Chemometric Characteristics of Soil Carbon, Nitrogen, and Phosphorus in *Quercus variabilis* Forest Land in the Funiu Mountain Area

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Abstract By measuring the contents of soil organic carbon (SOC), total nitrogen (TN), and total phosphorus (P) in rhizosphere and non-rhizosphere soils of *Quercus variabilis* forests with different ages (22, 35, and 45 a) and origins (natural forests and planted forests) in the Funiu Mountain area, the stoichiometric characteristics of carbon, nitrogen, and phosphorus in *Q. variabilis* forest soils were evaluated. The research results indicated that: (i) there were significant differences ($P<0.05$) in the SOC content between the rhizosphere and non-rhizosphere soils of *Q. variabilis* planted forests at different stand ages. Moreover, as the stand age increased, the SOC content in both the rhizosphere and non-rhizosphere soils of the planted forests initially decreased and then increased. The TN and TP contents in the rhizosphere and non-rhizosphere soils of *Q. variabilis* planted forests did not show significant differences across different stand ages, and they exhibited different trends as the stand age increased. (ii) The SOC and nitrogen to phosphorus ratio (N∶P) exhibited significant rhizosphere effects, whereas the rhizosphere effects of the TN, TP, carbon to nitrogen ratio (C∶N), and carbon to phosphorus ratio (C∶P) were not prominent. In terms of the influence of stand age, the SOC, TN, TP, C∶P, and N∶P were significantly affected. However, the effect of the C∶N at the stand age level was not significant. The interactive effects of soil carbon, nitrogen, phosphorus, and their stoichiometric ratios were all significant. It is recommended to appropriately supplement soil nitrogen and phosphorus to ensure the sustainable development of *Q. variabilis* forest stand.

Keywords *Quercus variabilis*, Ecological stoichiometry, Soil, Rhizosphere effect, Forest age
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The contents of soil organic carbon (SOC), total nitrogen (TN), and total phosphorus (TP) in soil can reflect the supply of soil nutrients and its potential role as a nutrient reservoir[1-2]. By utilizing soil ecological stoichiometry, it can clarify the availability of soil carbon, nitrogen, and phosphorus, and grasp the development trend of nutrient metabolism in plants. This theoretical approach has been applied in studies on ecosystem nutrient cycling[3], determination of limiting elements[4], nutrient use efficiency, and resource competition[5]. Generally, the nitrogen to phosphorus ratio (N∶P) serves as a good predictor of the nutrient type limiting plant growth in soil[6]. The carbon to nitrogen ratio (C∶N) reflects the relative abundance of SOC and TN[7]. The carbon to phosphorus ratio (C∶P) is often used to assess the potential of microorganisms to absorb or release phosphorus during the mineralization of soil organic matter[6]. Therefore, it is of crucial value for clarifying the nutrient balance mechanism of soil ecosystems and understanding the nutrient limitation status of plants by exploring the stoichiometric characteristics of soil C, N, and P elements[8]. The rhizosphere refers to the soil microenvironment that influences the vital activities of plant roots. The degradation of plant root epidermal exudates and the influx of a large amount of root secretions (such as organic acids, sugars, phenolic compounds, and amino acids) induce the rhizosphere effect[9]. The age of the forest stand also profoundly affects the nutrient status and stoichiometric characteristics of the soil rhizosphere and non-rhizosphere regions[10-12].

Since the mid-20th century, due to the long-term interference of human abnormal activities, the forest ecosystem in the Funiu Mountain area has suffered unprecedented damage. Natural forests have been largely replaced by planted forests, leading to a significant decline in biodiversity, and the ecological environment has consequently become sensitive and fragile[13-14]. The age and rhizosphere effect of *Quercus variabilis* forests have an impact on their soil nutrients and biological characteristics[15-17]. In this paper, the natural and planted forests of *Q. variabilis* in the Funiu Mountain area were taken as the research objects. The study on the differences in the stoichiometric characteristics of carbon, nitrogen, and phosphorus in the rhizosphere and non-rhizosphere soils of *Q. variabilis* contributed to a deeper understanding of the ecological functions of *Q. variabilis* planted forests, and could provide a basis for ecological restoration and the scientific and sustainable management of planted forests in the Funiu Mountain area.

1 Research area and methods

1.1 Sample collection

Soil sample collection began in August 2023. Planted forests of *Q. variabilis* aged 22, 35, and 45 years, as well as natural forests of *Q. variabilis*, were selected as sampling areas in Wangou Forest Farm, Neixiang County, within the Funiu Mountain (Fig.1). In each sampling area, it was randomly divided into three 20 m×20 m plots, with a spacing of 50–100 m between plots. That is, there were 12 plots in total, with 4 treatments (forest ages) and 3 replicates. In each sample plot, 3 to 5 standard trees were randomly selected. The litter within the projection area of the standard tree canopy was removed, and soil from the 0–20 cm soil layer was collected. The roots with smaller diameters were selected, and rhizosphere soil from the 0–20 cm soil layer was collected using the shaking method. Then, five sampling points were arranged in an "S" shape in each plot[19]. The samples from the 0–20 cm soil layer were collected, and gravel and plant roots were removed from the samples, resulting in a total of 12 rhizosphere soil samples and 12 non-rhizosphere soil samples. The contents of carbon, nitrogen, and phosphorus elements, as well as the physical properties of soil in each sample were measured.

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1.2 Indicator measurement

The content of SOC was determined using the potassium dichromate oxidation-dilution calorimetric method[20], TN was determined using the Kjeldahl method, and TP was determined using the NaOH alkali digestion-molybdenum antimony colorimetric method. The soil pH was measured using the water extraction potentiometric method, and the moisture content was determined using the EM-50 method[21-22].

1.3 Data processing

Using SPSS 27.0 software, one-way ANOVA and nested two-way ANOVA were employed to evaluate the significant effects of stand age, rhizosphere effect, and their interaction on the stoichiometric characteristics of soil carbon, nitrogen, and phosphorus (α=0.05).

2 Results and analysis

2.1 Soil carbon, nitrogen, and phosphorus contents and their chemometric characteristics in *Q. variabilis* forests

The research results indicated that the SOC content in the rhizosphere and non-rhizosphere soils of natural *Q. variabilis* forests was generally higher than that in planted forests (Fig.1). There was a significant difference between the rhizosphere and non-rhizosphere (Table 2). The data indicated that there was a significant rhizosphere effect on SOC, and the content of SOC in the rhizosphere and non-rhizosphere soils of planted forests decreased initially with the increase of forest age, and then showed an upward trend. Moreover, the differences in SOC content among forests of different ages were extremely significant (P<0.001) (Table 2). The interaction between stand age and rhizosphere also had a highly significant effect on SOC content (P<0.01).

Similar to the distribution pattern of carbon content, the TN content in the rhizosphere and non-rhizosphere soils of natural *Q. variabilis* forests was higher than that in planted forests (Fig.2), but the difference between the two was not significant (Table 2). The TN content in the rhizosphere soil of planted forests gradually decreased with the increase of stand age, while the TN content in the non-rhizosphere soil exhibited a trend of decreasing first and then increasing with the increase of stand age. The differences in TN content in soils from stands of different forest ages were extremely significant (P<0.001) (Table 2). The rhizosphere effect of TN was not prominent, while the interaction between stand age and rhizosphere had a significant impact on the content of TN (P<0.01).

Unlike the variation patterns of soil carbon and nitrogen content, the TP content in the rhizosphere soil of a 45-year-old *Q. variabilis* planted forest was the highest (Fig.3), showing significant differences from other forest stands. In non-rhizosphere soil, the TP content reached its highest level at 35 years old. There was no significant difference in TP content between rhizosphere and non-rhizosphere soils (Table 2). In non-rhizosphere soil, the TP content was the highest in soil from 35-year-old forests and the lowest in soil from 45-year-old forests, with significant differences between different forest ages (P<0.05) (Table 2). The interaction between stand age and rhizosphere also had a highly significant effect on TP content (P<0.01).

As shown in Fig.4, the C∶N in the rhizosphere soil of natural *Q. variabilis* forests was the highest, while the ratio in 35-year-old planted forests was the lowest. In non-rhizosphere soil, the C∶N was the highest in 35-year-old planted forests and the lowest in 22-year-old planted forests. The differences in the C∶N between different forest ages, as well as between rhizosphere and non-rhizosphere samples, were not significant (Table 2). The interaction between stand age and rhizosphere had a highly significant effect on the C∶N (P<0.01).

As shown in Fig.5, the natural forest of *Q. variabilis* had the highest C∶P in its rhizosphere soil, while the 45-year-old planted forest had the lowest ratio. In non-rhizosphere soil, the C∶P was the highest in 45-year-old planted forests and the lowest in 35-year-old planted forests. Significant differences were observed in the C∶N and C∶P among different forest ages (P<0.01), while no significant differences were found in the C∶P between rhizosphere and

Table 1 Overview of the *Quercus variabilis* sample plot in Wangou Forest Farm, the Funiu Mountain area

Sample plot	Type	Average age//a	Tree height//m	DBH//cm	Understory characteristics
1	Planted forest	22	13.50±1.71	17.3±1.70	Litter is 0.2 to 0.3 cm thick, with a coverage of 78%
2	Planted forest	22	13.10±1.34	18.5±2.70	Litter is 0.5 cm thick, with a coverage of 45%
3	Planted forest	22	12.90±1.22	16.9±2.30	Litter is 0.6 to 0.8 cm thick, with a coverage of 68%
4	Natural forest	–	12.50±1.24	21.5±2.10	Litter is 1.2 to 1.3 cm thick, with a coverage of 36%
5	Planted forest	35	14.52±1.19	22.5±2.10	Litter is 0.6 to 0.9 cm thick, with a coverage of 38%
6	Planted forest	35	13.80±1.24	23.5±2.20	Litter is 0.2 to 0.4 cm thick, with a coverage of 42%
7	Planted forest	35	14.80±1.35	24.8±2.40	Litter is 0.3 to 0.8 cm thick, with a coverage of 45%
8	Natural forest	–	13.50±1.45	19.6±2.80	Litter is 1.2 to 1.3 cm thick, with a coverage of 44%
9	Planted forest	45	15.13±1.39	25.8±1.90	Litter is 0.4 to 0.5 cm thick, with a coverage of 61%
10	Planted forest	45	15.60±1.23	24.5±1.80	Litter is 0.6 to 0.8 cm thick, with a coverage of 56%
11	Planted forest	45	14.90±1.45	27.8±2.70	Litter is 0.5 to 0.7 cm thick, with a coverage of 50%
12	Natural forest	–	10.20±1.23	23.6±3.87	Litter is 1.4 to 1.7 cm thick, with a coverage of 47%

Table 2 Variance analysis on the impacts of rhizosphere effect and stand age on soil carbon, nitrogen, and phosphorus stoichiometric characteristics

Item	Rhizosphere and non-rhizosphere (R)		Forest age (A)		Interaction (A × R)	
	F	*P*	*F*	*P*	*F*	*P*
SOC	16.203	0.001**	51.987	0.000**	11.280	0.000**
TN	0.168	0.687	49.492	0.000**	11.243	0.000**
TP	0.741	0.402	4.202	0.023*	23.614	0.000**
C ∶ N	0.865	0.366	3.250	0.050	10.960	0.000**
C ∶ P	0.029	0.867	5.835	0.007**	9.640	0.001**
N ∶ P	18.521	0.001**	10.118	0.001**	9.183	0.001**

Note: * shows P<0.05; ** shows P<0.01. SOC: soil organic carbon; TN: total nitrogen; TP: total phosphorus. The same below.

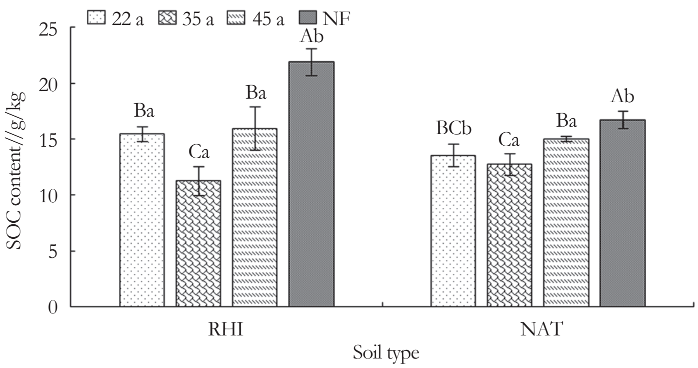
non-rhizosphere soils (Table 2). However, the interaction between stand age and rhizosphere had a highly significant effect on the C∶P (P<0.01).

As shown in Fig.6, the natural forest of *Q. variabilis* had the highest N∶P in its rhizosphere soil, while the 45-year-old planted forest had the lowest ratio. In non-rhizosphere soil, the N∶P was the highest in 45-year-old planted forests and the lowest in 35-year-old planted forests. The differences in C∶N and N∶P among different forest ages were extremely significant (P<0.01), as were the differences in C∶P between rhizosphere and non-rhizosphere soils (P<0.01) (Table 2). The interaction between stand age and rhizosphere also had a significant effect on the N∶P (P<0.01).

Fig.1–6 and Table 2 demonstrated that SOC and N∶P exhibited significant rhizosphere effects, whereas the rhizosphere effects of TN, TP, C∶N, and C∶P were not prominent. The stand age effect on SOC, TN, TP, C∶P, and N∶P was significant, while the stand age effect on C∶N was not significant. The interactive effects of soil carbon, nitrogen, phosphorus, and their stoichiometric ratios were all significant, with a higher degree of synergy observed in nitrogen and phosphorus limitations in rhizosphere soil.

2.2 Correlation between soil carbon, nitrogen, and phosphorus content and carbon–nitrogen–phosphorus stoichiometric ratios in *Q. variabilis* forests

Table 3 presented detailed analysis results



Note: RHI: rhizosphere soil; NAT: non-rhizosphere soil; NF: natural forest; SOC: soil organic carbon; 22a, 35a, 45a: planted forests with 22, 35, and 45 years of age. Different capital letters indicate significant differences between different forest ages, while lowercase letters indicate significant differences between rhizosphere and non-rhizosphere samples (P<0.05) . The same below.

Fig.1 Soil carbon content in natural and planted forests of *Quercus variabilis* in the Funiu Mountain area

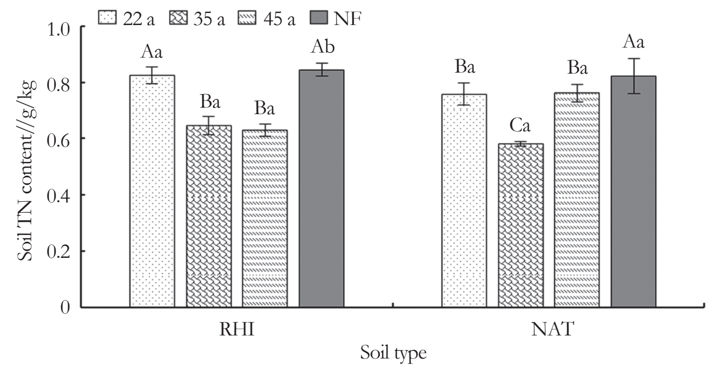


Fig.2 TN content in soil of natural and planted forests of *Quercus variabilis* in the Funiu Mountain area

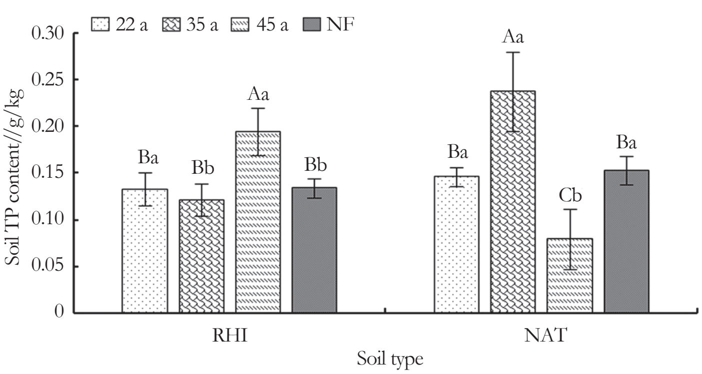


Fig.3 TP content in soil of natural and planted forests of *Quercus variabilis* in the Funiu Mountain area

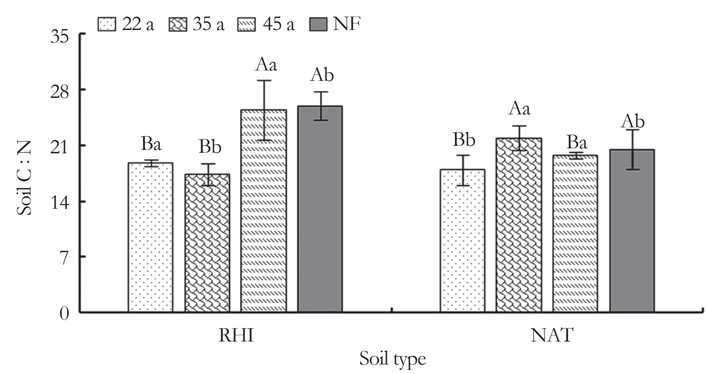


Fig.4 Stoichiometric ratio of carbon and nitrogen elements in the soil of *Quercus variabilis* forest

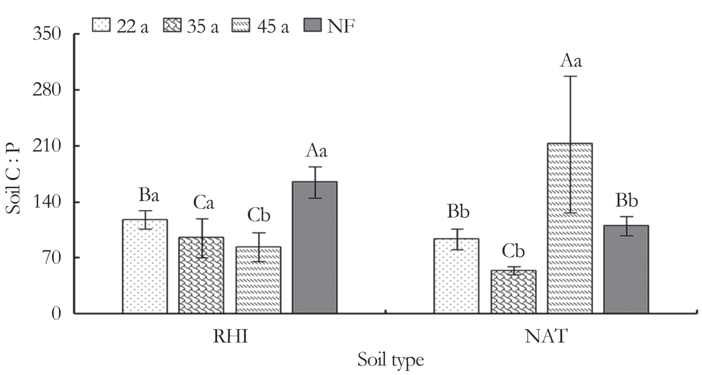


Fig.5 The stoichiometric ratio of carbon and phosphorus elements in the soil of *Quercus variabilis* forest

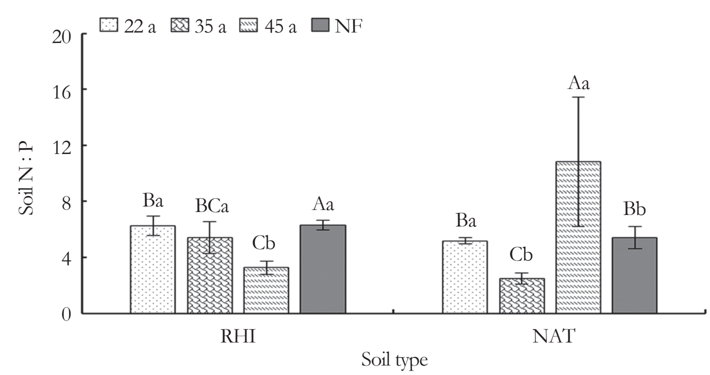


Fig.6 Stoichiometric ratios of nitrogen and phosphorus elements in the soil of *Quercus variabilis* forest

Table 3 Correlation among the stoichiometric characteristics of soil carbon, nitrogen, and phosphorus in *Quercus variabilis* forests

Factor	SOC_Rhi	TN_Rhi	TP_Rhi	C : N_Rhi	C : P_Rhi	N : P_Rhi	SOC_Non	TN_Non	TP_Non	C : N_Non	C : P_Non	N : P_Non
SOC_Rhi	1											
TN_Rhi	0.653	1										
TP_Rhi	0.010	−0.530	1									
C:N_Rhi	0.539	−0.277	0.627	1								
C:P_Rhi	0.255	0.536	−0.885**	−0.297	1							
N:P_Rhi	0.052	0.507	−0.952**	−0.515	0.967**	1						
SOC_Non	0.939**	0.750*	−0.147	0.324	0.380	0.201	1					
TN_Non	0.709*	0.919**	−0.423	−0.110	0.455	0.390	0.730*	1				
TP_Non	−0.181	−0.658	0.960**	0.529	−0.888**	−0.919**	−0.357	−0.515	1			
C:N_Non	0.701	0.198	0.239	0.614	0.091	−0.114	0.755*	0.107	0.024	1		
C:P_Non	0.744*	0.809*	−0.559	0.002	0.730*	0.607	0.888**	0.713*	−0.730*	0.587	1	
N:P_Non	0.492	0.872**	−0.826*	−0.361	0.849**	0.821*	0.629	0.814*	−0.904**	0.108	0.868**	1

Note: * shows P<0.05; ** shows P<0.01.

on the correlation between the contents of carbon, nitrogen, and phosphorus in the soil of the *Q. variabilis* forest stand and the stoichiometric ratios of carbon, nitrogen, and phosphorus. The SOC content in rhizosphere and non-rhizosphere soil exhibited a highly significant correlation (P<0.01) with each other, and was significantly correlated with the non-rhizosphere TN content and the non-rhizosphere soil C : P (P<0.05). The TN content in rhizosphere soil was significantly and extremely correlated with the TN content and N : P in non-rhizosphere soil (P<0.01), and was significantly correlated with the C : P in non-rhizosphere soil (P<0.05). The TP content in rhizosphere soil not only exhibited a highly significant positive correlation with the C : P and N : P in rhizosphere soil (P<0.01), but also showed a similar highly significant positive correlation with the TP content in non-rhizosphere soil (P<0.05). The correlation between the C : N in rhizosphere soil and other items was not significant; the C : P in rhizosphere soil was significantly and extremely correlated with the N : P, TP, and N : P (P<0.01), and was significantly correlated with the C : P in non-rhizosphere soil (P<0.05). The N : P in rhizosphere soil was significantly and extremely correlated with the TP content in non-rhizosphere soil (P<0.01), and was significantly correlated with the N : P in non-rhizosphere soil (P<0.05).

3 Discussion

The soil carbon, nitrogen, and phosphorus contents in the planted forest of *Q. variabilis* in the study area were 14.0, 0.70, and 0.15 g/kg, respectively, belonging to levels 4 (medium), 5 (low), and 6 (very low) according to the *Classification Standards for Soil Nutrient Grades in China*. Whereas the soil carbon (19.3 g/kg), nitrogen (0.83 g/kg), and phosphorus (0.14 g/kg) contents in the natural forest of *Q. variabilis* belonged to levels 2 (very high), 4 (medium), and 6 (very low), respectively. According to the soil nutrient classification standards established by the second national soil survey in 1992, the average contents of carbon, nitrogen, and phosphorus in the soil of *Q. variabilis* plantations were all lower than the average nutrient level of Chinese soil. The contents of SOC and TN in natural forests were at a relatively high level. The biomass and productivity of forest ecosystems, as well as the decomposition ability of forest soil microorganisms towards organic matter such as litter, are all crucial ecological factors influencing the carbon, nitrogen, and phosphorus cycles in ecosystems and the levels of these elements in soil[23]. The Funiu Mountain has a high altitude and low temperature, and the leaves of *Q. variabilis* are thick and leathery, making them difficult to decompose, which inhibits microbial activity and is not conducive to nutrient accumulation[17].

The rhizosphere positive effect of SOC content in the *Q. variabilis* forest ecosystem in the Funiu Mountain area was significant, but the rhizosphere effects of TN and TP were not significant. The research results were not completely consistent with those of Wu Xiaosheng et al.[24], who believed that there was no significant difference in the contents of C, N, and P between rhizosphere soil and non-rhizosphere soil. Moreover, as the forest ages, the TP content in the soil tended to decrease significantly, while the stoichiometric ratio of C, N, and P showed a notable upward trend. Comparing natural and planted forests of *Q. variabilis*, it can be found that the content of SOC in the rhizosphere soil of both types of forests was significantly higher than that in the non-rhizosphere soil. This was primarily due to the differences in nutrient cycling mechanisms between natural and planted forests. In the rhizosphere soil, the microenvironment, composed of substances secreted by numerous root hairs, shed cells, and microorganisms, provides a rich carbon source for the decomposition and transformation of carbon, thereby facilitating the accumulation of SOC[25-26]. The TN content in the rhizosphere soil of planted forests gradually decreased with the increase of stand age, while the TN content in non-rhizosphere soil showed a trend of decreasing first and then increasing. Overall, the contents of SOC and TN in the rhizosphere and non-rhizosphere soils of *Q. variabilis* forests showed a trend of initial decrease followed by increase with changes in forest age. In the young forest stage, trees grow rapidly, and the cycling speed of nitrogen and phosphorus elements also increases accordingly, making it have a large demand for various nutrients. For this reason, nutrients in the soil are more prone to decomposition, consumption, and transformation. As the forest ages, the input of nutrients into the soil gradually increases, leading to accumulation[27].

When the soil N : P is less than 10, plants are primarily limited by N; when the N : P exceeds 20, they are limited by P; when the N : P falls between 10 and 20, vegetation is jointly limited by both N and P[23]. The N : P in the rhizosphere and non-rhizosphere soils of the *Q. variabilis* forest in the Funiu Mountain area were 5.3 and 5.9, respectively, indicating that both rhizosphere and non-rhizosphere soils were limited by N for the growth of *Q. variabilis*. The average C : N in the rhizosphere and non-rhizosphere soils of the Funiu Mountain area was 20.9, while the C : P was as high as 116.4, significantly higher than the average ratio of Chinese soils. This further indicated that the soil under the *Q. variabilis* forest in the Funiu Mountain area had not only an extremely low TP content, but also a low rate of organic matter mineralization and a low level of P availability[25]. This was similar to the research results of Liu Yilin et al[23]. The reason may be that plant roots secrete a large amount of organic acids and other organic substances into the rhizosphere soil, leading to a decrease in the mineralization rate of organic matter. Plant roots and microorganisms also secrete a large amount of organic substances such as enzymes, which help to separate P from soil minerals and organic matter. This may be the direct reason for the higher availability of P in rhizosphere soil.

4 Conclusions

(1) In the natural forests of *Q. variabilis* in the Funiu Mountain area, the content of SOC in the rhizosphere and non-rhizosphere soils was significantly higher than that in the corresponding values of planted forests at different forest age stages. Within planted forests, significant differences ($P<0.05$) existed in the SOC content between rhizosphere and non-rhizosphere soils across different forest ages.

(2) The content of SOC and N : P both exhibited distinct rhizosphere effect characteristics. The interaction between carbon, nitrogen, and phosphorus content and their stoichiometric ratios has reached a significant level, indicating that nitrogen and phosphorus limitations in rhizosphere soil exhibited stronger synergistic effects.

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