

Soil with deficient inorganic elements promotes the accumulation of hesperidin , nobiletin and tangeretin in pericarps of Citrus reticulata ‘Chachi’

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Abstract

Aims

Environmental conditions, such as soil stress, are crucial for the accumulation of secondary metabolites in medicinal plants. This study aims to determine which soil type and environmental factors can promote the accumulation of *hesperidin*, *nobiletin* and *tangeretin* in the pericarps of *Citrus reticulata* 'Chachi' (*Citrus reticulata* cv. Chachiensis).

Methods

This study selected *Citrus reticulata* 'Chachi' planted in paddy soil, red soil, alluvial soil and conducted correlation between meteorological, soil factors and the three components. Then, linear regression models (LRMs) and random forest models (RFMs) were used to screen the soil factors affecting accumulation of the three components. Structural equation models (SEMs) were established to further explore how key soil factors affect the accumulation of the three components.

Results

Our results indicated that there was no significant difference in meteorological factors among the three regions ($p > 0.05$), and no correlation was found between the meteorological factors and the three components. Soil factors in three soil types, such as K, Ca, Mg, Mn, salinity and pH, have significant impacts on the content variation of *hesperidin*, *nobiletin* and *tangeretin*. The deficiency of K and Ca, play a crucial role in the accumulation of the three components.

Conclusions

Citrus reticulata 'Chachi' cultivated in soil with deficient K and Ca is more inclined to accumulate *hesperidin*, *nobiletin* and *tangeretin* in pericarps, which provides guidance for reasonable cultivation and paddy, red and alluvial soil improvement to promote the contents of medicinal components and the quality of medicinal plants.

Introduction

Citrus reticulata 'Chachi' (*Citrus reticulata* cv. Chachiensis) is a medicinal plant, which belongs to the Rutaceae and is one of the varieties of *Citrus reticulata* Blanco. It is predominately cultivated in Guangdong Province, China, especially in Xinhui District, Jiangmen city. *Hesperidin*, *nobiletin* and *tangeretin* are important polyphenolic secondary metabolites, which belong to flavonoid (Tripoli et al. 2007; Sun et al. 2010; Alam et al. 2021), and are one of the major medicinal components and official chemical references for quality control of dry pericarp of *Citrus reticulata* 'Chachi' in the Chinese Pharmacopoeia owing to its relatively high concentration in peels (In terms of dry sample: *hesperidin*, over 2.0%; *nobiletin* and *tangeretin*, over 0.42%) (Committee of National Pharmacopoeia 2020). *Hesperidin*, *nobiletin* and *tangeretin* possess various biological activities for human health, such as

antioxidant, antidiabetic, antiallergic, anti-inflammatory, anti-carcinogenic, antiallergic and anti-atherosclerosis properties (Garg et al. 2001; Ho et al. 2014; Roohbakhsh et al. 2014; Devi et al. 2015; Cui et al. 2019; Liu et al. 2020a; Zou et al. 2022).

Previous studies have reported that external environmental conditions, such as temperature, humidity, light, soil, abiotic biotic and stresses, and endogenous factors such as genetics, ontogeny, morphogenesis, hormone and maturity, commonly influenced the accumulation and biosynthesis of secondary metabolism, for example, flavonoid. (Naik et al. 2022; Shen et al. 2022). Temperature and humidity can affect the enzyme activity of secondary metabolism (Liang et al. 2008; Verma and Shukla 2015; Li et al. 2022). Light intensity, wavelength, color and photoperiod affect the net photosynthetic rate and the synthesis of sugar and phenylalanine in secondary metabolic processes (Zeng et al. 2010; Yang et al. 2018; Li et al. 2020). Soil environmental stresses, such as soil type, mechanical composition, salinity, moisture, heavy metal, and nutrition deficiency, also influence the accumulation of secondary metabolism in medical plants (Verma and Shukla 2015; Dar et al 2016; Ma et al 2018). However, the relationship between environmental conditions and *hesperidin*, *nobiletin*, *tangeretin* in *Citrus reticulata* 'Chachi' is poorly understood. In order to effectively improve the three medicinal components in the dry pericarp of *Citrus reticulata* 'Chachi', it is necessary to study how environmental conditions influence the contents of *hesperidin*, *nobiletin* and *tangeretin*.

Soil provides a rooting environment, an important medium for germination, growth, development, and fruit formation of medical plants during life cycles (Yuan et al. 2020; Pant et al. 2021). It also supplies a necessary medium for plants to undergo water and nutrient absorption, material and energy exchange (Liu et al. 2020b). The previous field investigation in the study found that *Citrus reticulata* 'Chachi' in Guangdong Province is mainly rooted in paddy, red and alluvial soils. The three soils are different in soil formation mechanism and may have quite different nutritional status. Paddy soil and alluvial soil are commonly initially formed by sediment deposition in river channels, mainly distributed along the downstream riverbanks of Xijiang and Tanjiang rivers. *Citrus reticulata* 'Chachi' cultivated in these types of soil is called "Paddy Field Citrus" ("Shuitian-gan" in Chinese). Red soil is formed by weathering of rocks, mainly distributed in the surface layer of hilly slopes. *Citrus reticulata* 'Chachi' cultivated in this type of soil is called "Montane Citrus" ("Shandi-gan" in Chinese). Meteorological conditions affect the weathering process and hydrothermal conditions of soil, playing a crucial role in the material cycle and energy flow of soil nutrition and microbial activity (Hu et al. 2021; Dong et al. 2022). Moreover, meteorological factors can also directly affect fruit quality of higher plants, such as citrus and apple (Zhang et al. 2018a; He et al. 2022). However, little is known about which major environmental factors induce concentration differences in *hesperidin*, *nobiletin* and *tangeretin* of *Citrus reticulata* 'Chachi' at different types of soils, and whether different soils formed environmental stresses that affects the content of the three components. Hence, the purpose of this study is to clarify which type of soil is suitable for the accumulation of *hesperidin*, *nobiletin* and *tangeretin* in the pericarps, and which key environmental factors promote the accumulation of the three components, in order to facilitate high-quality growth of *Citrus reticulata* 'Chachi'. The research simultaneously has guiding significance for the sustainable and ecological agriculture construction of *Citrus reticulata* 'Chachi'.

Materials and Methods

Survey of the research area

Xinhui District is the traditional cultivation region of *Citrus reticulata* 'Chachi' (*Citrus reticulata* cv. Chachiensis), which is situated in the eastern coastal zone of the Eurasian continent, the southwest of the Pearl River Delta and lower reaches of Tanjiang and Xijiang rivers, Jiangmen City, Guangdong Province, China. The geographical location is between 22°5'15" N-22°35'01" N and 112°46'55" E-113°15'43" E. Xinhui District is surrounded by mountains on three sides, namely Gudou Mountain on West side, Guifeng Mountain on North side and Niugu Mountain on East side, simultaneously facing the South China Sea on South side. Deqing County is the administrative area of Zhaoqing city, Guangdong Province. It is located in the northwest orientation of Xinhui District and on the north bank of the middle reaches of the Xijiang River. The geographical location is between 23°04' N-23°30' N and 111°32' E-112°17' E. Deqing County belongs to hilly and low mountain and river alluvial landform, surrounded by mountains in the west, north and east.

Paddy, red and alluvial soil are the three main soil types in *Citrus reticulata* 'Chachi' orchards. The climate type is Subtropical Marine Monsoon Climate. The soft branch with large and thin seed oil bodies is currently the main two *Citrus reticulata* 'Chachi' cultivars.

Soil sampling

During the semi-mature period (November, 2021) of *Citrus reticulata* 'Chachi' pericarp, soil samples were collected from Dongjia (DJ, 22°31'26" N, 113°3'55" E), Gujing (GJ, 22°19'13" N, 113°5'27" E) and Deqing (DQ, 23°16'15" N, 111°47'50" E) (Fig. 1) according to the soil sampling principle of random multi-point mixing (Lu 2000). The sample points of Dongjia (DJ) and Gujing (GJ) are located in Xinhui District. The soil type in Dongjia (DJ) orchard is paddy soil, Gujing (GJ) and Deqing (DQ) are red soil and alluvial soil, respectively. Other detailed information about the three sampling orchards was exhibited in Table S1. A total of five *Citrus reticulata* 'Chachi' trees (The soft branch with large seed oil body) with the same shape and size were randomly selected to operate sampling work at each site.

To measure topsoil (0–20 cm) volume weight and total porosity, cutting ring (volume = 100 cm³) was used in field work. Soil profile samples within canopy of *Citrus reticulata* 'Chachi' trees were collected respectively at the depths of 0–10 cm, 10–20 cm, 20–30 cm, 30–40 cm, 40–50 cm, 50–60 cm, 60–70 cm, 70–80 cm, 80–90 cm, 90–100 cm and 0–20 cm rhizosphere soil samples were simultaneously collected. All the soil samples were naturally air-dried in the laboratory. All soil samples firstly passed through 2 mm soil sieves to remove large pieces of gravels and plant root residues. The filtered samples were chosen to detect soil mechanical compositions and active acidity (pH). Then all samples passed through 100 mesh soil sieves for measurements of soil organic matter (SOM), cation exchange capacity (CEC), salinity, total nitrogen (TN), total phosphorus (TP), total potassium (TK) and other inorganic elements.

Pericarp of *Citrus reticulata* 'Chachi' sampling

During soil samples collection, the pericarp samples were simultaneously collected on the same trees. Fruits around the north, west, south and east positions of the tree were respectively collected at a distance of 1.5 m from the ground. The fruits at different positions were mixed and regarded as a fruit sample of the same tree. The pericarps were taken by three stripping method, then low temperature (45°C) dried, ground and bagged in the laboratory, and stored in a desiccator for subsequent experimental testing.

Sample analysis

Topsoil bulk density, total porosity and field capacity were determined by the ring knife method. Soil mechanical composition was determined by a laser particle analyzer (Beckman LS13 320, USA). Total nitrogen (TN) was determined by the modified kjeldahl method (China national standard: HJ 717–2014), and total phosphorus (TP) was determined by the alkali melting method and acid dissolution method (China national standard: LY/T 1232–2015). Soil organic matter (SOM) was measured by a total organic carbon analyzer (Elemaenter VARIO, Germany). Cation exchange capacity (CEC) was determined by the ammonium acetate exchange method (China local standard: DB33/T 966–2015). Salinity was measured by the gravimetric method (China local standard: DB37/T 1303–2009). Active acidity (pH) was determined by an automatic soil pH analyzer (water: soil = 2.5:1; Skalar SP2000-1, Netherlands). The contents of other inorganic elements were determined by the inductively coupled plasma-optical emission spectroscopy (ICP-OES, Optima 8000, USA). The operating parameters of ICP-OES were as follows: Plasma power was 1300 Watts, plasma gas flow rate was 0.70 L/min, flow rate of the auxiliary device was 0.40 L/min, atomizer flow rate was 0.55 L/min, pump speed was 1.50 L/min, lifting delay was 40s, stabilization time was 15 s.

An ultra-performance liquid chromatography (UPLC) method was used to detect concentrations of *hesperidin*, *nobiletin* and *tangeretin* in pericarp samples of *Citrus reticulata* 'Chachi'. Chromatographic separation was achieved on a ACQUITY UPLC BEH C18 column (2.1 mm×100 mm, 1.7 µm). The gradient elution system was consisted of (A) 0.1% formic acid and (C) acetonitrile as the mobile phase, (B) water and (D) methanol as the wash column. The gradients were as follows: 0–10 min: 90% A, 10% C; 10–25 min: 75% A, 25% C; 25–30 min: 40% A, 60% C; 30 min–33 min: 90% A, 10% C. The flow-rate was 0.3 mL/min and the sample injection volume was 1 µL. The temperature of the column was maintained at 35°C.

Meteorological data

Monthly meteorological data from March (florescence of *Citrus reticulata* 'Chachi') to November 2021 (semi-mature period of *Citrus reticulata* 'Chachi') in three sampling sites were downloaded from China Meteorological Data Service Centre (<http://data.cma.cn>). Eight meteorological factors were selected: monthly average temperature (MAVT), monthly maximum temperature (MMAT), monthly minimum

temperature (MMIT), monthly accumulative temperature (MACT), monthly cumulative precipitation (MCP), monthly relative humidity (MRH), monthly light intensity (MLI), and monthly light hour (MLH).

Statistical analysis

The sampling map was draw by the software ArcGIS 10.6. Correlation and linear regression analyses were performed using IBM SPSS Statistics 24.0 and Origin Pro 2021. Redundancy analysis was carried out using CANOCO 5.0 statistical software. Scatter diagram was generated using Python Project for Statistical and Computing. Random forest model was executed under Random Forest package in Python Project. Establishment of structural equation model was conducted by R software (4.0.1).

Parameters of random forest model (RFM) were defined as follows: ntree, which the meaning is number of trees in forest, was set as 500; mtry, which the meaning is number of variables per tree, was set as one-third of the total number of soil independent variables (19 soil independent variables in this study and mtry value was 6); nodesize, which represents the minimum amount of data at per terminal node, was considered to set as 5 (default value). A total of 150 data records were randomly assigned to two sections: the 120 calibration data records (80% of the total data) were selected to establish RFM, the remaining 30 validation data records (remaining 20% of the total data) were selected to evaluate and validate the performance of the model. The predicted values of the RFM obtained from the training set need to be compared with the data from the validation set (observed values), which is used to verify the model's accuracy. Three indexes were used to conduct accuracy evaluation: Pearson correlation coefficient (r), determination coefficient (R^2), mean squared error ($RMSE$). The importance (VI) of independent variables was calculated by Feature Importance parameter. Higher Importance value manifested a more important independent variable. Generally, an independent variable is important if $VI > 0.15$ in Python. This study used mean values and standard deviation (SDs) of r , R^2 , and $RMSE$ from a total of 200 repeated models (RFM trained 200 times) to evaluate the model accuracy and then used VI value to determine the importance of independent variables. All input data has been standardized using a $\log$ function with a base of 10.

Results

Comparison of *hesperidin*, *nobiletin* and *tangeretin* contents under different soil types

The contents of *hesperidin*, *nobiletin* and *tangeretin* in the pericarp of *Citrus reticulata* 'Chachi' in three different soil types were compared (Fig. 2). It was noted that *hesperidin* contents under the three soil types were significantly higher ($p = 0.00 < 0.01$) than *nobiletin* and *tangeretin*. *Hesperidin*, *nobiletin* and *tangeretin* contents under paddy soil (Dongjia, DJ) were the lowest. The pericarp of *Citrus reticulata* 'Chachi' under red soil (Gujing, GJ) showed higher contents of the three components than DJ (*hesperidin*: $p = 0.059$; *nobiletin*: $p = 0.080$; *tangeretin*: $p = 0.003 < 0.01$). *Nobiletin* and *tangeretin* contents under alluvial soil (Deqing, DQ) were between DJ and GJ, while *hesperidin* content in DQ displayed the highest (26.54 mg/g).

Comparison of meteorological factors, topsoil basic physicochemical properties in three regions

Meteorological factors, such as MAVT, MMAT, MMIT, MACT, MCP, MRH, MLI and MLH, were compared in three regions (Fig. 3). It was found that no significant differences ($p > 0.05$) was observed in the above eight factors between the three regions.

Topsoil (0–20 cm) physiochemical properties were compared in three regions (Table 1, Fig. 4). From bulk density and total porosity, it was found that the degree of surface soil tightness was moderate in Dongjia (DJ), but loose in Gujing (GJ) and Deqing (DQ). Field capacity result showed that the order of surface soil water retention from high to low was: DJ > DQ > GJ. According to the International classification of texture, the orchard soils between 0–20 cm depth in DJ and DQ was silty loam, while the soil in the same depth range of GJ orchard was silty clay loam. Compared with the three producing regions, TP, CEC and pH values showed the highest in DJ orchard, GJ orchard showed the lowest values in TN, TP, TK, CEC, salinity and pH. Furthermore, SOM concentration in DJ showed the lowest median value compared with GJ and DQ.

Table 1
Topsoil physical properties of three regions

Location	Bulk density (g/cm ³)	Total porosity (%)	Field capacity (%)	Mechanical composition		
				Sand (%)	Silt (%)	Clay (%)
DJ	1.208 ± 0.097a	50.99 ± 6.66a	34.13 ± 5.64a	15.90 ± 4.16a	72.79 ± 4.30a	11.41 ± 0.99ab
GJ	1.042 ± 0.053ab	44.79 ± 1.44a	23.26 ± 3.26b	18.97 ± 11.73a	64.14 ± 11.94a	16.88 ± 2.89a
DQ	1.003 ± 0.029b	49.18 ± 3.12a	33.52 ± 2.22a	29.08 ± 10.91a	63.94 ± 10.16a	6.98 ± 1.00b
Values are means ± standard deviation, n = 5. Different letters (a, b) indicate significant differences at $p < 0.05$ level according to Kruskal-Wallis one-way ANOVA test.						

Correlation between meteorological factors, topsoil physicochemical properties and *hesperidin*, *nobiletin*, *tangeretin* in pericarps

Spearman correlation (Fig. 5a) showed that *hesperidin*, *nobiletin* and *tangeretin* were no significantly correlated with the eight meteorological factors ($p > 0.05$).

Pearson correlation showed that rhizosphere soil elements were negatively correlated with *hesperidin*, *nobiletin* and *tangeretin* in three regions (Fig. 5b). In particular, P, Ca, Mg and Mn were significantly correlated with *hesperidin*; N, K, Mn and Zn were significantly correlated with *nobiletin*; N, P, K, Ca, Mg, Mn and Zn were significantly correlated with *tangeretin* ($p < 0.05$ or $p < 0.01$). The correlation between soil elements, similar to *hesperidin*, *nobiletin* and *tangeretin*, presented positive correlation.

From Fig. 5c, it was found that CEC, pH and bulk density were significantly and negatively correlated with *hesperidin* ($p < 0.05$ or $p < 0.01$); pH was significantly and negatively correlated with *nobiletin* ($p < 0.05$); SOM, CEC, salinity, pH and field capacity were significantly and negatively correlated with *tangeretin* ($p < 0.05$ or $p < 0.01$). Furthermore, clay significantly and positively correlated with *nobiletin* and *tangeretin* ($p < 0.05$ or $p < 0.01$). According to the result of correlation analysis between soil physical and chemical properties, it was found that CEC showed significantly positive correlations with salinity, pH, total porosity and field capacity ($p < 0.05$ or $p < 0.01$). Salinity was significantly and positively correlated with field capacity ($p < 0.01$), but negatively correlated with clay ($p < 0.05$). pH was significantly and positively correlated with bulk density ($p < 0.01$) and field capacity ($p < 0.05$). Sand and clay were negatively correlated with silt ($p < 0.01$) and field capacity ($p < 0.05$), respectively.

Redundancy analysis (RDA) was simultaneously employed to further understand the influence of rhizosphere soil elements and soil physicochemical properties on *hesperidin*, *nobiletin* and *tangeretin*. As shown in Fig. 5d and e, the full RDA models respectively explained 98.2% and 97.3% of the response variables, with the first and second axes explaining 87.1% and 90.0%. Figure 5d and e also exhibited that the RDA effectively separated the sampling points of the three regions. *Hesperidin*, *nobiletin* and *tangeretin* presented negative correlations with N, K, Zn, Cu, Mn, P, Mg, Ca and Fe (Fig. 5d). *Hesperidin* was positively correlated with sand and negatively correlated with field capacity, total porosity, salinity, SOM, CEC, pH, silt, bulk density; In addition, there was no correlation between *hesperidin* and clay; *Nobiletin* and *tangeretin* were positively correlated with clay, but negatively correlated with sand, field capacity, total porosity, salinity, SOM, CEC, pH, silt, bulk density (Fig. 5e). As for rhizosphere soil elements, K, Ca, Mg, N, Mn, Zn and P presented relatively stronger influences on the contents of *hesperidin*, *nobiletin* and *tangeretin*, while Cu and Fe had relatively weaker effects on *hesperidin*, *nobiletin* and *tangeretin*. Concerning soil physicochemical properties, clay, pH, CEC, bulk density and field capacity presented relatively stronger influences on *hesperidin*, *nobiletin* and *tangeretin*, while salinity, total porosity, SOM, sand and silt had relatively weaker effects on *hesperidin*, *nobiletin* and *tangeretin*. In brief, the relation revealed by RDA was consistent with Pearson correlation analysis, RDA had preliminarily identified some key soil factors that affect the contents of *hesperidin*, *nobiletin* and *tangeretin* in the pericarps of *Citrus reticulata* 'Chachi'.

Distribution of physicochemical properties and inorganic elements in soil profiles

Vertical distribution of soil physicochemical properties

Soil pH was compared along the vertical depth in all soil layers with ranges of 7.83–8.60 (paddy soil, DJ), 4.48–5.08 (red soil, GJ) and 5.79–6.13 (alluvial soil, DQ) (Fig. 6). The pH in red soil gradually decreased from the top down to the 1 m depth, while values in paddy and alluvial soil fluctuated within a certain range and exhibited no significant difference between each layer ($p > 0.05$). As shown in Table S2, mechanical composition in paddy soil (DJ) from all layers was with mean values of 8.12%–20.31% sand, 68.87%–79.87% silt and 10.81%–13.14% clay; red soil (GJ) was 10.73%–21.91% sand, 60.53%–75.68% silt and 13.02%–22.13% clay; alluvial soil (DQ) was 19.96%–32.38% sand, 59.94%–71.59% silt and

6.74%-9.07% clay. Salinity content in paddy soil (DJ) decreased from 0.1635–0.1515% in 0–30 cm soil depth, then sharply rose to within the range of 0.2965%-0.3106% in 30–60 cm depth, finally dropped within 0.2061%-0.2460% in 60–100 cm depth. Salinity content in alluvial soil (DQ) gradually decreased from 0.1703–0.0457% with increasing soil depth. Salinity content in red soil (GJ) showed no significant variation in 1-100 cm depth ($p > 0.05$) and fluctuated between 0.0198%-0.0349%. Salinity in paddy soil (DJ) was significantly higher than red soil (GJ) ($p < 0.01$) in all depth. Salinity in alluvial soil (DQ) was significantly higher than red soil (GJ) ($p < 0.01$) in 0–70 cm depth, but no significant difference was found in 70–100 cm soil layers ($p > 0.05$). The order of mean CEC contents in all depth was paddy soil (DJ, 12.93 cmol/kg) > alluvial soil (DQ, 7.49 cmol/kg) > red soil (GJ, 4.34 cmol/kg). Soil SOM in red soil (GJ) and alluvial soil (DQ) showed decreasing trends with increasing soil depths. SOM in paddy soil (DJ) decreased from 30.65 g/kg to 18.25 g/kg from surface to 20 cm soil depth, while fluctuating within the 14.84 g/kg-19.15 g/kg range in 20–100 cm soil layers.

Vertical distribution of soil inorganic elements

The distribution of inorganic elements across soil profiles were presented in Fig. 7. The concentration comparisons of Ca, Mg, Fe, Mn and Cu in 0-100 cm depth showed paddy soil (DJ) > alluvial soil (DQ) > red soil (GJ), K concentration displayed alluvial soil (DQ) > paddy soil (DJ) > red soil (GJ). N in 80–100 cm, P in 0–50 cm and 90–100 cm, Zn in 0–30 cm and 50–100 cm depths showed paddy soil (DJ) > alluvial soil (DQ) > red soil (GJ). N in 0–70 cm, Zn in 30–50 cm showed alluvial soil (DQ) > paddy soil (DJ) > red soil (GJ). N in 70–80 cm and P in 50–90 cm showed paddy soil (DJ) > red soil (GJ) > alluvial soil (DQ). Decreased content trends from top to 1 m soil depth ($p < 0.05$) were observed in the following elements and regions: N in red soil (GJ) and alluvial soil (DQ), P in paddy soil (DJ) and alluvial soil (DQ), Cu and Zn in alluvial soil (DQ). In addition, N in paddy soil (DJ), P in red soil (GJ), Cu and Zn in paddy soil (DJ) and red soil (GJ), together with K, Ca, Mg, Fe, Mn in three regions, fluctuated irregularly with no obvious trend of increase or decrease ($p > 0.05$) as soil profile depth increased. The order of element abundance in 0–1 m depth from the three regions was as follows: Fe (27.26 g/kg) > K (18.79 g/kg) > Ca (5.32 g/kg) > Mg (2.26 g/kg) > N (0.565 g/kg) > P (0.47 g/kg) > Mn (0.31 g/kg) > Zn (74.70 mg/kg) > Cu (17.05 mg/kg).

The linear regression model (LRM) establishment between *hesperidin*, *nobiletin*, *tangeretin* and inorganic elements, physicochemical indexes in different profile depths

To find out which key inorganic elements play important roles in the accumulation of *hesperidin*, *nobiletin* and *tangeretin* in the pericarp of *Citrus reticulata* 'Chachi', linear regression models (LRMs) were established across different soil profile depths (Table 2). It was noted that *hesperidin* was significantly and negatively affected by soil Ca at 0–10 cm, 10–20 cm, 20–30 cm, 30–40 cm, 40–50 cm, 50–60 cm, 60–70 cm, 70–80 cm and 80–90 cm depths ($p < 0.01$); *Nobiletin* was significantly and negatively affected by soil K at every layer within 0–1 m depth ($p < 0.01$); *Tangeretin* was significantly and negatively affected by soil Ca at 10–20 cm, 50–60 cm, 60–70 cm, 70–80 cm depths ($p < 0.01$), while affected by K at 10–20 cm, 20–30 cm, 30–40 cm, 40–50 cm, 50–60 cm, 60–70 cm, 70–80 cm, 80–90 cm and 90–100 cm depths ($p < 0.01$). In addition, other inorganic elements, such as Cu, Mn, P, Mg and N,

were also showed significantly and negatively correlations with *hesperidin* or *tangeretin* at certain depths, such as Cu-*hesperidin* at 90–100 cm, Mn and P-*tangeretin* at 0–10 cm, etc. Scatter plots (Fig. S1) further displayed the trend of data changes between significantly correlated components and key inorganic elements in each soil layer. It could be seen that there was a negative correlation and a trend toward linear changes between components and key elements.

Table 2

Linear stepwise regression equations between *hesperidin*, *nobiletin*, *tangeretin* and soil elements across different soil depths. r^2 is determination coefficient which reflects the fitting effect of the model. Meaning

of abbreviations: "Hes" is *Hesperidin*, "Nob" is *Nobiletin* and "Tan" is *Tangeretin*. Y_1 : $\log_{10}Hes$; Y_2 :

$\log_{10}Nob$; Y_3 : $\log_{10}Tan$; X_1 : $\log_{10}N$; X_2 : $\log_{10}P$; X_3 : $\log_{10}K$; X_4 : $\log_{10}Ca$; X_5 : $\log_{10}Mg$; X_6 : $\log_{10}Fe$; X_7 :

$\log_{10}Mn$; X_8 : $\log_{10}Cu$; X_9 : $\log_{10}Zn$; X_{10} : $\log_{10}Pb$; X_{11} : $\log_{10}Ni$; X_{12} : $\log_{10}Cr$.

Depths	Stepwise regression models	r^2	ANOVA		Pearson correlation coefficients	Key affecting elements
			F	p		
0-10cm	$Y_1 = 11.823 - 0.771X_4$	0.562	18.995	0.001	Y_1-X_4 : -0.771 ($p < 0.01$)	Ca
	$Y_2 = 6.990 - 0.746X_3$	0.522	16.298	0.001	Y_2-X_3 : -0.746 ($p < 0.01$)	K
	$Y_3 = -7.889 - 0.959X_7 + 0.624X_6 - 0.356X_2$	0.914	50.354	0.000	Y_3-X_7 : -0.852 ($p < 0.01$) Y_3-X_6 : -0.247 ($p > 0.05$) Y_3-X_2 : -0.757 ($p < 0.01$)	Mn P
10-20cm	$Y_1 = 6.632 - 1.203X_4 + 0.613X_9$	0.645	13.728	0.001	Y_1-X_4 : -0.734 ($p < 0.01$) Y_1-X_9 : -0.306 ($p > 0.05$)	Ca
	$Y_2 = 6.973 - 0.756X_3$	0.539	17.387	0.001	Y_2-X_3 : -0.756 ($p < 0.01$)	K
	$Y_3 = 6.088 - 0.539X_3 - 0.454X_4$	0.814	31.548	0.000	Y_3-X_3 : -0.858 ($p < 0.01$) Y_3-X_4 : -0.833 ($p < 0.01$)	K Ca
20-30cm	$Y_1 = 11.020 - 0.714X_4$	0.472	13.494	0.003	Y_1-X_4 : -0.714 ($p < 0.01$)	Ca
	$Y_2 = 6.944 - 0.774X_3$	0.567	19.369	0.001	Y_2-X_3 : -0.774 ($p < 0.01$)	K
	$Y_3 = 5.204 - 0.875X_3$	0.747	42.276	0.000	Y_3-X_3 : -0.875 ($p < 0.01$)	K
30-		0.428	11.482	0.005		Ca

40cm	$Y_1 = 10.988 - 0.685X_4$				$Y_1X_4: -0.685 (p < 0.01)$	
	$Y_2 = 6.264 - 0.769X_3$	0.560	18.839	0.001	$Y_2X_3: -0.769 (p < 0.01)$	K
	$Y_3 = 6.745 - 0.517X_5 - 0.454X_3$	0.764	23.629	0.000	$Y_3X_5: -0.830 (p < 0.01)$ $Y_3X_3: -0.810 (p < 0.01)$	Mg K
40-50cm	$Y_1 = 13.808 - 0.929X_4 + 0.543X_1$	0.770	24.422	0.000	$Y_1X_4: -0.738 (p < 0.01)$ $Y_1X_1: 0.216 (p > 0.05)$	Ca
	$Y_2 = 8.681 - 0.954X_3 + 0.396X_1$	0.716	18.606	0.000	$Y_2X_3: -0.791 (p < 0.01)$ $Y_2X_1: 0.003 (p > 0.05)$	K
	$Y_3 = 5.268 - 0.883X_3$	0.763	46.120	0.000	$Y_3X_3: -0.883 (p < 0.01)$	K
50-60cm	$Y_1 = 11.120 - 0.748X_4$	0.525	16.475	0.001	$Y_1X_4: -0.748 (p < 0.01)$	Ca
	$Y_2 = 2.498 - 1.361X_3 + 0.682X_9$	0.670	15.198	0.001	$Y_2X_3: -0.773 (p < 0.01)$ $Y_2X_9: -0.490 (p > 0.05)$	K
	$Y_3 = 5.824 - 0.633X_3 - 0.360X_4$	0.781	25.950	0.000	$Y_3X_3: -0.856 (p < 0.01)$ $Y_3X_4: -0.752 (p < 0.01)$	K Ca
60-70cm	$Y_1 = 11.549 - 0.796X_4$	0.606	22.544	0.000	$Y_1X_4: -0.796 (p < 0.01)$	Ca
	$Y_2 = 6.994 - 0.760X_3$	0.545	17.786	0.001	$Y_2X_3: -0.760 (p < 0.01)$	K
	$Y_3 = 4.653 - 0.976X_3 - 0.986X_4 + 0.872X_7$	0.866	31.275	0.000	$Y_3X_3: -0.838 (p < 0.01)$ $Y_3X_4: -0.798 (p < 0.01)$	K Ca Mn

					$Y_3-X_7: -0.813 (p < 0.01)$	
70-80cm	$Y_1 = 11.412-0.816X_4$	0.640	25.858	0.000	$Y_1-X_4: -0.816 (p < 0.01)$	Ca
	$Y_2 = 7.024-0.769X_3$	0.560	18.850	0.001	$Y_2-X_3: -0.769 (p < 0.01)$	K
	$Y_3 = 5.971-0.550X_3-0.439X_4$	0.766	23.954	0.000	$Y_3-X_3: -0.827 (p < 0.01)$ $Y_3-X_4: -0.786 (p < 0.01)$	K Ca
80-90cm	$Y_1 = 11.344-0.810X_4$	0.629	24.784	0.000	$Y_1-X_4: -0.810 (p < 0.01)$	Ca
	$Y_2 = 7.096-0.799X_3$	0.610	22.880	0.000	$Y_2-X_3: -0.799 (p < 0.01)$	K
	$Y_3 = 4.217-0.753X_3-0.350X_1$	0.783	26.278	0.000	$Y_3-X_3: -0.836 (p < 0.01)$ $Y_3-X_1: -0.529 (p < 0.05)$	K N
90-100cm	$Y_1 = 10.822-0.660X_8$	0.392	10.038	0.007	$Y_1-X_8: -0.660 (p < 0.01)$	Cu
	$Y_2 = 6.396-0.819X_3$	0.646	26.499	0.000	$Y_2-X_3: -0.819 (p < 0.01)$	K
	$Y_3 = 3.326-0.658X_3-0.429X_1$	0.835	36.514	0.000	$Y_3-X_3: -0.842 (p < 0.01)$ $Y_3-X_1: -0.711 (p < 0.01)$	K N

According to correlation analysis (Fig. 5c and e) and linear regression (Table S3) between *hesperidin*, *nobiletin*, *tangeretin* and physicochemical indexes, it was found that soil pH, salinity and CEC were the major physicochemical factors that affect *hesperidin*, *nobiletin* and *tangeretin* contents, respectively. *Hesperidin* was significantly and negatively affected by soil pH at 0–10 cm, 10–20 cm, 20–30 cm, 30–40 cm, 40–50 cm and 50–60 cm ($p < 0.01$); *Nobiletin* was significantly and negatively affected by soil salinity at 0–10 cm, 10–20 cm, 20–30 cm, 30–40 cm, 50–60 cm and 60–70 cm ($p < 0.01$ or $p < 0.05$); *Tangeretin* was significantly and negatively affected by soil CEC at 0–10 cm, 10–20 cm, 20–30 cm, 30–40 cm, 40–50 cm, 50–60 cm and 80–90 cm ($p < 0.01$).

The random forest model (RFM) establishment to predict the importance rank of variables in soil profiles affecting *hesperidin*, *nobiletin* and *tangeretin* concentrations

The performance of RFM in predicting $\log_{10}Tan$ was highest ($r = 0.9140 \pm 0.0291$, $R^2 = 0.8220 \pm 0.0523$, $RMSE = 0.4049 \pm 0.0462$) (data format: mean $\pm$ SD), followed by $\log_{10}Hes$ ($r = 0.8327 \pm 0.0453$, $R^2 = 0.6697 \pm 0.0745$, $RMSE = 0.5616 \pm 0.0697$) and $\log_{10}Nob$ ($r = 0.8140 \pm 0.0611$, $R^2 = 0.6391 \pm 0.0934$, $RMSE = 0.5821 \pm 0.0583$). All the three performance was comparatively acceptable and reliable, which partly confirmed the model accuracy. Figure 8 displayed the order of the important soil variables affecting peel flavonoid concentrations. For *hesperidin*, it showed that soil Mg and Ca ($VI > 0.15$) were ranking the top two key factors. Soil K ($VI > 0.15$) was the key factor that affects the *nobiletin* concentration. Soil K and Mg ($VI > 0.15$) were the top two factors that influence *tangeretin* content. Moreover, compared with soil elements and other physicochemical properties, it was found that VI values of soil pH, sand, silt, clay, SOM, CEC, salinity were less than 0.15, and VI values of a number of soil elements were higher than 0.15, which illustrated that soil elements played major roles in variation of *hesperidin*, *nobiletin* and *tangeretin* in the pericarp of *Citrus reticulata* 'Chachi'.

The structural equation model (SEM) establishment to reveal the causal relationships between different soil variables and *hesperidin*, *nobiletin*, *tangeretin* in pericarps

The structural equation model (SEM) showed that soil Ca ($p < 0.001$) have a significant and direct negative effect on *hesperidin*, with an absolute value of standard coefficient ($|\beta|$) higher than 1.000 (Fig. 9a). Soil K ($p < 0.001$) had significant and direct negative effects on *nobiletin* and *tangeretin*, with a standard coefficient (β) of -0.664 and -0.536, respectively. Soil Mg, Mn and K presented indirect effects on *hesperidin* mainly by positively interact with soil Ca ($\beta = 0.944, 0.877$ and 0.574 , respectively, $p < 0.001$). Similarly, Soil Mn, Mg and Ca displayed indirect effects on *nobiletin* and *tangeretin* mainly by positively interact with soil K ($\beta = 0.749, 0.610$ and 0.574 , respectively, $p < 0.001$). The three components in the pericarps had a significantly and positively correlated interaction relationship (Fig. 9a and b). For soil physicochemical properties, it showed that salinity and pH were the top two stronger indexes that affect the accumulation of *hesperidin*, *nobiletin* and *tangeretin* (Fig. 9b). Soil salinity exhibited negatively and directly influence on *nobiletin* ($\beta = -0.444$, $p < 0.001$) and *tangeretin* ($\beta = -0.413$, $p < 0.001$), and soil pH negatively and directly affected *hesperidin* ($\beta = -0.431$, $p < 0.001$) and *tangeretin* ($\beta = -0.300$, $p < 0.001$). Furthermore, soil sand showed positively and directly effect on *hesperidin* ($\beta = 0.305$, $p < 0.001$), while soil CEC negatively and directly influenced *tangeretin* ($\beta = -0.228$, $p < 0.001$). Soil salinity and CEC indirectly affected *hesperidin* mainly by positively interacting with pH ($\beta = 0.623$ and 0.737 , respectively, $p < 0.001$). Soil pH and CEC indirectly affected *nobiletin* mainly by the positive interaction with salinity ($\beta = 0.623$ and 0.762 , respectively, $p < 0.001$). Additionally, soil sand showed no significant causal relationship with *nobiletin* and *tangeretin*. More information about causal relationship between soil physicochemical properties and soil elements was simultaneously obtained (Fig. 9c). It should be noted that soil Mg and K made important contribution to soil sand ($\beta = -1.092$ and 0.398 , respectively, $p < 0.001$). Soil pH was mainly directly controlled by soil Ca ($\beta = 0.672$, $p < 0.001$), soil CEC and salinity was mainly directly controlled by soil Mn ($\beta = 0.565$ and 0.541 , $p < 0.001$). Moreover, soil salinity and CEC positively and directly influenced each other ($\beta = 0.279$, $p < 0.001$). The four soil elements (K, Ca, Mg and Mn) interacted with each other and exhibited strong causal relationships ($p < 0.001$ between every two elements).

Discussion

Influence mechanism of environmental factors on accumulation of *hesperidin*, *nobiletin* and *tangeretin* in pericarps of *Citrus reticulata* 'Chachi'

Dongjia Town is located on the plain, where an artificial river channel of the Xijiang River situated at the east side of town. Tanjiang main stream is far away from Dongjia Town. Both the trunk stream of Tanjiang River and artificial river channel of the Xijiang River flow into the Yamen Waterway and discharge into the South China Sea. The sample site of DJ was surrounded by urban facilities, as well as close to the downstream of artificial river channel of the Xijiang River. The main source of orchard irrigation water derives from the Xijiang artificial river channel. Gujing Town is located on the east side of Yamen Waterway and northwest of Niugu Mountain. The sample site of GJ was located at hillside of Niugu Mountain, with the orchard irrigation water comes from groundwater. Deqing County is located on the north side of the Xijiang River and surrounded by hills. The sampling orchard of DQ is selected at the northwest of Deqing County, where geomorphologic type is floodplain. The irrigation water for orchard mainly comes from the Panlongxia Reservoir, which the reservoir water flows into the Xijiang River and belongs to the water resources of the Xijiang River Basin. Previous report (Su et al. 2023) pointed out that due to the special geographical location at the intersection of sea and the two rivers, accompanied by the backflow in tides, the soil in Xinhui core region (such as Shuangshui) was detected to contain a large amount of salt and absorbed abundant inorganic element contents (such as Mg, Mn and K) compare with non-Xinhui regions (such as Taishan, Longmen and Pingnan). This research showed that paddy soil (DJ) and alluvial soil (DQ) contains higher salinity and inorganic elements than red soil (GJ) (Table S2, Fig. 4 and Fig. 7), which was partially consistent with Su et al. (2023) owing to DJ is located at the Xinhui core region. Plants of the same species growing in different environments may have differences in the concentration of the same secondary metabolites (Verma and Shukla 2015; Li et al 2020), which was consistent with the result that the contents of *hesperidin*, *nobiletin* and *tangeretin* at three soil environments were different (Fig. 2).

The meteorological factors showed no significant differences between the three regions (Fig. 3) and *hesperidin*, *nobiletin*, *tangeretin* showed no significant correlation with the eight meteorological factors (Fig. 5a), which demonstrated that the meteorological factors have little impact on the changes of the three medical compositions in the three regions. Soil is the resource that provides many important ecosystem services which are the foundation of life on earth (Salomom and Cavagnaro 2022). Soil is also the medium through which citrus roots absorb nutrients and exchange substances, providing the vast majority of water and nutrients required for citrus growth and development. This research found that compared with meteorological condition, soil physicochemical properties and elemental abundance may play key or more important roles in content variation of the three components in *Citrus reticulata* 'Chachi' between the three sites.

The LRM, RFM and SEM models (Table 2, Table S3, Fig. 8 and Fig. 9) comprehensively showed that soil K, Ca, Mg, Mn, salinity and pH are the main factors that affect *hesperidin*, *nobiletin* and *tangeretin*, in

which the soil element, such as K, was relatively important. In order to verify the accuracy of model results and confirm the effect of inorganic element on the components in the pericarps of *Citrus reticulata* 'Chachi', this study used K element as an example to design a field control experiment of foliar fertilization to verify this impact. The experiment site was located at the Orchard of *Citrus reticulata* 'Chachi' in Muzhou Town, Xinhui District, Jiangmen City, China. The experiment was conducted from 7 October to 11 November, 2022, and with intervention treatment of foliar fertilization once a week. Potassium foliar fertilizers of experimental groups were selected as KH_2PO_4 and K_2SO_4 , with mass concentration gradients set at 0.3%, 0.6%, and 0.9%, respectively. The control group was set to spray equal amounts of clean water on the leaves. The pericarp samples of each experimental group and control group were collected on 11 November, 2022, which was the semi-mature period. Fig. S2 showed the result of the field control experiment. It was noted that the contents of *nobiletin* and *tangeretin* (median values) showed a decrease trend with the increase of potassium fertilizer concentration, which demonstrated that potassium indeed has negatively impacts on the levels of *nobiletin* and *tangeretin*.

Supplementing plant mineral nutrients can not only stimulate plant growth, but also affect the content of secondary metabolites. The availability of nutrients by plants may be an important factor determining their secondary metabolism and antioxidant activity. Moreover, the characteristic of plant nutrient deficiency is the accumulation of flavonoids, such as anthocyanins (Lillo et al. 2008; Yang et al. 2018). In this study, soil elements were negatively correlated with *hesperidin*, *nobiletin* and *tangeretin* (Fig. 5b and d), indicating that the pericarps may be more inclined to accumulate the three components when *Citrus reticulata* 'Chachi' grows in soil with lower mineral nutrients. Potassium (K) plays a crucial role in regulation of enzymes activation, synthesis and transport of carbohydrates (Kumar et al. 2020; Wu et al. 2021). Carbohydrate metabolism is an important primary metabolic pathway in plants, and the primary metabolism provides energy and some small molecule compounds (such as acetyl-CoA, Malonyl-CoA, shikimic acid and other amino acid) as raw materials for secondary metabolism processes. This study found that K significantly, negatively and directly affected *nobiletin* and *tangeretin*, which may due to K has impacts on the carbohydrate metabolism process in primary metabolism, such as the activation of key enzyme activities (Oosterhuis et al. 2014) and the balance of electrical charge at the site of the ATP production (Al-Younis et al. 2018; Zhang et al. 2018b), thereby affecting the secondary metabolism process of polymethoxylated flavone through the bridging effect of key products in primary metabolism. Calcium (Ca) plays an important role in the structure and physiological function of plant cells. Calcium (Ca^{2+}) signal is an important regulatory factor for plant growth and development. When plants are stimulated by the external environment, specific changes in the concentration of calcium ions in cells occur, causing a series of protective physiological reactions. Ca also has regulatory effects on the activity of intracellular enzymes. Xu et al. (2014) reported that Ca affected the expression of flavonoid pathway genes and anthocyanin accumulation in strawberry fruit. Foliar spray of calcium fertilizer with a certain concentration promoted the total phenolics content of fruit, such as anthocyanins, which are precursors of flavonoids. Moreover, the changes in endogenous calcium levels may be regulated by activating flavonoid pathway genes such as *DFR* (Vitrac et al. 2000; Gollop et al. 2002), thereby regulating sucrose induced sugar absorption and anthocyanin accumulation (Shin et al. 2013). Zhang et al. (2021) also

reported that application of calcium fertilizer mainly regulates the occurrence of grape fruit cracking by regulating the content of secondary metabolites related to flavonoid biosynthesis pathway genes. This study indicated that Ca mainly significantly, negatively and directly affected *hesperidin*, of which the physiological mechanism may be originated from interaction between synthesis of anthocyanins and expression of flavonoid pathway genes, as well as calcium, as a cell second messenger (Kour et al. 2023), participates in the regulation of intracellular enzyme activity. Magnesium (Mg) is involved in photosynthesis, glycolysis, phosphorylation of tricarboxylic acid cycle, enzyme catalysis and transport of metabolites (Li et al. 2017; Chen et al. 2018; Wang et al. 2022). Besides, Mg^{2+} serve as cofactor or enzyme activator in carbohydrate metabolism (Guo et al. 2016). The result of this study discovered that Mg showed indirect effects on *hesperidin*, *nobiletin* and *tangeretin*. Previous research has demonstrated that high K^+ and Ca^{2+} inhibit the absorption of Mg^{2+} by plant roots (Grauer et al. 1992; Shabala et al. 2005; Hermans et al. 2010a; Hermans et al. 2010b; Chou et al. 2011; Horie et al. 2011; Guo et al. 2016), which may interpret the indirect effect of Mg owing to the direct impact of K on *nobiletin* and *tangeretin*, and Ca on *hesperidin*. Manganese (Mn) is an activator of plant enzymes, especially those related to phenylpropane metabolic pathways, which lead to the biosynthesis of some secondary metabolites, such as flavonoid (Burnell 1988). Chen et al. (2020) reported that foliar spraying $MnSO_4 \cdot H_2O$ improves flavonoid contents, as well as the acetylated, methylated, and total anthocyanins in grape berry skin of Cabernet Sauvignon (*Vitis vinifera* L.) grown on alkaline soil. It was found in this research that Mn had an indirect influence on *hesperidin*, *nobiletin* and *tangeretin*, which the possible reason may be that manganese is involved in the composition and activity regulation of enzymes involved in flavonoid metabolism. But based on above analysis, there are few studies on the regulation of *hesperidin*, *nobiletin* and *tangeretin* metabolism in *Citrus reticulata* 'Chachi' by soil potassium, calcium, magnesium and manganese, more detailed research associated with transcriptome and metabolome and direct evidence about flavonoid pathway gene expression are required to support this.

Su et al. (2022) summarized the flavonoid contents in pericarps of *Citrus reticulata* 'Chachi' at Xinhui District (Guangdong province) are relatively higher compared with non-Xinhui District (Guangxi province), which may be due to salt stress caused by soil conditions in Xinhui District. But the flavonoids reported by Su et al. (2022) did not involve in *hesperidin*, *nobiletin*, *tangeretin*. This study found that salinity significantly, negatively and directly affects the accumulation of *nobiletin* and *tangeretin*. The total salinity contents (dry soil weight) were at ranges of 0.083%-0.376% (paddy soil, DJ), 0.009%-0.047% (red soil, GJ), 0.099%-0.245% (alluvial soil, DQ) on surface soils (Fig. 3) and 0.1515%-0.3106% (range of mean value, paddy soil, DJ), 0.0198%-0.0349% (range of mean value, red soil, GJ), 0.0457%-0.1703% (range of mean value, alluvial soil, DQ) on profile soils (Table S2), which illustrated that paddy soil (DJ) in surface and profile is non-saline soil (total salinity contents (dry soil weight) < 0.3%) and weakly-saline soil (0.3% < total salinity contents (dry soil weight) < 0.5%), red soil (GJ) and alluvial soil (DQ) are non-saline soil. It can be speculated that high concentration salt stress may not play a crucial role in accumulation of *nobiletin* and *tangeretin*, the nutrient absorption of low concentrations of inorganic salts and elements by the root system of *Citrus reticulata* 'Chachi' may be one of the reasons for promoting the content increase of the two components. Soil pH is also an important index for regulating the content of secondary

metabolites in plants. The active acidity changes the micro-environment of soil liquid phase and availability of soil mineral nutrients, thereby affecting the nutrient absorption by roots and the accumulation of *hesperidin* and *tangeretin*. CEC is the total amount of various cations that can be adsorbed by soil colloids, which is closely associated with variation of soil pH, inorganic cation and soil texture (sand, silt and clay). It was found that CEC was significantly influenced by pH, salinity and inorganic elements (Fig. 9b and c). Soil particle is the basic unit of soil structure, and the composition of soil particle affects soil water retention, fertilizer retention and erosion resistance. This research found that sand is more important than silt and clay in the "soil - *Citrus reticulata* 'Chachi' - *hesperidin*, *nobiletin* and *tangeretin*" system. The above soil factors interact or have one-way causal effects (Fig. 9c), jointly forming a complex micro-physicochemical environment closed to *Citrus reticulata* 'Chachi' root system, then affecting accumulation of the three components in pericarps.

Soil characteristics under the cultivation of *Citrus reticulata* 'Chachi'

At the horizontal distribution of soil, topsoil tightness, water retention and texture analysis (Table 1) reflected that these three soil types, namely paddy soil, red soil and alluvial soil, are suitable for the aerobic respiration and water absorption of the roots of *Citrus reticulata* 'Chachi'. In addition, the contrast of chemical factors, including inorganic elements, at horizontal and vertical distribution in three regions (Fig. 4, Fig. 7 and Table S2) indicated that red soil on the hillside is relatively nutrient poor compared with paddy soil and alluvial soil. The pH values of soil profiles in three regions were at range of 4.48–8.60 (Fig. 6), which proved that *Citrus reticulata* 'Chachi' has strong adaptability to grow in strongly acidic, acidic, neutral, alkaline, and strongly alkaline soils. It should also be noted that the south of the Five Ridges in China mainly distributes acidic and strongly acidic soil. However, this research found alkaline and strongly alkaline soil in the south of the Five Ridges at Dongjia (DJ) orchard cultivated *Citrus reticulata* 'Chachi', which the main reason of this soil formation may be the soil matrix formed by sediment deposition from the Xijiang and Tanjiang rivers, and the impact of past rice cultivation on the soil properties. The alkaline and strongly alkaline soil here is a precious natural resource and needs to be protected by the local government in future.

This research established different linear regression equations at different soil profile depths (Table 2 and Table S3). According to comprehensive evaluation of sum of all r^2 values in each depth and referred to each result of ANOVA test (p value must lower 0.01), equations in 40–50 cm and 10–20 cm were well fitted compared with other depths. 10–20 cm is close to soil surface and is susceptible to human activities such as cultivation, fertilization and irrigation, while 40–50 cm is far from the surface of the soil. Then, it could be speculated that the 10–20 cm soil layer is the optimal depth for the interaction between soil and roots under the premise of human interference; And 40–50 cm is the optimal depth for the interaction between soil and roots with no obvious human interference. This could provide new insights into the impact of soil properties at different soil profiles on the accumulation of *hesperidin*, *nobiletin*, *tangeretin* in *Citrus reticulata* 'Chachi', and guide its daily agricultural management and production.

Limitations of the current research and prospect

Some limitations are needed to be taken into consideration in this research. The one is limitation of sample numbers. More orchards are required to be selected, more soil and pericarp samples are simultaneously needed to be collected in each soil type. Data can better reflect soil and component characteristics with the sample numbers increase. The established mathematical models will also be more stable, reliable and show more accurate goodness-of-fit as the amount of data increases. Unfortunately, due to the limitation of time, personnel, funds and sampling difficulty, the sampling work of this study paid more attention to ensure the reasonable distribution of samples among different soil types (Jiang et al. 2022), and choosing the sampling orchards which are with the same cultivation method, in order to prevent the disturbing influence of different variants on the key environmental variables and component contents in this study. Therefore, some shortcomings in sampling are inevitable. The other limitation is the duration of field control experiment was relatively short. The field experiment in this study only lasted for one month, and the variation character in results between different treatment just could be reflected by the median values (Fig. S2). If the intervention treatment could be carried out in advance, the differences of the two flavonoid contents between different treatment groups may be significant. Therefore, we must clarify that the results of this study are based on the current sampling and field experimental designs.

In consideration the future study, further in-depth research is needed to continue concentrating on the influence mechanism of soil inorganic elements on accumulation of *hesperidin*, *nobiletin* and *tangeretin* in *Citrus reticulata* 'Chachi' based the transcriptome and metabolome approaches, and attempt to discover key enzymes or genes that affect the metabolism.

Conclusion

This study investigated the horizontal and vertical changes of soil physicochemical properties and inorganic elements in paddy, red and alluvial soils cultivated *Citrus reticulata* 'Chachi', and analyzed the meteorological conditions of the three regions. It was found that difference in meteorological factors between the three regions was not significant. The 9 inorganic elements, namely N, P, K, Ca, Mg, Fe, Mn, Cu and Zn, in red soil are deficient compared with that in paddy and alluvial soil. The LRMs, RFMs and SEMs were shown that soil K, Ca, Mg, Mn, salinity and pH are the main factors influencing the content variation of the three medicinal components, among which the deficient soil elements, such as K and Ca, play a direct and vital role. The pericarp of *Citrus reticulata* 'Chachi' cultivated in soil with lower K and Ca is easier to accumulate *hesperidin*, *nobiletin* and *tangeretin*. Our observations and experiments in this research provide new judgment for improvement of medicinal ingredients of *Citrus reticulata* 'Chachi' and daily cultivation management of paddy, red and alluvial soil.

Abbreviations

DJ Dongjia Town

GJ Gujing Town

DQ Deqing County

Declarations

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Author Contributions Ruifei Ma contributed to experimental design, field survey and sample collection, laboratory analysis and testing of samples, data organization and analysis, graph making, manuscript writing, submission and edit of paper. Zhongming Xu contributed to methodology guidance and Python/R code provision for using mathematical models. Lili Ming provided experimental platform support and methodological guidance for inorganic element testing. Xiaoshuang Liu contributed to experimental support of soil sample analysis. Zhanming Tang gave support and cooperation of field work and sample collection. Yanyan Miao also gave support and cooperation of field work and sample collection. Yinghua Zheng gave support and guidance of soil research related to field work and sample collection. Chao Chen contributed to funding acquisition, resource support and cooperation of field work and sample collection, manuscript review and edit. Wensheng Zhang contributed to funding acquisition, project administration, resources, supervision, manuscript review, final interpretation of this research. All authors approved the final version of the manuscript.

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Figures

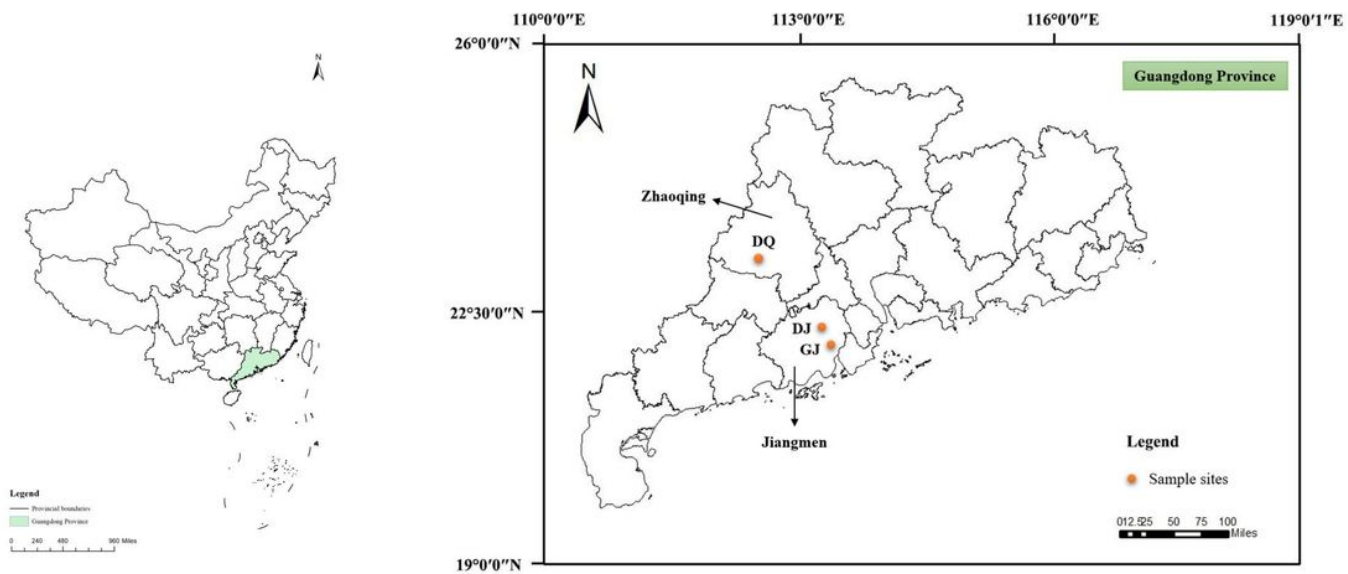


Figure 1

Sample sites of the research

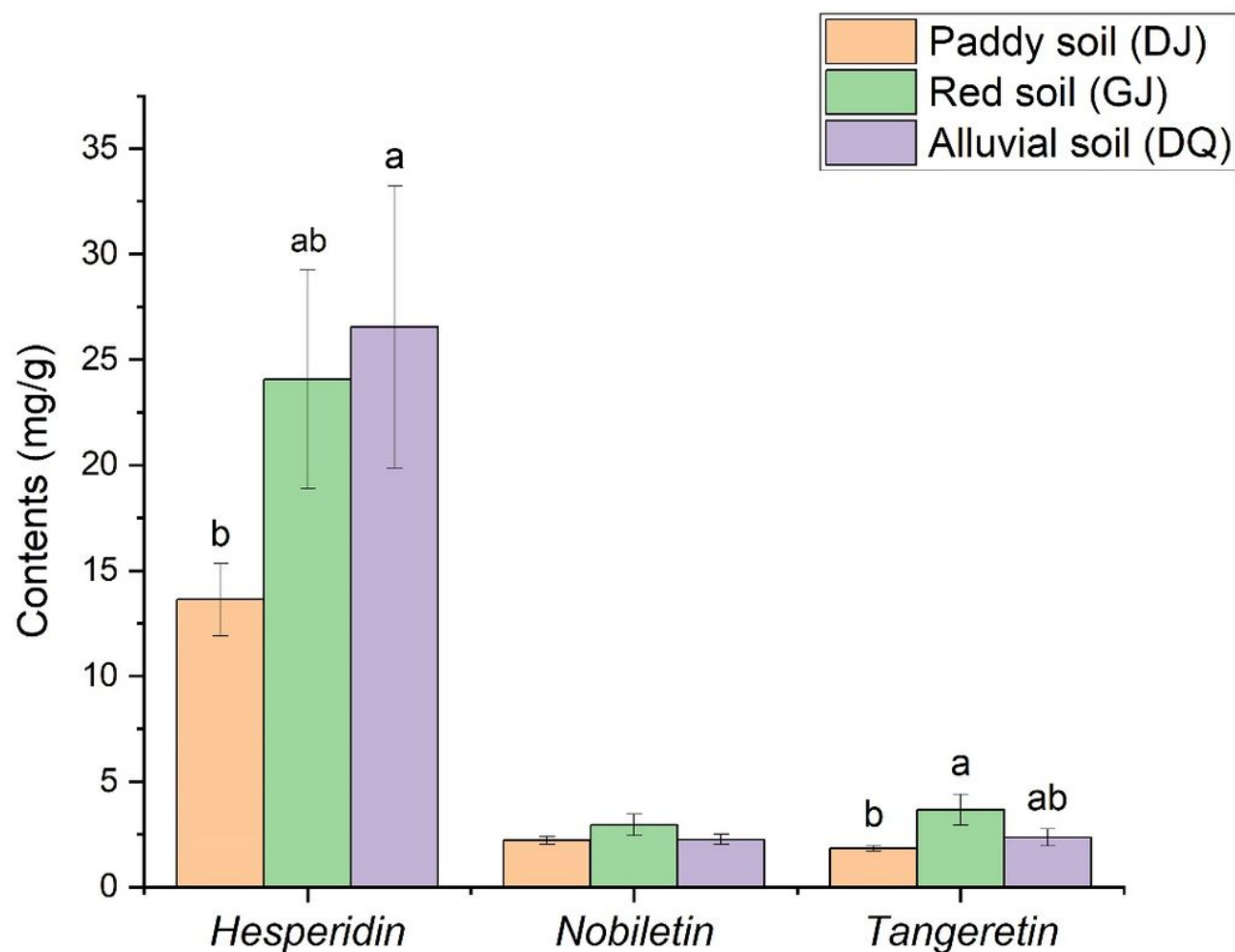


Figure 2

Hesperidin, nobiletin and tangeretin contents under different soil types. Values are mean $\pm$ standard deviation, $n=5$. Different letters (a, b) indicate significant differences at $p<0.05$ level according to Kruskal-Wallis one-way ANOVA test.

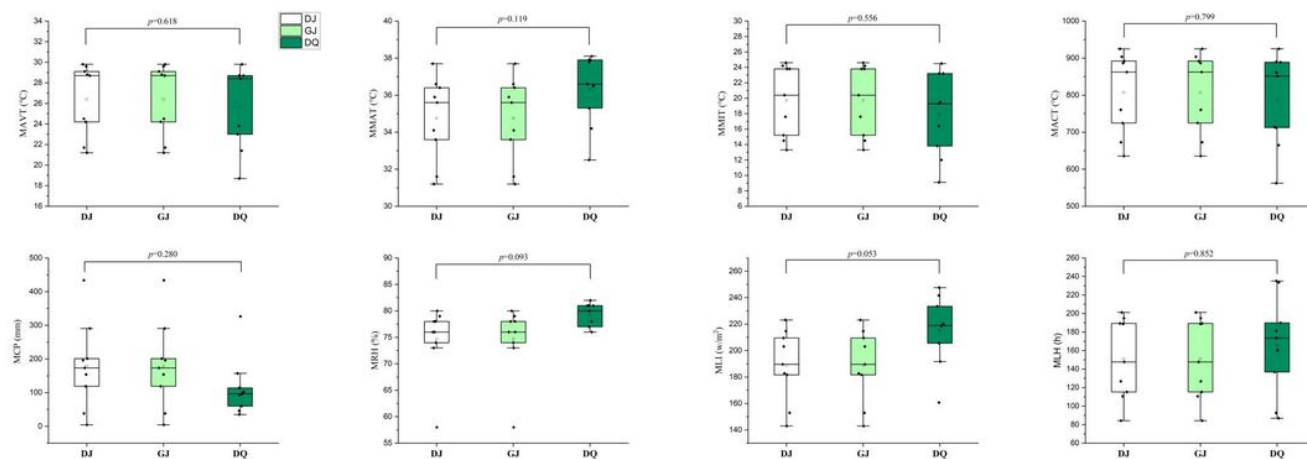


Figure 3

Meteorological factors of three regions. The significance test was based on Kruskal-Wallis one-way ANOVA test.

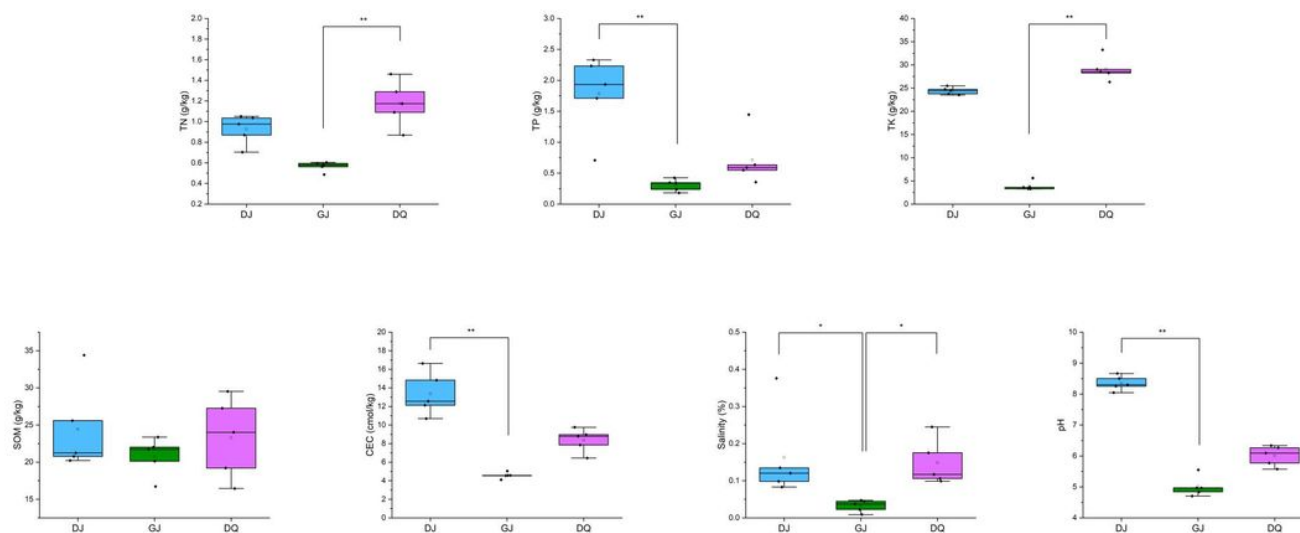


Figure 4

Topsoil chemical properties of three regions. The significance test was based on Kruskal-Wallis one-way ANOVA test, * represents $p < 0.05$, ** represents $p < 0.01$.

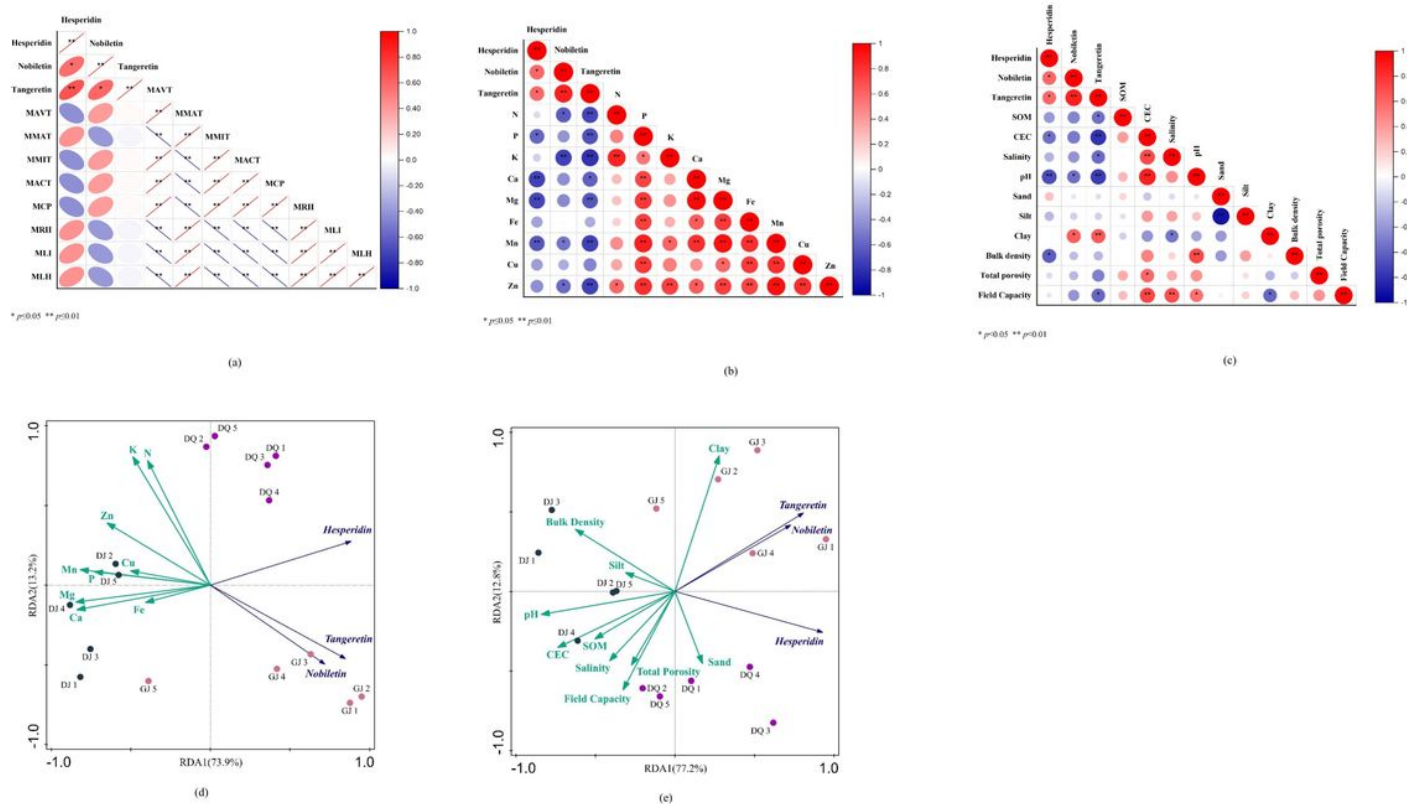


Figure 5

Correlation between meteorological factors, topsoil physicochemical properties and *hesperidin*, *nobiletin*, *tangeretin* in pericarps. (a) Spearman correlation between meteorological factors and *hesperidin*, *nobiletin*, *tangeretin* in pericarps; (b) Pearson correlation between rhizosphere soil elements and *hesperidin*, *nobiletin*, *tangeretin* in pericarps; (c) Pearson correlation between topsoil major physiochemical properties and *hesperidin*, *nobiletin*, *tangeretin* in pericarps; (d) Redundancy analysis between rhizosphere soil elements and *hesperidin*, *nobiletin*, *tangeretin* in pericarps; (e) Redundancy analysis between topsoil major physiochemical properties and *hesperidin*, *nobiletin*, *tangeretin* in pericarps. The different color circles in RDA ordinations from figure (d) and (e) represent different sampling sites. In figure (a), (b) and (c), red represents positive correlation, blue represents negative correlation. The size of the ellipse in figure (a) is inversely proportional to the size of the correlation coefficient and indicates the strength of the correlation. The size of the circle in figure (b) and (c) represents the strength of the correlation, the larger the circle, the stronger the correlation. * $p \leq 0.05$, ** $p \leq 0.01$, no* means $p > 0.05$.

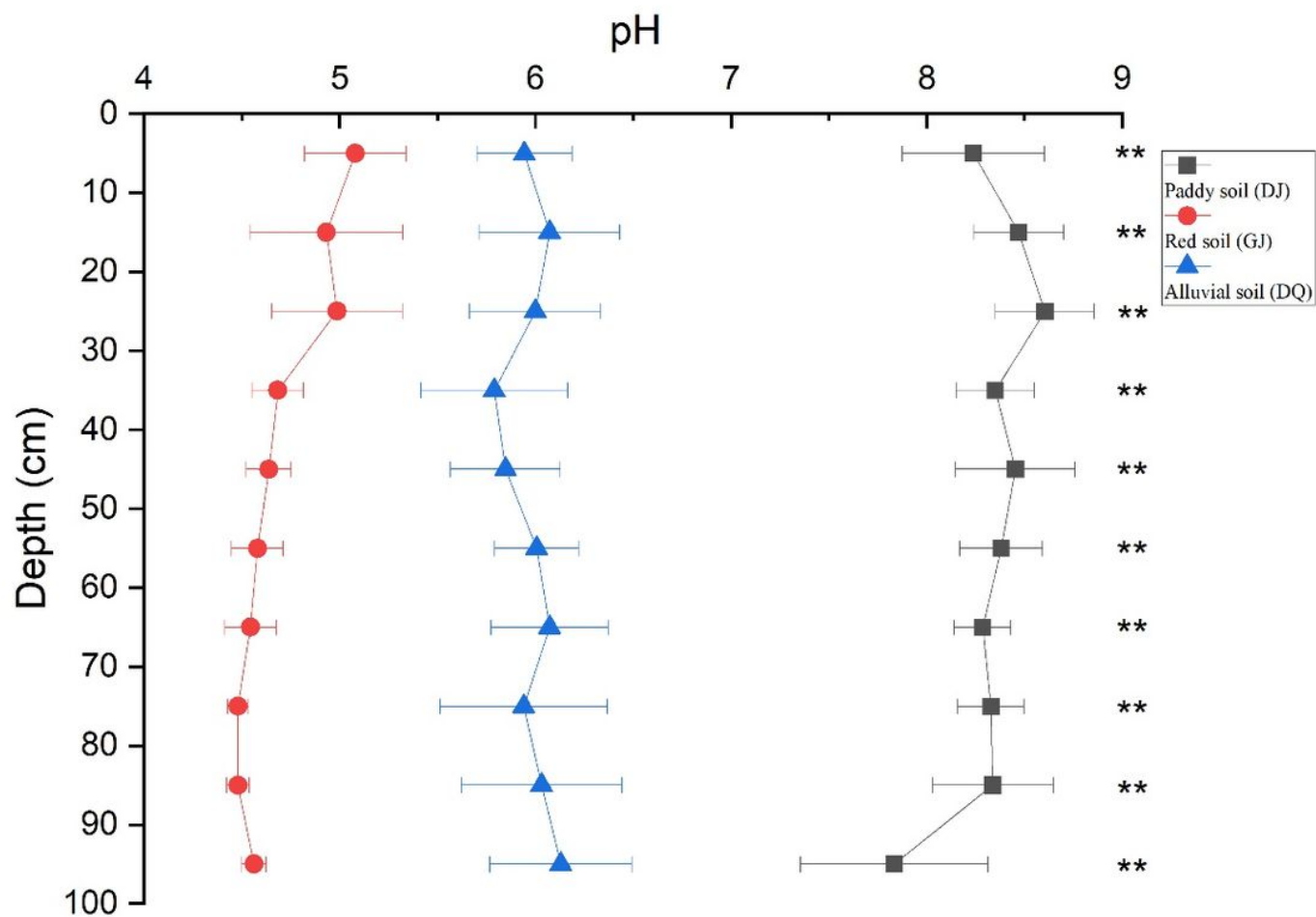


Figure 6

Vertical distribution of pH in different soil types. Values are means ($n=5$) with standard deviation. The stars indicate significant differences in the same layer among the three regions. * $p<0.05$, ** $p<0.01$.

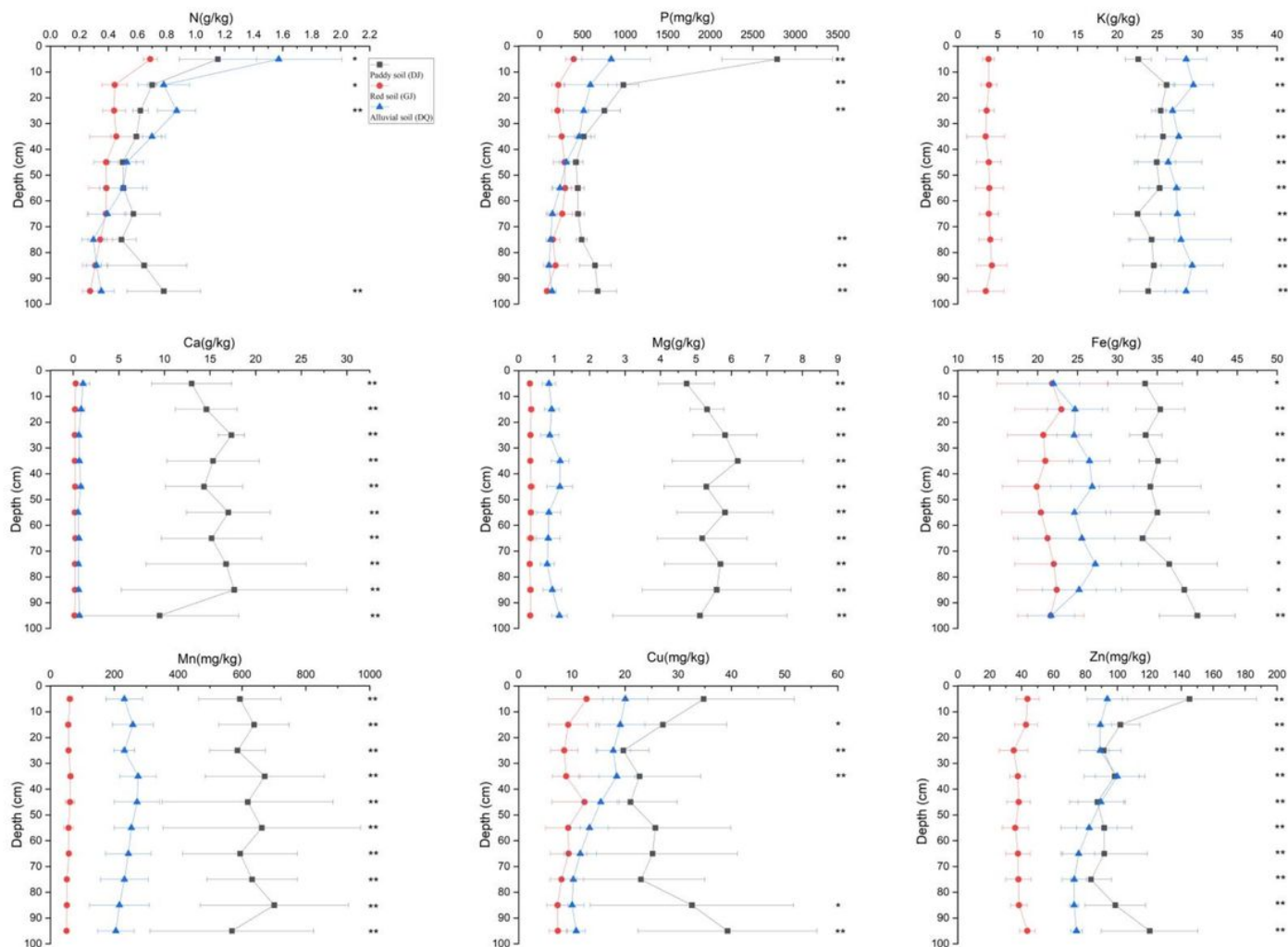


Figure 7

Vertical distribution of inorganic elements in different soil types. Values are means ($n=5$) with standard deviation. The stars indicate significant differences of soil inorganic elements in the same layer among the three regions. * $p < 0.05$, ** $p < 0.01$.

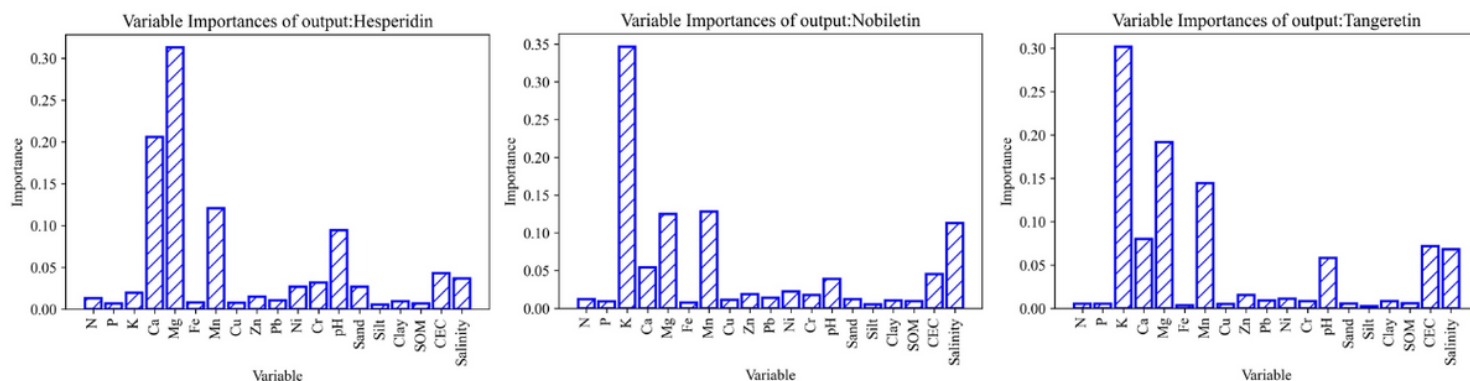


Figure 8

The variable importance rank of soil factors affecting *hesperidin*, *nobiletin* and *tangeretin* in pericarps selected by random forest model.

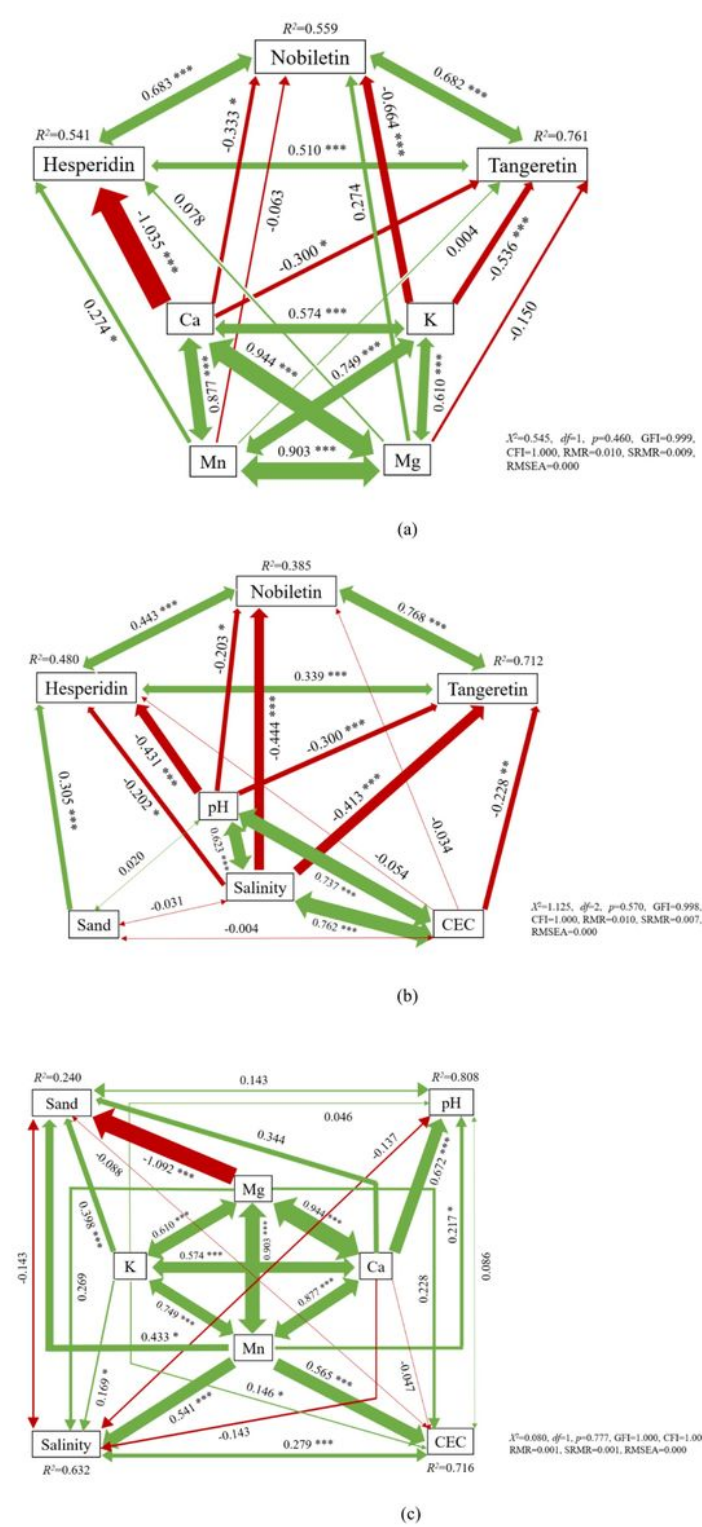


Figure 9

Structural equation model revealing the causal effects of key soil indexes on the accumulation of *hesperidin*, *nobiletin* and *tangeretin* in pericarps of *Citrus reticulata* 'Chachi'. (a) The effects of key soil

element on the accumulation of *hesperidin*, *nobiletin* and *tangeretin*; (b) The effects of key soil physicochemical properties on the accumulation of *hesperidin*, *nobiletin* and *tangeretin*; (c) The causal relationship between different key soil indexes. The coefficient is standardized prediction for each causal path. Solid green arrow indicates positive effect, solid red arrow indicates negative effect. The thickness of the arrow is proportional to the size of the standardized path coefficient and represents the strength of the relationship. The thicker the arrow, the stronger the relationship. The asterisk represents the significance level of each coefficient: * means $p < 0.05$, ** means $p < 0.01$, and *** means $p < 0.001$. The indicators for evaluating model quality: R^2 is determination coefficient, χ^2 is chi-square value, df is degree of freedom, p reflects significance of chi-square test, GFI is fit index, CFI is relative fit index, RMR is unstandardized residual, SRMR is standardized residual, RMSEA is root mean squared error of approximation. The structural equation models are accepted when $p > 0.05$, GFI > 0.90 , CFI > 0.95 , RMR value is smaller, SRMR < 0.08 and RMSEA < 0.05 . The path coefficient represents the standardized multiple linear regression coefficient. The standardized coefficients are listed on each path. Unidirectional arrow indicates that one variable has an impact on another variable, and the bidirectional arrow represents the (co) variance between two variables.

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