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Original Research

Correlation between Soil Physicochemical Factors and the Accumulation of Functional Components of *Camellia Tetracocca* in Karst Areas and Non Karst Areas

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Abstract

C. tetracocca are local characteristic plants with significant medicinal and economic values. However, little is known about the relationship of soil nutrient contents and the accumulation of primary and secondary metabolites from *C. tetracocca*. The metabolic accumulation of *C. tetracocca* grown in karst areas and non karst areas have not been compared. In this study, we sampled soil and leaves of *C. tetracocca* in karst areas and non karst areas to compare the relationship between soil nutrients (soil organic carbon, total nitrogen, total phosphorus, total potassium, calcium, magnesium) and organic components (organic carbon, total nitrogen, total phosphorus, total potassium, calcium, magnesium), free amino acids, functional components (catechin (C), caffeine (CAFF), gallic acid (GA), epicatechin (EC), (-)-epicatechin gallate (ECG), (-)-epigallocatechin (EGC), and epigallocatechin gallate (EGCG)) of *C. tetracocca*. The results showed that there was a significant positive correlation between K and EC in soil and plants and a significant negative correlation between P and EC. There was a negative correlation between Mg and EGC in soil and plants. Therefore, increasing the content of K and reducing the content of Mg and P can improve the functional components of *C. tetracocca*. The *C. tetracocca* in karst areas and non karst areas share excellent quality and have not been severely affected by

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the harsh environment. Therefore, these findings emphasized the significance of ancient tea trees and laid a solid foundation for the promotion and planting of *C. tetracocca*.

Keywords: *C. tetracocca*, soil, functional components, karst, non karst

Introduction

The tea plant is an important economic plant in China, which belongs to the family Theaceae, genus Camellia [1]. There are 23 genera and 749 species of Camellia plants in the world, of which 15 genera and 398 species are found in China. Tea plants are native to the Yunnan-Guizhou Plateau of China, and mostly distributed in Yunnan, Guizhou, Sichuan and other provinces [2]. Recently, people's health awareness gradually shifts to high-quality ecological, natural organic direction, and tea beverages produced by ancient tea trees are widely concerned by domestic and international markets [3]. Ancient tea plants are natural-grown in natural forests, covering wild trees, half-cultivated trees, and cultivated trees that are more than a century, without any manual interventions like frequent clipping or excessive fertilization [4]. They play a vital role in ecological environmental protection, health care and fitness and treatment of diseases [5]. Their unique natural landscape can also be viewed, explored, and inspected by tourists. *Camellia tetracocca* Zhang is one of the typical representatives of ancient tea trees [6], three Late Tertiary to Quaternary "Fossils of *C. tetracocca* Seed" dating from 1 million years ago were discovered at Shanjiaqing, Yuntou Dashan, the junction of Pu'an and Qinglong, Guizhou. Pu'an, Guizhou, located in the center of tea tree origin, has the world's largest population of wild *C. tetracocca*. In 2016, it was approved as a "Pu'an *C. tetracocca* geographical indication product" [7].

It is well known that natural substances provide us with the essential elements for living. The beneficial effects of tea on people are mainly based on the chemical ingredients in tea [8]. L-theanine, tea polyphenols, and caffeine are vital indicators for evaluation of tea quality, and are also the main taste substances of tea soup [9]. In the recent years, the understanding and research on the functional chemical components of tea have become a hot topic of domestic and foreign experts [10, 11]. Tea polyphenols are the products of carbohydrate metabolism, the main components of tea inclusion and functional components [12, 13]. Catechin is a kind of phenolic active substance extracted from tea including catechin (C), gallocatechin (GC), epicatechin (EC), epicatechin gallate (ECG), epigallocatechin (EGC), and epigallocatechin gallate (EGCG) [14, 15], which shares a variety of pharmacological effects, like antitumor [16, 17], antioxidant [11, 18, 19], anti-virus [20], and protecting cardio organs. L-Theanine, as one of the main substances in tea, its inclusion, composition, degradation and transformation products also have a direct impact on the quality of tea. What is more, it plays a crucial role in the formation of taste, aroma and color of tea soup [21].

The content of functional components in tea is related to a series of factors, including genetic characteristics [6], tree age, geographical environment of tea producing area [2]. Climate, soil altitude and other environmental factors can affect the growth conditions of tea plants, thus affecting the quality of tea [22, 23]. Soil is the necessary site condition for the survival and growth of tea trees and the place for their growth, development and absorption of nutrients [24, 25]. Soil contains non-essential mineral elements for the growth of tea trees, a large number of elements including N, P and K, medium elements consisting of Ca and Mg, and trace elements containing Fe and Zn. As an important influencing factor, soil properties can significantly affect the formation of tea quality at different altitudes. Previous studies have shown that phosphorus fertilizer can increase the content of tea polyphenols in fresh tea leaves, and potassium fertilizer can significantly increase the contents of amino acids in tea, which are conducive to ameliorating and improving the quality of tea [26]. In addition, the difference of nutrient content in soil will also affect the quality of tea by affecting the content of amino acids, tea polyphenols, caffeine and other inclusions in tea.

As the only tea area with low latitude, high altitude and little sunshine, Guizhou Province is suitable for tea growth in terms of altitude, annual temperature, sunshine hours, air humidity, annual rainfall and soil pH [27]. Most tea grows in non karst areas, but our previous investigation of ancient tea resources found that *C. tetracocca* also grows in Wangjiazhai in the karst area of Qianxinan Prefecture, Guizhou Province. In order to protect the wild *C. tetracocca* ancient tea resources, increase the development and utilization of *C. tetracocca*, accelerate the development of tea industry, improve the taste of tea culture, and promote the rapid and healthy economic and social development of Guizhou Province, therefore, growth environment of tea trees in karst and non karst areas was compared, we further evaluate the impact of soil nutrients on the quality of ancient tea trees, and analyze the effect of soil elements on the content of primary metabolites and secondary metabolites of tea. Studying the relationship between plant functional components and environmental factors is an important idea to understand the suitable growth environment of *C. tetracocca*, providing a reference for their protection, comprehensive utilization, introduction and cultivation, and ensuring their excellent quality.

Materials and Methods

Sample Collection

All studied leaves of *C. tetracocca* were collected in Pu'an and Xingren (JD, PB, SD, W1, W2, W3), Guizhou in August 2021 (Figs 1, 2). JD, PB, SD are located in non karst areas, and the other are located in karst areas. Voucher specimens have been deposited at the school of karst science, Guizhou Normal University. The leaves of *C. tetracocca* were cut into small pieces and dried in a blast drying oven at 60ºC until they were easily ground. After cooling, samples were crushed and passed through a 0.25 mm sieve. Then, the powder was sealed for later use. The plantation soil was selected and collected at the depths of 10~20 cm in August 2021, corresponding to the collected plant samples. A total of 6 sampled soil and plants were obtained according to the five-point sampling method. The soil samples were quickly packed and brought back to the laboratory. After air drying, the soil was ground using a 2 mm sieve and stored in a self-sealing bag.

Chemicals Reagents

Aspartic acid (Asp), Glutamic acid (Glu), Serine (Ser), Histidine (His), Glycine (Gly), Threonine (Thr), Arginine (Arg), Alanine (Ala), Tyrosine (Tyr), Cysteine (Cys), Valine (Val), Methionine (Met), Phenylalanine (Phe), Isoleucine (Ile), Leucine (Leu), Lysine (Lys), Proline (Pro) were obtained from Sigma-Aldrich (USA). L-Theanine was obtained by Shanghai Yuanye Bio-Technology Co., Ltd (China). The HPLC grade of methanol and acetonitrile were purchased from CNW (Germany). Sodium hydroxide, O-phthalaldehyde (OPA), FMOC and 3-mercaptopropionic acid were obtained from Sigma-Aldrich (USA). Hydrochloric acid, boric acid, sodium phosphate monobasic dihydrate, and disodium hydrogen phosphate dodecahydrate were purchased from Guangzhou Chemical Reagent Factory (China).

The main functional components used in the experiment are listed in Table 1. HPLC grade water and methanol were obtained from Merck (Germany). Remaining analytical reagents were purchased from Tianjin Kemiou Chemical Reagent Co., Ltd (China).

Sample Preparation and Analytical Methods

Analysis of SOC, N, P, K, Ca, Mg and pH in Soil

Soil organic carbon (SOC) was analyzed by the dichromate oxidation approach [28]. Total nitrogen (TN) was determined using an automatic Kjeldahl apparatus (Shandong Haineng Scientific Instrument, Shandong, China) [29]. Total phosphorus (TP) was analyzed by the molybdenum blue method [30]. Contents of total potassium (TK), calcium (Ca) and magnesium (Mg) were obtained by sulfuric digestion through iCAP-7200 (Thermo Fisher Scientific, MA, USA), according to Vettori (1969) [31]. The contents of Ca and Mg were measured separately in the supernatant by atomic absorption spectrometry. The soil pH was determined using the electrode potential method, and the water-soil ratio was mixed at a ratio of 2.5:1. In additon, C/N C/P C/K C/Mg N/P N/K N/Mg P/K P/Mg Ca/Mg as important indicators were calculated using Excel 2021.

Analysis of SOC, N, P, K, Ca, Mg in Plants

The method for determining plant organic carbon was consistent with the method for determining soil organic matter [28]. Total nitrogen (TN) was analyzed by automatic nitrogen determinator method using the Kjeldahl technique [29]. Total potassium (TK), total phosphorus (TP), and calcium (Ca) were determined in reference to [32], and magnesium (Mg) was measured by microwave digestion and inductively coupled plasma-atomic emission spectrometry (ICP-AES). Additionally, C/N C/P C/K C/Mg N/P N/K N/Mg P/K P/Mg Ca/Mg as important indicators were calculated using Excel 2021.

Determination of L-Theanine Content in C. tetracocca

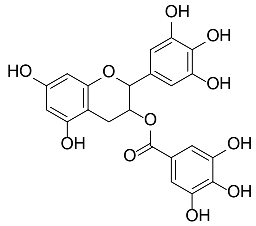
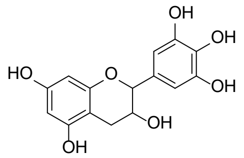
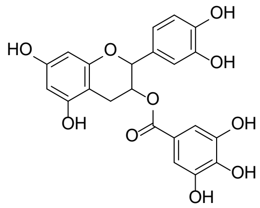
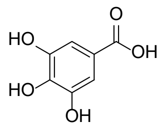
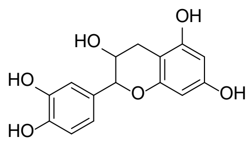
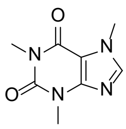
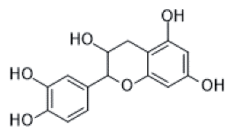
According to the method of GB/T 23193-2008 (China), L-theanine was extracted from leaves separately, and then theanine content was determined by HPLC. The Agilent 1200 Series (Agilent Technologies Co., CA, USA) a Zorbax Eclipse XDB-C18 analytical column (250 mm × 4.6 mm inner diameter, 5 μm nominal particle size) and DAD detector were used for chromatographic separation and detection. Mobile phase A was 20 $mmol·L^{-1}$ ammonium, and mobile phase A 20 $mmol·L^{-1}$ ammonium acetate: methanol: acetonitrile = 1: 2: 2, the ratio of A to B was set to 1:1. The flow rate is 1.0 mL/min, the column temperature is 40ºC, the injection volume is 10 μL, the detection wavelength is 338 nm.

Determination of Amino Acid Content in C. tetracocca

Tea sample powder of 100.0 mg was weighed in sealed a bottle, and 10 mL HCl (6 mol·L-1 containing 1% phenol) was added, then the bottle was flushed with N2 for 1 min and the bottle was sealed. The reaction hydrolyzed was conducted at 110ºC for 22 h. The product was taken out to cool down water was added to make up to 50 ml. 1 mL sample was blown dry at 95ºC, 1 mL HCl dissolution (0.01 mmol) was accurately added and then filtered out. The primary amino acids was derivatized with O-phthalaldehyde (OPA) and the secondary amino acids were derivatized with fluorene methoxycarbonyl chloride (FMOC) and then passed through the column (ZORBAX Eclipse AAA, 4.6 × 75 mm, 3.5 μm) for detection.

HPLC system (Agilent Technology-Series 1100) was equipped with a VWD detector and a binary pump with a column temperature regulator. Chromatography Chemstation software (Agilent, German) was used for data processing. Pre-column derivatization and

Table 1. The information of chemicals used in the present study.

Chemicals	Abbreviations	CAS No.	Molecular structure	Supplier	Purity (%)
Epigallocatechin gallate	EGCG	989-51-5		Bomei biochemical technology Co., Ltd., Anhui China	≥98
(-)-Epigallocatechin	EGC	970-74-1		Bomei biochemical technology Co., Ltd., Anhui China	≥98
(-)-Epicatechin gallate	ECG	1257-08-5		Bomei biochemical technology Co., Ltd., Anhui China	≥98
Gallic acid	GA	149-91-7		Bomei biochemical technology Co.Ltd. Anhui China	≥98
Epicatechin	EC	490-46-0		Bomei biochemical technology Co., Ltd., Anhui China	≥98
Caffeine	CAFF	58-08-2		Bomei biochemical technology Co., Ltd., Anhui China	≥98
Catechin	C	7295-85-4		Bomei biochemical technology Co., Ltd., Anhui China	≥98

filtration were detected at 338 nm and 266 nm. A ZORBAX Eclipse AAA (4.6 x 75 mm, 3.5 μm) was used for chromatographic analysis. Mobile phase A was mM sodium dihydrogen phosphate (pH 7.8), and mobile phase B was methanol with acetonitrile, water (45:45:10). The flow rate was 1.0 mL/min. The gradient elution was programmed as Table 2. The solutions were filtered through a 0.45 μm membrane filter before use.

Determination of C CAFF GC EC ECG EGC EGCG in C. tetracocca

Samples were air dried under natural conditions and grinded into a powder, then extracted with 70% methanol aqueous solution assisted by ultrasonic waves.

The Agilent 1200 (USA) equipped with G1311A pump, G1315 DAD detector and Agilent chromatography workstation were used for chromatographic analysis. The mobile phase A was (1000 mL) was the mixture of acetonitrile (90 mL), acetic acid (20 mL) and EDTA-2Na (2 mL), and the mobile phase B was (1000 mL) with acetonitrile (800 mL), acetic acid (20 mL) and EDTA-2Na (2 mL). The flow rate was at 1 mL/min, and column temperature was set to 35ºC. The gradient elution program was 100% A containing for 10 min, and decreased linearly to 68% A within 15 min, 68% A maintained for 10 min, and then increased 100% A. The injection volume was 10 μL. The chemicals were detected at a wavelength of 278 nm. An ultrasonic cleaning machine was used for sample extraction.

Table 2. Gradient elution conditions of 17 amino acids. A B.

Time (min)	A (%) (40 mM phosphate butter)	B (%) (methanol: acetonitrile: water = 45:45:10)
0	100	0
1	100	0
23	46	57
27	0	100
34	0	100
40	100	0
41	100	0

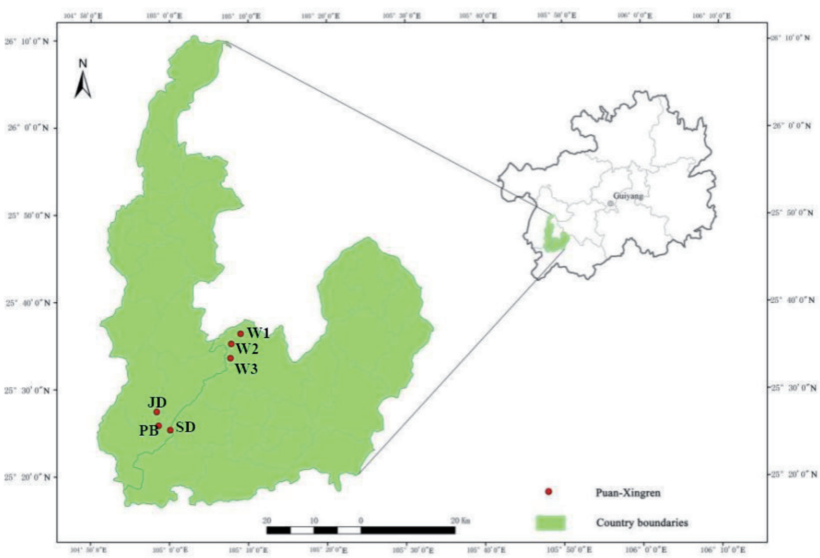


Fig. 1. Location of the study site in South China Karst.

Statistical Analysis

In order to compare amino acids and secondary metabolites in relation to that of the soil and plant samples, correlation coefficients were calculated in the different areas. The analysis of the relationship between plants, soil and amino acids and secondary metabolites was performed using the Pearson method. The data distribution was examined with an application of normality test. Kolmogorov-Smirnov test value (sig.= 0.24) greater than 0.05. The data belonged to normal distribution. Statistic package SPSS 22.0 was used for the correlation analysis. In this study, the Figures were drawn with Origin 2021 (OriginLab Inc., Northampton, MA, USA).

Results

Analysis of SOC, N, P, K, Ca, Mg and pH of Soil

Under different geological backgrounds, the pH value of soil, the content of nutrient elements, and the availability of plants are different. Soil pH directly affects the existing forms of various nutrient elements in soil and their availability to plants [33]. There were significant differences in soil pH among the six different habitat types, and the overall trend was that the soil pH in karst areas was higher than that in non karst areas. JD, PB and SD were 4.8, 4.9 and 4.63 respectively. The pH of W1, W2, and W3 are 5.6, 5.0 and 5.63, respectively. Previous studies have shown that when the pH value is in the range of 5.0~6.0, it is most conducive for tea plants to absorb calcium. If the soil acidity is enhanced, the leaching amount of mobile Mg will also be significantly increased [34]. This might be a notable tea production areas in China. The results further indicate that the soil in karst areas is suitable for the growth of *C. tetracocca*.

SOC, N, P, K, Ca and Mg, as irreplaceable biogenic elements in soil are important factors to characterize the quality of soil fertility [35]. The content of total K in non karst areas is higher than that in karst areas. The highest content of total K appears in JD, and the lowest content occurs in SD. The content range of total K from JD, PB and SD is 10.63 g/kg~16.91 g/kg, and the content range from W1-W3 is 5.26 g/kg~10.85 g/kg.

Fig. 2. a) The seeds of *C. tetracocca*; b) *C. tetracocca* and soils in non karst areas; cd) *C. tetracocca* and soils in karst areas.

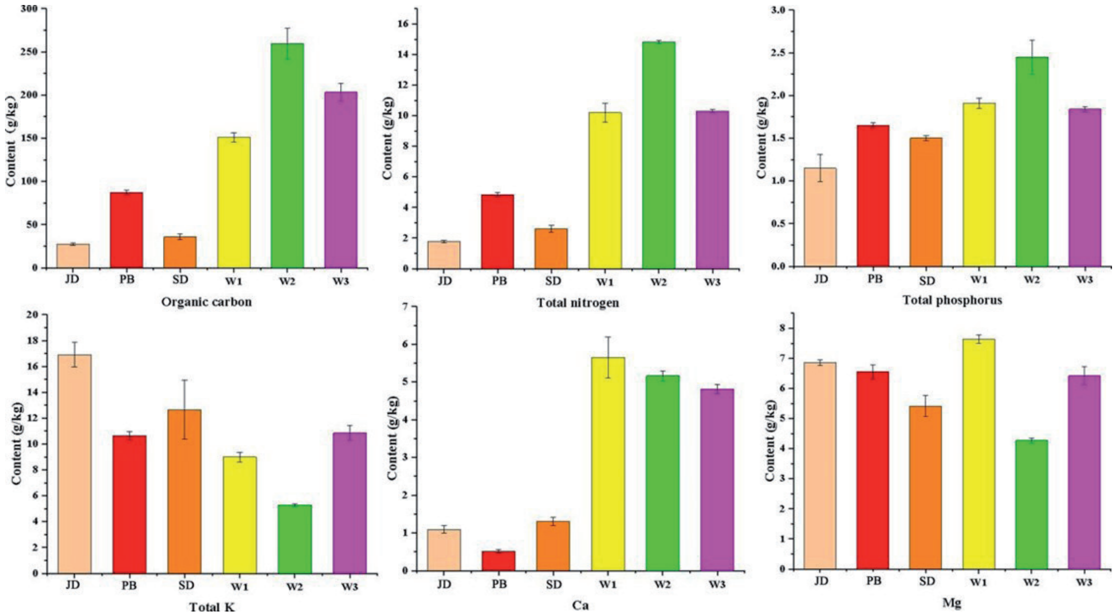


Fig. 3. The content of each element in the soil.

There is a significant difference in the overall trend of Mg content. Among them, SOC, N, P and Ca in W (1-3) are higher than those in JD, PB, SD. There is a significant difference in the content of organic carbon in the range of 27.21 g/kg~86.9 g/kg (Fig. 3). The soil SOC, N and Ca are significantly higher than those in non karst areas, and the content of P is slightly higher than those in karst areas. Due to the high calcium of karst areas [36], the content of Ca is much higher than those in the other three regions, about 5 to 7 times of its content. However, the content of K is just the opposite. Mg content differed rarely between the two soils.

Stoichiometric equilibrium is a powerful tool that has been proved to provide a quantitative understanding and analysis of ecosystem balance and process. The C: N: P ratio can be used to reflect the growth of plants, and the N:P critical value can be used to judge the effect of soil on plant growth, as indicators of long-term nutrient supply. The ratio of C/P, C/K, C/Mg, N/P, N/K, N/Mg, P/K, P/Mg, Ca/Mg in karst areas is higher than that in non karst areas, except C/N and C/Ca (Fig. 4).

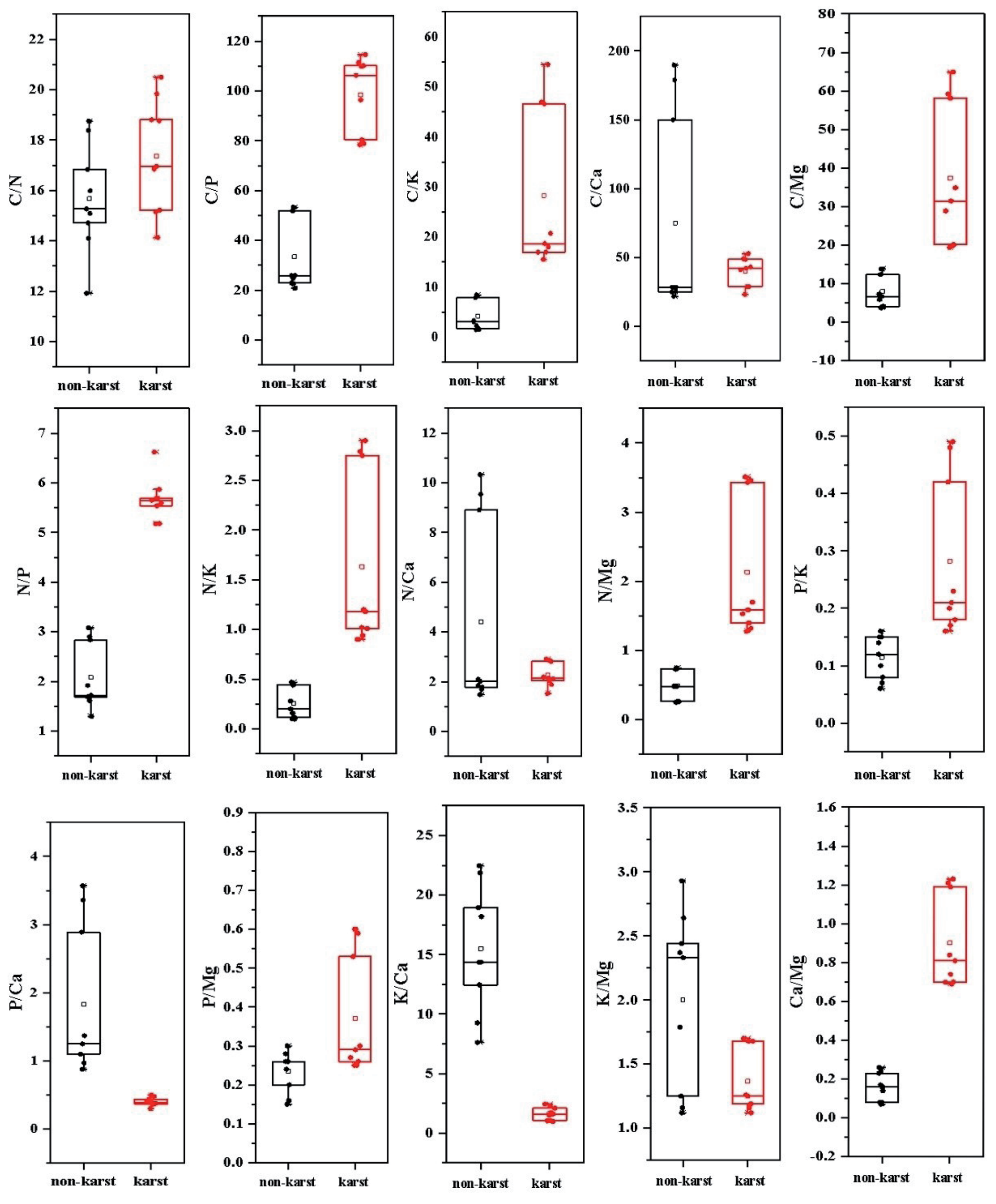


Fig. 4. Ecological stoichiometry of different soil elements.

The range of C/N is 13.77~19.72, with the minimum value appearing in non karst areas and the maximum value appearing in karst areas. The range of C/P is 23.84~110.24. There is a significant difference between the two regions. The value of P in karst areas is 2-5 times that in non karst areas, and the value of P in non karst areas is higher than that of JD and SD. The value of C/K is 1.61-49.33, with a significant difference. The ratio of karst areas is about 25 times that of non karst areas, and the value of P is the same as that of C/P, which is significantly higher than that of JD and SD. The content of C/Mg ranges from 3.94~60.76, and the highest value is located in W2 in karst areas. Although there is a difference in N/P, the difference is not significant. The content range of N/K is 0.11~2.81, the content range of N/Mg is 0.26-3.47, the content range of P/K is 0.07~0.47, and the content range of Ca/Mg is 0.16~0.75. The trend ranges of these four ratios are relatively consistent. The ratio of C/Ca in PB area is significantly higher than that in other areas and the ratio of N/Ca is consistent with that of C/Ca, which may be related to the unique environment in PB. The ratio of K/Ca in non karst areas is significantly higher than that in karst areas, the highest value also appears in PB and the lowest value appears in W2.

Analysis of SOC N P K Ca Mg of *C. tetracocca*

The range of C in *C. tetracocca* is 365.98 g/kg 435.84 g/kg, the average value in non karst areas is 423.63 g/kg, and the average value in karst areas is 368.69 g/kg, which is lower than the content of SOC in non karst areas. The content of N ranges from 16.52 g/kg to 26.64 g/kg. The content of N in areas JD and SD is similar, lower than in karst areas. The highest value (24.7 g/kg) of P appears in SD and the other five regions are basically consistent. The content of K tends to rise at the position of point P then decreases linearly, and an inflection point appears at W2. There are two higher points of Ca content in karst areas and non karst areas SD and W2, respectively. The content of Mg is the lowest at PB and the highest at W1. The general trend is that karst areas are higher than non karst areas. C, P and K in the non karst areas of *C. tetracocca* are higher than those in the karst areas. However, N, Ca and Mg are just the opposite (Fig. 5).

The range of C/P in non karst areas is 21.77~26.63, the range of C/K is 174.97~259.96, the range of N/K is 1.07~1.37, the range of N/P is 6.71~10.82, and the range of N/Ca is 3.11~4.07. In karst areas the range of C/P is 14.61~21.4, the range of C/K is 207.04~289.49, the range of N/K is 1.43~2.77, the range of N/P is 12.25~14.96, and the range of N/Ca is 3.11~4.59. The C/P C/K N/K N/P N/Ca in non karst areas are lower than those in karst areas, which indicates that the ancient tea trees in karst areas have sufficient C and high content of N. The ratios of other physical and chemical properties C/N, C/Ca, C/Mg, N/Mg, P/Ca, P/Mg, K/Ca, K/Mg, Ca/Mg in non karst areas are lower than those in karst areas (Fig. 6). These results can be seen that the nature of high Ca in karst areas, especially the content of Mg and Ca is higher than that in non karst areas which may be due to the quality of Ca enrichment of ancient tea trees in *C. tetracocca* and the fact that Mg is in the middle of chloroplast structure which can better carry out photosynthesis.

Determination of L-Theanine Content

As the most abundant non-structural amino acid in tea plant, L-Theanine has potential medicinal value in calming nerves, improving cognitive ability, reducing

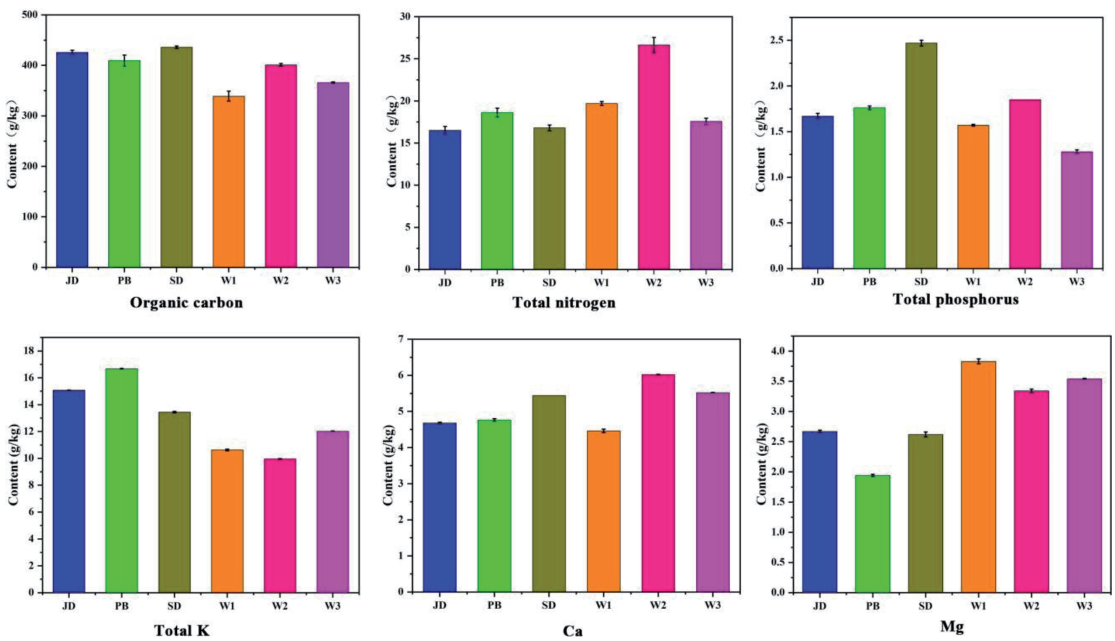


Fig. 5. The content of each element in the *C. tetracocca*.

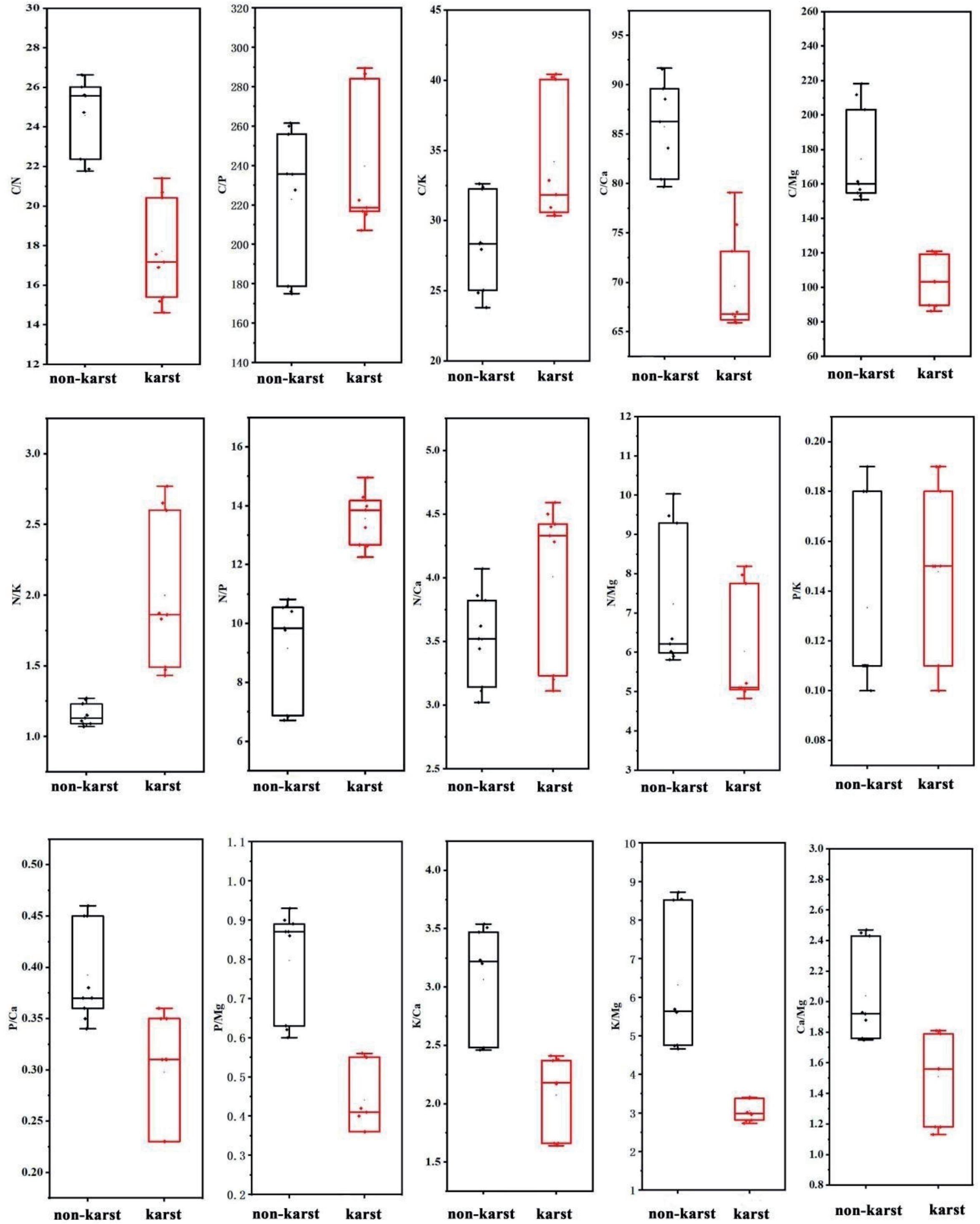


Fig. 6. Ecological stoichiometry of element in the *C. tetracocca*.

weight, it has anti-cancer properties, and helps prevent cardiovascular diseases. In non karst areas, theanine content of JD is 1.72 mg/g, and the content of PB and SD is equivalent, which are 0.59 mg/g and 0.55 mg/g, respectively (Fig. 7). In karst areas, the content in the three areas is higher than that in non karst areas, which are 1.75 mg/g, 2.64 mg/g and 2.09 mg/g, respectively. Special adversity conditions contribute to the increase of amino acid content. Therefore, *C. tetracocca* with high theanine content can be cultivated by simulating the relevant soil chemical properties.

Amino Acid Composition and Nutritional Value Evaluation of *C. tetracocca*

Tea contains significant amounts of amino acids, which can not only provide essential nutrients for the human body, but are also the main component of tea's

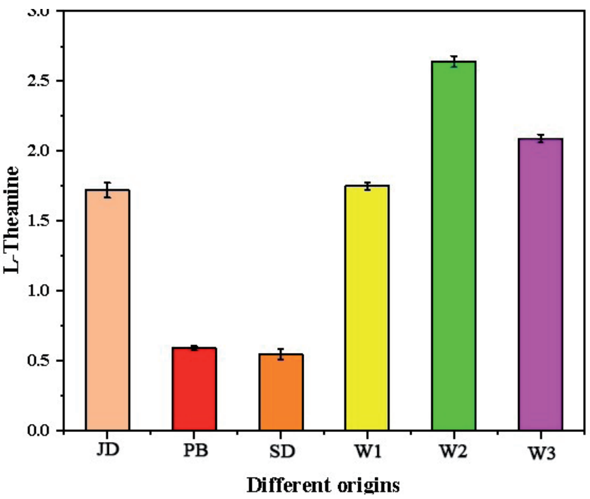


Fig. 7. Content of L-Theanine in different origins.

taste. It is an extremely important part of tea quality. Ile, Leu Lys, Phe, Thr and Val are essential amino acids for the human body and His is essential for infant growth and development. 17 kinds of free amino acids have been detected in the *C. tetracocca* from different habitats (Fig. 8). The content of Glu and Asp with fresh taste is significantly higher than that of other free amino acids, and the content of Gly, Ser and Pro with sweet taste is slightly lower. The range of total essential amino acids in six different producing areas is 80.74 mg/g~115.37 mg/g, among which W2 has the highest content of amino acids and JD has the lowest content (Figs 9, 10). Among the 17 kinds of amino acids in the 6 samples, the contents of Asp, Glu, Ala, Phe, Leu and Lys were higher than 5.00 mg/g. The content of amino acids in green tea is higher than that in other green tea, such as Wuling Mountain selenium rich green tea in Western Hubei, Yichang Three Gorges famous green tea, Qinba mountain high flavor green tea in Northwest Hubei. The content of essential amino acids ranged from 32.18 mg/g to 43.32 mg/g, among which W2 had the highest content of amino acids and JD had the lowest content. The content range of non essential amino acids is 48.56 mg/g~72.05 mg/g. The content of non-essential amino acids is higher than that of essential amino acids, which is consistent with other green tea studies.

The content of GA and ECG in all functional components is at a low level, lower than that of other

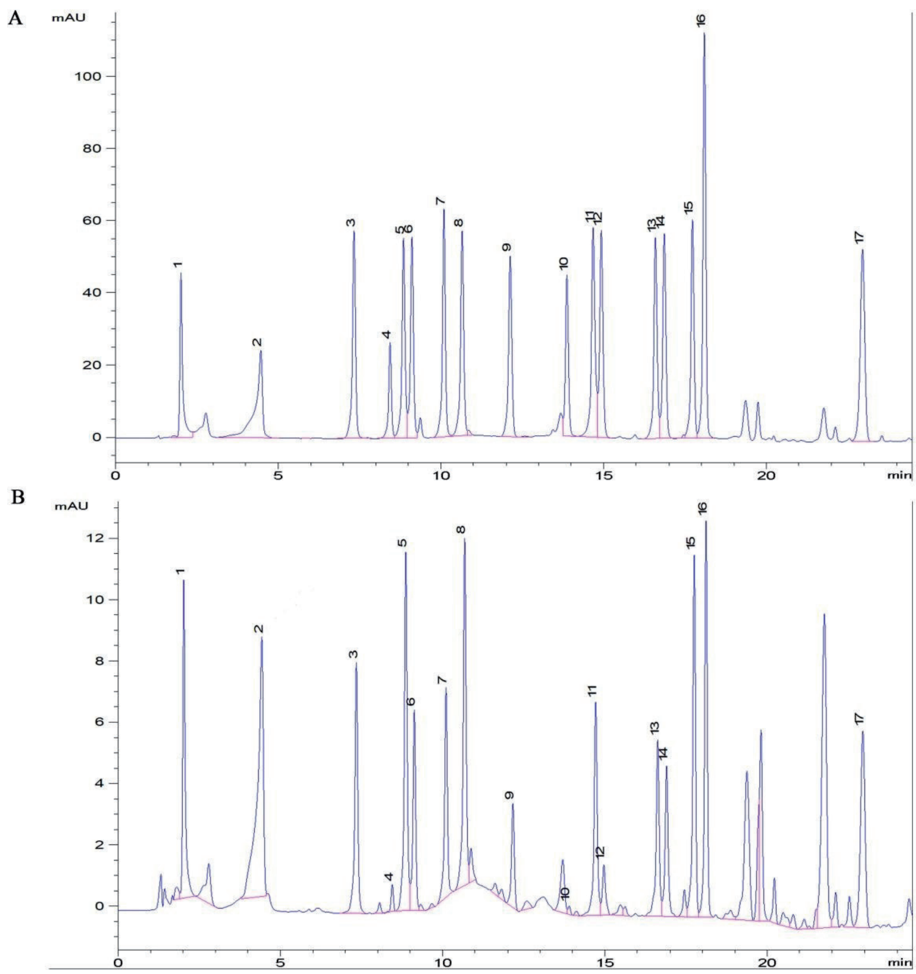


Fig. 8. Chromatograms of 17 free amino acids from Standards. a) and samples; b) in *C. tetracocca*.
Note: 1-Asp, 2-Glu, 3- Ser, 4-His, 5-Gly, 6-Thr, 7-Arg, 8-Ala, 9-Tyr, 10-Cys, 11-Val, 12-Met, 13-Phe, 14-Ile, 15-Leu, 16-Lys, 17-Pro.

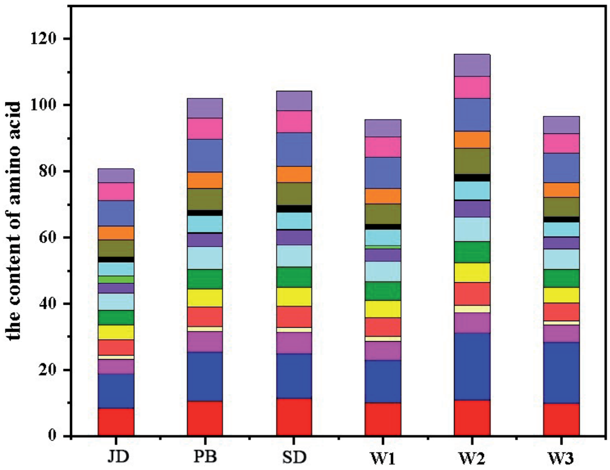


Fig. 9. Content of 17 amino acids of *C. tetracocca* in different origins.

Note: red: Asp; blue: Glu; dark pink: Ser; light yellow: His; dark orange: Gly; yellow: Thr; green: Arg; gray: Ala; purple: Tyr; grass green: Cys; sky blue: Val; black: Met; chartreuse: Phe; light orange: Ile; light blue: Leu; light pink: Lys; light purple: Pro.

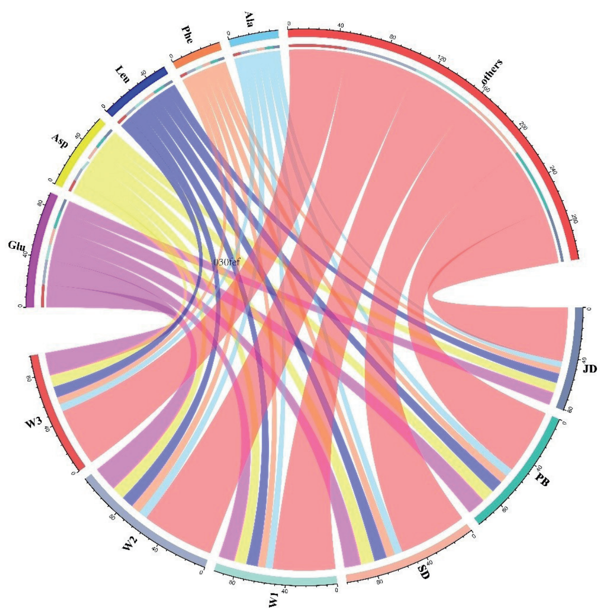


Fig. 10. Main amino acids from different origins.

compounds (Fig. 11). The content of CAFF ranges from 0.095%~1.628%, and the lowest and highest values occur in karst areas. This result reflects the heterogeneity of karst areas to a certain extent. The content of C ranges from 0.262% to 1.174%, and the highest value still occurs in karst areas. The content of EC and ECG is indeed the highest in non karst areas. However, the content of EGC and EGCG is opposite to that of EC and ECG, and the maximum values appear in W2 in karst areas which are 0.049% and 0.466%, respectively.

In the non karst area, the content of EC is the highest in area JD, and the content of CAFF and ECG in area PB is the same, higher than that of other compounds (Fig. 11). The content of ECG in SD is the highest, followed by C and EC. The content of other compounds is lower than that of these three compounds. The content of compounds in W1 in karst area is similar to that in SD. ECG, C and EC were higher. However, CAFF is the highest in W2, the content of other compounds is low, and the content of C is the highest in W3. Therefore, the accumulation of secondary metabolites in different habitats is quite different, and there may be great differences in their biosynthetic pathways.

Correlation Analysis

Relationship between Non-Mineral Elements in Soil and Chemical Composition of C. tetracocca

Various catechins were the key contributors to the flavor and quality of tea. EC is the main component of catechins, EC can inhibit the proliferation of prostate stromal cells. The correlation analysis between secondary metabolites and physical and chemical properties in soil shows that the content of EC is significantly correlated with the content of K and P, positively correlated with K and negatively correlated with P (Fig. 12a). The correlation analysis between the secondary metabolites in *C. tetracocca* and C, N, P, K, Ca and Mg in the plant shows that the content of EC is significantly correlated with K and P (Fig. 12b). In addition, EGC is negatively correlated with Mg in soil and Mg in plants. These results are consistent with previous studies on green tea and nutrients.

Relationship between Non-Mineral Elements in Soil and Amino Acid of C. tetracocca

The analysis of elements and amino acids in soil and plants showed that Ca in soil was consistent with Ca in plants, showing a significant positive correlation with Glu, and N was correlated with His to a certain extent (Fig. 13a, Fig. 13b). His is not an essential amino acid for adults, but it is an essential amino acid for children and uremic patients. Therefore, the increase of N content in soil can increase the histidine content in the leaves of *C. tetracocca*. Ca, P, N showed positive correlation with amino acids except Cys, while K and Mg showed negative correlation. Both C in soil and C in plants showed low correlation. Overall, in the promotion and planting of *C. tetracocca*, the amino acid composition of *C. tetracocca* can be improved by improving the addition ratio of each original element [37].

Discussion

Soil pH value is of great significance to the growth and development of soil microorganisms, soil animals and plants, which will be affected by bedrock climate and surface litter [38, 39]. In the area where ancient

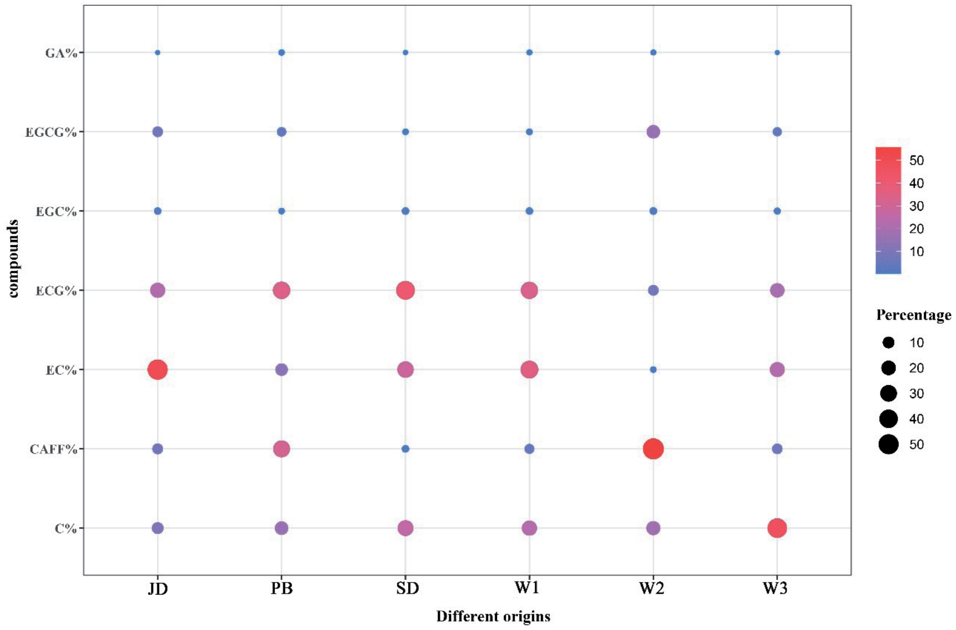


Fig 11. The contents of C, CAFF, GC, EC, ECG, EGC, EGCG in *C. tetracocca*. The size of the circles represents the content of different compounds, red indicates high content of compounds, and blue indicates a low content of compounds.

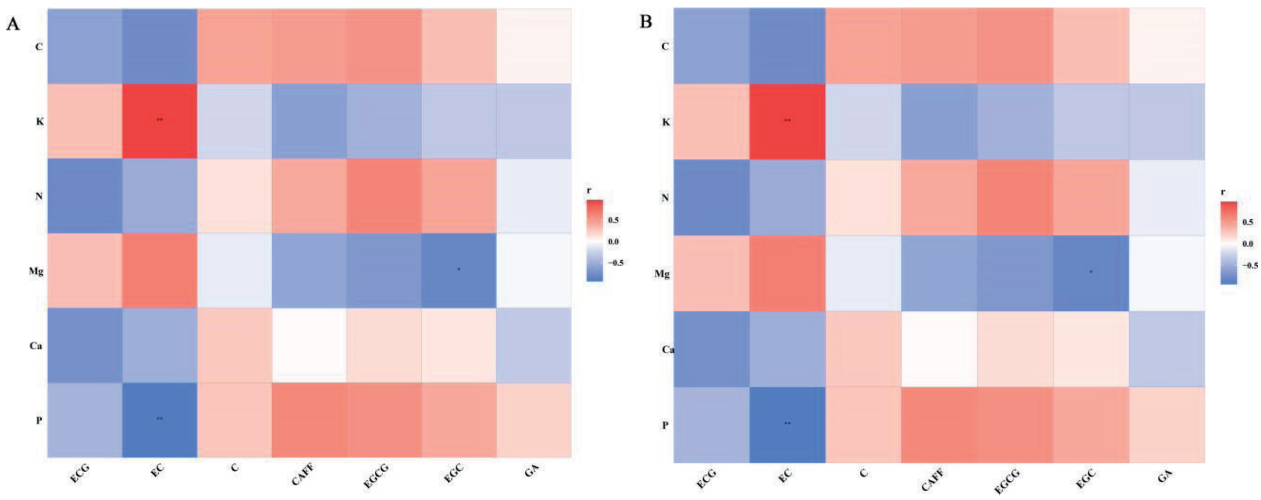


Fig. 12. The relationship of functional components and C, N, P, K, Ca, Mg in soil and *C. tetracocca*, respectively.
The colors from red to blue range from positive correlation to negative correlation.

tea trees grow in *C. tetracocca*, the pH value in karst area and non karst area changes little, but the pH value in karst area is still higher than that in non karst area, which may be related to the special environment of karst area, and due to the influence of rock cracks, deep-seated soil may not be collected in karst area. Soil nitrogen is very important to maintain plant growth and basic metabolism. Its main sources are biological nitrogen fixation and atmospheric deposition [38]. The soil N in karst area is significantly higher than that in non karst area. Because the altitude of Wangjiazhai karst area is higher than that in Pu'an, it indicates that the altitude may have a direct impact on the soil nitrogen content. P element is an important component element of adenosine triphosphate and nucleic acid. It is one of a large number of elements for plant growth and development. It affects plant genetics, respiration, photosynthesis, and thus affects plant growth [40]. It has been found that the P element accounts for 2% of the dry weight of the plant, and the P content of SD in non karst areas is significantly higher than that in other areas. This may be due to the absorption of P in the corresponding soil. Soil K element is mainly obtained through atmospheric precipitation, karstification and other means, especially in the places where Wangjiazhai is rarely visited by people, it is impossible to obtain it through soil fertilization [41]. In the karst rocky desertification area, the karstification is strong and the terrain space is developed. Therefore, the nutrient factors in the soil flow to the low-lying areas with the

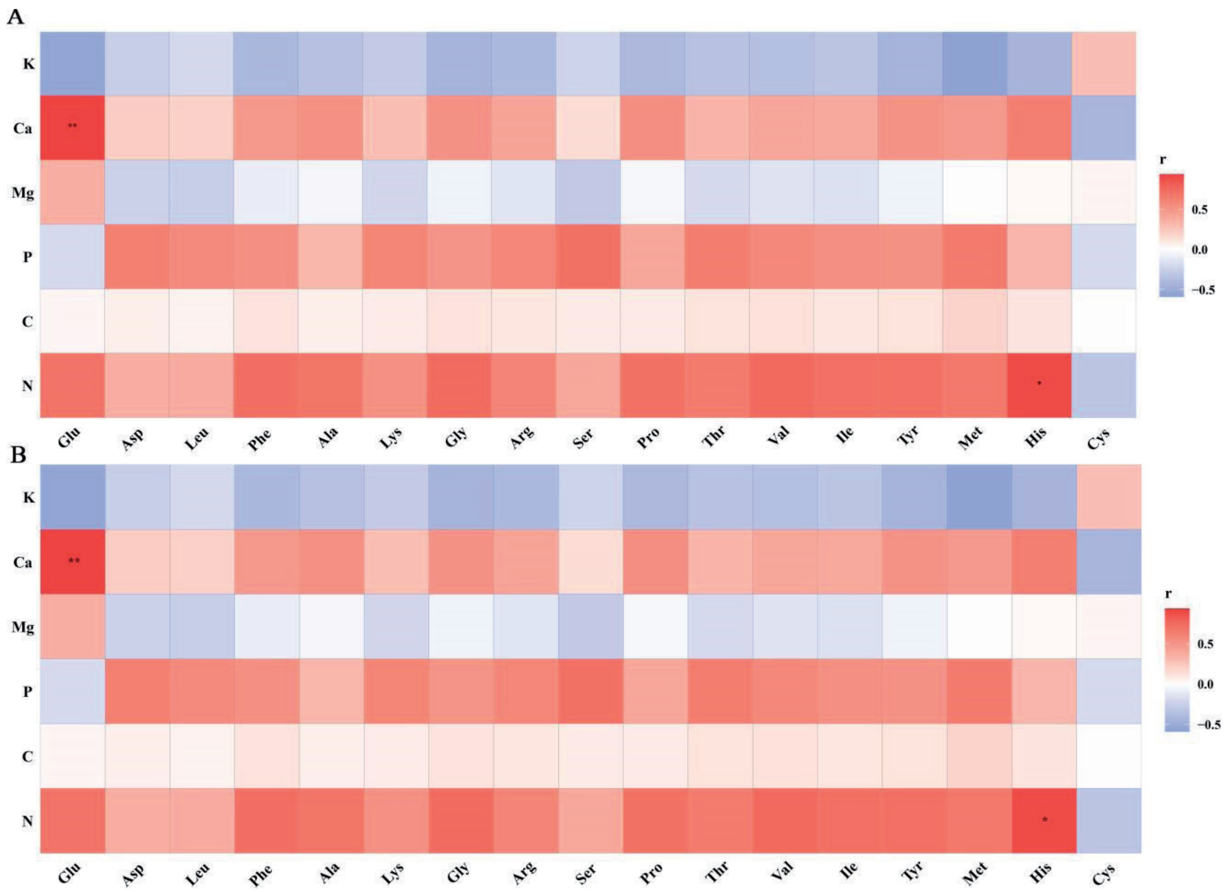


Fig. 13. The relationship of amino acid and C, N, P, K, Ca, Mg in soil. a) and *C. tetracocca*: b), respectively.
Note: *P<0.05 **P<0.1 The colors from red to blue range from positive correlation to negative correlation .

surface runoff generated by precipitation. Therefore, the content of the K element is low.

N, P, K, Ca, Mg and Zn play an important role in the synthesis of polyphenols and free amino acids in spring leaves [42]. N can not only promote the growth of tea plants but also indirectly affect the synthesis and accumulation of theanine by inducing the activity of phenylalanine ammonia lyase and increasing the activity of glutamate synthase responsible for theanine production [43]. Magnesium is located in the center of chloroplast and can indirectly affect the biosynthesis of polyphenols, flavonoids and free amino acids [44]. Similarly, K has not only been shown to improve drought stress, but also induce more free amino acids. In addition, our results may indicate that there is a threshold for P level to increase the contents of catechins and flavonoids. Due to the negative correlation with soil P and plant P and flavonoids, this is consistent with the results of previous study [37] and contradicts the previous research results. Early studies suggested that inhibition of cinnamic acid biosynthesis was the precursor of flavonoid metabolism, which occurred under the condition of phosphorus deficiency.

Tea polyphenols and amino acids are important secondary metabolites of tea plant and also important evaluation criteria of tea quality, L-theanine is mainly composed of theanine synthetase (TS) glutamine synthetase (GS) arginine decarboxylation (ADC) alanine aminotransferase (ALT) and glutamic acid synthase/glutamine-α-Ketoglutarate synthase/glutamine-α-Ketoglutarate aminotransferase [45]. L-Theanine is the main component of the flavor substance in tea soup [46]. Its content accounts for 1-2% of the dry weight of tea and 40-70% of the total amino acids. However, theanine content in *C. tetracocca* is lower than that of other amino acids, except His. This may be due to the fact that glutamic acid is the precursor of theanine biosynthesis, and the glutamic acid content of *C. tetracocca* is at a high level. Moreover, the content of glutamic acid in karst areas is also higher than that in non karst areas. Under adverse conditions, glutamic acid is conducive to the accumulation of glutamic acid, which may be converted into more theanine after a long time of growth. The *C. tetracocca* overcomes the drought growth conditions in karst areas by regulating relevant genes and signal networks, so as to regulate the production of more amino acids beneficial to the human body.

Conclusions

We measured SOC, N, P, K, Ca, Mg in soil and SOC, N, P, K, Ca, Mg in *C. tetracocca*. The elements in karst areas and non karst areas showed differences. The catechins, theanine and 17 kinds of free amino acids in *C. tetracocca* were also determined. There was a significant positive correlation between K and EC in soil and plants and a significant negative correlation between P and EC. There was a negative correlation between Mg and EGC in soil and plants. In addition, the pH of soil is also an important index affecting catechins and amino acids. Therefore, increasing the content of k and reducing the content of Mg and P can improve the functional components of *C. tetracocca*. Although the harsh environment in karst areas, *C. tetracocca* still grows well and its functional components are not lower than those in non karst areas, but they can still be promoted. The result is of great significance for the protection of *C. tetracocca* trees in karst areas and promoting the planting of *C. tetracocca* in karst areas of Guizhou Province.

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Conflicts of Interest

The authors declare no conflict of interest.

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