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Influence of Different Planting Combinations on the Amino Acid Concentration in Pericarp of *Zanthoxylum planispinum* 'Dintanensis' and Soil

Yitong Li [1], Yanghua Yu [1,*], Yanping Song [1] and Changsheng Wei [2]

[1] School of Karst Science, State Engineering Technology Institute for Karst Decertification Control, Guizhou Normal University, Guiyang 550001, China
[2] Forestry Bureau of Zhenfeng County, Zhenfeng 562200, China
* Correspondence: yuyanghua2003@163.com

Abstract: In this study, the effect of different planting combinations on the amino acid concentration in the pericarp of *Zanthoxylum planispinum* 'dintanensis' (hereafter referred to as *Z. planispinum*) was studied, and the response of amino acid concentration to soil factors was clarified. The aim of this study was to screen optimal planting combinations and provide a theoretical basis for improving pericarp quality. Five planting combinations of *Z. planispinum* in a karst rocky desertification area were selected as the research objects, and the concentration and accumulation of free amino acids in the pericarp of *Z. planispinum* were analyzed. Then, combined with existing soil quality data, the pericarp quality of *Z. planispinum* was comprehensively evaluated by principal component analysis, and the effect of soil factors on amino acid concentrations was clarified by redundancy analysis. The results are as follows: (1) except for arginine, serine, proline, alanine, tyrosine and cystine, the concentrations of other free amino acids significantly differed among the five planting combinations. In general, the planting combination has a great influence on the concentration of free amino acids in the pericarp of *Z. planispinum*, especially essential amino acids; (2) free amino acid concentration in the pericarp of *Z. planispinum* mostly increased in combination with *Sophora tonkinensis* Gagnep. (hereafter referred to as *S. tonkinensis*) and decreased in combination with *Prunus salicina* Lindl; (3) principal component analysis showed that the concentration of free amino acid in the pericarp of *Z. planispinum* was generally at a high level when combined with *S. tonkinensis* or *Lonicera japonica* Thunb. (hereafter referred to as *L. japonica*). Among them, the amino acids in the pericarp of *Z. planispinum* with *S. tonkinensis* were closer to the ideal protein standard of FAO/WHO; (4) soil-available potassium, available phosphorus, microbial biomass nitrogen, available calcium and microbial biomass phosphorus in soil factors had significant effects on amino acid concentration after a redundancy analysis. It can be seen that the available nutrients and soil microbial biomass contribute greatly to the amino acid concentration of the pericarp. According to the soil quality and the amino acid quality of the pericarp, planting with *L. japonica* can improve the amino acid quality of the pericarp of *Z. planispinum*, as well as selecting *Z. planispinum* + *L. japonica* as the optimal planting combination.

Keywords: principal component analysis; redundancy analysis; karst rocky desertification area

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1. Introduction

As a type of organic nitrogen, amino acids are the most basic element of proteins and are an important primary metabolite for maintaining plant growth, reproduction and development [1]. Amino acids are also common small molecular precursors of synthesized organic compounds, such as nucleic acid, chlorophyll and hormone and secondary metabolism in plants [2]. In addition, as one of the existing forms of amino acids, free amino acids (FAAs) are important flavoring substances. Their types and concentrations are often used as important indicators to evaluate the nutritional value and taste of fruits and have an important impact on the formation of fruit quality [3,4]. Free amino acids are generally grouped according to their taste as bitter (valine, leucine, isoleucine, methionine and arginine), sweet (glycine, alanine, serine, threonine, proline and histidine), delicious (lysine, glutamate and aspartate) and aromatic (phenylalanine, tyrosine and cystine) [5]. Therefore, the study of amino acid concentration is particularly important to further identify the flavor of *Zanthoxylum planispinum* 'dintanensis' (hereafter referred to as *Z. planispinum*).

The chemical elements in the soil are the direct source of nutrients for fruit formation, the raw material storage for nutrients and determine the main chemical reaction processes and rates such as photosynthesis, respiration and transpiration [6]. Nitrogen (N), phosphorus (P) and other macro elements play an important role in the growth and development of plants, as well as the regulation of various physiological functions [7,8]. Nitrogen is an important component of protein and other metabolites, participating in the amino acid synthesis of secondary metabolites [9,10]. Phosphorus is an important chemical element that constitutes the metabolites of deoxyribonucleic acid, ribonucleic acid and adenosine triphosphate. It also directly participates in the biochemical reaction of some primary metabolite synthesis [11,12]. In addition, calcium, as the second messenger, is used to activate the resistance gene of plant cells and regulate the response to the stimulation signal of whole plant somatic cells [13,14].

Microorganisms in soil can decompose plant residues into humus, thus participating in the assimilation of secondary metabolism in plants [15]. In addition, the decomposition and mineralization of organic matter by soil microorganisms affect soil nutrient cycling, thereby changing the balance of soil nutrient elements. Ecological stoichiometry is a combination of the basic principles of ecology and stoichiometry to explore the energy balance and nutrient element balance of the ecosystem [16]. The change in element stoichiometry has an obvious correlation with the change in metabolites, which can regulate molecular synthesis in the organism and further affect the metabolic reaction [17]. It can be seen that the changes in soil elements, microorganisms and their stoichiometry are of great significance to the synthesis of metabolites.

Z. planispinum is a small deciduous tree of *Zanthoxylum planispinum* in the family Rutaceae. It is a medicinal and edible homologous plant with rich protein and complete amino acid species [18]. It has become a unique dominant tree species for karst rocky desertification control in Guizhou due to its calcium (Ca) preference and drought-resistant and lithogenic characteristics. However, in recent years, due to the continuous planting of monoculture forests, soil fertility decline, improper management measures and other reasons, the pericarp quality of *Z. planispinum* has decreased, and plant growth has declined. Adopting the combination planting method of different plants can effectively increase the diversity of plant communities, make full use of space and resources [19] and have the following advantages: increased crop yield; optimized crop quality; efficient use of nutrients; increased biodiversity; and reduced disease [20,21]. Based on this, this study selected four common planting combinations of *Z. planispinum* and compared them with a *Z. planispinum* monoculture forest. The effects of different planting combinations on the amino acid quality in the pericarp of *Z. planispinum* and their response to soil were studied. The purpose of this is to solve the following problems: (1) concentration characteristics, flavor contribution and quality evaluation of FAAs in the pericarp of *Z. planispinum* with different planting combinations; and (2) effects of soil properties on the pericarp quality of *Z. planispinum*. To do so, the plants suitable for planting with *Z. planispinum* were selected, and the basis for the selection of planting combinations and the cultivation management of *Z. planispinum* was provided.

2. Materials and Methods

2.1. Overview of the Research Site

The research area is located in Beipanjiang Town, Zhenfeng County, Guizhou Province, China (105°38′35″ E, 25°39′37″ N). It belongs to a subtropical humid monsoon climate, with rainfall concentrated from May to October and an annual average rainfall of about

1100 mm. The annual average temperature is 18.4 °C. It is a valley terrain with an altitude of 370–1473 m. The soil type is mainly lime soil with carbonate rock accounting for 78.45%, pH > 6.5. The soil is rich in Ca. Most of the study area is moderately and intensively a rocky desertification area, with a rock exposure rate of 50%–80%. The environment is highly heterogeneous, with many kinds of niche types, such as stone surface, stone ditch, stone crevice, stone groove, stone cave, etc. *Z. planispinum* has the largest planting area in the study area. In addition, there were *Zea mays* L. (hereafter referred to as *Z. mays*), *Lonicera japonica* Thunb. (hereafter referred to as *L. japonica*), *Prunus salicina* Lindl. (hereafter referred to as *P. salicina*), *Sophora tonkinensis* Gagnep. (hereafter referred to as *S. tonkinensis*), *Arachis hypogaea* L. (hereafter *A. hypogaea*) and other associated species.

2.2. Treatment Setting

In order to ensure the typicality, representativeness and comparability of the selected sample plots, some common local planting combinations were screened. The final allocation of tree species determined that *P. salicina* represented the arbor, *S. tonkinensis* represented the dwarf medicinal material, *A. hydropogaea* represented the legume plant, and *L. japonica* represented the liana plant (*Z. planispinum* + *P. salicina*; *Z. planispinum* + *S. tonkinensis*; *Z. planispinum* + *A. hydropogaea*; *Z. planispinum* + *L. japonica*). The *Z. planispinum* monoculture forest was used as a control. One treatment with similar environmental elements was set for each plantation (Figure 1 & Table 1). Before planting research plantations, all treatments were mainly planted with *Z. mays*, and the management measures were the same in order to make the soil background value similar. In 2012, *Z. planispinum* was planted in 5 treatments. After 2018, *L. japonica*, *S. tonkinensis*, *A. hydropogaea* and *L. japonica* were planted around *Z. planispinum*. The annual plant *A. hydropogaea* was continuously planted according to the phenological season. Other perennial plants were regularly maintained to maintain the dynamic stability of the community. The age of *Z. planispinum* in the 5 treatments was 8 years, and the slopes were 10°. Li et al. [22] introduced the planting density of interplanting plants and forest land management measures in detail.

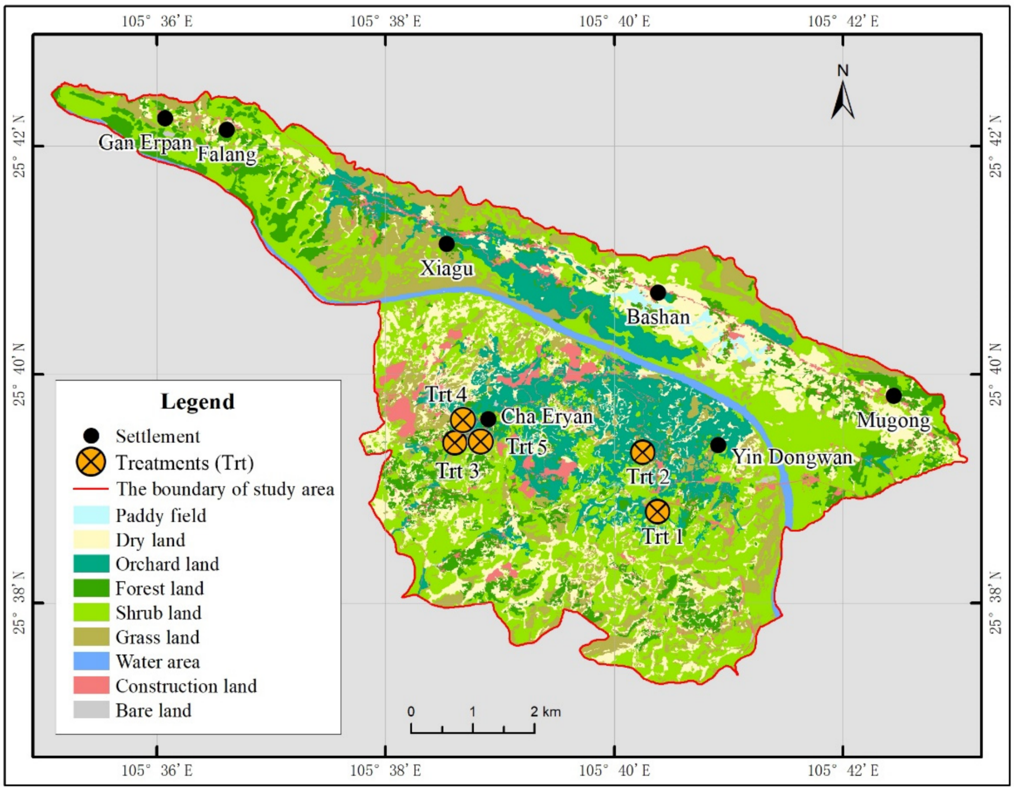

Figure 1. Distribution of treatments (Trt).

Table 1. Descriptions of the plantation types.

Plantation Types	Species Combinations	Longitude	Latitude	Growing Area (ha)	Altitude (m asl)	Density (m)	Height (m)	Crown Width (m)	Coverage (%)
Trt 1	*Z. planispinum* + *P. salicina*	105°40′28.33″ E	25°37′57.41″ N	1.34	764	3 × 3	3.5	2 × 2.3	70
Trt 2	*Z. planispinum* + *S. tonkinensis*	105°40′19.79″ E	25°39′25.75″ N	0.67	728	2 × 2	2.0	1.2 × 1.8	60
Trt 3	*Z. planispinum* + *A. hypogaea*	105°38′36.32″ E	25°39′23.64″ N	0.67	791	2 × 2	2.5	2.5 × 2.8	85
Trt 4	*Z. planispinum* + *L. japonica*	105°38′36.35″ E	25°39′22.29″ N	6.67	814	3.5 × 3	2.5	1.5 × 2.5	70
Trt 5	*Z. planispinum*	105°38′35.64″ E	25°39′23.35″ N	33.35	788	3 × 4	2.2	2.5 × 2.3	65

2.3. Soil Sample Collection and Soil Parameters

Three 10 m × 10 m sample squares were arranged in each treatment, and multiple sampling points were arranged within each sample square. At each sampling point, equal amounts of soil were collected in layers of 0–10 cm and 10–20 cm, and samples of the same soil layer were uniformly mixed. The soil sample parameters in this study were taken as the average of the data from 2 soil layers to ensure that the number of observations of soil samples was consistent with the number of observations of the FAAs (15 observations in total over 5 treatments).

The soil water content of Trt 5 was significantly lower than that of other treatments, indicating that combined planting can significantly improve soil water content. Soil organic carbon (SOC), total N and total P were the highest in Trt 4. Total N, total P and total potassium (K) were significantly lower in Trt 2. Trt 5 had the highest concentration of total Ca. The concentration of available N and available Ca in Trt 4 was significantly higher than that in other treatments. There was no significant change in available P concentration among treatments. Available K concentration in Trt 1 was significantly higher than that in other treatments. There was no significant difference in the C:N value among the 5 treatments. C:P and N:P values of Trt 2 were significantly higher than that of Trt 5. The values of microbial biomass carbon, microbial biomass nitrogen (MBN) and microbial biomass phosphorus (MBP) had no significant difference among the 5 treatments, indicating that soil microorganisms have strong stability. Relevant data are shown in Table 2. Li et al. [23] described the soil sample collection, index determination and data analysis in detail.

Table 2. Soil parameters in different planting combinations.

Soil Parameters	Trt 1	Trt 2	Trt 3	Trt 4	Trt 5
Soil water content	31.60 ± 6.29 ab	36.73 ± 2.65 a	25.05 ± 1.38 bc	28.69 ± 0.30 bc	21.15 ± 0.14 c
Soil organic carbon concentration	37.73 ± 7.32 ab	29.40 ± 0.57 ab	28.68 ± 12.62 ab	50.83 ± 13.33 a	26.50 ± 2.19 b
Total nitrogen concentration	3.53 ± 0.46 ab	2.64 ± 0.07 b	2.78 ± 0.74 b	4.60 ± 0.44 a	2.76 ± 0.23 b
Total phosphorus concentration	1.37 ± 0.02 a	0.82 ± 0.03 b	1.10 ± 0.43 ab	1.52 ± 0.17 a	1.26 ± 0.04 ab
Total potassium concentration	6.95 ± 0.34 b	6.11 ± 1.51 b	12.33 ± 0.25 a	11.88 ± 0.53 a	10.88 ± 0.03 a
Total calcium concentration	0.95 ± 0.28 b	1.48 ± 0.39 b	1.85 ± 0.71 b	1.88 ± 0.18 b	6.05 ± 0.21 a
Available nitrogen concentration	275.00 ± 74.25 ab	160.00 ± 5.66 b	161.75 ± 61.87 b	350.00 ± 55.15 a	153.75 ± 15.91 b

Table 2. *Cont.*

Soil Parameters	Trt 1	Trt 2	Trt 3	Trt 4	Trt 5
Available phosphorus concentration	45.80 ± 13.29 a	23.38 ± 11.63 a	26.55 ± 10.54 a	36.68 ± 10.01 a	20.08 ± 2.44 a
Available potassium concentration	393.00 ± 107.48 a	195.85 ± 32.03 b	172.75 ± 57.63 b	223.75 ± 98.64 ab	141.25 ± 2.47 b
Available calcium concentration	317.50 ± 14.85 b	334.75 ± 0.35 b	347.75 ± 24.40 ab	371.00 ± 8.49 a	350.50 ± 7.07 ab
Soil C:N ratio	10.65 ± 0.70 a	11.13 ± 0.53 a	10.07 ± 1.85 a	10.97 ± 1.85 a	9.59 ± 0.00 a
Soil C:P ratio	27.68 ± 5.79 abc	35.83 ± 0.79 a	25.82 ± 1.33 bc	33.10 ± 4.99 ab	21.03 ± 2.38 c
Soil N:P ratio	2.59 ± 0.37 ab	3.22 ± 0.22 a	2.60 ± 0.34 ab	3.02 ± 0.05 a	2.19 ± 0.25 b
Microbial biomass carbon	243.00 ± 4.95 a	254.75 ± 2.47 a	252.00 ± 2.83 a	262.75 ± 21.57 a	262.25 ± 26.52 a
Microbial biomass nitrogen	12.40 ± 1.70 a	13.58 ± 1.31 a	14.38 ± 0.60 a	13.90 ± 1.06 a	14.08 ± 0.18 a
Microbial biomass phosphorus	128.00 ± 23.33 a	144.50 ± 4.95 a	148.00 ± 8.49 a	154.50 ± 13.44 a	139.00 ± 3.54 a

Trts 1–5, five plantations, representing the research objectives of this article. Means followed by the same lowercase letter are not significantly different ($p > 0.05$) among root types as determined by the least significance difference test. Data are presented as mean ± standard deviation.

2.4. Fruit Sample Collection

In the first ten days of June 2021, the fresh fruit of *Z. planispinum* was collected in 5 treatments, at which time the fruit was mature. We selected 5 healthy-growing *Z. planispinum* from each sample square discussed above and collected about 200 g of mature and disease-free *Z. planispinum* fruit from different directions, taking care to eliminate the edge effect. The collected fruits were naturally air-dried in nylon bags, and 5 plant fruit samples from each sample square were mixed into 1 (15 observations in total over 5 treatments). After the pericarps were separated, dried at 45 °C, crushed and screened for the determination of FAAs. Because the pericarp of *Z. planispinum* was rich in amino acids, aromatic oil, fatty acids and other substances and was the main part of utilization, this paper studied the changes in free amino acid concentration in its pericarp.

2.5. Free Amino Acid Analysis

The free amino acids were determined by high-performance liquid chromatography [24,25], and phenylisothiocyanate acetonitrile solution was used as the pre-column derivatization reagent. The UV detector wavelength was 254 nm; the chromatographic column was C18SHISEIDO (4.6 mm × 250 mm × 5 µm). The column temperature was 40 °C, the flow rate was 1 mL/min, the injection volume was 10 µL, mobile phase A was sodium acetate-acetonitrile solution, mobile phase B was 80% acetonitrile aqueous solution and the mobile phase was gradient-eluted. N-leucine was used as the internal standard. The accumulation of essential and nonessential amino acids was calculated by reference.

2.6. Data Analysis

A 1-way analysis of variance and Duncan's method were used for multiple comparisons to analyze the difference in free amino acid concentration in the pericarp of *Z. planispinum* with different planting combinations. The data of free amino acid concentration were standardized and then analyzed by principal component analysis to evaluate the quality of FAAs in the pericarp of *Z. planispinum*. Redundancy analysis was used to reveal the influence mechanism of soil properties on pericarp quality. The data are presented as mean ± standard deviation. Microsoft Excel 2013 (version 2013, Microsoft, Redmond, WA, USA), SPSS 20.0 (version 20.0, IBM SPSS, Armonk, NY, USA) and Origin 8.6 (version 8.6, OriginLab Corporation, Northampton, MA, USA) software were used to complete data sorting, analysis and mapping.

3. Results

3.1. Amino Acid Concentrations in the Pericarp of *Z. planispinum* in Different Planting Combinations

As shown in Table 3, 17 kinds of FAAs were detected in the pericarp of *Z. planispinum* from 5 planting combinations. Among them, arginine, serine, proline, alanine, tyrosine and cystine had no significant differences among the five plantations, while the concentration of the remaining 11 FAAs had significant differences in different degrees. The results showed that the combination planting had a great influence on the free amino acid concentration of the peel of *Z. planispinum*, especially the essential amino acids. Among the 11 amino acids with significant differences mentioned above, the FAAs in the pericarp of *Z. planispinum* were lowest at Trt 1 and highest at Trt 2 (except phenylalanine).

Table 3. Amino acid concentrations in the pericarp of *Z. planispinum* in different planting combinations.

Amino Acid		Trt 1	Trt 2	Trt 3	Trt 4	Trt 5
Essential amino acids (mg/kg)	Valine	376.51 ± 10.86 b	500.15 ± 87.33 a	420.97 ± 10.85 ab	438.20 ± 8.85 ab	409.29 ± 22.74 ab
	Threonine	282.72 ± 7.50 b	368.39 ± 58.89 a	314.29 ± 6.22 ab	337.44 ± 16.76 ab	304.01 ± 22.22 ab
	Phenylalanine	8.46 ± 1.40 c	33.19 ± 2.62 b	23.96 ± 8.93 bc	20.92 ± 8.22 bc	55.35 ± 11.82 a
	Methionine	33.71 ± 0.04 b	46.81 ± 8.85 a	41.84 ± 3.45 ab	34.09 ± 4.92 ab	41.17 ± 3.22 ab
	Leucine	471.19 ± 10.18 b	608.99 ± 98.29 a	514.84 ± 1.06 ab	555.49 ± 24.27 ab	507.19 ± 25.65 ab
	Lysine	347.38 ± 0.42 b	470.78 ± 90.79 a	361.81 ± 47.02 ab	384.33 ± 16.50 ab	386.29 ± 2.37 ab
	Isoleucine	43.18 ± 6.89 b	458.46 ± 90.71 a	375.71 ± 12.23 a	370.20 ± 25.94 a	380.95 ± 12.11 a
	Total	1563.14 ± 36.47 b	2486.75 ± 437.47 a	2053.41 ± 68.29 ab	2140.67 ± 27.30 a	2084.22 ± 70.05 a
Nonessential amino acids (mg/kg)	Histidine	175.01 ± 1.83 b	224.90 ± 36.95 a	189.15 ± 11.31 ab	205.69 ± 7.85 ab	187.80 ± 10.38 ab
	Arginine	367.86 ± 21.83 a	496.41 ± 125.63 a	439.46 ± 43.59 a	420.37 ± 6.47 a	396.40 ± 12.56 a
	Serine	325.79 ± 12.94 a	447.59 ± 98.81 a	380.91 ± 22.85 a	406.63 ± 16.93 a	366.69 ± 25.24 a
	Proline	427.79 ± 36.36 a	608.59 ± 128.90 a	458.82 ± 88.71 a	601.17 ± 21.00 a	533.05 ± 87.49 a
	Glycine	381.66 ± 17.76 b	491.17 ± 83.21 a	422.02 ± 14.86 ab	437.38 ± 9.76 ab	416.49 ± 0.92 ab
	Glutamate	738.39 ± 30.78 b	1012.02 ± 203.02 a	863.35 ± 9.32 ab	899.38 ± 18.59 ab	846.80 ± 22.80 ab
	Aspartate	477.48 ± 48.72 b	744.83 ± 181.96 a	646.66 ± 126.96 ab	597.16 ± 8.89 ab	578.21 ± 1.11 ab
	Alanine	363.17 ± 9.57 a	454.11 ± 69.92 a	386.75 ± 0.01 a	406.52 ± 20.65 a	377.00 ± 29.29 a
	Tyrosine	232.20 ± 8.64 a	270.88 ± 19.56 a	245.27 ± 11.75 a	273.96 ± 10.92 a	221.14 ± 37.96 a
	Cystine	52.02 ± 4.85 a	48.37 ± 2.22 a	39.48 ± 8.34 a	54.93 ± 12.49 a	37.41 ± 4.61 a
	Total	2998.50 ± 87.20 b	4077.54 ± 783.16 a	3443.26 ± 236.39 ab	3677.10 ± 59.48 ab	3376.77 ± 209.43 ab
Total free amino acids (mg/kg)		5104.49 ± 147.33 b	7285.59 ± 1383.21 a	6125.28 ± 359.58 ab	6443.82 ± 88.16 ab	6045.19 ± 302.42 ab
EAAs/TFAAs (%)		30.62	34.13	33.52	33.22	34.48
EAAs/NEAAs (%)		44.14	51.82	50.43	49.75	52.62

Trts 1–5, five plantations, representing the research objectives of this article. EAAs/TFAAs, the ratio of essential amino acids to total amino acids; EAAs/NEAAs, the ratio of essential and nonessential amino acids. Means followed by the same lowercase letter are not significantly different ($p > 0.05$) among root types as determined by the least significance difference test. Data are presented as mean ± standard deviation.

The concentration of essential amino acids (EAAs), nonessential amino acids (NAAs) and total free amino acids (TAAs) in the pericarp of *Z. planispinum* in different planting combinations were 1563.12–2486.75, 2998.50–4077.54 and 5104–7285.59 mg/kg, respectively, with Trt 2 being the highest, significantly higher than Trt 1, in which the lowest amino acid concentration was found. The ideal protein standard proposed by the Food and Agriculture Organization of the United Nations/World Health Organization (FAO/WHO) in 1973 is that the essential amino acid divided by the total amino acid (EAAs/TFAAs) is 40.00%, and the essential amino acid divided by the nonessential amino acid (EAAs/NEAAs) is higher than 60.00%. The EAAs/TFAAs of *Z. planispinum* in each plantation were 30.62%–34.48%, and the EAAs/NEAAs were 44.14%–52.62%, of which *Z. planispinum* + *S. tonkinensis* and *Z. planispinum* monoculture forest were closest to this standard.

3.2. Accumulation of Flavoring Amino Acids in Pericarp of *Z. planispinum* in Different Planting Combinations

According to Table 4, in general, the concentration of sweet amino acids (SAAs) (1956.13–2594.73 mg/kg) was the highest, and the concentration of aromatic amino acids (AAAs) (292.68–352.43 mg/kg) was the lowest. The concentration of bitter amino acids (BAAs) (1292.44–2110.81 mg/kg) was similar to that of delicious amino acids (DAAs) (1563.25–2227.62 mg/kg). The concentration of various flavoring amino acids of *Z. planispinum* in all planting combinations was SAAs > DAAs > BAAs > AAAs. The concentration of four kinds of flavored amino acids in Trt 2 was the highest, and that in Trt 1 was the lowest, indicating that planting with *S. tonkinensis* is the most beneficial to the accumulation of amino acids and the formation of the special flavor in *Z. planispinum*.

Table 4. Accumulation of flavoring amino acids in the pericarp of *Z. planispinum* in different planting combinations.

Amino Acid	Trt 1	Trt 2	Trt 3	Trt 4	Trt 5
Bitter amino acid (mg/kg)	1292.44 ± 49.82 b	2110.81 ± 410.80 a	1792.82 ± 62.15 a	1818.35 ± 4.21 a	1734.99 ± 69.83 ab
Sweet amino acid (mg/kg)	1956.13 ± 13.25 b	2594.73 ± 476.69 a	2151.94 ± 101.79 ab	2394.82 ± 50.96 ab	2185.03 ± 175.55 ab
Delicious amino acid (mg/kg)	1563.25 ± 79.08 b	2227.62 ± 475.77 a	1871.82 ± 183.31 ab	1880.86 ± 26.21 ab	1811.29 ± 26.28 ab
Aromatic amino acid (mg/kg)	292.68 ± 5.19 b	352.43 ± 19.97 a	308.71 ± 12.34 ab	349.79 ± 15.20 a	313.89 ± 30.76 ab

Trts 1–5, five plantations, representing the research objectives of this article. Means followed by the same lowercase letter are not significantly different ($p > 0.05$) among root types as determined by the least significance difference test. Data are presented as mean ± standard deviation.

Due to the different taste perception thresholds of different amino acids, a higher concentration of amino acids does not necessarily correlate to a greater contribution to food flavor. Therefore, the taste activity value (TAV), calculated as the ratio of concentration to its taste threshold, was used to further analyze the impact of flavoring amino acids on the pericarp flavor of *Z. planispinum* [26]. The TAVs of each flavoring amino acid in the five planting combinations are shown in Table 5. When the TAV ≥ 1, it indicates that the amino acid contributes to the flavor effect. The TAVs of arginine (3.68–4.96) in BAAs, glutamate (14.77–20.24) and aspartate (15.92–24.83) in DAAs and cystine (1.87–2.75) in AAAs were all greater than 1. In addition, the TAVs of histidine were both greater than 1 at Trt 2 (1.03) and Trt 4 (1.12). Therefore, aspartate, glutamate, arginine, cystine and histidine played an important role in the formation of the unique flavor of *Z. planispinum*. In addition, the combination of *Z. planispinum* with *S. tonkinensis* (Trt 2) or *L. japonica* (Trt 4) was more conducive to the accumulation of amino acids and the formation of the special flavor. The delicious amino acids among the four flavoring amino acids had the highest contribution rate to the flavor of *Z. planispinum*, which was more consistent with the unique freshening flavor of *Z. planispinum*.

Table 5. Taste activity values of flavoring amino acids in the pericarp of *Z. planispinum* in different planting combinations.

Amino Acid		Taste Threshold [27] (mg/g)	Taste Activity Value				
			Trt 1	Trt 2	Trt 3	Trt 4	Trt 5
Bitter amino acid	Valine	1.50	0.25	0.33	0.28	0.29	0.27
	Leucine	3.80	0.12	0.16	0.14	0.15	0.13
	Isoleucine	0.90	0.05	0.51	0.42	0.41	0.42
	Methionine	0.30	0.11	0.16	0.14	0.11	0.14
	Arginine	0.10	3.68	4.96	4.39	4.20	3.96
Sweet amino acid	Glycine	1.10	0.35	0.45	0.38	0.40	0.38
	Alanine	0.60	0.61	0.76	0.64	0.68	0.63
	Serine	1.50	0.22	0.30	0.25	0.27	0.24
	Threonine	2.60	0.11	0.14	0.12	0.13	0.12
	Proline	3.00	0.14	0.20	0.15	0.20	0.18
	Histidine	0.20	0.88	1.12	0.95	1.03	0.94

Table 5. *Cont.*

Amino Acid		Taste Threshold [27] (mg/g)	Taste Activity Value				
			Trt 1	Trt 2	Trt 3	Trt 4	Trt 5
Delicate amino acid	Lysine	0.50	0.69	0.94	0.72	0.77	0.77
	Glutamate	0.05	14.77	20.24	17.27	17.99	16.94
	Aspartate	0.03	15.92	24.83	21.56	19.91	19.27
Aromatic amino acid	Phenylalanine	1.50	0.01	0.02	0.02	0.01	0.04
	Tyrosine	2.60	0.09	0.10	0.09	0.11	0.09
	Cystine	0.02	2.60	2.42	1.97	2.75	1.87

3.3. Principal Component Analysis of Free Amino Acids in Pericarp of Z. planispinum

The free amino acids in the pericarp of *Z. planispinum* were analyzed by principal component analysis. It can be seen from Table 6 that the cumulative variance contribution rate of two principal component eigenvalues greater than 1 was 89.63%. Therefore, selecting these two main components as effective components for data analysis can reflect most of the amino acid information and can characterize the quality of amino acids. The variance contribution rate of the first principal component was 72.56%, which had the greatest impact on the accumulation of amino acid concentration in the pericarp of *Z. planispinum*. Among them, except cystine, methionine and phenylalanine, the load values of amino acids were higher and positively correlated. The variance contribution rate of the second principal component was 17.08%, which indicated that it had a certain impact on the peel of *Z. planispinum*, but this effect was small. Cystine had a greater negative impact, while phenylalanine had a greater positive impact.

Table 6. Principal component load matrix and coefficient.

Factors	Principal Component Load Matrix	
	PC1	PC2
Aspartate	**0.866**	0.378
Glutamate	**0.971**	0.212
Serine	**0.988**	0.102
Histidine	**0.993**	0.031
Glycine	**0.951**	0.174
Threonine	**0.989**	−0.021
Arginine	**0.916**	0.249
Alanine	**0.986**	−0.034
Tyrosine	**0.811**	−0.458
Cystine	0.157	**−0.915**
Valine	**0.989**	0.123
Methionine	0.563	0.673
Phenylalanine	0.111	**0.881**
Isoleucine	**0.702**	0.513
Leucine	**0.993**	0.019
Lysine	**0.901**	0.231
Proline	**0.838**	0.055
Eigenvalue	12.639	2.599
Variance contribution rate/%	72.557	17.076
Cumulative variance contribution rate/%	72.557	89.633

The bold font is the relatively large influence factor of each principal component load factor.

The variance contribution rate (W_i) and factor score (F_i) of the two principal component factors extracted were further weighted. Finally, the comprehensive score (CS) and ranking of *Z. planispinum* plantation of five planting combinations were obtained, and the CS reflected the comprehensive quality level of FAAs in the pericarp of *Z. planispinum* under each combination. The comprehensive score calculation was combined with the weighted method using the following formula:

$$CS = \sum W_i \times F_i$$

where W_i is the contribution rate of each principal component, and F_i is the principal component score of each plantation type. By weighting the variance contribution rate (W_i) and factor score (F_i) of each principal component factor, the CSs of different plantation types are obtained.

It can be seen from Table 7 that the CS was ranked from high to low as Trt 2 > Trt 4 > Trt 3 > Trt 5 > Trt 1. Among them, after planting with *S. tonkinensis* (Trt 2) or *L. japonica* (Trt 4), the CS of fruit pericarp quality of *Z. planispinum* was positive, indicating that the FAAs in the pericarp of *Z. planispinum* in these two treatments were higher than the average level.

Table 7. Factor score and comprehensive evaluation of *Z. planispinum* in different planting combinations.

Plantation Types	Factor Score		Comprehensive Score	Ranking
	PC1	PC2		
Trt 1	−3.78	−2.71	−3.20	5
Trt 2	4.46	1.69	3.52	1
Trt 3	−0.52	0.56	−0.28	3
Trt 4	0.94	−1.23	0.47	2
Trt 5	−1.10	1.70	−0.51	4

Trts 1–5, five plantations, representing the research objectives of this article.

3.4. Effects of Soil Properties on Amino Acid Concentration of Pericarp

Soil factors were used as explanatory variables (red arrows), and amino acid concentrations were used as response variables (blue arrows) to carry out redundancy analysis to reveal the interaction rules between them. According to Figure 2, the cumulative interpretation rates of the first and second ranking axes were 83.60% and 10.20%, respectively, with the cumulative contribution rate as high as 93.80%. Among them, the effect of soil factors on the concentration of FAAs in the pericarp of *Z. planispinum* was available K > available P > MBN > available Ca > MBP, reaching a significant level (Table 8). In general, the contribution rate of available nutrients was higher than that of total nutrients, while the contribution rate of stoichiometric ratio and soil water content was lower.

Effects of available K, total P and available P on amino acids were all negative (except cystine), reflecting the inhibition of K and P on the accumulation of amino acids (Figure 2). Total Ca had a strong negative effect on cystine and a positive effect on phenylalanine, but it had little effect on other amino acids, while available Ca had a significant enhancement effect on amino acids, indicating that available forms of Ca had different effects on amino acids and available Ca was beneficial to the accumulation of amino acids. Soil water content, SOC, total N, available N and element stoichiometry had a great influence on cystine and tyrosine in aromatic amino acids, and the influence of the stoichiometric ratio on amino acids was mostly positive. The effect of microbial biomass on amino acids was one of enhancement.

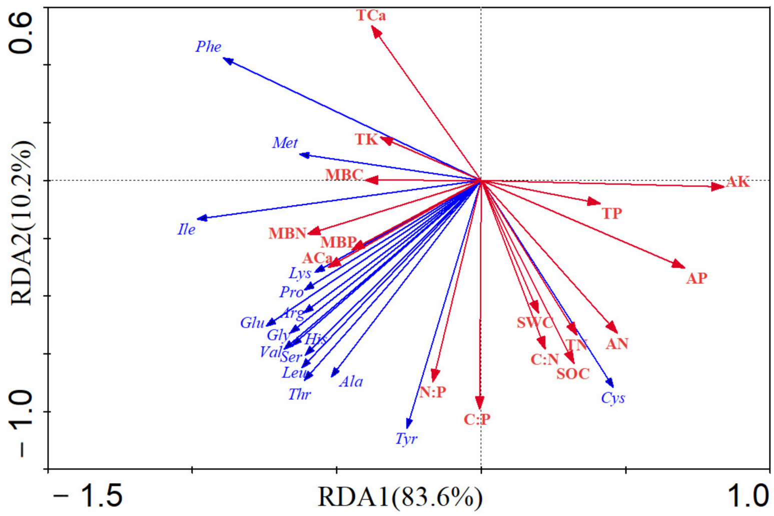

Figure 2. Redundancy analysis of free amino acids and soil. SWC, soil water content; SOC, soil organic carbon; TN, total nitrogen; TP, total phosphorus; TK, total potassium; TCa, total calcium; AN, available nitrogen; AP, available phosphorus; AK, available potassium; ACa, available calcium; MBC, microbial biomass carbon; MBN, microbial biomass nitrogen; MBP, microbial biomass phosphorus; Val, valine; Thr, threonine; Phe, phenylalanine; Met, methionine; Leu, leucine; Lys, lysine; Iso, isoleucine; His, histidine; Arg, arginine; Ser, serine; Pro, proline; Gly, glycine; Glu, glutamate; Asp, aspartate; Ala, alanine; Tyr, tyrosine; Cys, cystine.

Table 8. Importance sequencing and significance test of soil factors.

Soil Factors	Contribution/%	Pseudo-*F*	*p*
Available potassium	0.646	23.763	0.002
Available phosphorus	0.472	11.626	0.002
Microbial biomass nitrogen	0.365	7.458	0.012
Available calcium	0.286	5.217	0.018
Microbial biomass phosphorus	0.235	3.989	0.038
Available nitrogen	0.218	3.633	0.052
Microbial biomass carbon	0.191	3.062	0.076
Total calcium	0.171	2.691	0.066
Total phosphorus	0.156	2.402	0.15
Total potassium	0.134	2.02	0.174
Soil organic carbon	0.131	1.955	0.146
Total nitrogen	0.125	1.854	0.15
Soil N:P ratio	0.087	1.233	0.276
Soil C:N ratio	0.078	1.106	0.354
Soil water content	0.072	1.012	0.354
Soil C:P ratio	0.071	0.995	0.364

4. Discussion

4.1. Effects of Planting Combinations on Amino Acids in the Pericarp of Z. planispinum

Previous studies have shown that plant varieties [28], altitude [29], environmental stress [30], water and fertilizer coupling treatment [31], season [32], etc. affect the concentration and accumulation of FAAs in the pericarp. In this study, it was found that the planting combination had a greater impact on the concentration of FAAs in the pericarp of *Z. planispinum*, especially on essential amino acids. This is because the combination of planting can effectively reduce the leaching and loss of water and fertilizer in the soil. It can also keep the soil loose, and its thermal insulation effect creates a good environment for the survival of microorganisms. The litter and root exudates produced by combined plants change the soil's micro-ecological environment and soil nutrient status [33,34]. Secondly, the combination of plant species, planting density and other factors can cause spatial niche differences; directly affect the air temperature and humidity, CO_2 concentration, wind speed, light intensity and light quality distribution; and form different field microclimates [35]. Different planting combinations change the soil water status, fertilizer status and microenvironment; affect the acquisition and utilization of plant resources; lead to changes in the stress factors of *Z. planispinum*; and ultimately affect its C and N metabolism balance and amino acid accumulation. Cannabinoid has a significant contribution to the spicy taste of *Z. planispinum*, and valine and leucine are considered to be precursors for the synthesis of the nitrogen-containing portion of cannabinoid [36]. In this study, the concentration of valine and leucine in the pericarp of *Z. planispinum* with *S. tonkinensis* (Trt 2) was significantly the highest, followed by *L. japonica* (Trt 4). From the perspective of TAV value, aspartate, glutamate, arginine, cystine and histidine had a significant impact on the formation of the flavor. In summary, the combination of *Z. planispinum* with *S. tonkinensis* (Trt 2) or *L. japonica* (Trt 4) was more conducive to the accumulation of amino acids and the formation of the special flavor.

Principal component analysis showed that the amino acid quality of the pericarp of *Z. planispinum* was the best and was closest to the standard of ideal protein after planting with *S. tonkinensis* (Trt 2). However, soil nutrient concentration under this mode was generally low. This is similar to the conclusion of Lin et al. [37], who found that adverse environmental factors can improve the quality of *Ganoderma lucidum* to some extent. It is speculated that when faced with environmental stress, *Z. planispinum* can promote the accumulation of FAAs and the synthesis of secondary metabolites by reducing yield and improving nutrient reabsorption, finally improving the quality of the pericarp. In addition, when plants are stressed by adverse environmental factors, they can inhibit the growth of other plants by releasing secondary metabolites to the external environment to improve their competitiveness [38], which may limit the N fixation effect of *S. tonkinensis*, which is not conducive to the improvement in soil quality. The concentration of FAAs in the pericarp of *Z. planispinum* after planting with *L. japonica* (Trt 4) was also relatively rich, ranking second in the comprehensive score. In addition, previous research showed that soil quality under this mode was the best [23]. This is because *L. japonica* produces a rich decomposable litter, which provides rich materials for the C and N metabolism of *Z. planispinum*, and promotes the formation of *Z. planispinum* pericarp quality. *Z. planispinum* has a large root system and strong ability to sprout, which improves soil conditions in the rhizosphere, thus enhancing the ability of its root system to absorb nutrients and water in the lower layer, increasing the supply of the N metabolism substrate in the upper part, which is conducive to the improvement in N-metabolism-related enzyme activities, such as glutamine synthetase activity, thus affecting the accumulation of FAAs [39]. It can be seen from Table 2 that the soil water content of *Z. planispinum* + *L. japonica* plantation was significantly lower than that of *Z. planispinum* + *P. salicina*/*S. tonkinensis* (Trt 1 or Trt 2). It is speculated that under the condition of relative water deficit, FAAs are synthesized and accumulated in large quantities to improve the osmoregulation ability of *Z. planispinum*. In addition, FAAs also protect plants from water stress by participating in redox balance and energy metabolism, as well as regulating mitochondrial function as signal molecules [40,41]. The pericarp quality of *Z. planispinum* after planting with *P. salicina* (Trt 1) was the lowest in this study. This is due to the fact that *P. salicina* is a tall tree, which has formed a strong nutrient competition with *Z. planispinum*, limiting the accumulation of amino acids in *Z. planispinum* [42]. Moreover, *Z. planispinum* is a photophilic plant. After being blocked by *P. salicina*, the photosynthesis of *Z. planispinum* is inhibited, resulting in a lower C assimilation rate of leaves, which in turn leads to a lower amino acid assimilation rate, further leading to the reduction of FAAs [43,44]. In the future, based on this research, we need to pay more attention to secondary metabolites and their formation mechanism that forms the aroma and hemp of *Z. planispinum* to lay a theoretical basis for comprehensive quality control.

4.2. Relationship between Soil Nutrients and Pericarp Quality

Soil nutrient stress affects the secondary metabolism process of plants and changes the accumulation of C-based secondary metabolites in plants, but different nutrient elements have different effects on the secondary metabolism process of plants [45]. This study showed that soil-available K contributed the most to the accumulation of FAAs in fruit pericarp and showed a negative effect. This is because K can significantly affect N metabolism, especially amino acid and protein metabolism. K deficiency leads to an increase in protease and peptidase activity and promotes protein degradation, which leads to the accumulation of low-molecular-weight substances, such as FAAs, and confirms that the accumulation of metabolites is the result of environmental adaptation [46,47]. In this study, soil-available P had a significant negative impact on the concentration of FAAs in the pericarp of *Z. planispinum*, and the previous study showed that the soil of five planting combinations had phosphorus saturation [22]. This may be due to excessive soil available phosphorus leading to the consumption of beneficial microorganisms in plants and increasing the abundance of pathogenic microorganisms, which ultimately affects the growth of *Z. planispinum* [48]. The number of beneficial microorganisms in soil can be increased by adding organic fertilizer or soil conditioner to promote the coordinated development of soil microbial flora of *Z. planispinum* [49]. In this study, the contribution rate of soil-available Ca to FAAs was large and had a promoting effect. It indicated that Ca could improve the quality and stress resistance of crops, which is consistent with the research conducted by Li et al. [50]. The reason for this is that Ca is one of the essential nutrients for plant growth and development, which can promote the transport and transformation of plant carbohydrates and the absorption of mineral elements [51]. Ca deficiency can cause the vacuolar membrane of mesophyll cells to break, destroy the lamellar structure of thylakoids and inhibit the photosynthetic capacity of plants. Moreover, Ca, as the second messenger in the process of cell signal transduction, is involved in the regulation of the synthesis and metabolism of amino acids and proteins [52,53]. It can accelerate the absorption and metabolism of N by plants, as well as promote the growth and development of plants and the formation of fruit quality [54]. This study found that soil microbial biomass nitrogen and microbial biomass phosphorus had a significant positive effect on the accumulation of free amino acid concentration in the pericarp of *Z. planispinum*. This is because soil microorganisms can secrete a variety of enzymes to decompose animal and plant residues and other organic substances, as well as accelerate the transformation and transportation of carbon. Some metabolites can promote the decomposition of minerals to help plants absorb and use them [55]. This study also found that the contribution rate of the soil stoichiometric ratio to the concentration of amino acids in the pericarp was low, which was related to the extremely strong internal stability of the soil-nutrient-element stoichiometric ratio of five plantations [22]. In the future, it is worth further studying the influence of different element components on amino acid accumulation in the pericarp of *Z. planispinum*, especially the determination of the threshold value of the influence direction. Based on the mechanism of the soil microbial community improving soil nutrient status and plant nutrient absorption, as well as the cascade relationship of the soil microorganism and the nutrient element, fruit quality is further constructed to provide a scientific basis to formulate a soil-nutrient-optimization plan. It is also necessary to further study the effects of total nutrients and available nutrients on the FAAs in the pericarp and explore the specific reasons. The mechanisms of soil microbial community-improving plant nutrition should also be deeply explored. Furthermore, the mechanisms of planting combinations improving mineral nutrition should be fully and comprehensively understood, and targeted soil-nutrient-optimization programs for different planting combinations should be formulated.

5. Conclusions

(1) Planting with *S. tonkinensis* or *L. japonica* can significantly increase the concentration of FAAs in the pericarp of *Z. planispinum*, which is conducive to the formation of pericarp quality. Based on the results of soil quality analysis in the early research and

the amino acids in the pericarp, the optimal planting combination was found to be *Z. planispinum* + *L. japonica*;

(2) As a characteristic element of karst, available Ca had a high contribution rate to the accumulation of FAAs in the pericarp, which had a positive impact;

(3) The effect of available nutrients on FAAs in the pericarp was greater than that of total nutrients. Soil management in plantations should involve paying more attention to the concentration and proportion of available nutrients.

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