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Article

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Comprehensive Effects and Evaluation of Ecological Factors on the Quality of *Citrus reticulata 'Tangerina'*

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Abstract

Citrus reticulata 'Tangerina', a characteristic citrus cultivar native to Fujian Province, is widely cultivated locally. Its dried ripe fruit pericarp constitutes *Citri Reticulatae Pericarpium* (Chenpi) derived from *C. reticulata 'Tangerina'*, a traditional Chinese medicinal and edible homologous material with both medicinal and edible values.Modern studies on the formation mechanism of the genuineness of Chenpi and on *C. reticulata 'Tangerina'* remain scarce. This study focuses on the key factors underlying the genuineness of *C. reticulata 'Tangerina'*. By investigating the effects of climate, rhizosphere soil elements, and rhizosphere microorganisms on the quality of this *C. reticulata 'Tangerina'* , we aim to elucidate the basis of its genuineness, thereby providing a scientific theoretical foundation for the screening of high-quality producing areas of *C. reticulata 'Tangerina'*, the high-quality cultivation of citrus, and the evaluation of Chenpi genuineness.Correlation analysis revealed that rhizosphere soil elements total phosphorus（TP）, organic matter(OM), available phosphorus(AP), available manganese(AMn), available boro(AB), total potassium(TK), pH, rapidly available potassium(AK), and exchangeable magnesium(EMg), rhizosphere microorganisms at the genus level *Dongia, Pseudolabrys*, *Bacillus*, *Sphingomonas, Hawksworthiomyces*, *unidentified_Cyanobacteria*, *Mycena*, *Jatrophihabitans*, *Fusarium*, *Mycobacterium*, and *Occallatibacter*, and environmental factors Mean temperature of coldest quarter (bio11), Annual precipitation (bio12), Precipitation of warmest quarter (bio18), Precipitation seasonality (bio15), Mean diurnal range (bio2), Isothermality (bio3), and Temperature annual range (bio7) were all key factors driving the accumulation of secondary metabolites in *Citrus reticulata 'Tangerina'*.This study demonstrates that differences in ecological factors across distinct producing regions drive the differentiation of secondary metabolite accumulation patterns in *Citrus reticulata 'Tangerina'*, which serves as the underlying cause for the formation of Chenpi genuineness. Furthermore, this study provides a crucial basis for the scientific cultivation and rational fertilization of citrus, while also enriching research on Chenpi genuineness and the theory of ecological adaptability of genuine medicinal materials.

Keywords: *Citrus reticulata 'Tangerina'*; ecological environment; climate; rhizosphere microorganisms; rhizosphere soil elements

Introduction

Chenpi is the dried ripe pericarp of *Citrus reticulata Blanco* and its cultivated varieties belonging to the Rutaceae family. It possesses the effects of regulating qi and invigorating the spleen, as well as drying dampness and resolving phlegm (Cheng et al. 2024), and is commonly used for the treatment of vomiting, diarrhea, and respiratory diseases (Yang et al. 2022; Zheng et al. 2020). In folk practice, it is also used as tea to achieve the purpose of health preservation while drinking; additionally, people add it as a condiment when cooking. In other words, Chenpi is a medicinal material that can be used both medicinally and as food. *Citrus reticulata 'Tangerina'* is one of its cultivated varieties, which is widely planted in Fujian Province. The fruit of *Citrus reticulata 'Tangerina'* is regarded as the city fruit of Fuzhou City, Fujian Province, and its dried pericarp is also a genuine medicinal material of Fujian. The fruit of *Citrus reticulata 'Tangerina'* is moderate in size, oblate with a slightly concave top; its epidermis is vermilion, thin and easy to peel off; the pulp is juicy, sweet with a slight astringency, and rich in nutrients such as VC, citric acid, and dietary fiber.

The ecological environment is a key factor underlying the formation of a genuine medicinal material (Chen et al. 2022). Traditional Chinese medicines (TCMs) are derived from nature. Having existed for billions of years, animals, plants, and minerals have formed intimate relationships with their natural environments, and both their healthy growth and accumulation of pharmacodynamic constituents require suitable ecological conditions (Hao et al. 2024). The genuine medicinal material represent high-quality TCMs preferentially selected through long-term clinical practice in traditional Chinese medicine (Luo et al. 2021). They serve as the core benchmark for the quality and therapeutic efficacy of TCMs, defined by their key attributes: production in specific geographical regions, superior quality and efficacy, stable quality, and a long cultivation history (Wang et al. 2025; Xu et al. 2022). These medicinal plants have broad distributions, however, influenced by ecological factors, their morphological traits and pharmacological efficacy are markedly inferior when grown in other regions. Even when transplanted to alternative areas, their quality and therapeutic effects remain inferior to those of specimens grown in their native habitats.

Climatic factors (e.g., light, temperature, and precipitation) are major determinants of the quality of

medicinal plants (Liu et al. 2023a). They not only shape their geographical distribution and growth and development processes but also regulate the biosynthesis and accumulation of secondary metabolites. Favorable climatic conditions can significantly improve the yield and pharmacodynamic quality of medicinal materials (C. He et al. 2023; Zheng et al. 2022). For instance, light intensity and photoperiod can coordinate metabolic rhythms in plants by modulating photosynthetic efficiency and the expression of related genes (Wu et al. 2025). Hao et al. (2024) predicted that the suitable habitats of Hypericum perforatum would show an overall expansion trend under future climate scenarios, with its distribution centroid shifting toward the northwest and higher-altitude regions; Liu et al. (2023b) found that mean annual temperature and annual temperature range were negatively correlated with saponin contents in *Panax notoginseng*. High annual precipitation and high humidity favored the accumulation of NG-R1, G-Rg1, and G-Rb1, but were not conducive to that of G-R1.

Soil elements are also crucial ecological factors and serve as the material basis for plant growth and development, yield formation, and accumulation of medicinal active ingredients (Liu et al. 2022). Macronutrients determine plant growth and yield (Champati et al. 2022), while meso- and micronutrients regulate metabolism, stress resistance, and medicinal quality (Da Costa Santos et al. 2024). The contents and proportions of soil elements vary considerably among different producing regions. A balanced and appropriate soil element profile is essential for sustaining normal growth, achieving high yield, and improving the efficacy of medicinal plants. In contrast, an imbalanced soil element status impairs nutrient uptake, thereby hindering plant growth and development. For example, Liao et al. (2019) reported that moderate nitrogen application significantly enhanced nitrogen metabolic enzyme activities and related gene expression, root vigor, yield, and fruit quality in citrus, whereas excessive nitrogen application exerted significant inhibitory effects. Excessive total nitrogen (TN) leads to overgrowth in tobacco and reduces the accumulation of secondary metabolites (Wang et al. 2019).

Rhizosphere microorganisms also represent a crucial factor influencing the quality of medicinal plants. They assist plant roots in uptaking key soil nutrients. For example, nitrogen-fixing bacteria can convert atmospheric N_2 into plant-available ammonium (NH_4^+), thereby improving soil nitrogen availability (Faoro et al. 2019). Phosphate-solubilizing bacteria are able to mobilize insoluble

phosphorus accumulated in soils over long periods (Santoyo et al. 2026). These processes collectively promote plant growth and development, boost both the yield and quality of medicinal plants, and enhance their disease resistance.

In this study, GC-MS was employed to analyze the volatile components of *Citrus reticulata 'Tangerina'* from three different production regions, while HPLC was used to quantitatively determine seven compounds, namely Hesperidin, Sinensetin, 4',5,7,8-Tetramethoxyflavone, Nobiletin, 3,5,6,7,8,3',4'-Heptamethoxyflavone, Tangeretin, and Demethylnobiletin. Multivariate statistical analyses including PCA and PLS-DA were applied to screen differential volatile components of *Citrus reticulata 'Tangerina'* samples from various origins, providing a reference for quality evaluation. Rhizosphere soil samples of *Citrus reticulata 'Tangerina'* were collected from each production region, and 15 rhizosphere soil elements and soil microorganisms were determined, respectively. In addition, 20 environmental factors of *Citrus reticulata 'Tangerina'* from different regions were obtained via GMPGIS. Correlation analysis was performed with the detected chemical components of *Citrus reticulata 'Tangerina'* to identify the key environmental factors affecting the quality of *Citrus reticulata 'Tangerina'*. This study provides an effective reference and data support for the quality control, optimized planting site selection, and rational fertilization of *Citrus reticulata 'Tangerina'*.

Results

Results of Active Component Determination by HPLC

The contents of seven active components in samples from the three production regions were as follows: Hesperidin(HPRD) 5.99–8.91%, Sinensetin(SNST) 0.09–0.12%, 4',5,7,8-Tetramethoxyflavone(4TFV) 0.06–0.08%, 3,5,6,7,8,3',4'-Heptamethoxyflavone(7TFV) 0.04–0.05%, Demethylnobiletin(DTBT) 0.05–0.06%, Nobiletin(NBLT) 0.52–0.74%, and Tangeretin(TGRT) 0.25–0.41%.The contents of all seven active components in samples from FJDZ were higher than those from the other two regions.Compared with FJJY, FJLQ exhibited higher contents of Hesperidin, Sinensetin, Nobiletin, 3,5,6,7,8,3',4'-Heptamethoxyflavone, and Demethylnobiletin, whereas FJJY contained higher levels of 4',5,7,8-Tetramethoxyflavone and

Tangeretin(**Figure1.** b and **Supplementary Table S**1). Principal component analysis (PCA) was performed on the active component data from the three regions (**Figure1.** c). PC1 accounted for 84.24% and PC2 for 15.68% of the total variance, with a cumulative contribution rate of 99.92%. Distinct separation was observed among the three groups, indicating significant differences in the active component profiles across the three production regions.

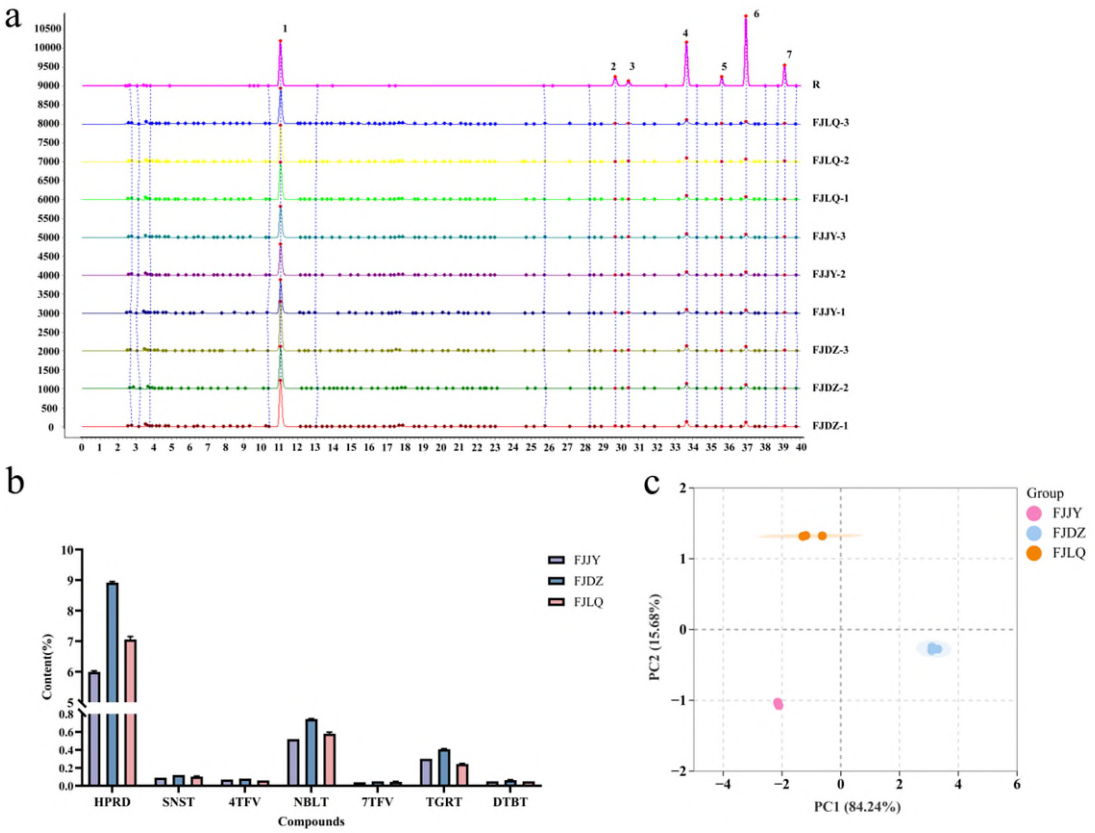


Figure1. Chromatographic fingerprint:1. Hesperidin; 2. Sinensetin. 3. 4',5,7,8-Tetramethoxyflavone; 4. Nobiletin; 5. 3,5,6,7,8,3',4'-Heptamethoxyflavone; 6. Tangeretin; 7. Demethylnobiletin (a); Histogram comparison of bioactive components in samples from different producing regions (b); PCA score plots (c).

Results of Volatile Component Determination by GC-MS

To investigate the volatile components in *Citrus reticulata 'Tangerina'* from different production regions, gas chromatography-mass spectrometry (GC-MS) was employed to identify the volatile compounds and their relative contents in *Citrus reticulata 'Tangerina'*. A total of 54 compounds were identified after screening, including 29 alkenes (53.70%), 5 alcohols (9.26%), 11 aldehydes (20.37%), 3 esters (5.56%), and 11 others (11.11%) (**Figure2.** a).The relative contents of compounds

from different regions are also presented in the figure.The six most abundant compounds among all identified components were D-Limonene (55.89%–71.15%), Linalool (6.99%–17.07%), .gamma.-Terpinene (4.03%–7.80%), .beta.-Myrcene (1.52%–3.43%), and alpha,4-trimethyl-alph(thet (0.69%–2.39%) (**Figure2.** b and **Supplementary Table S**2).

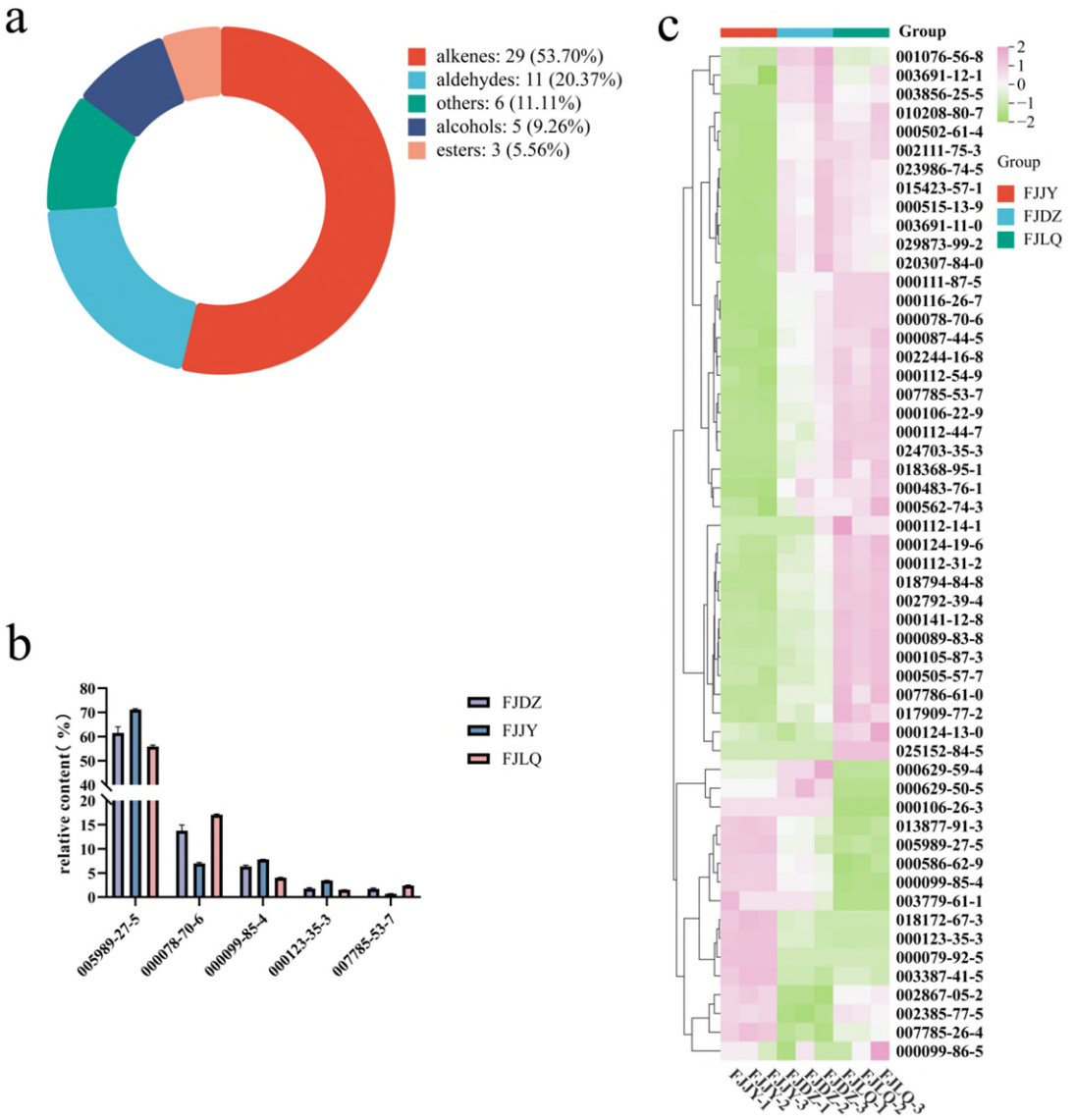


Figure2. Pie chart (a); Bar chart (b); Clustered heatmap (c).

Unsupervised principal component analysis (PCA) was performed on *Citrus reticulata 'Tangerina'* samples from different production regions using 54 volatile compounds with a match degree ⩾ 80% from the NIST17.L database as dependent variables (**Figure3.** a). PC1 accounted for 79.4% and PC2 for 17.01% of the total variance, with a cumulative explanatory power of 96.41%. Clear

separation was observed among the three groups, indicating significant differences in volatile components across the three production regions.Supervised partial least squares discriminant analysis (PLS-DA) was subsequently conducted for further analysis, which also effectively distinguished samples from the three regions (**Figure3.** b). The model exhibited good fitness with R2X = 0.964, R2Y = 0.991, and Q2 = 0.982, indicating that the model was reliable and acceptable (**Figure3.** c).A 200-permutation test was performed for the PLS-DA model. The intersection of the Q2 regression line with the vertical axis was below zero, verifying that the model was not overfitted (**Figure3.** d).

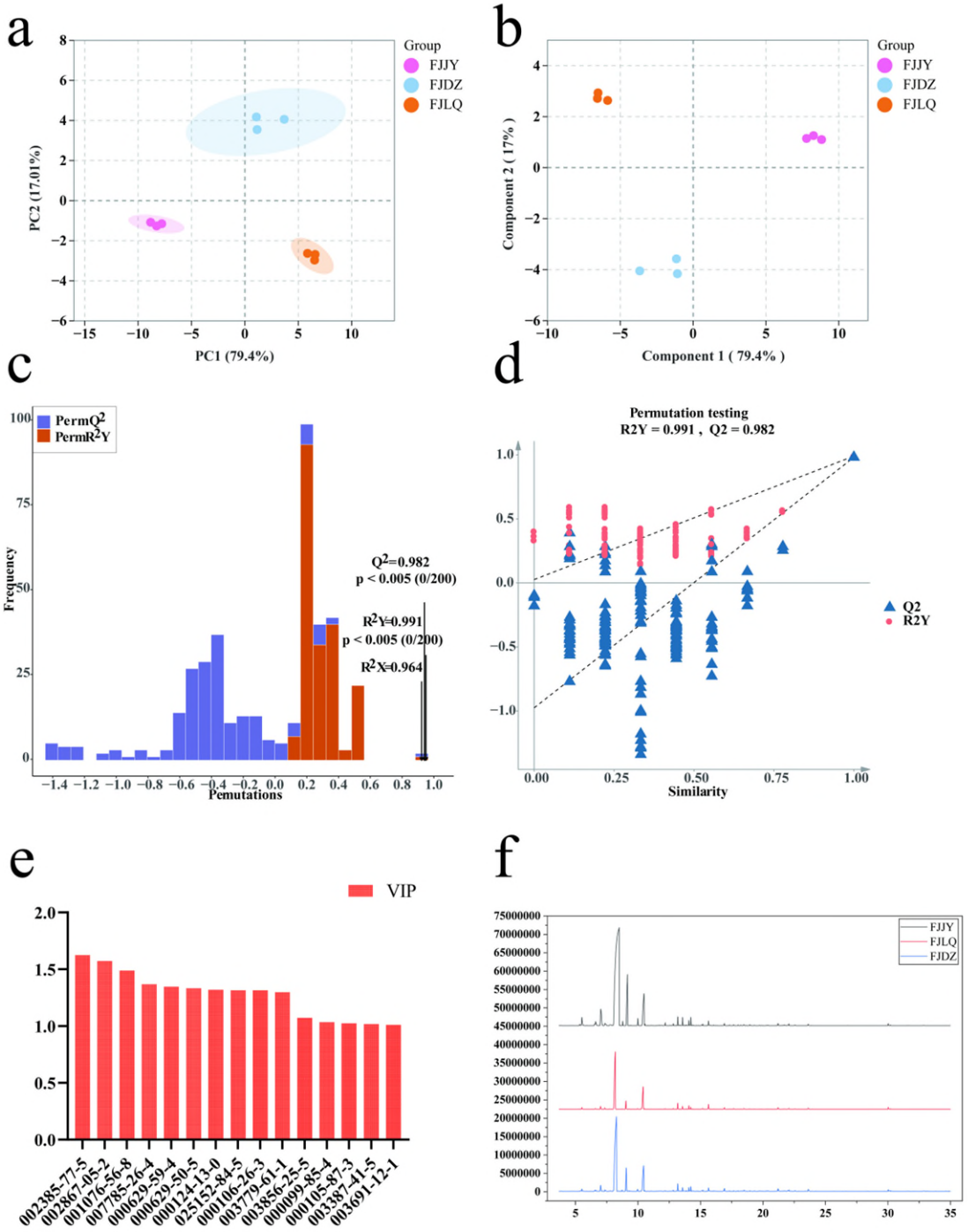


Figure3. PCA score plot (a); PLS-DA score plot (b); PLS-DA validation plot (c); Chromatographic fingerprint (d); VIP score plot (e); Permutation test plot (f).

Given the differences in volatile compounds among *Citrus reticulata 'Tangerina'* samples from three distinct production regions, the PLS-DA model was employed for data analysis. Differential volatile components were screened using the criteria of VIP > 1. A total of 15 differential metabolites were identified, including 7 alkenes, 4 aldehydes, 2 alkanes, 1 ether, and 1 ester (**Figure3.** e).

Analysis Results of Climatic Factors in Each Producing Region

Comparative analysis was performed on 20 Climatic factors across different production regions (Figure a). Among the three regions, FJLQ exhibited higher values of Bio1, Bio4, Bio5, Bio6, Bio7, Bio10, Bio14, Bio17, and Bio19, but lower values of Bio2, Bio3, Bio9, Bio13, Bio15, Bio16, and Elev than the other two regions;FJJY showed higher values of Bio2, Bio3, Bio12, Bio13, Bio16, and Bio18, but lower values of Bio1, Bio4, Bio5, Bio6, Bio8, Bio10, and Bio11 than the other two regions;FJDZ presented higher values of Bio8, Bio9, Bio11, Bio15, and Elev, but lower values of Bio7, Bio12, Bio14, Bio17, Bio18, and Bio19 than the other two regions (**Figure4.** a and **Supplementary Table S**3).

Determination Results of Soil Elements in Each Producing Region

To investigate the effects of rhizosphere soil differences and rhizosphere soil nutrient elements on the quality of *Citrus reticulata 'Tangerina'* from different production regions, fifteen soil physicochemical indicators were determined, including total nitrogen (TN, g/kg), total phosphorus (TP, g/kg), total potassium (TK, g/kg), available phosphorus (AP, mg/kg), rapidly available potassium (AK, mg/kg), hydrolyzable nitrogen (HN, g/kg), organic matter (OM, g/kg), available zinc (AZn, mg/kg), available iron (AFe, mg/kg), available manganese (AMn, mg/kg), available boron (AB, mg/kg), exchangeable calcium (ECa, cmol(1/2Ca^{2+})/kg), exchangeable magnesium (EMg, cmol(1/2Mg^{2+})/kg), soil pH, and electrical conductivity of soil water (ECsw).

Comparison of soil nutrient element contents among the three producing regions is shown in the figure b. Organic matter (OM, 35.64 – 55.24 g/kg) exhibited the highest content in all soils, followed by TK (15.19 – 20.22 g/kg), TN (1.27 – 2.30 g/kg), and TP (0.77 – 1.73 g/kg), with AK, AMn, HN, AP, AB, AFe, AZn, EMg, and ECa in descending order.All soil indicators in FJJY were higher than those in the other two regions except for AB, which may be related to its geographical condition as it is closest to the sea.Compared with FJLQ, FJDZ showed higher contents of AP, HN, AZn, AMn, ECsw, ECa, OM, TN, and TP, whereas FJLQ displayed higher levels of the remaining indicators (**Figure4.** b and **Supplementary Table S**4).

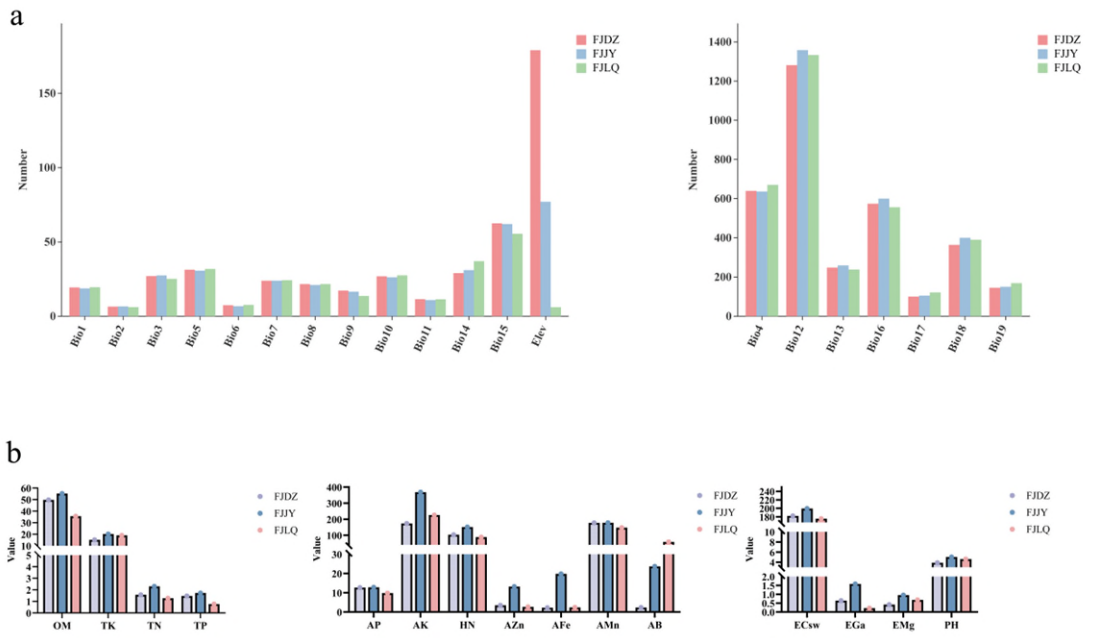


Figure4. Bar charts of climatic factors (a); Soil elements (b).

Determination Results of Soil Microorganisms in Each Producing Region

Rhizosphere microorganisms serve as crucial regulators in the quality formation of medicinal plants.To investigate the differences in rhizosphere microorganisms and their effects on the quality of *Citrus reticulata* 'Tangerina' from different regions, rhizosphere microbes (bacteria and fungi) were analyzed (**Supplementary Table S**5). In total, 11 fungal phyla, 18 bacterial phyla, 226 fungal genera, and 174 bacterial genera were identified.*Proteobacteria* was the most dominant bacterial phylum, accounting for 14.95% – 43.55%, followed by *Actinobacteriota* (10.14% – 37.08%) and *unidentified_Bacteria* (4.72% – 16.07%);*Ascomycota* was the most abundant fungal phylum, accounting for 77.43% – 84.80%, followed by *Basidiomycota* (3.39% – 16.42%) and *Mortierellomycota* (0.16% – 1.19%) (**Figure5.** a and b). Unconstrained principal coordinate analysis (PCoA) was performed on bacterial and fungal communities. The total explanatory power of PCoA1 and PCoA2 reached 94.17% for bacteria and 97% for fungi (**Figure5.** c and d).Alpha diversity of fungal and bacterial communities was evaluated using multiple indices (**Figure5.** e and f). Multiple indices were used to analyze the alpha diversity of bacterial and fungal communities in different samples. Tukey's test showed that all indicators differed significantly ($P < 0.05$), with the exception of fungal Good's coverage, demonstrating significant differences in microbial species composition and abundance among samples from different habitats (**Supplementary Table S6**).

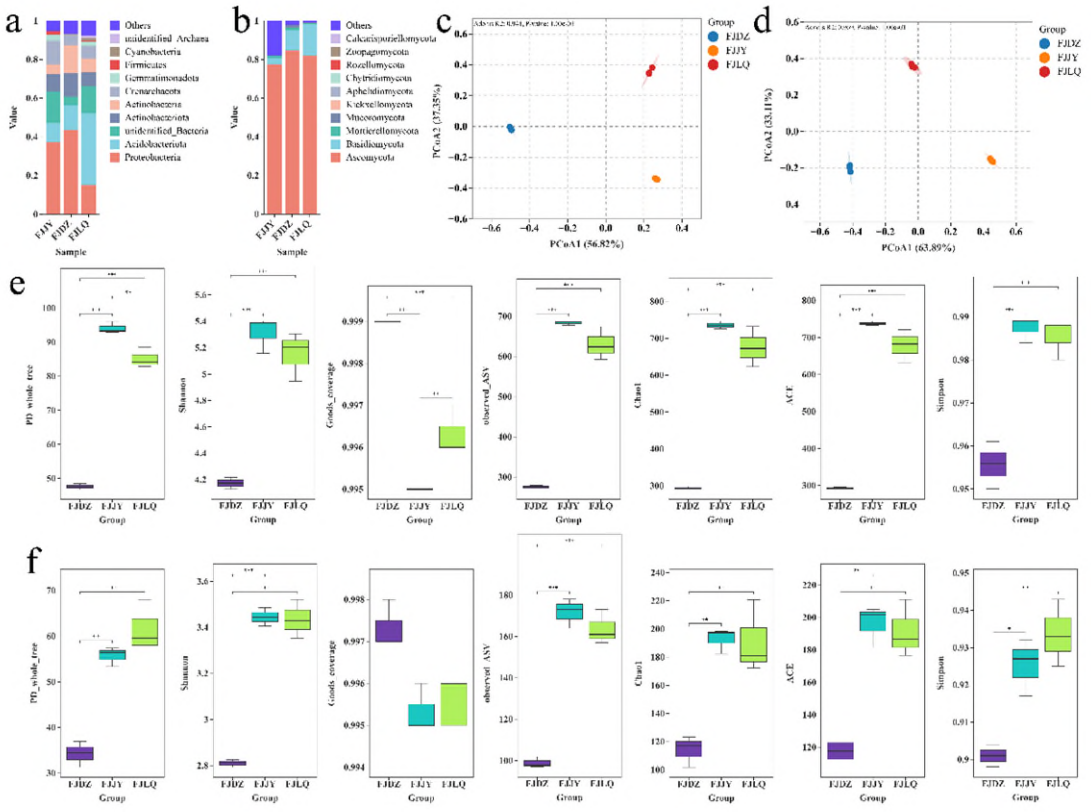


Figure5. Stacked bar graph of the top 10 dominant bacterial phyla (a); Stacked bar graph of the top 10 dominant fungal phyla (b); Bacterial PCoA plot (c); Fungal PCoA plot (d); Bacterial alpha diversity (e); Fungal alpha diversity (f).

Correlation Analysis of Climatic Factors with Chemical Components of *Citrus reticulata 'Tangerina'*

Based on the GMPGIS system, correlation analysis between climatic factors and chemical constituents of *C. reticulata 'Tangerina'* was performed, as shown in the figures a.Correlation analysis between active compounds of *C. reticulata 'Tangerina'* revealed that 4',5,7,8-Tetramethoxyflavone was highly significantly positively correlated with elevation; 3,5,6,7,8,3',4'-heptamethoxyflavone was significantly positively correlated with Bio11; Nobiletin was significantly negatively correlated with Bio12 and Bio18; Hesperidin was significantly negatively correlated with Bio12; and Sinensetin was significantly negatively correlated with Bio12 (**Figure6.** a).

Correlation analysis between volatile components of *Citrus reticulata 'Tangerina'* showed that Neral was significantly positively correlated with Bio15;1,3,6-Octatriene,3,7-dimethyl- was

significantly positively correlated with Bio2;2-isopropyl-5-methyl-Anisole was significantly negatively correlated with Bio12 and highly significantly negatively correlated with Bio18;Tetradecane was significantly positively correlated with elevation;Geranylacetate was significantly negatively correlated with Bio2 and Bio3;Octanal was highly significantly negatively correlated with Bio15 and significantly positively correlated with Bio7;2,4-Decadienal,(E,E)- was significantly negatively correlated with Bio15;3-Thujene was significantly positively correlated with Bio12 and Bio18;Sabinene was significantly negatively correlated with Bio11 (**Figure6.** b).

Correlation between Citrus reticulata 'Tangerina' and Rhizosphere Soil Elements

Correlation analysis was conducted between soil elements and active compound data (**Figure6.** c). TK was significantly negatively correlated with Demethylnobiletin and Nobiletin. Soil pH was significantly negatively correlated with Nobiletin, and highly significantly negatively correlated with Hesperidin and Sinensetin.

Correlation analysis with volatile components was performed (**Figure6.** d).2,4-Decadienal,(E,E)- was significantly negatively correlated with AP and AMn; Geranylacetate was significantly negatively correlated with TP and OM; 2-isopropyl-5-methyl-Anisole was significantly negatively correlated with TK; Copaene was significantly negatively correlated with AK; (1S)-(-)-alpha-Pinene was significantly positively correlated with EMg; 6-Octenal,3,7-dimethyl-,(R)- was significantly positively correlated with TK; 3-Thujene was significantly positively correlated with Soil pH; Tridecane was significantly negatively correlated with AB;Neral was significantly positively correlated with AP and AMn; 1,3,6-Octatriene,3,7-dimethyl- was significantly positively correlated with AP and highly significantly positively correlated with OM.

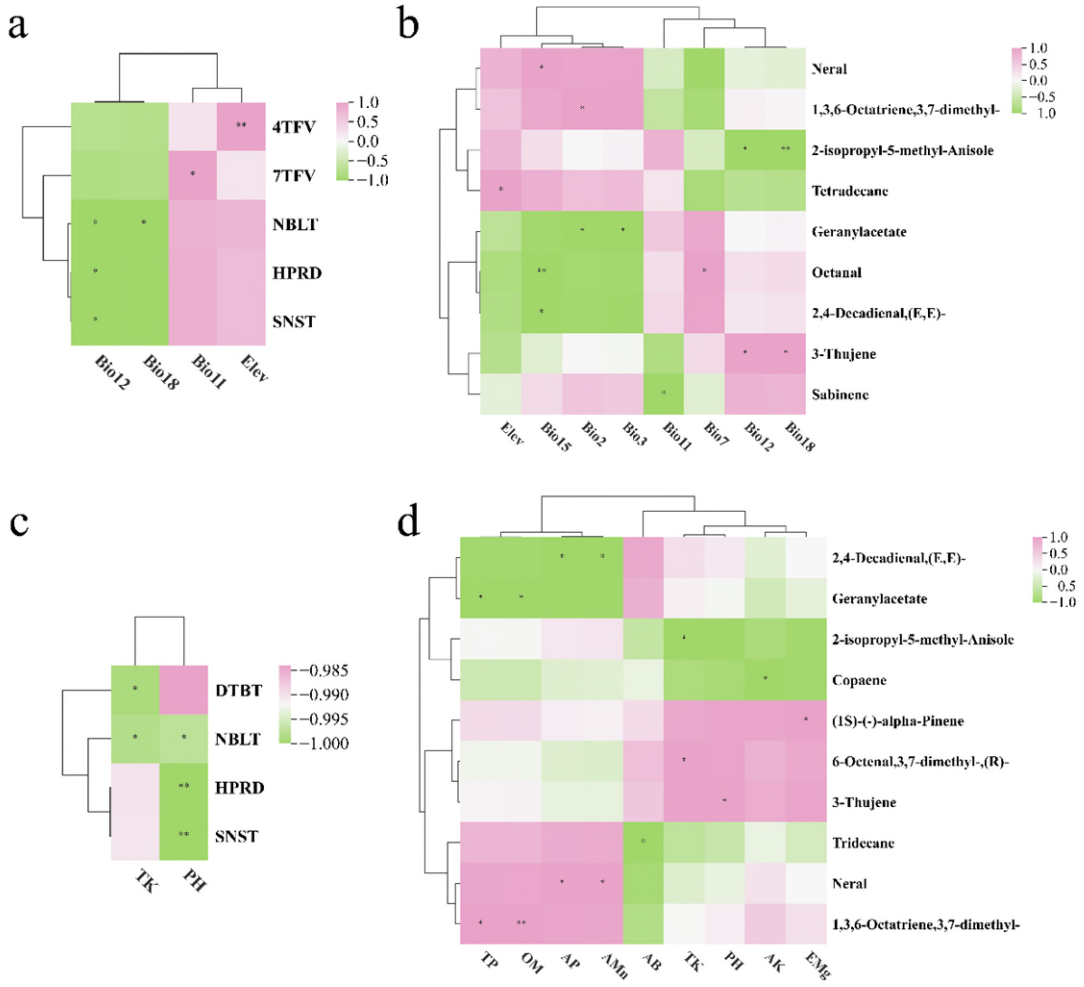


Figure6. Clustered heatmap of correlations between climatic factors and bioactive components (a); Clustered heatmap of correlations between climatic factors and volatile components (b); Clustered heatmap of correlations between root soil elements and bioactive components (c); Clustered heatmap of correlations between root soil elements and volatile components (d).

Correlation between *Citrus reticulata 'Tangerina'* and Rhizosphere Soil Microorganisms

Rhizosphere microorganisms play a vital role in the normal growth and development of medicinal plants and act as key regulators in the formation of their quality.To investigate the differences in rhizosphere microorganisms among *Citrus reticulata 'Tangerina'* producing regions and their effects on fruit quality, correlation analysis was performed between the top 10 most abundant

bacterial and fungal genera and chemical compounds in each region, as shown in the figure.

In the correlation analysis between microorganisms and active components of *Citrus reticulata 'Tangerina'* (**Figure7. a**), 3,5,6,7,8,3',4'-Heptamethoxyflavone was significantly negatively correlated with *Dongia*, and also significantly negatively correlated with *Pseudolabrys*, *Bacillus*, and *Sphingomonas*; Demethylnobiletin was significantly positively correlated with *Hawksworthiomyces*.

In the correlation analysis between microorganisms and volatile components (Figure b), Neral was significantly negatively correlated with *unidentified_Cyanobacteria* and extremely significantly negatively correlated with *Mycena*;(R)-3,7-dimethyl-6-octenal was significantly negatively correlated with *Hawksworthiomyces*;(1S)-(-)-alpha-Pinene was significantly negatively correlated with *Jatrophihabitans;*Sabinene was significantly positively correlated with *Pseudolabrys*, *Bacillus*, and *Sphingomonas*;Octanal was highly significantly positively correlated with *unidentified_Cyanobacteria* and significantly positively correlated with *Mycena*;2,4-Decadienal,(E,E)- was significantly positively correlated with *unidentified_Cyanobacteria* and extremely significantly positively correlated with *Mycena*;2-isopropyl-5-methylanisole was significantly positively correlated with *Hawksworthiomyces*;Copaene was significantly negatively correlated with *Fusarium*, significantly positively correlated with *Mycobacterium*, and highly significantly positively correlated with *Occallatibacter*(**Figure7. b**).

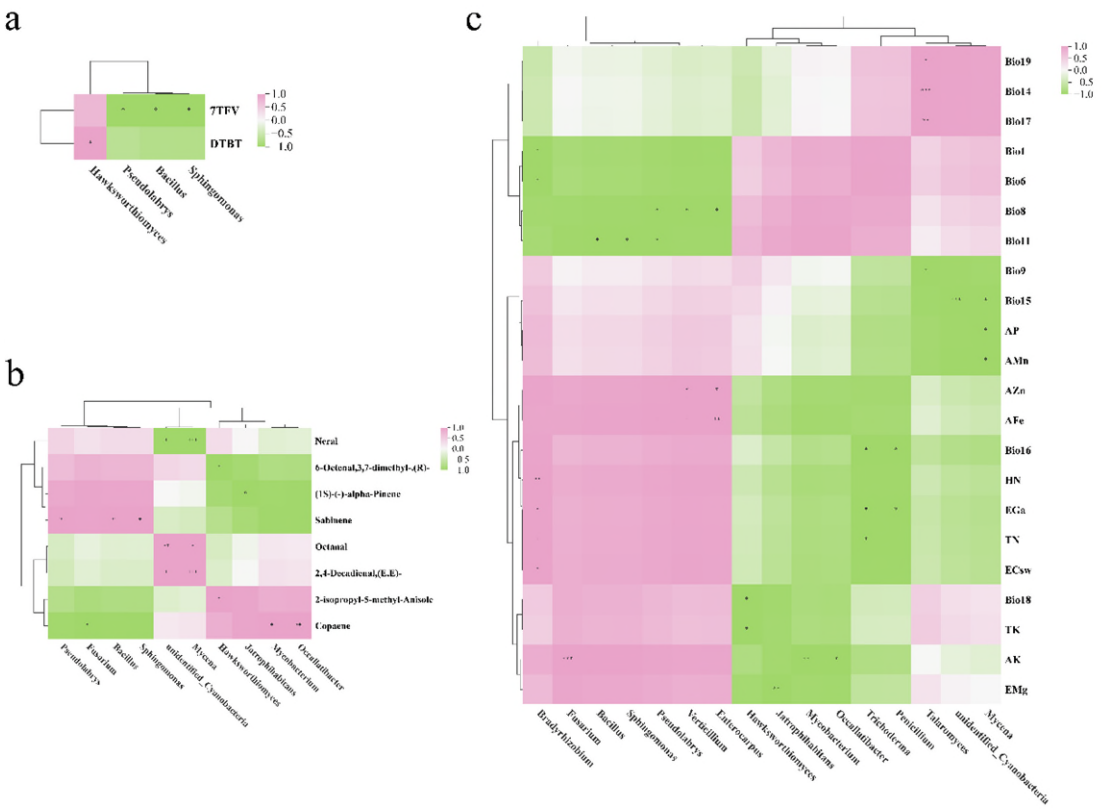


Figure7. Clustered heatmap of correlations between microorganisms and bioactive components (a); Clustered heatmap of correlations between microorganisms and volatile components (b); Clustered heatmap of correlations of root soil elements and climatic factors with microorganisms (c).

Discussion and Conclusions

Discussion

Active ingredients, mainly secondary metabolites, are crucial for the quality evaluation of medicinal herbs (Luo et al. 2023). As one of the four major types of Chenpi, *Citrus reticulata 'Tangerina'* contains bioactive components dominated by flavonoids and volatile oils. Flavonoids exert important effects in anticancer, antioxidant activities, and immune regulation (Liskova et al. 2021). Among them, Hesperidin, Nobiletin, and Tangeretin are the most critical active ingredients, and their contents are used as the quality criteria for Chenpi in the Chinese Pharmacopoeia. In this study, HPLC analysis of *Citrus reticulata 'Tangerina'* from three producing regions showed that Hesperidin content ranged from 5.99% to 8.91%, and the total content of Nobiletin and Tangeretin ranged from 0.83% to 1.15%, all of which met the quality standards specified in the "Chinese

Pharmacopoeia" (2025 Edition). Samples from FJDZ exhibited the highest overall component contents, suggesting that geographical origin is a key factor affecting the quality of *Citrus reticulata 'Tangerina'*. A total of 54 volatile compounds were identified by GC-MS, with D-Limonene, Linalool and .gamma.-Terpinene as the dominant components, consistent with the traditional aroma characteristics of Chenpi (Shi et al. 2024). PCA and PLS-DA revealed clear discrimination among samples from different regions, indicating that the ecological environment regulates secondary metabolism and ultimately contributes to the geographically specific quality of Chenpi.

Climatic environment not only affects the distribution, growth and development of crops, but also influences the accumulation of their secondary metabolites. The present study showed that elevation, Bio11, Bio12, and Bio18 were significantly correlated with flavonoids in *Citrus reticulata 'Tangerina'*, whereas Bio11, Bio12, Bio18, Bio15, Bio2, Bio3, and Bio7 were significantly correlated with volatile components. Precipitation was negatively correlated with flavonoid accumulation. Excessive precipitation may increase soil saturation, reduce soil aeration (He et al. 2024) and temperature (Costa et al. 2023), impairing nutrient uptake efficiency by crop roots and thereby inhibiting flavonoid biosynthesis. Correlation analysis revealed that elevation was significantly correlated with 4',5,7,8-Tetramethoxyflavone, and appropriate altitude could promote the accumulation of flavonoids (Zhou et al. 2025). Temperature affects enzyme activities in crops (Guo et al. 2025), as well as rhizosphere soil enzymes and microorganisms (Zhao et al. 2023), thus regulating metabolic accumulation and growth. Suitable temperatures promote the biosynthesis of secondary metabolites such as flavonoids and terpenoids (J. He et al. 2023), enhancing the medicinal efficacy and aroma quality of *Citrus reticulata 'Tangerina'*. These results indicate that *Citrus reticulata 'Tangerina'* exhibits specific adaptation to warm, humid habitats with mild seasonal variations.

Rhizosphere soil serves as the growth matrix for plants, and its nutrient status and physicochemical properties directly impact the quality of medicinal materials. The present study revealed that soil total potassium (TK) and soil pH significantly affected flavonoid accumulation in *Citrus reticulata 'Tangerina'*, whereas total phosphorus (TP), organic matter (OM), available phosphorus (AP), available manganese (AMn), available boron (AB), TK, soil pH, available potassium (AK), and exchangeable magnesium (EMg) significantly influenced volatile oil accumulation. Crop growth

relies on soil nutrients; for instance, nitrogen supply is essential for metabolic activities, protein synthesis, and vegetative growth (Zhang et al. 2020). Appropriate potassium supply promotes crop growth, enhances drought resistance, disease resistance, and salt tolerance, and facilitates nitrogen uptake and transport (Wang et al. 2023). Potassium application under potassium-deficient conditions elevates photosynthetic rate, promotes CO_2 assimilation, increases the partitioning of assimilates to spikes and roots, thereby accelerating grain growth rate and yield, and supporting robust plant growth (Li et al. 2022; Liu et al. 2024). However, excessive soil nutrients tend to cause excessive vegetative growth (Cai et al. 2025; Won et al. 2025a), poor fruit coloration (Mo et al. 2022), and reduced leaf chlorophyll content (Won et al. 2025b), which are detrimental to secondary metabolite accumulation. Additionally, soil pH exhibited significant negative correlations with Hesperidin, Nobiletin, and Sinensetin, suggesting that *Citrus reticulata 'Tangerina'* prefers slightly acidic to neutral soils.

Rhizosphere microorganisms are regarded as the "second genome" of medicinal plants (Jia et al. 2020; Jiang et al. 2023). They interact with plants directly and indirectly, and participate in quality formation by regulating nutrient uptake by plant roots, modulating phytohormones, and inhibiting pathogens. The present study revealed that the rhizosphere microbial genera *Dongia*, *Pseudolabrys*, *Bacillus*, *Sphingomonas*, and *Hawksworthiomyces* were significantly correlated with flavonoid components in *Citrus reticulata 'Tangerina'*. The genera *Pseudolabrys*, *Bacillus*, *Sphingomonas*, *unidentified_Cyanobacteria*, *Mycena*, J*atrophihabitans*, *Fusarium*, *Mycobacterium*, and *Occallatibacter* were significantly correlated with volatile components. Su et al. (2023) reported that *Streptomyces strain Strep-4* and *SynCom* promoted the accumulation of terpenoids in citrus peels. Rhizosphere microorganisms can colonize using root exudates (sugars and organic acids). They not only promote humus accumulation and carbon sequestration in rhizosphere soil (Yang et al. 2025), but also stimulate lateral root growth (Li et al. 2026). thereby improving nutrient uptake efficiency (Qiu et al. n.d.), regulating soil pH (Santoyo et al. 2026), remediating contaminated soil (Xie et al. 2022), and enhancing crop stress resistance (Ren et al. 2023). Consequently, they exert crucial regulatory effects on crop growth and development, the accumulation of secondary metabolites, and plant health.

Correlation analysis was performed between the top 10 most abundant bacterial and fungal genera

and both climatic factors and soil elements (**Figure7.** c). Most microorganisms showed significant correlations with environmental and soil variables. In general, microorganisms were significantly negatively correlated with climatic factors, suggesting a preference for dry and cool environments, with the exception of the fungal genus *Talaromyces*, which may favor humid conditions. For example, Neral was significantly positively correlated with soil AP and AMn, as well as climatic factor Bio15, and extremely significantly negatively correlated with the microbial genus *Mycena*. Further analysis revealed that AP, AMn, and Bio15 were all significantly negatively correlated with *Mycena*, indicating that AP, AMn, and precipitation may inhibit the growth of *Mycena*, thereby promoting Neral accumulation in *Citrus reticulata 'Tangerina'*. Demethylnobiletin was significantly positively correlated with the genus *Hawksworthiomyces* and significantly negatively correlated with soil TK. In turn, *Hawksworthiomyces* was significantly negatively correlated with TK. These results suggest that TK may indirectly negatively regulate Demethylnobiletin accumulation by inhibiting the growth and colonization of *Hawksworthiomyces*. Copaene was significantly negatively correlated with the genus *Fusarium* and soil AK, while *Fusarium* was highly significantly positively correlated with AK. High AK levels may enrich *Fusarium*, which may preferentially occupy rhizosphere nutrient niches and compete for potassium resources, reducing the efficiency of potassium uptake and utilization by plants, thereby interfering with secondary metabolic pathways and inhibiting Copaene biosynthesis. Conversely, under moderate AK conditions, the abundance of *Fusarium* is restricted, and root potassium uptake and transport are more efficient, favoring the flux of terpenoid metabolism toward Copaene synthesis and thus promoting its accumulation.

Taken together, the quality of *Citrus reticulata 'Tangerina'* is the result of the synergistic effects of climate, rhizosphere soil elements, and rhizosphere microorganisms. Climate determines habitat suitability, soil elements provide the material basis, and rhizosphere microorganisms regulate metabolism at the rhizosphere level. Differences in ecological factors among different producing regions further drive the differentiation of secondary metabolic accumulation patterns in *Citrus reticulata 'Tangerina'*, which is the reason for the formation of its authenticity. From the perspective of multi-omics ecology, this study analyzed the effects of ecological factors on the quality of *Citrus reticulata 'Tangerina'* and provided a reference for the quality evaluation of other authentic citrus

medicinal materials. However, genetic factors were not investigated, so their impact on *Citrus reticulata 'Tangerina'* quality cannot be excluded. In addition, the producing regions selected in this study have a small geographical span, making it difficult to fully reflect the quality response rules under ecological differences. Future studies should expand the sampling scope and geographical span, combine genetic data such as genomics and transcriptomics, and conduct in-depth multi-dimensional correlation research on "genetics-environment-microorganisms", so as to more systematically and comprehensively clarify the intrinsic mechanism of the formation of *Citrus reticulata 'Tangerina'* authenticity.

Conclusions

The results showed that the chemical components of *Citrus reticulata 'Tangerina'* were significantly correlated with rhizosphere soil elements (TP, OM, AP, AMn, AB, TK, pH, AK, and EMg), rhizosphere microorganisms (genera *Dongia, Pseudolabrys*, *Bacillus*, *Sphingomonas*, *Hawksworthiomyces*, *unidentified_Cyanobacteria*, *Mycena*, *Jatrophihabitans*, *Fusarium*, *Mycobacterium*, and *Occallatibacter*), and environmental factors (elevation, Bio11, Bio12, Bio18, Bio15, Bio2, Bio3, and Bio7). In other words, these ecological factors can drive the accumulation of metabolic components in *Citrus reticulata 'Tangerina'*, thereby affecting its quality. This study identified the key ecological factors driving the quality formation of *Citrus reticulata 'Tangerina'*, providing a scientific basis for the quality regulation, producing region suitability evaluation, and standardized cultivation of authentic Chenpi.

Materials and Methods

Sample Collection and Processing Methods

The experimental samples were *Citrus reticulata 'Tangerina'*, collected from three producing regions within Fujian Province.The collected samples included *Citrus reticulata 'Tangerina'* fruits, *Citrus reticulata 'Tangerina'* rhizosphere soil, and *Citrus reticulata 'Tangerina'* rhizosphere microorganisms.

Three individual trees were randomly selected at each production site, and 30 citrus fruits were randomly picked from each tree, yielding a total of 90 fruits per site. The harvested fruits were peeled, air-dried, ground, and sieved through a 40-mesh sieve, then stored at −20 °C until further analysis. Rhizosphere soil samples were collected from the three selected trees at three different directions, 1 m away from the trunk and 30 cm above the ground. Soils were air-dried in the shade, cleaned of impurities such as stones, residual roots, and fallen leaves, then sieved through a 40-mesh sieve. For microbial analysis, rhizosphere samples were collected and placed in 15 mL centrifuge tubes, with one tube per tree. All samples were stored on dry ice during transportation and transferred to a −80 °C freezer upon arrival at the laboratory, and thoroughly homogenized before detection.

Chemical Substances

Reagents: Chromatographic grade acetonitrile was purchased from Merck Co., Ltd.; chromatographic grade formic acid was purchased from Shanghai Yien Chemical Technology Co., Ltd.; experimental water was ultrapure water (Millipore Corporation, Bedford, MA, USA); and analytical grade methanol was purchased from Xilong Scientific Co., Ltd. Standards: Hesperidin (Batch No.: JN269789), Sinensetin (Batch No.: J27IB221137), 4',5,7,8-Tetramethoxyflavone (Batch No.: P09M10F82674), nobiletin (Batch No.: JB256254), 3,5,6,7,8,3',4'-Heptamethoxyflavone (Batch No.: J21HB173466), Tangeretin (Batch No.: H24F11K108893), and Demethylnobiletin (Batch No.: N29IB233903) were all purchased from Shanghai yuanye Bio-Technology Co., Ltd..

Collection of Climatic Factor Data

Nineteen Climatic factors for each production region were obtained from the WorldClim (World Clim-global climate data) and the CliMond (Cli Mond: global climatologies for bioclimatic modelling）.Based on the longitude and latitude coordinates recorded during sample collection, the 19 Climatic factors for *Citrus reticulata 'Tangerina'* from different regions were extracted using GMPGIS: Annual mean temperature (bio1), Mean diurnal range (bio2), Isothermality (bio3), Temperature seasonality (bio4), Max temperature of warmest month (bio5), Min temperature of coldest month (bio6), Temperature annual range (bio7), Mean temperature of wettest quarter (bio8),

Mean temperature of driest quarter (bio9), Mean temperature of warmest quarter (bio10), Mean temperature of coldest quarter (bio11), Annual precipitation (bio12), Precipitation of wettest month (bio13), Precipitation of driest month (bio14), Precipitation seasonality (bio15), Precipitation of wettest quarter (bio16), Precipitation of driest quarter (bio17), Precipitation of warmest quarter (bio18), and Precipitation of coldest quarter (bio19). Elevation (Elev) was obtained via GPS positioning during sampling.

Determination of Rhizosphere Soil Elements

Fifteen key soil physicochemical indicators were determined in accordance with Chinese national standards, with the specific methods and standard codes listed as follows: total nitrogen (Kjeldahl method, LY/T 1228-2015); total phosphorus/available phosphorus (molybdenum-antimony colorimetric method, LY/T 1232-2015/HJ 704-2014, respectively); total potassium/available potassium (flame photometric method, LY/T 1234-2015/NY/T 889-2004, respectively); organic matter (potassium dichromate volumetric method, LY/T 1237-1999); available Zn/Fe/Cu/Mn (DTPA extraction method, NY/T 890-2004); available boron (curcumin colorimetric method, LY/T 1258-1999); exchangeable Ca/Mg (atomic absorption spectrophotometry, LY/T 1245-1999); pH (potentiometric method, HJ 962-2018); and electrical conductivity (electrode method, HJ 802-2016).

Amplicon Sequencing of Root Soil Microorganisms

Total genome DNA from samples was extracted using CTAB method. DNA concentration and purity was monitored on 1% agarose gels. According to the concentration, DNA was diluted to 1ng/μL using sterile water.

The bacterial 16S rRNA genes of the V3-V4 region were amplified using specific primers 314F （CCTAYGGGRBGCASCAGwith the barcode）and 806R(GGACTACNNGGGTATCTAAT) . The fungal ITS genes of the ITS1-1F region were amplified using specific primers ITS1-1F-F （CTTGGTCATTTAGAGGAAGTAA） and ITS1-1F-R (GCTGCGTTCTTCATCGATGC) with the barcode. All PCR reactions were carried out with 15 μL of Phusion® High-Fidelity PCR Master Mix (New England Biolabs); 2μM of forward and reverse primers, and about 10 ng template DNA. Thermal cycling consisted of initial denaturation at 98 °C for 1 min, followed by 30 cycles of denaturation at 98 °C for 10 s, annealing at 50°C for 30 s, and elongation at 72°C for 30 s. Finally

72°C for 5 min.

Mix same volume of 1X loading buffer (contained SYBR Green) with PCR products and operate electrophoresis on 2% agarose gel for detection. PCR products was mixed in equidensity ratios. Then, mixture PCR products was purified with Qiagen Gel Extraction Kit (Qiagen, Germany).

Sequencing libraries were generated using TruSeq® DNA PCR-Free Sample Preparation Kit (Illumina, USA) following manufacturer's recommendations and index codes were added. The library quality was assessed on the Qubit@ 2.0 Fluorometer (Thermo Scientific) and Agilent Bioanalyzer 2100 system. At last, the library was sequenced on an Illumina NovaSeq platform and 250 bp paired-end reads were generated.

HPLC Determination Method of Active Components

Samples were ground and sieved through a 40-mesh sieve. Exactly 0.2 g of each sample was weighed in triplicate and transferred into 50 mL Erlenmeyer flasks, followed by the addition of 25 mL of methanol. Ultrasonic extraction was performed for 45 min. After cooling to room temperature, the supernatant was collected with a syringe, filtered through a 0.22 μm organic-phase syringe filter, transferred to LC sample vials, and stored for subsequent injection.

HPLC analysis was performed on an LC-20AT high-performance liquid chromatography system (Shimadzu, Japan) equipped with an Eclipse XDB-C18 column (250 mm × 4.6 mm, 5 μm).The mobile phase consisted of pump A (0.1% formic acid in water) and pump B (acetonitrile).Gradient elution was applied as follows: 0–10 min, 20%–30% B; 10–25 min, 30%–42% B; 25–37 min, 42%–70% B; 37–40 min, 70% B.Detection wavelengths were set at 283 nm (Hesperidin) and 330 nm (Sinensetin, 4',5,7,8-Tetramethoxyflavone, Nobiletin, 3,5,6,7,8,3',4'-Heptamethoxyflavone, Tangeretin, and Demethylnobiletin).The flow rate was 1.0 $mL \cdot min^{-1}$, the column temperature was maintained at 30 ℃, and the injection volume was 10 μL.

Determination of Volatile Components by GC-MS

Exactly 0.2 g of *Citrus reticulata 'Tangerina'* powder (sieved through a 40-mesh sieve) from each production region was accurately weighed into 20 mL headspace vials and sealed with aluminum caps, with three replicates per sample.The sealed vials were placed in the sample tray for analysis

under the following conditions: vial heating temperature 120 ℃, vial equilibration time 10 min, loop temperature 130 ℃, injection time 0.5 min, transfer line temperature 140 ℃, and pressure equilibration time 0.5 min.

Volatile compounds were analyzed using an Agilent 7890A GC system coupled with a 5975C MS detector equipped with a MassHunter workstation. Separation was performed on an Agilent 19091S-433 (HP-5MS) capillary column (30 m × 250 μm × 0.25 μm). Headspace injection was adopted with an injection volume of 1 μL, injector temperature of 220 ℃, and MSD transfer line temperature of 280 ℃. High-purity helium (>99.999%) was used as the carrier gas with a split ratio of 30:1.The oven temperature program was set as follows: initial temperature 50 ℃; ramped at 4 ℃/min to 90 ℃ and held for 1 min; then at 10 ℃/min to 120 ℃ and held for 1 min; then at 3 ℃/min to 160 ℃ and held for 1 min; then at 30 ℃/min to 200 ℃ and held for 1 min; and finally at 15 ℃/min to 250 ℃.An electron ionization (EI) source was used with an electron energy of 70 eV. The ion source temperature was 230 ℃ and the quadrupole temperature was set at a maximum of 150 ℃. The solvent delay time was 3.60 min, and the total run time was 35 min.The mass spectrometer was operated in full-scan mode with a mass range of m/z 40.0–450.0 Da.

Data Processing

HPLC data of batch samples from different production regions were imported into the “Similarity Evaluation System for Chromatographic Fingerprint of Traditional Chinese Medicine” (2012 Edition) with the following procedures: a reference chromatogram was set, the average method was adopted, the time window width was set to 0.1 min, and marker peak matching was performed via multi-point correction. Subsequently, the chromatographic peak areas of the seven target compounds and the corresponding fingerprint profiles of the samples were generated. The data were then imported into the Metware Cloud Platform for PCA analysis, and GraphPad Prism 10 software was used to draw bar charts for comprehensive analysis.

Each batch of samples was analyzed in triplicate, and the average response values determined by 14 sensors were selected as characteristic variables.GC-MS data were imported into DataAnalysis 2013 software for processing. The relative contents of each component were calculated from total ion chromatograms using the area normalization method. After peak integration, compound

identification was performed by matching mass spectra against the built-in NIST20.L standard mass spectral database (National Institute of Standards and Technology, USA), and the search reports were exported.Compounds with a match degree ≥ 80% were screened via CAS number search. Retention time alignment was conducted by matching retention times, accurate molecular masses, and fragment ions with reference compounds, combined with online databases and literature. The processed data were then subjected to multivariate statistical analysis.Bar charts were plotted using GraphPad Prism 10 software; fingerprint profiles and pie charts were generated using Origin 2025 software; and cluster heatmaps, correlation heatmaps, PCA, and PLS-DA analyses were performed on the Metware Cloud Platform.

Correlation Analysis

Correlation analysis was performed between climatic conditions, soil properties, soil microorganisms from different habitats and both the active components determined by HPLC and the differential volatile compounds identified and screened by GC-MS. Pearson's correlation analysis combined with heatmap visualization was further used to investigate the effects of these ecological factors on the chemical constituents of *Citrus reticulata 'Tangerina'*. Significance levels were defined as $P < 0.05$ (significant), $P < 0.01$ (highly significant), and $P < 0.001$ (extremely significant).

Data Availability

All data generated and analysed during this study are included in the supplementary tables. Further relevant raw data are available from the corresponding author upon reasonable request.

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Author Contributions

The research conception and manuscript writing were completed by Kailin Qiao. The experimental scheme was designed by Kailin Qiao, Luxin Xie and Shouwen Zhang. The collection, investigation and pretreatment of experimental materials were finished by Bo Wang and Xinlin Peng. Data analysis was performed by Yan Luo and Yu Zhu. Luxin Xie, Qian Qin and Shouwen Zhang provided the main guidance for this experiment. Qian Qin and Shouwen Zhang were responsible for manuscript revision and funding acquisition.

Data availability

We deposited sequencing data in the Sequence Read Archive (SRA), National Center for Biotechnology Information (NCBI; accession no. PRJNA1471510 and PRJNA1471612).

Competing interests

The authors declare no competing interests.

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