

Microbial fertilizer and organic manure combined fertilization changes the rhizosphere bacterial community and carotenoids of Citrus reticulata Blanco 'Orah'

Qichun Huang

Guangxi Academy of Agricultural Sciences

Wei Zhou

Guangxi Academy of Agricultural Sciences

Zhikang Zeng

Guangxi Academy of Agricultural Sciences

Nina Wang

Guangxi Academy of Agricultural Sciences

Yanxiao Huang

Guangxi Academy of Agricultural Sciences

Hao Cheng

Huazhong Agricultural University

Quyuan Huang

Guangxi Academy of Agricultural Sciences

Jimin Liu

Guangxi Academy of Agricultural Sciences

Fuping Lu

Guangxi Academy of Agricultural Sciences

Huihong Liao

Guangxi Academy of Agricultural Sciences

Chengxiao Hu

Huazhong Agricultural University

Dongkui Chen

Guangxi Academy of Agricultural Sciences

Shaolong Wei

Guangxi Academy of Agricultural Sciences

Chaosheng Li

Guangxi Academy of Agricultural Sciences

Zelin Qin

hqchun1288@163.com

Guangxi Academy of Agricultural Sciences

Research Article

Keywords: Citrus reticulata, Bacterial communities, Carotenoids, Fertilization, 16S rRNA

Posted Date: June 17th, 2024

DOI: <https://doi.org/10.21203/rs.3.rs-4376678/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Additional Declarations: No competing interests reported.

Version of Record: A version of this preprint was published at BMC Microbiology on November 14th, 2024. See the published version at <https://doi.org/10.1186/s12866-024-03607-8>.

Abstract

Background

Citrus reticulata Blanco 'Orah' is one of the most widely grown citrus varieties in southern China. It has been proven that microbial fertilizer and organic manure combined fertilization could improve the yields and appearances of 'Orah' fruits. However, details regarding the mechanisms underlying the effects of combined fertilization on the agronomic traits and rhizosphere bacterial community of plants still need to be elucidated.

Results

This study compared the rhizosphere bacterial community and carotenoids of 'Orah' with (WYT group) and without (WYCK group) combined fertilization. The WYT group was sprayed with 50 ml Stronggreen and 250 g of Yumeiren five times while WYCK group did not sprayed. Combined fertilization increased fruit weight and the *Citrus* color index (CCI) significantly ($P < 0.05$). By 16s rRNA sequencing, 7,126 operational taxonomic units (OTU) were obtained. A higher Shannon index was observed in the WYT group compared to that in the WYCK group. Comparison between the two groups showed that *Pseudomonas* was enriched in the WTY group, and *Cyanobacteria* was enriched in the WYCK group. At the family level, *Phyllobacteriaceae* was significantly abundant in the WTY group, whereas *Thermosporothrix* and *Sphingobium* were significantly abundant in the WYCK group. A total of 51 carotenoid components were tested using UPLC-MS/MS. In the pulp tissues, 37 carotenoid components were decreased in the WTY group compared to those in the WYCK group. In fruit skin, 24 significantly different components (7 downregulated and 17 upregulated) were identified in WTY compared to those in WYCK. Correlation analysis revealed that the network between OTUs and carotenoids contained seven carotenoid components and four OTUs. Four OTUs, strain TRA3-20 (a eubacterium), *Roseiflexus*, OPB35, and *Fictibacillus* contributed to carotenoid accumulation regulation in fruit skin.

Conclusions

By exploring the complex interactions between soil microbiota and fruit traits, our research has illuminated potential pathways through which these microbial communities influence the biosynthesis and accumulation of carotenoids. In conclusion, this study provides valuable information regarding soil bacterial communities related to carotenoid accumulation in 'Orah'.

1 Introduction

Soil health is crucial for plant growth because it provides a continuous living ecosystem and affects crop yield [1]. Fertilizers are used to improve the soil environment. However, long-term chemical use usually damages the soil environment and disrupts ecosystems [2]. For example, probiotics in the soil can be

dramatically changed by chemical fertilizers [3]. Recently, microbial fertilizers and organic manure have been developed to address these problems [4, 5]. The combined use of microbial fertilizers and organic manure leads to comprehensive changes in the soil ecosystem, contributing to high crop yields [6, 7]. The Stronggreen and Yumeiren used in this study contained various strains of microorganisms and fermented organic fertilizers developed by Jiangmen Jieshi Plant Nutrition Co., Ltd. and Guangxi Yuanrun Agricultural Development Co., Ltd., respectively. They can improve soil health by stimulating probiotic growth and depressing the growth of pathogenic bacteria [8]. Although the beneficial effects of the combined use of Stronggreen and Yumeiren on soil have been determined, more details regarding its mechanism are needed to guide its practical application.

The fruit color of *Citrus* affects the price and market competitiveness [9]. Thus, the cause of different fruit colors has received widespread attention. The fruits have vivid colors that are mainly determined by anthocyanins and carotenoids. Anthocyanins are a class of water-soluble flavonoids that are widely distributed in vegetables and fruits [10]. Plants rich in anthocyanins, such as grapes, apples, and cabbage, exhibit color diversity that may appear red, purple, blue, or black [10]. Carotenoids are produced by plants, bacteria, archaea, and fungi. They present as yellow, orange, and red organic pigments [11]. Citrus plants are a rich source of carotenoids. Carotenoids constitute a large family of compounds produced via comprehensive pathways. In addition, carotenoid concentrations are highly diverse among citrus varieties [12]. For example, satsuma mandarin (*Citrus unshiu* Marc.) However, violaxanthin isomers are enriched in Valencia oranges (*Citrus sinensis* Osbeck) [13, 14]. These different carotenoid components in fruits affect the nutritional composition of the juice sacs. Therefore, the carotenoid component is important for fruit color and juice sac quality.

Recent studies have shown that the rhizosphere bacterial community contributes to plant health and affects plant growth. Network interactions of the rhizosphere bacterial community can induce carotenoid production in plants. For *Citrus* planting, a combination of microbial fertilizer and organic manure fertilization has been widely used in recent years (Das et al., 2014). This combined fertilization increases fruit yield and optimizes fruit color. Unfortunately, we still lack information about the interaction between rhizosphere bacterial community and carotenoid accumulation driven by microbial fertilizer and organic manure combined fertilization. In this study, we used two fertilizers, Stronggreen and Yumeiren, to treat the *Citrus reticulata* Blanco 'Orah'. Stronggreen contains biological fulvic acid (BFA), organic matter, humic acid, manganese, boron, zinc (3–5%), and potassium oxide ($\geq 6\%$). Yumeiren contains organic fertilizers used for the enzymatic hydrolysis of fish proteins and peanut bran. These two fertilizers have been widely used in South China to promote plant growth for pitaya (*Hylocereus undatus*), banana (*Musa acuminata*), and 'Orah' [8].

Based on previous planting knowledge, we presumed that the combined fertilization of Stronggreen and Yumeiren might affect the rhizosphere bacterial community and lead to the accumulation of several carotenoids. In this study, we compared the fruit characteristics of Orah plants with and without combined fertilization of Stronggreen and Yumeiren. The rhizosphere bacterial community and carotenoids in the two groups were determined by 16S rRNA gene sequencing and ultra-performance

liquid chromatography tandem mass spectrometry (UPLC-MS/MS) analysis, respectively. Using bioinformatics analysis, the differences in diversity and taxa of the rhizosphere bacterial community, and carotenoid components, were identified. Finally, rhizosphere bacterial community–carotenoid pairs with high correlations were analyzed, and an interaction network was constructed. The details of the effects of combined fertilization on rhizosphere bacterial community and carotenoids in ‘Orah’ has not been unveiled yet. Thus, the results would provide valuable information for understanding the mechanism of microbial fertilizer and organic manure combined fertilizer.

2 Materials and methods

2.1 Field experiments and sampling

The studied ‘Orah’ in this study were growing in a local orchard in Wuming town from Nanning, Guangxi. The plants were grown from 2017 to 2022 and grown at the same management conditions as described previously [15]. The longitude and latitude positions of experimental place were 23°15'58" and 108°17'23"E, respectively. The annual average temperature was 22.3°C, the average temperature of the coldest month was 13.2°C, the average temperature of the hottest month was 28.8°C, the average annual precipitation was 1272.3 mm, and the average annual sunshine duration was 1509.3 hours. Lighting is natural light. All tested plants assigned to the groups were randomly selected. The 60 trees were randomly divided into two groups: WYT and WYCK groups. The WYT group was sprayed with 50 ml Stronggreen and 250 g of Yumeiren (diluted in 10 kg ddH₂O) five times every month from July to November from 2017 to 2022. Sampling and measurements were double-blind [16]. For each group, 100 g of rhizosphere soil samples from five trees with three replicates were collected using a sampling shovel for 16S rRNA gene sequencing. Briefly, sterilize shovels were used to sample the rhizosphere soil. After removing impurities such as plant roots, stones, etc., from the soil, a portion of the soil sample was preserved in an insulated box containing ice packs and brought back to the laboratory, where it was immediately stored in a -80°C freezer for microbial analysis. For each group, three fruit replicates were obtained in January 2023 for the UPLC-MS/MS analysis.

2.2 Measurements of fruit

The weight, cross diameter, and longitudinal diameter of the ‘Orah’ fruit were measured following the standard method as previously described on ripening stage. The color of the ‘Orah’ fruit was determined by UltraScan Pro (Hunter Lab, Reston, VA, USA) under room temperature per the L, a, b standard color. *Citrus* color index (CCI) was used to evaluate fruit color. The CCI was calculated as $CCI = (a \times 100) / L \times b$.

2.3 DNA extraction and sequencing

For 16s rRNA sequencing, DNA from the rhizosphere soil samples (5g for each sample) was extracted using the TIANamp Soil DNA Kit (TIANGEN, China) according to the manufacturer’s protocols. DNA was assayed using a 1% agarose gel and a NanoDrop 2000 spectrophotometer (NanoDrop, USA) to confirm its quality and calculate its concentration. Sequence fragments of bacterial DNA from the 16S rRNA

genes were amplified using polymerase chain reaction (PCR). The primers were 341F: 5'-CCTAYGGGRBGCASCAG-3' and 806R: 5'-GGACTACNNGGGTATCTAAT-3' as reported before [16]. Three biological replicates were used for each treatment group. The PCR reaction condition was: 98°C for 30 s, followed by 25 cycles (98°C for 10 s, 55°C for 30 s, and 72°C for 30 s), and a final extension at 72°C for 5 min. After PCR, the products were isolated by 2% agarose gel electrophoresis and Agencourt Ampure XP beads (Beckman, USA), following the PicoGreen dsDNA quantitation assay (Thermo Fisher, USA). The sequencing libraries were constructed by a TruSeq® DNA PCR-Free Sample Preparation Kit (Illumina, USA), determined by an Agilent Bioanalyzer 2100 (Agilent, USA), and sequenced using an Illumina NovoSeq 6000 platform (Illumina, USA). All the raw sequencing data have been deposited in the NCBI SRA database under the BioProject number PRJNA1090555.

2.4 Bioinformatic analysis of soil bacterial communities

The generated raw reads were filtered to eliminate adaptors, primers, low-quality sequences, nonbacterial ribosome sequences, and chimeras. The sequences were then assembled by FLASH v1.2.11 [17] following clustered into operational taxonomic units by CD-HIT algorithm using the UCLUST program (USEARCH V11, <https://www.drive5.com/usearch/>). Diversity comparisons were analyzed for alpha and beta diversity using QIIME2 plugins [18]. The Bray-Curtis distances and square-root transformed abundance data were analyzed and visualized by the “phyloseq,” “dplyr,” and “ggplot2” R packages.

2.5 Analysis of carotenoids in 'Orah'

Carotenoid content was analyzed using UPLC-MS/MS (Wuhan MetWare Biotechnology Co., Ltd., Wuhan, China). The fruit skin and pulp from WYCK and WYT groups were first crushed into powder by an MM 400 Grinding Mill (Retsch, Germany) at 4°C. The analyzed groups were named WYCKS (fruit skin of the WYCK group), WYCKP (fruit pulp of the WYCK group), WYTS (fruit skin of the WYT group), and WYTP (pulp of the WYT group). Then, 50 mg samples were dissolved in 0.6 ml extraction buffer (n-hexane/acetone/ethanol with a volume of 1:1:2 containing 0.01% BHT). After adding the standard substance and treated for 20 min at room temperature, the samples were isolated by 12000 r/min centrifugation for 5 min at 4°C twice. Samples were dissolved in a methanol/methyl tert-butyl ether (1:1) mixture. By filtration into 0.22 µm pore size, the samples were analyzed by a UPLC-MS/MS system. The system included an ExionLC™ AD (<https://sciex.com.cn/>) and a QTRAP® 6500+ (<https://sciex.com.cn/>) using the ESI mode. The condition for ExionLC™ AD was as follows: chromatographic column, YMC C30 (3 µm, 100 mm × 2.0 mm i.d.); mobile phase A was methanol:acetonitrile (1:3), 0.01% BHT and 0.1% formic acid and mobile phase B was methyl tert-butyl ether (containing 0.01% BHT); the gradient elution program was 0 min A/B 100:0 (V/V), 3 min 100:0 (V/V), 5 min 30:70 (V/V), 9 min 5:95 (V/V), 10 min 100:0 (V/V), 11 min 100:0 (V/V); the flow rate was 0.8 mL/min, column temperature was 28°C, and the injection volume was 2 µL. The mass spectrometry used a 350°C Atmospheric Pressure Chemical Ionization Source. In QTRAP® 6500+, the Declustering Potential and Collision Energy methods were used for the detection. The data were processed using the Analyst 1.6.3 software (Sciex). Scheduled multiple reaction monitoring was used for the analysis, and the Multiquant 3.0.3 software (Sciex) was

used to quantify the carotenoids. The declaration of potentials and collision energies as key mass spectrometer parameters were analyzed for optimization.

2.6 Statistical analysis

All statistical analyses and plots in this study were performed using R software. Alpha and beta diversities were calculated after normalization, and the Shannon diversity index was used to represent alpha diversity. Beta diversity was presented using a Bray–Curtis dissimilarity matrix and permutational analysis. Correlation analysis between microorganisms and carotenoids was performed using the correlation package of R.

3 Results

3.1 Fruit quality and soil content

The fruit quality of the two groups was assessed (Fig. 1A). The fruit weight, cross diameter, longitudinal diameter, and color were compared. Fruit weight was significantly higher in the WYT group than in the WYCK group ($P < 0.05$) (Fig. 1B). The fruit color differed significantly between the WYT and WYCK groups. The analysis results, including L, a, b, and CCI, were significantly different between the groups ($P < 0.05$) (Fig. 1C-F).

3.2 Analysis of alpha and beta diversity

The 16s rRNA sequencing generated 736,037 clean sequences, and 683,533 sequences (92.87%) were effective in generating 7,126 operational taxonomic units (OTU) (Supplementary Table S1). Shannon and Simpson indices were used to analyze the genus diversity of the WYT and WYCK groups. The WYT group showed a higher Shannon index than the WYCK group ($P < 0.05$, Fig. 2A), whereas similar Simpson indices were observed when comparing the WYT and WYCK groups ($P > 0.05$, Fig. 2B). The Curtis similarity and PCA were performed to show the overall divergence in bacterial community composition between the groups (Fig. 2C, D). The replicate samples from the WYT and WYCK groups demonstrated similar results; however, a significant separation between the two groups was observed.

3.3 Bacterial diversity of 'Orah'

Most of the OTU were assigned into 10 phyla, accounting for 95.41%, including Proteobacteria (30.70%), Chloroflexi (13.55%), Acidobacteria (14.96%), Actinobacteria (17.19%), Cyanobacteria (3.82%), Saccharibacteria (3.71%), Verrucomicrobia (2.82%), Gemmatimonadetes (3.35%), Firmicutes (2.30%) and Bacteroidetes (3.01%) (Supplementary Table 2, Fig. 3). The top three phyla in the WYT group were Proteobacteria (34.00%), Actinobacteria (16.77%), and Acidobacteria (15.69%), whereas those in the WYCK group were Proteobacteria (27.39%), Actinobacteria (17.61%), and Chloroflexi (16.02%). Only 4.59% of the taxa observed in the two groups were not assigned to the top 10 phyla.

3.4 Rhizosphere bacterial community changes in 'Orah' with microbial fertilizer and organic manure combined fertilization

The comparison of microbiota in the rhizosphere soil between the WYT and WYCK groups was performed by discriminant analysis of the effect size. The results demonstrated that *Pseudomonas* was significantly enriched in WYT, whereas *Cyanobacteria* were significantly enriched in WYCK ($P < 0.05$, Student's t-test) (Fig. 4). At the family level, *Phyllobacteriaceae* was significantly more abundant in the WTY group than in the WYCK group ($P < 0.05$). *Thermosporothrix* and *Sphingobium* were significantly more abundant in the WYCK group than in the WTY group ($P < 0.05$) (Fig. 5) (Supplementary Tables S3-4).

3.6 Carotenoid changes in 'Orah' with microbial fertilizer and organic manure combined fertilization

A total of 51 carotenoid components were identified using UPLC-MS/MS (Fig. 6A). Among the identified carotenoid components, 37 were downregulated in WYCKP compared to those in WYTP. The comparison between WYCKS and WYTS identified 24 significantly different components, including 7 downregulated and 17 upregulated carotenoid components (Fig. 6B). The top 10 components with the most changes in WYCKP compared to WYTP comparison were violaxanthin myristate, α -cryptoxanthin, lutein dimyristate, zeaxanthin palmitate, violaxanthin dipalmitate, 5,6epoxy-luttein dilaurate, lutein oleate, lutein palmitate, violaxanthin-myristate-laurate, and violaxanthin dimyristate (Fig. 6C). By WYCKS and WYTS comparison, the most up-regulated 5 carotenoid components were zeaxanthin-laurate-myristate, zeaxanthin dilaurate, zeaxanthin dimyristate, lutein dilaurate, and antheraxanthin while the most up-downregulated 5 carotenoid components were violaxanthin dioleate, 8'-apo-beta-carotenal, violaxanthin-myristate-oleate, rubixanthin palmitate and β -cryptoxanthin palmitate (Fig. 6D) (Supplementary Table S5).

3.7 Correlations between rhizosphere bacterial community and carotenoids

The interactions between the rhizosphere bacterial community and carotenoids were evaluated using correlation analysis. The threshold of the p-value was < 0.0001 in this study. A total of 113 OTU-carotenoid pairs with high correlations were identified in skin tissue (Fig. 7A). In the pulp tissue, 88 OTU-carotenoid pairs with high correlations were observed (Fig. 7B). The overlap of the OTU-carotenoid pairs from the skin and pulp tissues was identified. Four OTUs were correlated with seven carotenoid components in the two tissues. The four OTUs were annotated as TRA3-20 (order), *Roseiflexus* (genus), OPB35 (class), and *Fictibacillus* (genus). The results showed that these groups of rhizosphere bacterial affected carotenoid generation in the fruit of "Orah."

4 Discussion

In this study, we compared the “Orah” under different fertilization conditions to investigate the changes in microbial fertilizer and organic manure combined fertilization. The characteristics of the fruits were compared, and the results showed significant changes in fruit color after combined fertilization. A brighter color was detected in the WYT group. Similar results have been reported for other *Citrus* varieties. For example, Magnesium (Mg) application can alter fruit coloration and sugar accumulation in navel orange (*Citrus sinensis* Osb.) [19]. Using *Bacillus subtilis* biofertilizer, a simultaneous color change in the fruit skin and pulp of Tarocco blood orange (*Citrus sinensis* (L.) Osbeck) was observed [20]. Although the effects of fertilizer application on fruit color have been investigated previously, knowledge of the underlying mechanisms is limited. Undoubtedly, combined microbial fertilizer and organic manure fertilization changed the rhizosphere bacterial community. Therefore, we assayed the bacterial community by rhizosphere soil 16s rRNA sequencing. In addition, to evaluate carotenoid accumulation in the different groups, carotenoids were analyzed using UPLC-MS/MS. Interaction networks between rhizosphere bacterial community and carotenoid components were constructed and key bacterial related to carotenoid accumulation were identified. Therefore, this study investigated changes in fruit color and provided mechanistic details from rhizosphere bacterial community insights using microbial fertilizer and organic manure combined fertilization.

The top 10 phyla with OTU were identical for the 'Orah' in the two groups. Thus, at the phylum level, the rhizosphere bacterial composition was similar with and without combined fertilization of Stronggreen and Yumeiren. However, in the WYT group, *Pseudomonas* was enriched compared to the WYCK group. *Pseudomonas*, the major gram-negative bacteria phylum, contains several important genera and pathogenic bacteria. Some free-living bacteria of this phylum participate in nitrogen fixation. In this study, rhizosphere bacterial community, which are free-living bacteria, were obtained from the root microbial community. Palm oil seedlings (*Elaeis guineensis* Jacq.), sterilization, and chemical fertilizers change the *Pseudomonadota* community and contribute to the high nutrient transformation effectiveness [21]. Similar results were reported for *Myrothamnus flabellifolia* [22], winter wheat (*Triticum aestivum* L.) [23], and maize (*Zea mays* L.) [23]. Thus, it could be inferred that these enriched bacteria are responsible for nitrogen fixation and promote the production of the 'Orah' fruit. In the WYCK group, cyanobacteria, which are gram-negative bacteria, were enriched. Cyanobacteria can associate with other plants to fix nitrogen [24]. This study showed that the combined fertilization of Stronggreen and Yumeiren regulates cyanobacteria. The details and their effects remain unknown and require further investigation.

This study revealed that 37 of the 51 carotenoid components tested were downregulated in WYCKP compared to those in WYTP. Surprisingly, all carotenoid components in the pulp were decreased by combined fertilization. In contrast, 7 downregulated and 17 upregulated carotenoid components were identified in the skin. These results indicated that the effects of combined fertilization on carotenoid component accumulation were different between the pulp and skin in the 'Orah' fruit. To date, many reports have demonstrated conserved carotenoid metabolism in different plant species. However, the

carotenoid content under the different fertilization conditions was significantly different. For example, Magnesium treatment could increase the lutein, β -cryptoxanthin, zeaxanthin, and violaxanthin levels in the pulp of Satsuma mandarin (*C. unshiu* Marc.) [25]. The sulfur fertilization of spinach (*Spinacia oleracea*) can increase carotenoid levels [26]. The more vibrant colors of fruit skin in the combined fertilization 'Orah' are associated with the increasing of zeaxanthin-related components and lutein dilaurate and antheraxanthin. Carotenoids in the skin are yellow and red. Furthermore, the β -carotene pathway yielded a substantial amount of decomposition products among the decreased components. Thus, the results inferred that the more vibrant colors and higher juice yield by combined fertilization by increasing the products in the α -carotene pathway rather than the β -carotene pathway.

Correlation analysis identified an interacting network that included four OTUs and seven carotenoid components. The OTUs identified were TRA3-20 (order), *Roseiflexus* (genus), OPB35 (class), and *Fictibacillus* (genus). TRA3-20 is positively correlated with C and N metabolism [27]. *Roseiflexus* is the carotenoid source and grows photoheterotrophically [28]. OPB35 is a good predictor of total organic carbon (TOC), total nitrogen (TN), and total phosphorus (TP), which may contribute to soil health for plant growth [29]. *Fictibacillus* belongs to the Bacillaceae family. Several species of this genus can improve carbon metabolic properties and plays a role in promoting diversity in the rhizosphere microbial community [30, 31]. Soil health, including soil microbiome communication, is important for the interactions between microorganisms and plants. Rhizosphere bacterial community are crucial for soil metabolism. Our results demonstrated that the key microorganisms were strongly related to some carotenoid accumulation that participated in coloration of the 'Orah' skin. The seven carotenoid components that correlated with the OTUs were from both the lycopene ε -cyclase (LCYE)- and lycopene β -cyclase (LCYB)-mediated synthesis pathways. Microbial fertilizer and organic manure combined fertilization changed the bacterial community in the rhizosphere soil. Thus, we propose that the changes in carotenoids were driven by an improvement in rhizosphere bacterial community rather than by direct carotenoid synthesis pathway regulation.

5 Conclusion

In this study, we compared the fruit characteristics, rhizosphere bacterial community, and carotenoids in 'Orah'. These results indicated that combined fertilization improved the bacterial community in the rhizosphere soil, which affected the soil environment. These effects contributed to better nutrient absorption of 'Orah' resulting in changes in carotenoid accumulation. Finally, fruit characteristics, including yield and skin color, were altered by combined fertilization. These results provide evidence for elucidating the mechanism of improved agronomic characteristics of fruits by microbial fertilizer and organic manure combined fertilization and provide clues to the microorganism-carotenoid regulatory network. Taken together, these findings provide valuable information regarding the fertilization strategy of 'Orah'.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

All the raw sequencing data have been deposited in the NCBI SRA database under the BioProject number PRJNA1090555.

Competing interests

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Funding

This work was funded by Science and Technology Major Project of Guangxi (GuiKeAA22036002, GuiKeAA20108003); Science and Technology Major Project of Nanning (20212141, 20222065), Guangxi Characteristic crop experimental station, China (GuiTS202201); Project of Guangxi Academy of Agricultural Sciences (Guinongke2021YT051, 2022JM32, 2023YM73); Functional expert of "Cultivation and Pest Control" in Guangxi Citrus Innovation Team of National Modern Agricultural Industry System (nycytxgxcxtd-2021-05-02); Citrus Huanglongbing prevention and control engineering technology research center of Guangxi.

Authors' contributions

Qichun Huang designed and performed the experiment and wrote the manuscript. Wei Zhou, Zhikang Zeng, Nina Wang, Yanxiao Huang, Hao Cheng, Quyan Huang, Jimin Liu and Fuping Liu performed the experiment and analyzed the data. Huihong Liao performed the bioinformatic analysis. Chengxiao Hu edited the manuscript. Dongkui Chen confirmed the experiment. Shaolong Wei, Chaosheng Li, and Zelin Qin supervised the experiment and edited the manuscript. All authors contributed to the article and approved the submitted version.

References

1. Lehmann J, Bossio DA, Kögel-Knabner I, Rillig MC. The concept and future prospects of soil health. *Nat Reviews Earth Environ.* 2020;1(10):544–53.
2. Edwards CA. Assessing the effects of environmental pollutants on soil organisms, communities, processes and ecosystems. *Eur J Soil Biol.* 2002;38(3):225–31.

3. Molina-Romero D, Juárez-Sánchez S, Venegas B, Ortiz-González CS, Baez A, Morales-García YE, Muñoz-Rojas J. A Bacterial Consortium Interacts With Different Varieties of Maize, Promotes the Plant Growth, and Reduces the Application of Chemical Fertilizer Under Field Conditions. *Front Sustainable Food Syst* 2021, 4.
4. Ren J, Liu X, Yang W, Yang X, Li W, Xia Q, Li J, Gao Z, Yang Z. Rhizosphere soil properties, microbial community, and enzyme activities: Short-term responses to partial substitution of chemical fertilizer with organic manure. *J Environ Manage.* 2021;299:113650.
5. Zhang Q-C, Shamsi IH, Xu D-T, Wang G-H, Lin X-Y, Jilani G, Hussain N, Chaudhry AN. Chemical fertilizer and organic manure inputs in soil exhibit a vice versa pattern of microbial community structure. *Appl Soil Ecol.* 2012;57:1–8.
6. Das S, Adhya TK. Effect of combine application of organic manure and inorganic fertilizer on methane and nitrous oxide emissions from a tropical flooded soil planted to rice. *Geoderma.* 2014;213:185–92.
7. Iqbal A, Liang H, McBride SG, Yuan P, Ali I, Zaman M, Zeeshan M, Khan R, Akhtar K, Wei S, et al. Manure applications combined with chemical fertilizer improves soil functionality, microbial biomass and rice production in a paddy field. *Agron J.* 2022;114(2):1431–46.
8. Wu J-Q, Xiao-Hong Z, Zhao X-M, Li Z-Y, Huang Y-Q. Study on the Effects of Fertilizer Reduction with Efficiency Increase by Appling Strongreen on Rice. *China Rice.* 2014;20(6):46.
9. Hu Y, Wang G, Pan S, Wang L. Influence of ethylene and ethephon treatments on the peel color and carotenoids of Gannan Newhall navel orange during postharvest storage. *J Food Biochem.* 2018;42(5):e12534.
10. Kayesh E, Shangguan L, Korir NK, Sun X, Bilkish N, Zhang Y, Han J, Song C, Cheng Z-M, Fang J. Fruit skin color and the role of anthocyanin. *Acta Physiol Plant.* 2013;35(10):2879–90.
11. Schweiggert RM, Carle R. Carotenoid Production by Bacteria, Microalgae, and Fungi. In: *Carotenoids* 2016: 217–40.
12. Fanciullino A-L, Dhuique-Mayer C, Luro F, Casanova J, Morillon R, Ollitrault P. Carotenoid Diversity in Cultivated Citrus Is Highly Influenced by Genetic Factors. *J Agric Food Chem.* 2006;54(12):4397–406.
13. Absorption, Storage and Distribution of β -Cryptoxanthin in Rat after Chronic Administration of Satsuma Mandarin (< i > Citrus unshiu MARC.) Juice. *Biological and Pharmaceutical Bulletin* 2013, 36(1):147–151.
14. Lux PE, Carle R, Zacarías L, Rodrigo M-J, Schweiggert RM, Steingass CB. Genuine Carotenoid Profiles in Sweet Orange [Citrus sinensis (L.) Osbeck cv. Navel] Peel and Pulp at Different Maturity Stages. *J Agric Food Chem.* 2019;67(47):13164–75.
15. Huang Q, Wang N, Liu J, Liao H, Zeng Z, Hu C, Wei C, Tan S, Liu F, Li G et al. Soil bacterial communities associated with marbled fruit in Citrus reticulata Blanco 'Orah'. *Front Plant Sci* 2023, 14.

16. Chen C, Etemadi F, Franck W, Franck S, Abdelhamid MT, Ahmadi J, Mohammed YA, Lamb P, Miller J, Carr PM, et al. Evaluation of environment and cultivar impact on lentil protein, starch, mineral nutrients, and yield. *Crop Sci.* 2022;62(2):893–905.
17. Magoč T, Salzberg SL. FLASH: fast length adjustment of short reads to improve genome assemblies. *Bioinformatics.* 2011;27(21):2957–63.
18. Fung C, Rusling M, Lampeter T, Love C, Karim A, Bongiorno C, Yuan L-L. Automation of QIIME2 Metagenomic Analysis Platform. *Curr Protocols.* 2021;1(9):e254.
19. Liu X, Hu C, Liu X, Riaz M, Liu Y, Dong Z, Tan Q, Sun X, Wu S, Tan Z. Effect of magnesium application on the fruit coloration and sugar accumulation of navel orange (*Citrus sinensis* Osb). *Sci Hort.* 2022;304:111282.
20. Qiu F, Liu W, Chen L, Wang Y, Ma Y, Lyu Q, Yi S, Xie R, Zheng Y. *Bacillus subtilis* biofertilizer application reduces chemical fertilization and improves fruit quality in fertigated Tarocco blood orange groves. *Sci Hort.* 2021;281:110004.
21. Ding JYM, Ho LS, Ibrahim J, Teh CK, Goh KM. Impact of sterilization and chemical fertilizer on the microbiota of oil palm seedlings. *Front Microbiol* 2023, 14.
22. Tebele SM, Marks RA, Farrant JM. Exploring the root-associated microbiome of the resurrection plant *Myrothamnus flabellifolia*. *Plant and Soil*; 2023.
23. Wei D, Wei S, Peng A, Yang C, Chen C. Responses of Bacterial Communities in Soils under Winter Wheat to Nightly Warming and Nitrogen Addition. *Agronomy.* 2022;12(7):1616.
24. Rai AN, Singh AK, Syiem MB. Chap. 23 - Plant Growth-Promoting Abilities in Cyanobacteria. In: *Cyanobacteria*. Edited by Mishra AK, Tiwari DN, Rai AN: Academic Press; 2019: 459–476.
25. Liu X, Liu Y, Zhou Y, Hu C, Tan Q, Sun X, Wu S. Magnesium accelerates changes in the fruit ripening and carotenoid accumulation in Satsuma Mandarin pulp. *Plant Physiol Biochem.* 2023;204:108082.
26. Reif C, Arrigoni E, Neuweiler R, Baumgartner D, Nyström L, Hurrell RF. Effect of Sulfur and Nitrogen Fertilization on the Content of Nutritionally Relevant Carotenoids in Spinach (*Spinacia oleracea*). *J Agric Food Chem.* 2012;60(23):5819–24.
27. Xiao Z, Zhang S, Yan P, Huo J, Aurangzeib M. Microbial Community and Their Potential Functions after Natural Vegetation Restoration in Gullies of Farmland in Mollisols of Northeast China. *Land.* 2022;11(12):2231.
28. Takaichi S, Maoka T, Yamada M, Matsuura K, Haikawa Y, Hanada S. Absence of carotenes and presence of a tertiary methoxy group in a carotenoid from a thermophilic filamentous photosynthetic bacterium *Roseiflexus castenholzii*. *Plant Cell Physiol.* 2001;42(12):1355–62.
29. Yang L, Barnard R, Kuzyakov Y, Tian J. Bacterial communities drive the resistance of soil multifunctionality to land-use change in karst soils. *Eur J Soil Biol.* 2021;104:103313.
30. Chen Y, Wang W, Zhou D, Jing T, Li K, Zhao Y, Tang W, Qi D, Zhang M, Zang X, et al. Biodegradation of lignocellulosic agricultural residues by a newly isolated *Fictibacillus* sp. YS-26 improving carbon metabolic properties and functional diversity of the rhizosphere microbial community. *Bioresour Technol.* 2020;310:123381.

31. Wang L, Lin H, Dong Y, Li B, He Y. Effects of endophytes inoculation on rhizosphere and endosphere microecology of Indian mustard (*Brassica juncea*) grown in vanadium-contaminated soil and its enhancement on phytoremediation. *Chemosphere*. 2020;240:124891.

Figures

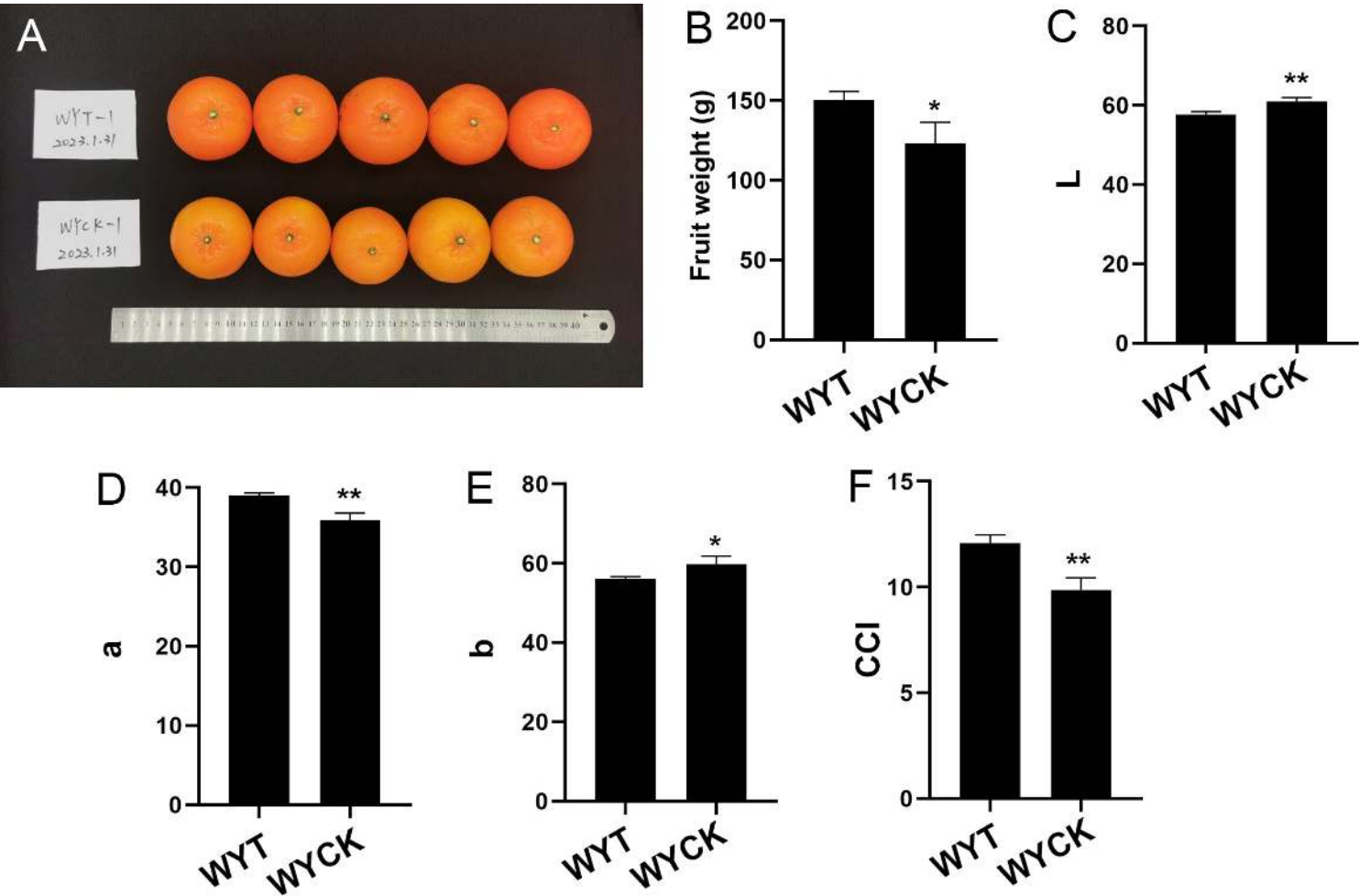


Figure 1

Comparisons of fruit appearances between the WYT and WYCK groups. (A) The fruits in the WYT and WYCK groups. (B) Fruit weight from the WYT and WYCK groups. (C), (D), (E) and (F) were L, a, b and CCI value tested using UltraScan Pro in the WYT and WYCK groups, respectively. The asterisks show the significant differences between the two groups ($P < 0.05$) by t-test.

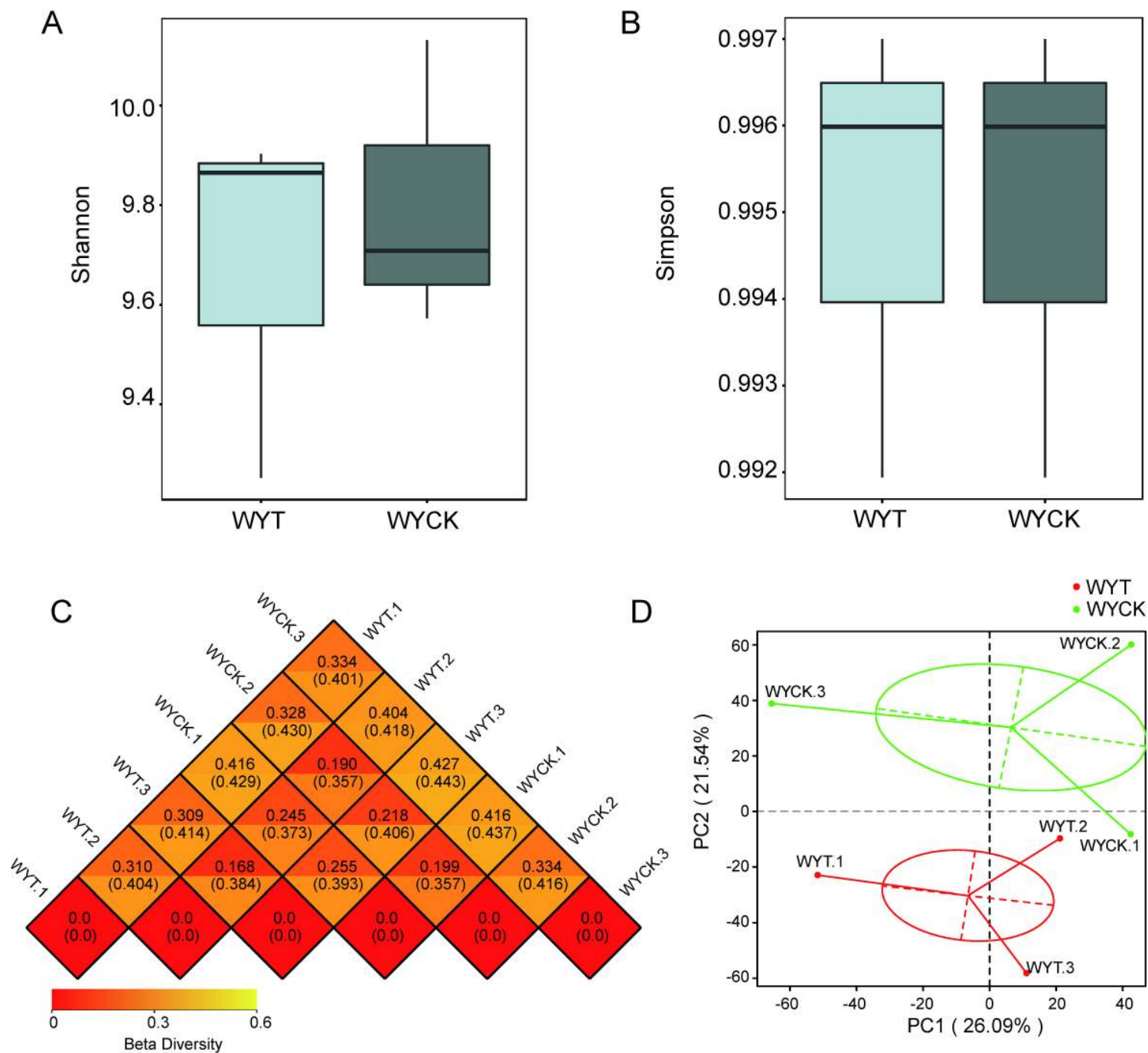


Figure 2

The analysis of alpha and beta diversity in the WYT and WYK groups. (A) and (B) were Shannon index and Simpson index of the alpha diversity of the samples in the WYT and WYK groups, respectively. (C) Correlations of beta diversity between the samples. (D) PCA among the samples in the WYT and WYK groups,

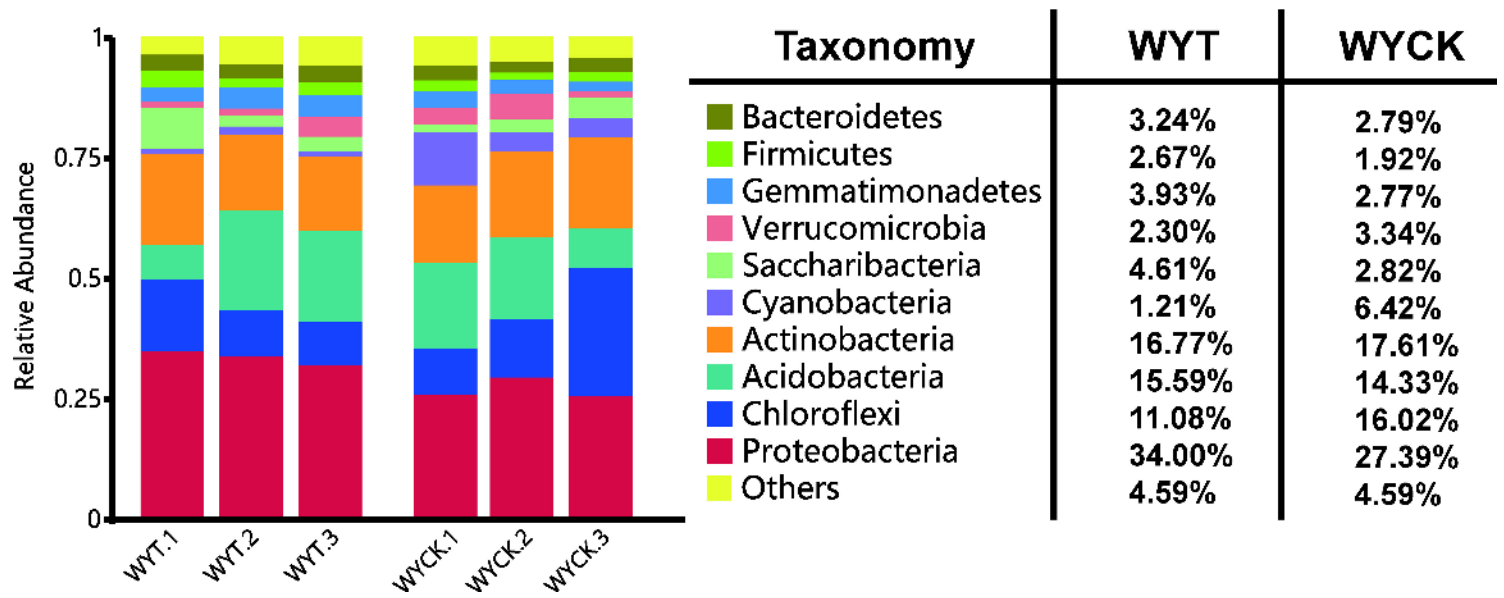


Figure 3

The distribution of most ten abundant phyla in the WYT and WYK groups

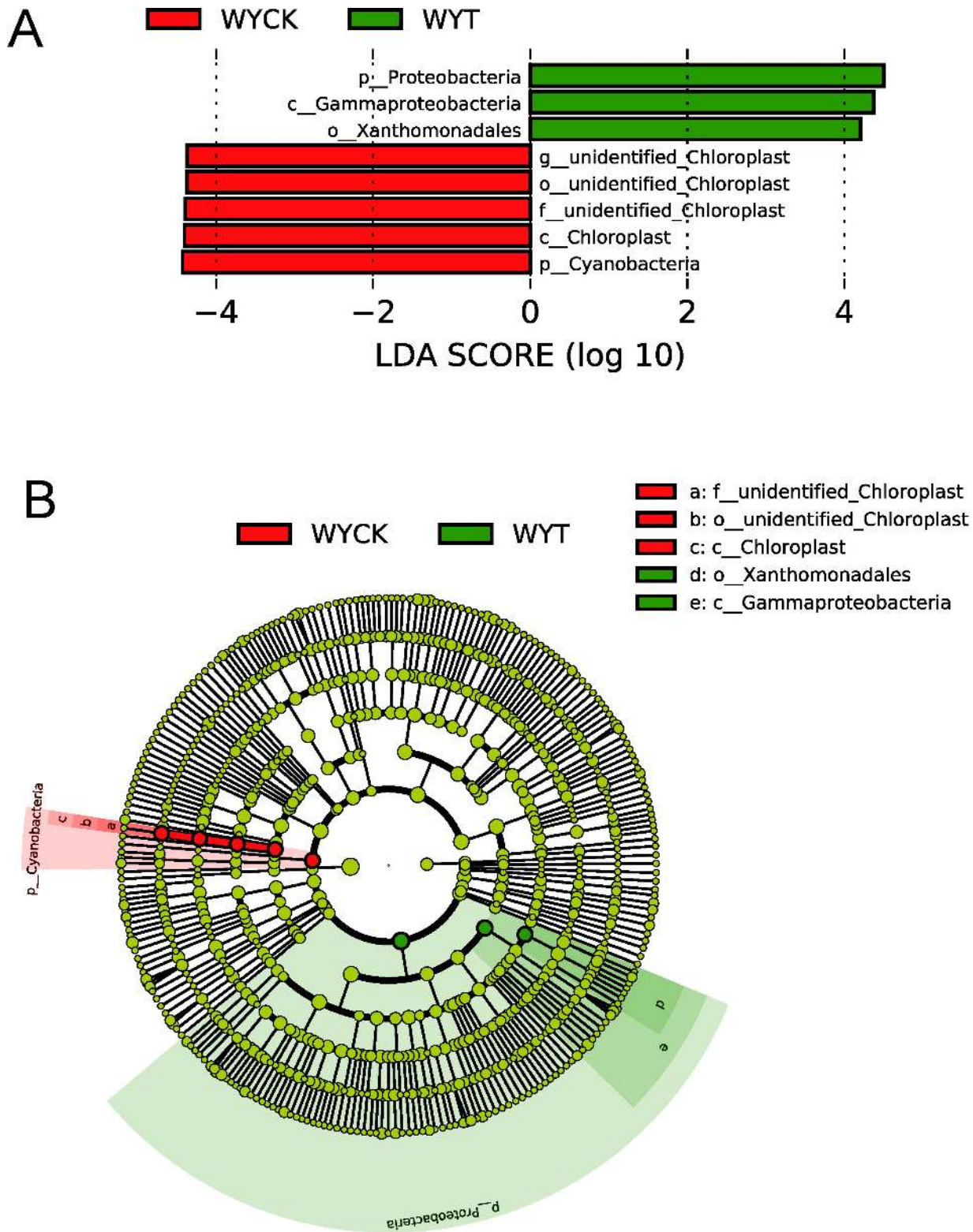


Figure 4

Differences in bacterial taxa abundances of the WYT and WYCK groups. (A) The forest plots show the linear discriminant analysis score (effect size) of the significant difference between the WYT and WYCK group. (B) The cladograms generated by linear discriminant analysis of effect size method indicated the phylogenetic distribution of microbes in the WYT and WYCK groups.

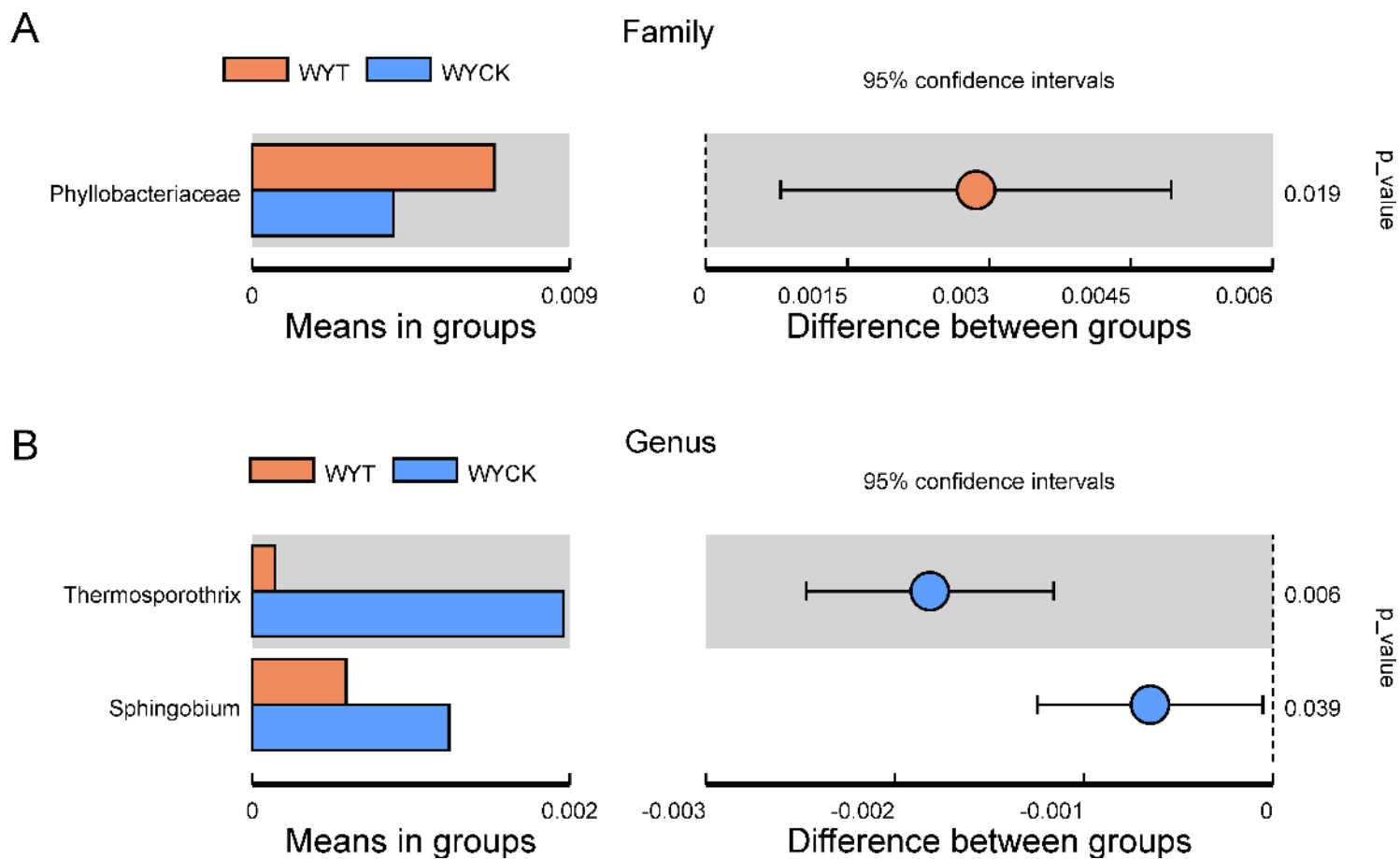


Figure 5

The significantly different taxa at the (A) family and (B) genus levels.

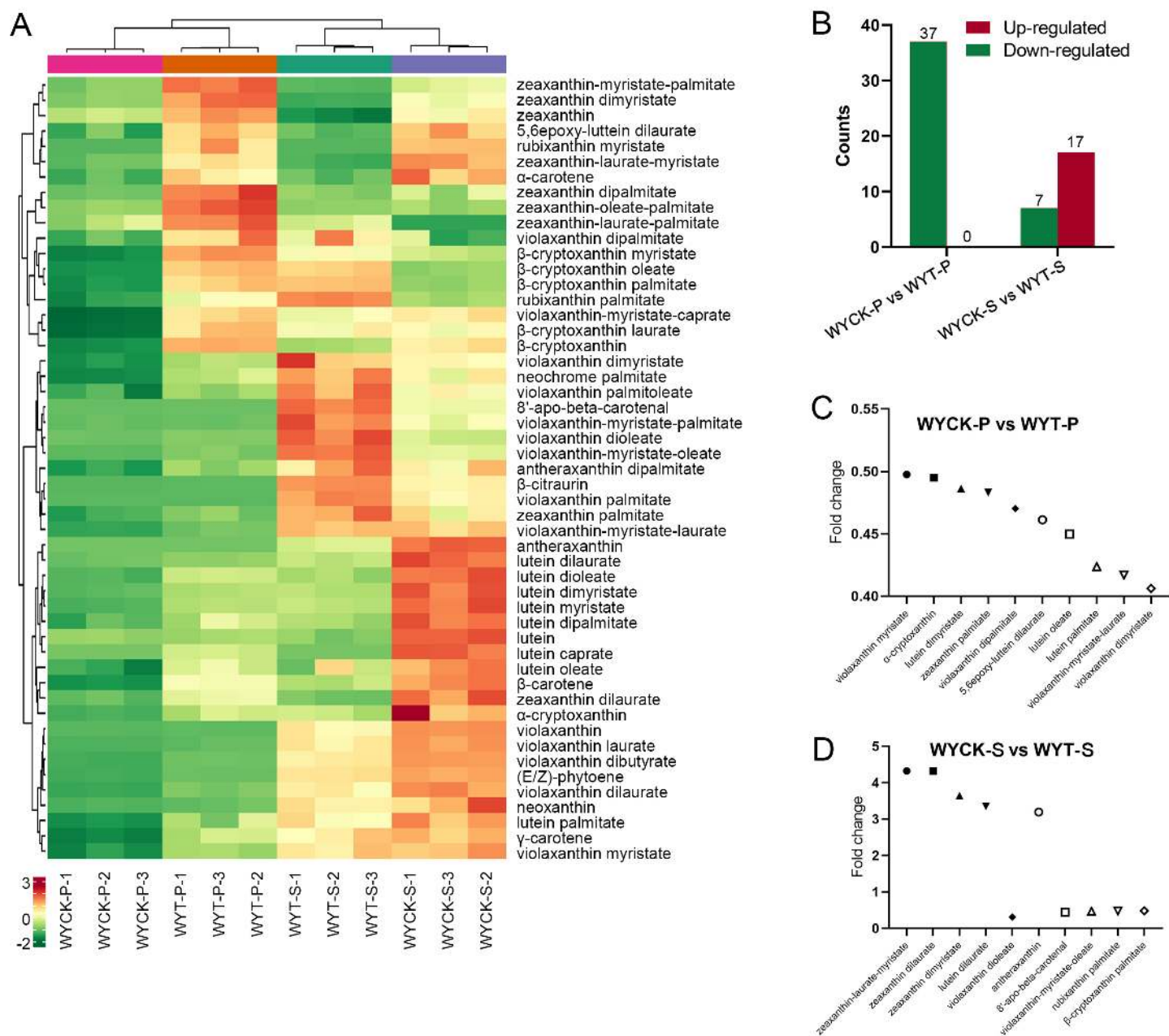


Figure 6

Carotenoids changes of fruit pulp and fruit skin from WYT and WYCK groups. (A) Heatmap of carotenoid levels in fruit pulp and fruit skin from WYT and WYCK groups. (B) Up-regulated and down-regulated carotenoid components compared the fruit skin and fruit pulp from WYT and WYCK groups. (C) Top ten differential carotenoid components between fruit pulp from WYT and WYCK groups. (D) Top ten differential carotenoid components between fruit skin from WYT and WYCK groups. WYCK-P, fruit pulp of the WYCK group; WYT-P, pulp of the WYT group; WYCK-S, fruit skin of the WYCK group; WYT-S, fruit skin of the WYT group.

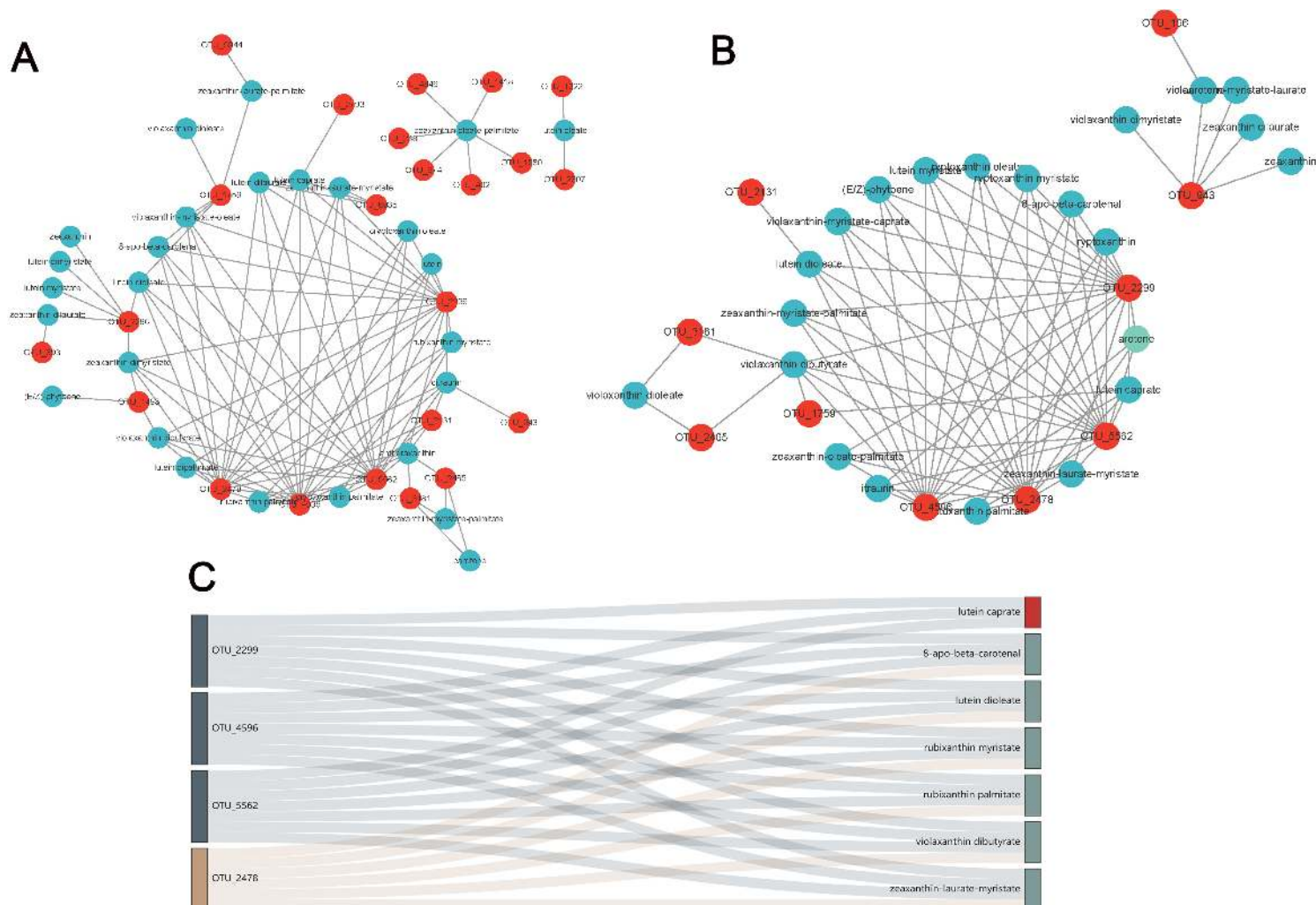


Figure 7

Correlation analysis of rhizosphere bacterial community and carotenoids. (A) The network of 113 OTU-carotenoid pairs with high correlations from fruit skin. (B) The network of 88 OTU-carotenoid pairs with high correlations from fruit pulp. (C) The four overlapped OTU-carotenoid pairs from the skin and pulp tissues.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [1353876Table1.xlsx](#)
- [1353876Table2.xlsx](#)
- [1353876Table3.xlsx](#)
- [1353876Table4.xlsx](#)
- [1353876Table5.xlsx](#)