups: the first group contained F2 and F3; second group contained the F1 treatment only; third group contained CK and OF; and fourth group contained SF only (Fig. 3a). PCoA analysis further confirmed the grouping results, the distribution of the two parts of the soil samples was relatively independent, suggesting that there were significant differences in soil community structure between the gradient test and the contrast test (Fig. 3b). These results showed that bacterial community structure was directly related to the fertilization treatments.

3.4 Random forest modeling and SEM of soil properties and rhizosphere bacterialdiversity

Soil OM, TN, and TP contents significantly affected the bacterial diversity. Random forest modeling indicated that OM, TN, and TP were the key drivers of bacterial alpha diversity (Fig. 4). Furthermore, SEM (probability level > 0.05) indicated that OM, TN, TP, and TK had significant and positive effects on the bacterial diversity (Fig. 6 and Table S1). Macro-elements and trace elements have no correlation. Macro-elements generally have more significant effects on bacterial diversity than trace elements. Macro-elements had a major impact on the overall bacterial community (0.669). Although trace elements had a moderate effect (0.354) on bacterial communities, the overall impact was not obvious.

3.5 Relationship between rhizosphere bacterial communities and soil properties

The RDA showed that the first and second axes of soil bacterial community structure and soil properties accounted for 72.5% and 13.0%, respectively, of the total variation in different fertilization measures, which together accounted for the 85.5% of the total variation explained. Soil bacterial community structure was strongly related to TN, TP, TK, OM, AFe, and ACu. At the phylum level (average abundance $> 1\%$), *Chloroflexi* ,*Acidobacteria* , and *Proteobacteria* were significantly related to soil OM, TN, and TP contents, whereas *Firmicutes* and *Actinobacteria* were related to soil TK (Fig. 5).

4. Discussion

4.1 Response of environmental factors to different fertilization treatments

We found that soil nutrient content differed significantly under varying fertilization treatments in *C. oleifera* plantation. Moreover, the soil nutrient content of gradient experiment (F1, F2, and F3) was higher than comparison experiment (SF and OF; Table 2), indicating that continuous fertilization could improve the soil nutrient content more than standard fertilization. The nutrient composition of the organic–inorganic compost was more comprehensive and balanced than those of other fertilizer applications; thus, organic–inorganic compost is highly conducive to the growth and reproduction of microbial colonies (Hong et al., 2021). Continuous fertilization conforms to the nutrient requirements of plants and microbial communities during different periods. In addition to N, P, and K fertilizers, trace elements such as Ca, Mg, and B had been added based on the local soil nutrient status (Zhou et al., 2021). Therefore, the soil nutrient content can significantly affect the activities of soil microorganisms (Nan et al., 2012). The nutrient composition of commercially available organic and inorganic mixed fertilizers is comprehensive and balanced, which is also conducive to the growth and reproduction of microbial colonies (Moritsuka et al., 2001). Continuous fertilization conforms to the nutrient requirements of plants and microbial communities at different growth stages (Littrell et al., 2021). This research also revealed that the organic fertilizer could slowly reduce the content of sand and increase the proportion of silt, which probably resulted from the ability of soil microorganisms to dissolve the insoluble fine sand.

4.2Effects of soil chemical properties on the structure and diversity of rhizosphere bacterial communities

The bacterial richness of OF and SF was higher than that of CK, as showed by the Chao1 and ACE indices, which suggests that organic fertilizers could increase the richness of soil bacterial communities. Similar results were reported in cropland studies (Shen et al., 2010). The application of organic fertilizers significantly increased the abundance of bacteria and the number of dominant species in the soil of the rhizosphere. Balanced fertilization of N, P and K can significantly increase soil nutrient content and increase the richness and diversity of soil bacteria (Ai et al., 2012). Geisseler and Scow (Geisseler & Scow, 2014) summarized the bacterial response to long-term application of mineral fertilizers, and showed that bacterial growth is generally inhibited by mineral fertilizers. The study indicating that organic–inorganic compost could enhance soil bacterial diversity (Table 3). The diversity of the soil bacterial community is directly affected by the addition of N and P and by the change in vegetation–soil interaction (Luo et al., 2018; Xu et al., 2020). Therefore, the diversity, abundance, and composition of bacterial communities are correlated with the type of fertilizer used.

We found that different dosages of fertilizer exerted varying degrees of remarkable effects, but the excessive application of fertilizer had negative effects. Soil bacterial community diversity in the gradient experiment was as follows: F2 > F1 > F3 (Table 3), indicating that F1 and F2 facilitated an increase in bacterial diversity. However, the diversity of bacteria decreased significantly with the duration of time, because the F3 treatment destroyed vegetation and soil microbes, caused minimal plant burns, and reduced the soil bacterial community diversity (Yanfang et al., 2008). A previous study reported that soil TN affects soil microorganisms by improving the functional soil microbial community diversity (Tsiknia et al., 2014). F2 had significantly higher TN content and bacterial community diversity than F1 and F3 (Table 2, Table 3), which verified the results of Tsiknia et al. (2014). Soil nutrient availability will change. After the application of N and P fertilizers, the number of cations (such as Ca^{2+} and Mg^{2+}) will decrease and the exchange capacity of H+ and Al3+ will increase, which can indirectly affect the soil microbial community (X. Lu et al., 2015). Organic fertilizer provides nutrients to plants and soil microbes, and are significantly vital for improving the productivity and soil fertility of *C. oleifera* forestland (J. Liu et al., 2018).

4.3 Effects of soil chemical properties on the rhizosphere bacterial community composition

Continuous application of organic and inorganic fertilizers could promote the bacterial diversity in the *C. oleifera* rhizosphere. Different treatments promote the richness of eutrophic bacteria and inhibit the richness of hypotrophic bacteria (Lijun et al., 2022). In this research, PCoA revealed that bacterial community composition had significant differences among the six fertilization treatments. Studies have shown that long-term application of organic fertilizers can promote microbial species richness and improve soil nutrient utilization

efficiency (Sharaf et al., 2021). The application of organic fertilizer associated with microbial richness increased as *Proteobacteria* ,*Gemmatimonadetes* , and *Cyanobacteria* increased (Fig. 2).*Proteobacteria* is a type of copiotrophic bacteria, adapted to soil conditions with a high content of organic matter and nutrients (Tao et al., 2020). The gradient fertilizer treatment significantly enhanced the relative abundances of *Acidobacteria* , *Verrucomicrobia,*and *GAL15* but significantly decreased the relative abundances of*Chloroflexi* and *Firmicutes* (Fig. 2; $p < 0.05$). Members of the *Firmicutes* phylum, including*Bacillus* , *Clostridium* , and *Erysipelas* , are often abundant in nutrient-poor soil. *Chloroflexi* , also known as green gliding bacteria, green phototrophs, and green non-sulfur bacteria, tend to be higher in soil with low organic matter content.*Verrucomicrobia* promote the P content of soil and are abundant in soil with high P content. The phosphorus content of quaternary red clay is low and coms mainly from external sources (Lindstrm et al., 2004), such as fertilizers, which is also the conclusion of this study. Gradient fertilizer treatment improves soil fertility and the growth environment of eutrophic microorganisms, thereby improving eutrophic bacteria.

Bacteria are among the most important decomposers in soil (Waring et al., 2013). On the basis of the relationship between soil bacterial community composition and soil environmental factors (Q. Li et al., 2021; Tu et al., 2019), we found that soil properties under different fertilizer treatments were significantly related to soil bacterial community structure and diversity. Through random forest models, RDA, SEM, and PCoA, we found that *Chloroflexi* , *Acidobacteria* , and *Proteobacteria* were significantly related to soil OM, TN, and TP levels in the soil, while *Firmicutes* and *Actinobacteria*were significantly related to OM, TP and TK in the *C. oleifera*rhizosphere. Soil organic matter has been proven to enhance the functional diversity of the soil microbial community (Bending et al., 2002; Zhu et al., 2020). The increase of soil organic matter content will increase the total nitrogen content, consistent with previous study (Prescott & Grayston, 2013). Moreover, F3 enhanced the abundance of*Acidobacteria* , a slow-growing and K-loving phylum (Zhang et al., 2014). Our research revealed that soil bacterial diversity increased with increasing soil pH and that there was a strong relationship between bacterial community structure and soil chemistry in forest ecosystems.. Previous studies showed that *acidophilic* bacteria are negatively related to soil pH (Jones et al., 2009). In this study, the richness and diversity of soil bacteria in *C. oleifera* forestland under continuous fertilization were significantly higher than those under ordinary fertilization, indicating that constant organic–inorganic compost application could increase microbial community diversity in quaternary red clay areas (S. Lu et al., 2022), and continuous application of moderate amounts, but not excessive amounts, of organic–inorganic compost could improve soil bacterial communities mainly by changing OM, TN, and TP contents, thus changing the abundance of copiotrophic bacteria.

5. Conclusions

The diversity and richness of the bacterial community in the F2 treatment was higher than in other treatments. Soil OM, TN, TP, TK, AK, and trace element contents also significantly increased in F1, F2 and F3. Application of organic–inorganic compost significantly enhanced the relative abundance of *Proteobacteria* , *Gemmatimonadetes* ,*Cyanobacteria* , *Acidobacteria* , and *Verrucomicrobia*but significantly decreased the relative abundance of *Chloroflexi*and *Firmicutes* . The gradient experiment clearly enhanced the relative abundance of most vegetative bacteria such as*Proteobacteria* and *Gemmatimonadetes* compared to the comparison experiment. Furthermore, environmental factors, namely, OM, TN, and TP, had the most significant impact on bacterial diversity, as revealed by random forest models, RDA, and SEM. Furthermore, the influence of macro-elements on bacterial communities was more significant than that of trace elements, with 66.9% direct effect. Therefore, the application of fertilizers, especially F2 treatment, to*C. oleifera* plantations in different seasons can significantly increase soil bacterial community structure and diversity. Future studies should pay more attention to changes in microbial communities, given its importance to *C. oleifera* plantations, and the method of no fertilization or only minimal winter fertilizer should be changed.

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Figure Legends

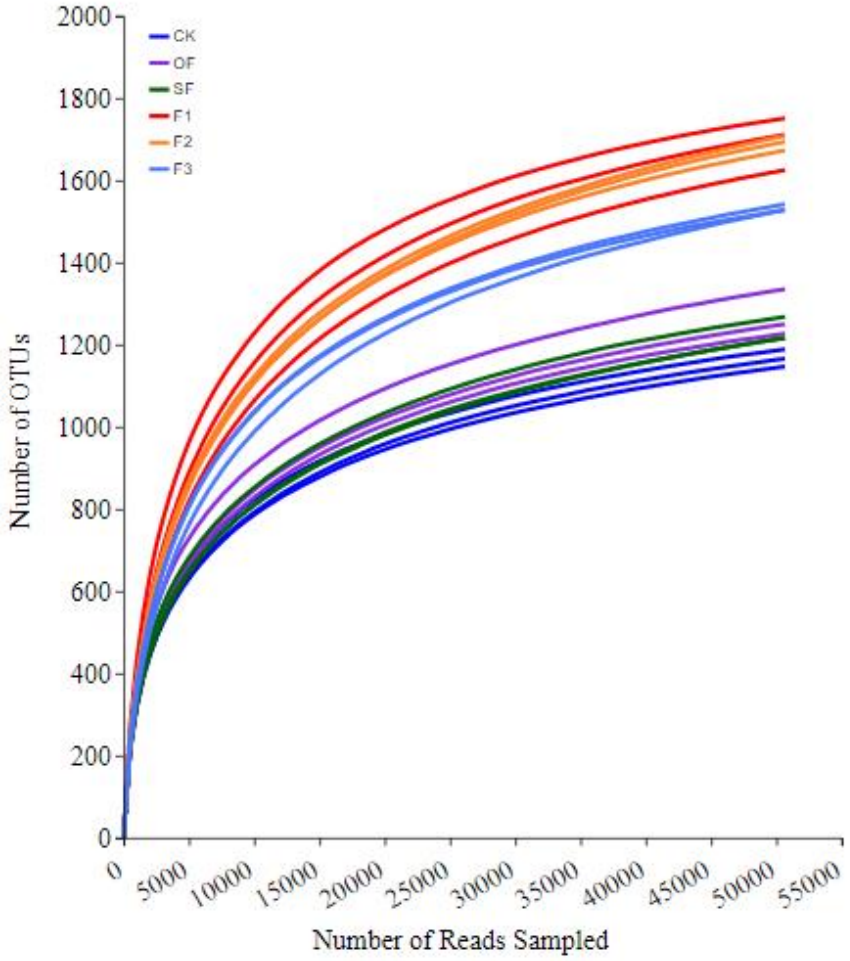


Fig. 1. Dilution curve showing the number of operational taxonomic units (OTUs) of soil bacteria in different treatments. CK, no fertilizer control; OF, organic fertilizer; SF, organic–inorganic compost; F1, F2, and F3 indicate low, medium, and high amounts of continuously applied organic–inorganic compost.

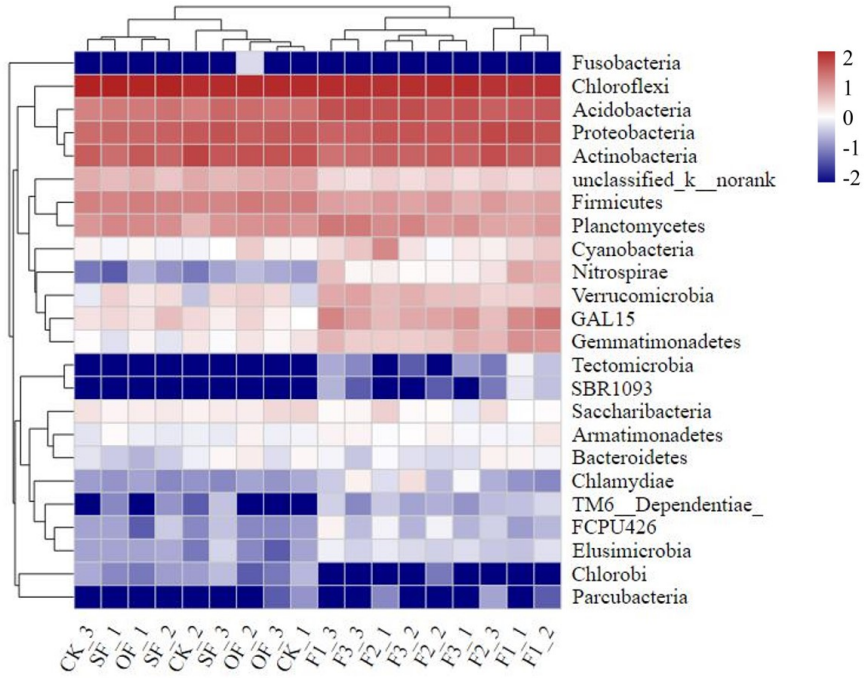


Fig. 2. Distribution of the bacteria at the phylum level in different fertilizing treatments. Different colors indicate the relative abundance of the individual samples. Red represents the most abundant feature and purple represents the least abundant feature. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

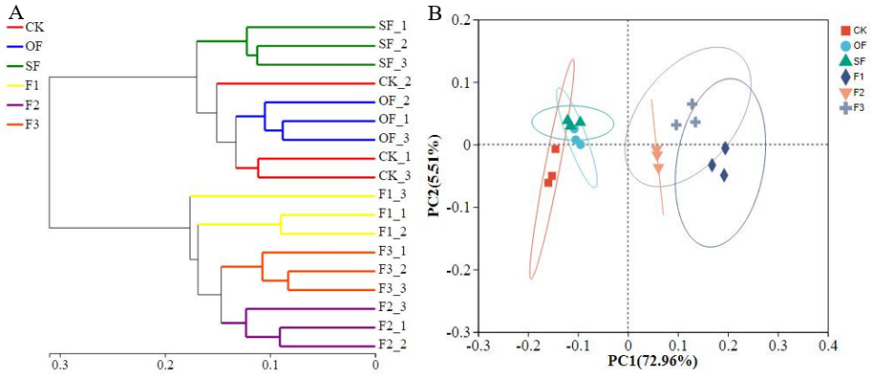


Fig. 3. (A) Weighted Unifrac UPGMA cluster of soil bacterial communities at the OTU level in different treatments. The figure was constructed on the basis of Illumina sequencing data. (B) Principal Coordinate analysis (PCoA) plot based on 16S rDNA gene sequences in soil samples at the phylum level. The X-axis and Y-axis of the scatter plot indicate principal component 1 (PC1) and principal component 2 (PC2). CK, no fertilizer control; OF, organic fertilizer; SF, organic–inorganic compost; F1, F2, and F3 indicate low, medium, and high amounts of continuously applied organic–inorganic compost.

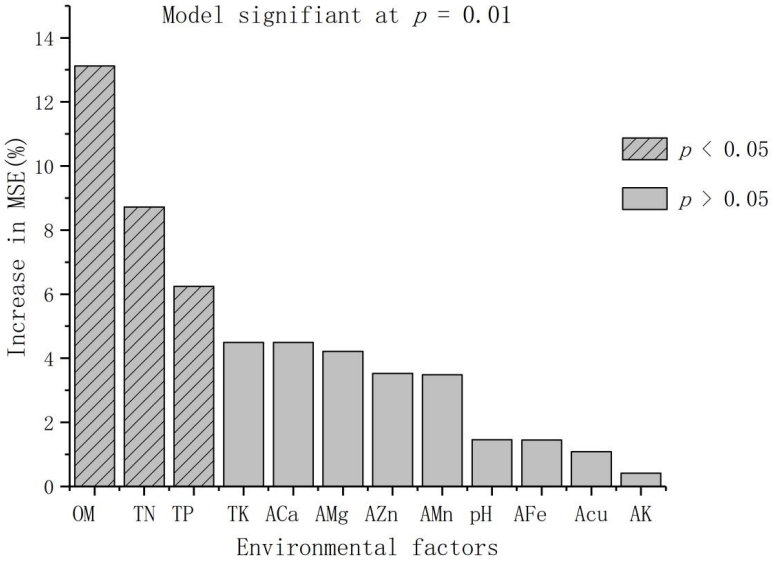


Fig. 4. Mean predictor importance (% of increased mean square error, MSE) of environmental factors for bacterial community diversity based on random forest modeling. OM, organic matter; TN, total nitrogen; TP, total phosphorus; TK, total potassium; AP, available phosphorus; AK, available potassium; pH, pH value; ACa, available calcium; AMg, available magnesium; AFe, available iron; AMn, available manganese; ACu, available copper; AZn, available zinc.

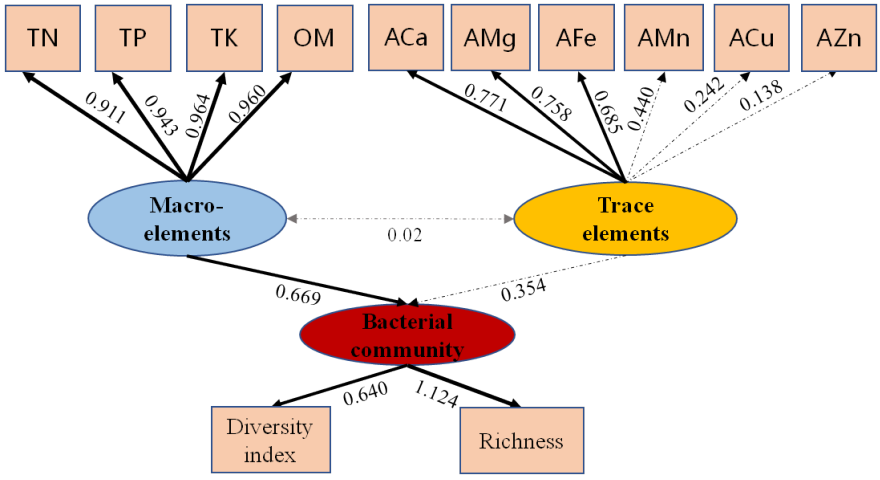


Fig. 5. Direct and indirect effects of environmental factors on bacterial community diversity based on structural equation modeling. Black arrows represent causal relationships. Ellipse represents a potential variable, and rectangle represents the observed variable. Gray arrows represent nonsignificant paths (p > 0.05). Numbers adjacent to arrows represent the total effect coefficients. The path widths are scaled proportionally to the path coefficient. OM, organic matter; TN, total nitrogen; TP, total phosphorus; TK, total potassium; AP, available phosphorus; AK, available potassium; pH, pH value; ACa, available calcium; AMg, available magnesium; AFe, available iron; AMn, available manganese; ACu, available copper; AZn,

available zinc. Diversity index and richness are the Shannon index and Chao1 index, respectively. (Please refer to the web version of this article to interpret the meaning of the different colors used in the figure)

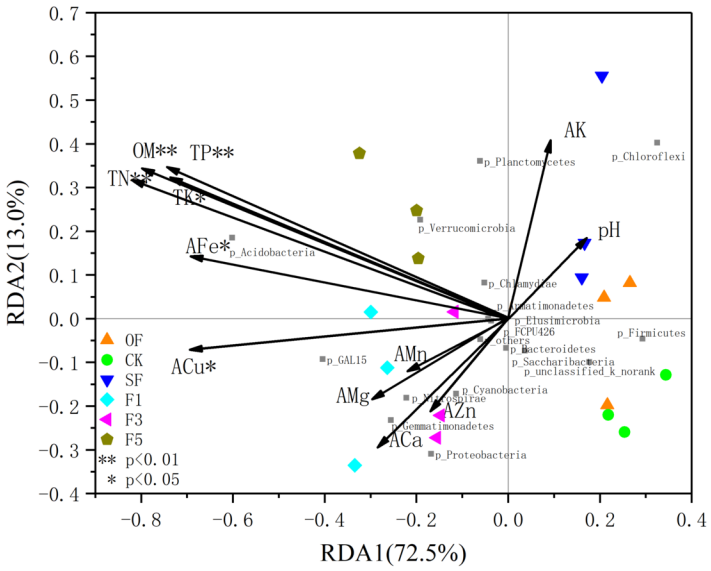


Fig. 6. Ecological correlation between the bacterial phylum abundance and environmental factors in Hunan Tianhua Camellia Base in China. OM, organic matter; TN, total nitrogen; TP, total phosphorus; TK, total potassium; AP, available phosphorus; AK, available potassium; pH, pH value; ACa, available calcium; AMg, available magnesium; AFe, available iron; AMn, available manganese; ACu, available copper; AZn, available zinc. $*p < 0.05$; $**p < 0.01$ (Mantel test).

Table 1. Fertilizer type and application rate of *Camellia oleifera* in 2015 and 2017 in Youxian, Hunan Province, China.

Fertilization regime	Fertilization regime	Fertilizer type	Application rate (kg/ ha^{-1})	Appl
			2015	2016
			Dec	Mar
Comparison experiment	CK	No Fertilizer	0	0
	SF	Organic-inorganic compound fertilizer	1111	0
	OF	Organic fertilizer	1667	0
Gradient experiment	F1	Organic-inorganic compound fertilizer	556	278
	F2		1667	833
	F3		2778	1389

SF and OF had the same total N, P, and K; F1, F2, and F3 were applied at a ratio of 1:3:5.

Table 2. Effects of different fertilizing treatments on soil physical and chemical properties in *Camellia oleifera* forest in Youxian, Hunan Province, China.

	F1	F2	F3	CK	OF	SF	p-value
Sand(%)	5.01±1.12ab	4.74±08b	4.82±0.46b	5.59±0.82a	5.42±0.17a	5.47±0.92a	0.179
Silt(%)	32.5±3.64ab	33.2±0.52a	33.1±2.42a	31.6±2.46a	31.8±0.46a	31.7±5.65a	0.349
Clay (%)	62.79±6.67a	62.06±5.57a	62.08±8.64a	62.81±3.32a	62.78±3.87a	62.93±6.70a	0.694
pH	4.72±0.57a	4.77±1.21a	4.78±0.27a	4.57±1.34a	4.66±0.97a	4.68±0.92a	0.206
OM(mg g^{-1})	19.04±0.86b	21.48±2.19b	24.36±1.35a	12.95±1.87d	13.86±0.96c	13.46±2.62c	0.000
TN(mg g^{-1})	1.64±0.17ab	1.69±0.82ab	1.77±0.42a	1.49±0.12b	1.58±0.11b	1.57±0.23b	0.000
TK(mg g^{-1})	24.26±3.87ab	27.69±4.17ab	27.84±1.27a	20.76±1.59b	22.38±3.17b	21.29±4.46b	0.000
TP(mg g^{-1})	0.37±0.017a	0.39±0.04a	0.39±0.018a	0.28±0.07b	0.29±0.04b	0.31±0.07b	0.000
AK(mg kg^{-1})	66.59±24.34a	68.59±19.48a	71.59±28.39a	50.51±12.58b	61.59±29.49ab	59.55±10.71ab	0.001
ACa(mg kg^{-1})	30.34±6.95a	35.52±2.41a	35.68±0.8a	28.57±0.77c	29.97±0.41c	29.98±0.75a	0.052
AMg(mg kg^{-1})	9.14±0.81a	8.95±0.12a	5.34±1.91a	3.65±0.03a	3.83±0.07a	5.04±0.07a	0.759
AFe(mg kg^{-1})	4.03±1.57b	6.01±0.39a	7.21±2.66a	3.71±0.08c	3.71±0.12c	5.96±0.34ab	0.000
AMn(mg kg^{-1})	0.51±0.07a	0.20±0.02a	0.23±0.13a	0.04±0.00a	0.11±0.01a	0.21±0.02a	0.122
ACu(mg kg^{-1})	0.7±0.05a	0.7±0.17a	0.75±0.16a	0.4±0.17a	0.4±0.17a	0.5±0.08a	0.217
AZn(mg kg^{-1})	0.27±0.15a	0.28±0.06a	0.27±0.03a	0.34±0.1a	1±0.1a	1.9±0.2a	0.801

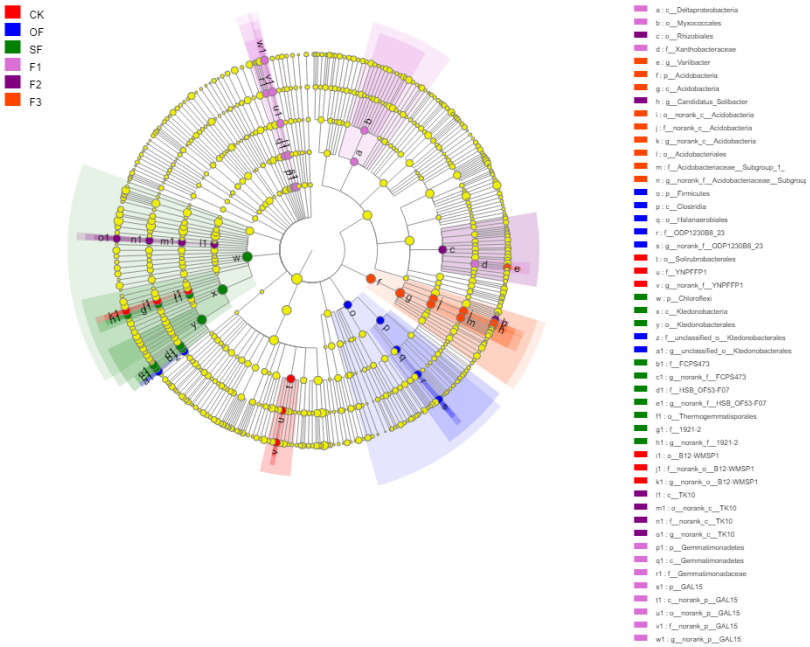
Data represent mean ± standard error of mean (SEM). Different lowercase letters (a, b, c) represent significant differences among the three sites ($p < 0.05$; Tukey's test). OM, organic matter; TN, total nitrogen; TP, total phosphorus; TK, total potassium; AK, available potassium; pH, pH value; ACa, available calcium; AMg, available magnesium; AFe, available iron; AMn, available manganese; ACu, available copper; AZn, available zinc.

Table 3. Soil bacterial richness and diversity indexes of different fertilizing treatments in *Camellia oleifera* forest in Youxian, Hunan Province, China.

Regimes	Sobs	Shannon	Simpson	Ace	Chao1	Coverage
F1	1735±46d	5.86±0.2b	0.009±0.0026a	1900±26d	1929±29d	0.995±0.0003a
F2	1754±27d	5.84±0.02b	0.008±0.0004a	1958±24d	1991±9d	0.995±0.0006a
F3	1583±15c	5.72±0.1ab	0.009±0.0017a	1772±14c	1807±12c	0.995±0.0002a
CK	1209±8a	5.53±0.11a	0.013±0.004a	1360±11a	1398±29a	0.997±0.0004b
OF	1295±48b	5.66±0.11ab	0.011±0.0026a	1468±52b	1491±48b	0.996±0.0004ab
SF	1250±25ab	5.42±0.14a	0.02±0.0051b	1441±28b	1490±47b	0.996±0.0003a
p-value	0.000	0.028	0.020	0.000	0.000	0.031

Data represent mean ± standard error of mean (SEM). Different lowercase letters (a, b, c) represent significant differences among the three sites ($p < 0.05$; Duncan test).

Supplementary materials



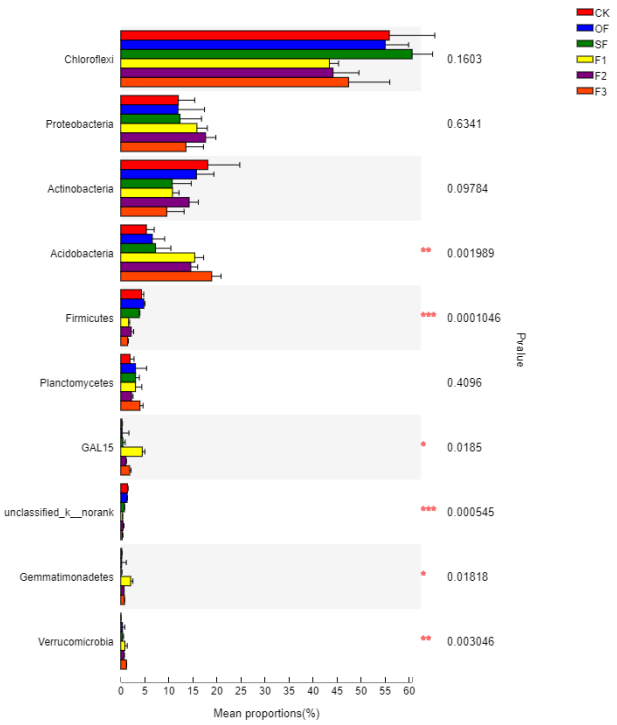


Fig. S1. Soil microbial community composition by LefSe analysis (LDA effect size analysis). Only taxa meeting a linear discriminant analysisignificance threshold of >4.0 were shown.

Fig. S2. Partial sequence distribution and abundance of different fertilizing treatments of soil bacteria at the phylum level. The top 10 species in different treatments are shown (*, $0.01 < p < 0.05$; **, $0.001 < p < 0.01$; ***,$p < 0.001$).

Table S1. Fitting indexes of the structural equation model.

Fitting index	**Reference**	**Value**
CMIN/DF	1–3	1.075
GFI	>0.90	0.743
RMSEA	<0.05, good; < 0.08, acceptable	0.066
PL	>0.05	0.335

CMIN/DF, GFI, RMSEA, and PL were calculated as the minimum discrepancy divided by the degree of freedom; GFI, Goodness-of-Fit; RMSEA, Root Mean Square Error of Approximation; PL, probability level.