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Microbial Nutrient Limitation of Different Tea Cultivars: Evidence from Five Representative Cultivars

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Abstract: Soil microbial activity is generally limited by the availability of carbon (C), nitrogen (N), or phosphorus (P) in agricultural ecosystems. Soil ecoenzymatic activity (EEA), ecoenzymatic stoichiometry (EES), and vector characteristics were examined to assess microbial nutrient limitation. Investigating soil microbial nutrient limitation can provide insight into nutrient cycling in tea plantations with different tea cultivars. However, the dynamics of different tea cultivars on soil microbial nutrient limitations and their effect on tea quality remains poor. To address this issue, soil and plant samples were collected from a tea plantation cultivating five representative tea cultivars in Hunan Province, China. Baojing Huangjincha No. 1 (HJC1) and Huangjincha No. 2 (HJC2) were the extra early-sprouting cultivars, Zhuyeqi (ZYQ) and Zijuan (ZJ) were the middle-sprouting cultivars, and Zhenghedabai (ZHDB) was the late-sprouting cultivar, respectively. The results indicated that differences in EEA and EES were significant among five treatments. Notably, ZYQ and ZJ exhibited markedly lower activities of carbon (C), nitrogen (N), and phosphorus (P) acquiring enzymes compared to HJC1 and HJC2, whereas ZHDB showed significantly higher ecoenzymatic activities. Despite a general limitation in C and P for soil microorganisms across all cultivars (VL ranging from 1.42 to 1.59 and VA ranging from 58.70° to 62.66°), the degree of microbial nutrient limitation varied. Specifically, ZYQ experienced a pronounced P limitation (VA = 62.66°, N:P enzyme = 0.52), as evidenced by increased vector angles and decreased N:P enzyme values. Although C limitation was most pronounced in ZYQ (VL = 1.59), it did not significantly differ among the cultivars. These findings suggest that tea cultivars can influence the P limitation of microbial communities. Further analysis revealed that microbial nutrient limitations might adversely affect tea quality via impeding enzyme secretion. This study highlights the critical role of nutrient cycling within the soil-microorganism-plant ecosystem and emphasizes the influence of soil microbial nutrient limitations on tea quality within tea plantations. It is recommended that in the management of tea plantation fertilization, managers need to consider the influence of cultivars and develop specialized cultivar fertilizers.

Keywords: ecoenzymatic stoichiometry; microbial nutrient limitation; tea cultivars; ecoenzymatic activity; tea quality

1. Introduction

Tea (*Camellia sinensis* L.) is extensively cultivated in subtropical and tropical regions globally, particularly in the south of China, due to its extremely high economic value [1,2].

Producers employ high doses of nitrogen (N) fertilizers to achieve high free amino acid (FAA) contents in the tea leaves. Consequently, the substantial N inputs and the growth characteristics of the tea plants cause tea plantations to differ from other agricultural ecosystems. Moreover, the tea cultivation process is fraught with issues, including soil acidification, nutrient imbalance, and reduction of beneficial bacteria [3,4]. These issues emerging during tea cultivation can influence soil microbial nutrient limitation patterns. Comprehending these patterns is vital for the sustainable development of tea plantations. However, current research on tea cultivars mainly focuses on genetic diversity, metabolomics, nutrient demand and cycling, and tea quality [5–7], and an understanding of the patterns of soil microbial metabolism and their effect on tea quality in tea plantations remains limited. It is imperative to investigate soil microbial nutrient limitation and its relationship with tea quality to advance the knowledge of nutrient cycling, transformation, and balance within the soil-microorganism-plant system in tea plantations. Given the importance of soil microbial nutrient limitation in maintaining equilibrium in biogeochemical processes of elements [8], investigating soil microbial nutrient limitation and its impact on tea quality is crucial for the sustainable development of tea plantations. This investigation is vital as it offers insights into how soil microbial nutrient limitation influences tea quality, ultimately contributing to the comprehension and enhancement of tea production. By understanding the nutrient limitations faced by soil microbes and their subsequent effects on tea quality, tea plantation managers can make informed decisions on soil management practices, fertilizer applications, and other interventions to maintain and enhance tea quality.

Soil extracellular enzymes, produced by microorganisms and plant roots, play a pivotal role in decomposing organic matter and cycling essential elements within the soil [9,10]. The breakdown of soil organic matter is critical for releasing energy and nutrients, which are indispensable for microbial metabolism [8,11]. Consequently, soil ecoenzymatic activity (EEA) serves as an indicator to measure soil microbial nutrient demand and utilization efficiency [12]. Specifically, enzymes such as β-1,4-glucosidase (BG) and β-D-cellobiohydrolase (CBH), which facilitate carbon acquisition, and β-1,4-N-acetylglucosaminidase (NAG) and acid phosphatase (ACP), which are involved in nitrogen and phosphorus acquisition, constitute the primary extracellular enzymes synthesized via soil microbes to procure C, N, and P [13]. Prior research has indicated that soil EEA can reflect the energetic efficiency of microbial communities and the extent of substrate limitation experienced by soil microorganisms [14,15].

The introduction of soil ecoenzymatic stoichiometry (EES) by Sinsabaugh et al. (2009) [11] has provided a method to investigate soil EEAs, allowing for deeper insights into the connection between microbial metabolic demand and soil nutrient supply [11,16,17]. This innovative approach has been widely embraced as a vital tool for discerning microbial nutrient limitation [17] and is recognized for its significance in elucidating the mechanisms pertaining microbial C, N, and P cycling [18]. Moorhead et al. (2013) [19] also suggested a method for understanding the characteristics of microbial metabolism by calculating the "length" and "angle" of vectors on a plot showing enzymatic C:N versus C:P acquisition activities. This approach has been corroborated under a variety of environmental conditions [20,21], enhancing its applicability and relevance in studying microbial nutrient limitation.

Recent investigations have extensively utilized soil EES and vector characteristics to explore soil microbial nutrient limitation [22,23]. The findings have emphasized the influence of various factors across diverse ecosystems. However, the majority of recent studies have concentrated on forest ecosystems [24,25], grassland ecosystems [26,27], and agricultural ecosystems [28,29], with a primary emphasis on vegetation restoration and succession [30], nutrient addition [31], and management practices [32]. Relatively limited attention has been directed towards establishing a connection between soil microbial nutrient limitation and crop yield or quality, yet the quality of tea is of paramount importance in the cultivation process. Building upon previous research, this study delves into the relationship between tea quality and soil microbial nutrient limitation.

Within the tea plantation of Hunan Province, China, various cultivars were cultivated to meet diverse production requirements and enhance economic benefits. Prior research has identified variations in soil properties within tea plantation associated with different tea cultivars [33,34]. Informed by these findings, this investigation extends to examining the impact of soil properties and microbial biomass on soil EEA and microbial nutrient limitation, and how soil microbial nutrient limitation in turn affects tea quality. To accomplish this, soil and fresh leaf samples were collected from five representative tea cultivars selected from a tea germplasm demonstration garden in Hunan Province, China. Analyses were conducted on soil properties, microbial biomass, EEA, EES, and tea quality. This study aimed to test the following hypotheses: (1) soil EEA would exhibit significant variability among different tea cultivars; (2) nutrient limitation status would differ significantly among different tea cultivars; and (3) soil microbial nutrient limitation may be detrimental to tea quality.

2. Materials and Methods

2.1. Study Site

This study was conducted in the tea plant resource plantation at Xiangxi Academy of Agricultural Sciences in Hunan Province, China. This area is known for its subtropical monsoon climate, with a mean annual temperature of 17 °C, an annual sunshine duration of 1262 h, and a frost-free period lasting 296 days. To ensure representative sampling, five different tea cultivars in Hunan Province were chosen based on their distinct sprouting stages. Baojing Huangjincha No. 1 (HJC1) and Huangjincha No. 2 (HJC2) were chosen to represent the extra early-sprouting cultivars, while Zhuyeqi (ZYQ) and Zijuan (ZJ) were selected as representatives of the middle-sprouting cultivars, and Zhenghedabai (ZHDB) was chosen to represent the late-sprouting cultivar. Each cultivar was planted in uniform plots comprising several rows of 11 m in length, spaced 1.4 m apart, and maintained for 3 years under consistent management practices and ecological conditions.

2.2. Soil Sampling

In April 2023, soil sample collection was undertaken within the tea plantation soils. Rhizosphere soil samples were collected from the firmly bound soil of the roots within each plot after removing surface litter and plant residue. To form a composite sample, three soil samples from around each tea plant were combined, and three replicates were prepared. The collected soil samples were then sieved through a 2-mm mesh to remove roots and stones. Subsequently, the soil samples were partitioned into two fractions. The first fraction was immediately put into the ice box and stored at 4 °C after being transported to the laboratory. The second fraction was air-dried for the subsequent chemical property analysis.

2.3. Plant Sampling

Tea shoots, consisting of one bud and two young expanding leaves, were harvested in April 2023. We then quickly froze the fresh tea leaves in liquid nitrogen and subsequently stored them at −80 °C. Subsequently, the samples were oven-dried at 105 °C for 20 min, followed by drying at 70 °C until a constant weight was achieved.

2.4. Assay of Soil Properties

Soil pH was determined using a glass electrode meter via agitating a soil-distilled water (1:5 *w/v*) suspension for 30 min. Soil-dissolved organic carbon (DOC) was quantified using a TOC-TN analyzer. After extracting fresh soil with 2M KCl, the continuous flow analyzer was used to determine the contents of NH_4^+-N and NO_3^--N to assess the available nitrogen (AN). The available phosphorus (AP) was extracted using 0.5 M $NaHCO_3$ in a soil-to-solution ratio of 1:20 on a rotary shaker at 130 rpm and 25 °C for 30 min. AP content was then quantified using the molybdenum blue method in an ultraviolet spectrophotometer. Stoichiometric ratios of carbon to nitrogen (C:N), carbon to phosphorus (C:P), and nitrogen to phosphorus (N:P) in the soil were derived as ratios of DOC:AN,

DOC:AP, and AN:AP, respectively. This approach utilized soil DOC, AN, and AP for stoichiometric calculations, given their greater bioavailable for microbial uptake [35]. A chloroform-fumigation extraction method was used to estimate soil microbial biomass carbon (MBC), nitrogen (MBN) [36], and phosphorus (MBP) with calculated values using conversion factors of 0.45 for MBC and MBN, and 0.40 for MBP [37].

2.5. Assay of Soil Ecoenzymatic Activity

The ecoenzymatic activities were measured using the microplate method described previously [11]. Specifically, the preparation of soil suspensions was to homogenize 1 g of fresh soil with 125 mL of sodium acetate buffer (at the approximate pH of the soil). The assays were conducted fluorometrically using black polystyrene 96-well microplates. The microplates were assigned to sample assay, sample control, quench standard, reference standard, negative control, and blank wells. There were eight replicate wells for each category of the assigned wells per soil sample. Subsequently, the microplates were incubated in the dark at 25 °C for up to 4 h. Once the incubation is completed, the microplate reader is used to measure the value of each well with excitation at 365 nm and emission at 450 nm.

2.6. Quantification of Microbial Nutrient Limitation

The stoichiometry of the EEAs was calculated using Equations (1)–(3).

$$C:N \; enzyme = \ln(BG + CBH) : \ln(NAG) \tag{1}$$

$$C:P \; enzyme = \ln(BG + CBH) : \ln(ACP) \tag{2}$$

$$N:P \; enzyme = \ln(NAG) : \ln(ACP) \tag{3}$$

The relative proportions of N to P (x-axis) versus C to N (y-axis) in a scatter plot of EES can indicate nutrient limitation among different microbial groups [38]. Lower ratios of C:N and C:P enzyme suggest greater N and P limitations, respectively [12].

The soil enzymatic activities' vector lengths and angles were calculated using Equations (4) and (5), respectively.

$$Vector \; length = SQRT\left\{[\ln(BG + CBH) / \ln(ACP)]^2 + [\ln(BG + CBH) / \ln(NAG)]^2\right\} \tag{4}$$

$$Vector \; angle = Degrees\{ATAN2[\ln(BG + CBH) / \ln(ACP), \ln(BG + CBH) / \ln(NAG)]\} \tag{5}$$

Vector length indicates soil microbial C limitation. An increased vector length corresponds to a greater soil microbial C limitation. A vector angle below 45 degrees suggests soil microbial N limitation, while an angle above 45 degrees suggests soil microbial P limitation [17].

2.7. Determination of Tea Quality

The quality indicators of green tea are represented by free amino acid (FAA), contributing to the umami taste, and tea polyphenol (TPP), contributing to the bitter and astringent flavor. Limiting tea polyphenol content is important to minimize bitterness in the tea [39]. Conversely, soluble sugar (SS), is a crucial component that enhances the sweet taste and aroma of green tea [40]. The concentration of FAA, TPP, and SS components were assessed in fresh leaves (one bud with two leaves) harvested from tea plants. According to national standards GB/T8314-2013 [41], free amino acids were determined using the ninhydrin colorimetry method. The extraction and detection methods of tea polyphenol were performed according to GB/T8313-2018 [42]. Soluble sugar content was determined using the anthrone-sulfuric acid colorimetric method [43].

2.8. Statistical Analysis

Statistical analysis was performed using SPSS 26.0 statistical software to calculate the standard deviation. The effects of different tea cultivars on soil properties, soil micro-bial biomass, soil EEA and EES, vector characteristics, and tea quality were assessed using

one-way analysis of variance (ANOVA). Redundancy analysis (RDA) was performed with CANOCO 5.0 to determine the relationships among soil EEA, vector characteristics and soil factors. Pearson's correlation analysis was conducted using the "ggcor", "ggplot2", and "ggpubr" packages in R to explore the relationships between soil factors, soil EEA and EES, and vector characteristics. Additionally, this study utilized partial least squares structural equation modeling (PLS-SEM) to delve into the influence of soil microbial nutrient limitation, EEA, EES, and soil available nutrients on tea quality. For visual representation of the data, GraphPad Prism 8.3.0 software was utilized.

3. Results

3.1. Soil Properties and Microbial Biomass

Significant variations in soil properties were observed among different tea cultivars ($p < 0.05$; Table 1). Specifically, soil pH values were generally acidic, ranging from 4.92 to 6.40, with significantly lower readings in ZHDB and ZJ. In terms of soil DOC content, ZHDB exhibited significantly higher levels, whereas HJC2 and ZYQ displayed significantly lower concentrations. Regarding soil AN, HJC1 and HJC2 showed significantly higher concentrations, while ZJ, ZHDB, and ZYQ had significantly lower ones. Moreover, ZHDB showed significantly higher levels of soil AP. Significant disparities in soil available nutrient stoichiometry were noted among different tea cultivars, with ZHDB presenting a significantly higher soil C:N ratio and HJC2 a significantly lower one. The soil C:P ratio was also significantly higher in ZYQ and lower in HJC2, and the soil N:P ratio was significantly higher in HJC2 and lower in ZHDB (Table 1).

Table 1. Soil properties and microbial biomass in different tea cultivars.

	HJC1	HJC2	ZYQ	ZJ	ZHDB
pH	5.99 ± 0.33 ab	6.12 ± 0.06 a	6.40 ± 0.06 a	5.45 ± 0.23 b	4.92 ± 0.17 b
DOC (mg kg^{-1})	56.54 ± 4.49 ab	39.55 ± 4.83 b	46.21 ± 2.73 b	53.07 ± 7.60 ab	66.38 ± 4.36 a
AN (mg kg^{-1})	8.70 ± 0.70 a	8.53 ± 0.42 a	5.29 ± 0.47 b	5.61 ± 0.45 b	5.58 ± 0.35 b
AP (mg kg^{-1})	45.66 ± 10.56 b	43.02 ± 6.75 b	27.32 ± 0.51 b	43.85 ± 3.90 b	75.90 ± 8.28 a
C:N	6.62 ± 0.87 bc	4.63 ± 0.45 c	8.86 ± 0.92 b	8.76 ± 1.19 b	12.03 ± 1.34 a
C:P	1.36 ± 0.28 ab	0.71 ± 0.03 c	1.69 ± 0.11 a	1.21 ± 0.12 b	0.89 ± 0.10 bc
N:P	0.15 ± 0.00 b	0.22 ± 0.04 a	0.20 ± 0.02 ab	0.13 ± 0.02 b	0.08 ± 0.02 b
MBC (mg kg^{-1})	241.22 ± 13.36 a	202.9 ± 28.79 ab	147.54 ± 12.1 ab	152.66 ± 22.06 b	180.93 ± 19.7 b
MBN (mg kg^{-1})	32.13 ± 3.70 a	28.95 ± 4.92 a	20.49 ± 2.55 a	32.43 ± 5.85 a	31.06 ± 4.53 a
MBP (mg kg^{-1})	9.92 ± 1.21 a	8.87 ± 1.58 a	4.67 ± 0.63 b	4.61 ± 0.78 b	2.75 ± 0.22 b
MBC:MBN	7.63 ± 0.56 a	7.11 ± 0.41 a	7.28 ± 0.31 a	4.94 ± 0.84 b	5.98 ± 0.67 ab
MBC:MBP	25.35 ± 4.42 b	23.35 ± 2.52 b	28.09 ± 1.86 b	34.61 ± 5.73 b	65.72 ± 4.58 a
MBN:MBP	3.36 ± 0.62 b	3.34 ± 0.54 b	3.79 ± 0.30 b	8.65 ± 1.55 a	11.43 ± 1.96 a

Different lowercase letters indicate significant differences between treatments at $p < 0.05$.

Regarding soil microbial properties, significant variations were found between different tea cultivars ($p < 0.05$; Table 1). HJC1 exhibited a significantly higher soil MBC level, while ZJ and ZHDB exhibited significantly lower ones. In addition, HJC1 and HJC2 exhibited significantly higher soil MBP levels, whereas ZYQ, ZJ, and ZHDB displayed significantly lower levels. Furthermore, HJC1, HJC2, and ZYQ showed significantly higher MBC:MBN, while ZJ had a significantly lower one. The soil MBC:MBP in ZHDB was notably higher. Lastly, ZJ and ZHDB revealed a significantly higher soil MBN:MBP, while HJC1, HJC2, and ZYQ displayed a lower one (Table 1).

3.2. Soil Ecoenzymatic Activity

In general, the ecoenzymatic activities associated with carbon (C), nitrogen (N), and phosphorus (P) acquisition and their stoichiometric ratios varied among the different tea cultivars ($p < 0.05$; Figure 1). Specifically, activity levels of soil enzymes involved in carbon acquisition (BG + CBH) peaked in ZHDB, while the minimum was noted in ZYQ and ZJ. The ecoenzymatic activity for nitrogen acquisition (NAG) was minimal in ZYQ and ZJ and was significantly lower than that in other cultivars. ACP activity exhibited a similar trend, with the highest levels in ZHDB, followed by HJC1 and HJC2, while the lowest levels were recorded in ZYQ and ZJ.
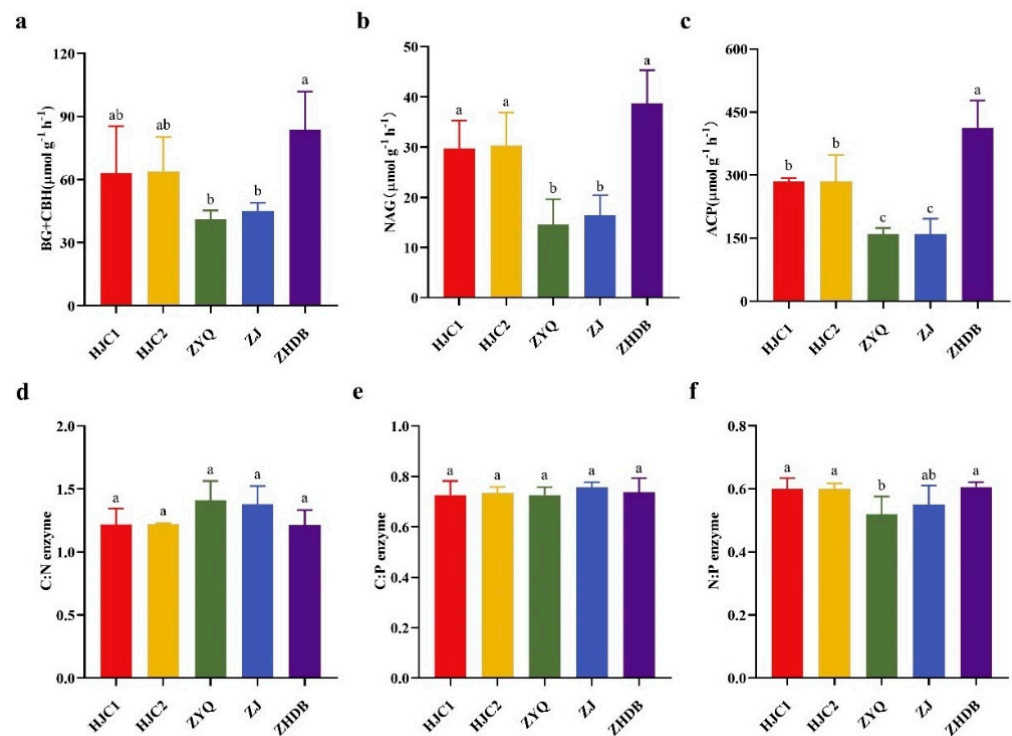
Figure 1. Soil ecoenzymatic activities (**a**–**c**) and stoichiometries (**d**–**f**) in different tea cultivars. Different letters indicate significant variation among the tea cultivars according to one-way ANOVA (LSD, $p < 0.05$). BG, β-1,4-glucosidase; CBH, β-D-cellobiohydrolase; NAG, β-1,4-N-acetylglucosaminidase; ACP, acid phosphatase; C:N enzyme, ln(BG + CBH):ln(NAG); C:P enzyme, ln(BG +CBH):ln(ACP); N:P enzyme, ln(NAG):ln (ACP); HJC1: Baojing Huangjincha NO. 1; HJC2: Huangjincha NO. 2; ZYQ: Zhuyeqi; ZJ: Zijuan; ZHDB: Zhenghedabai.

3.3. Soil Microbial Nutrient Limitation

The C:N enzyme ratio ranged from 1.21 to 1.42, indicating microbial C limitation, while the C:P and N:P enzyme ratios varied between 0.73 to 0.76 and 0.52 to 0.61, respec-tively, suggesting that all the studied tea cultivars were limited by microbial P (Figure 1d–f). Sig-nificantly, a lower N:P enzyme ratio was observed in ZYQ ($p < 0.05$; Figure 1f), highlighting a higher microbial P limitation. Notably, all data points in Figure 2a were positioned above the 1:1 line, showing a pronounced microbial P limitation in this study. Additionally, the vector length exceeding 1 indicated microbial C limitation across all cultivars, with no significant differences among them ($p > 0.05$; Figure 2b). Furthermore, vector angles greater than 45° for all cultivars indicated microbial P limitation (Figure 2c). Particularly, ZYQ exhibited elevated vector angle values, indicating a higher microbial P limitation. Notably, a linear regression analysis demonstrated that vector length was positively correlated with vector angle ($p < 0.001$; Figure 2d).
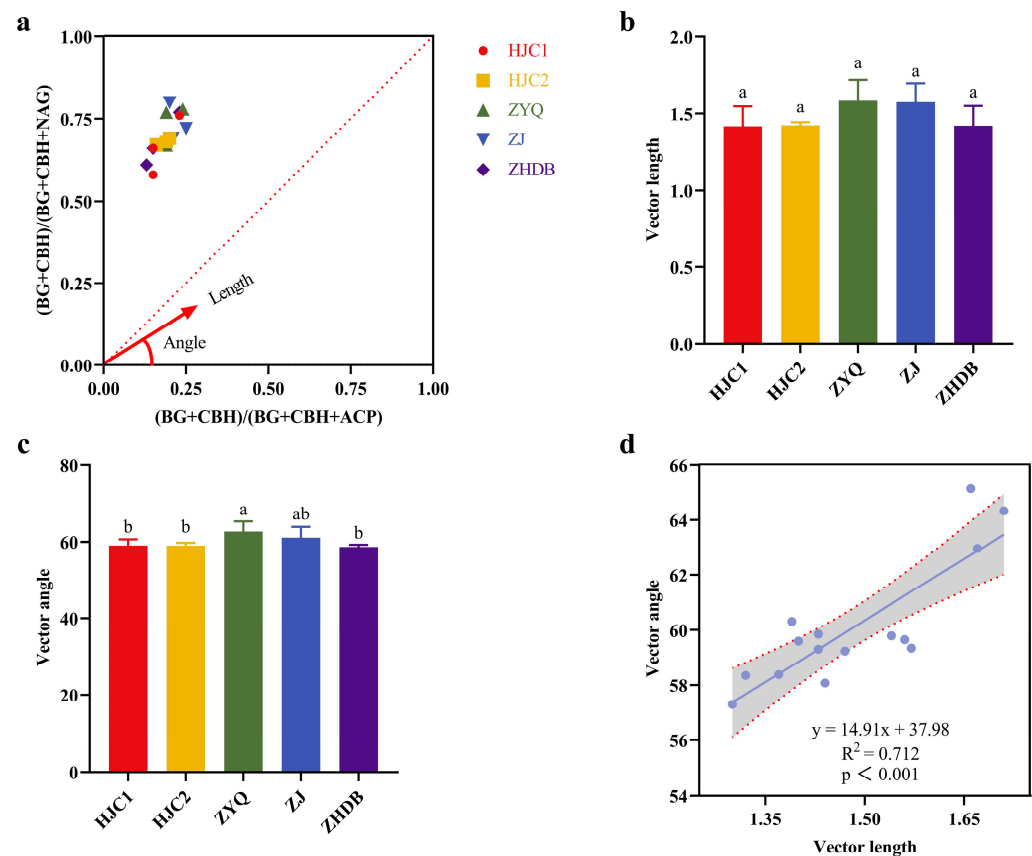
Figure 2. Ecoenzymatic stoichiometry of the relative proportions of C to N acquisition versus C to P acquisition (**a**), the variation in vector length and angle (**b**,**c**) and their relationships (**d**) in different tea cultivars. Different letters indicate significant variation among the tea cultivars according to one-way ANOVA (LSD, *p* < 0.05). BG, β-1,4-glucosidase; CBH, β-D-cellobiohydrolase; NAG, β-1,4-N-acetylglucosaminidase; ACP, acid phosphatase; C:N enzyme, ln(BG + CBH):ln(NAG); C:P enzyme, ln(BG + CBH):ln(ACP); N:P enzyme, ln(NAG):ln (ACP); HJC1: Baojing Huangjincha NO. 1; HJC2: Huangjincha NO. 2; ZYQ: Zhuyeqi; ZJ: Zijuan; ZHDB: Zhenghedabai.

3.4. Relationships among Soil Properties, Ecoenzymatic Activities, and Microbial Nutrient Limitation

The constrained axes of the RDA explained 96.23% of the relationship between soil properties and microbial biomass, soil EEAs, and vector characteristics. The first and second axes accounted for the variance of 94.63% and 1.60%, respectively (Figure 3). Soil AP was identified as the most influential factor, with a 49.9% explanatory power for soil ecoenzymatic activities and microbial nutrient limitation, showing significant associations with these variables (*p* < 0.01). Additionally, the MBC:MBP ratio was the second most explanatory factor, contributing 20.3% to soil ecoenzymatic activities and microbial nutrient limitation (*p* < 0.01). Pearson's correlation analysis revealed that ACP activity was positively correlated with soil AP and negatively correlated with soil C:P. The C:N enzyme ratio showed a positive correlation with soil C:P and a negative correlation with soil AP, whereas the N:P enzyme ratio displayed a positive correlation with soil AP and a negative correlation with soil C:P. Furthermore, the activity levels of BG + CBH, NAG, and ACP were positively correlated with soil MBC:MBP, while the C:N enzyme ratio was inversely related to soil MBC and the N:P enzyme ratio showed a positive correlation with soil MBC (Figure 4).
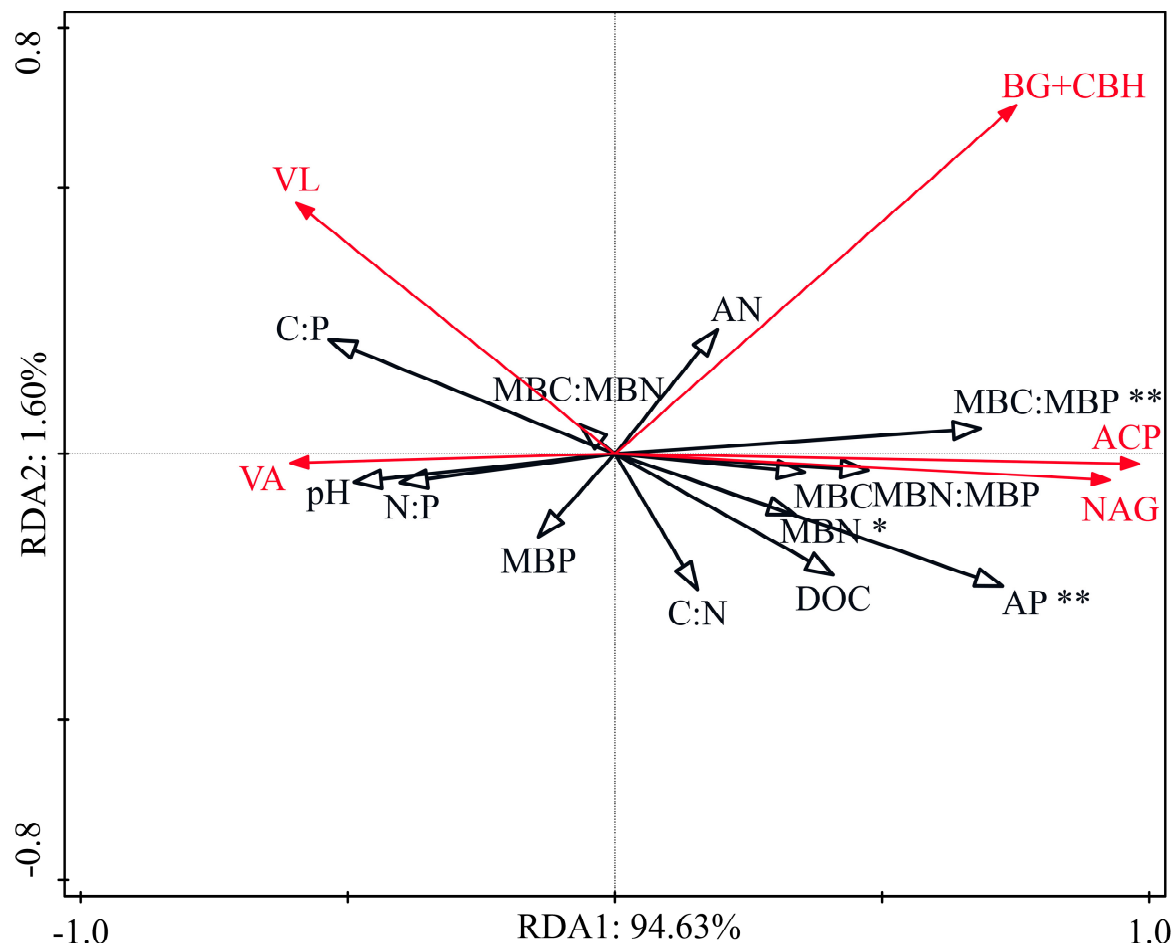
Figure 3. The redundancy analysis (RDA) used to identify the relationships among the soil properties and microbial biomass, ecoenzymatic activities, and microbial nutrient limitation. DOC, dissolved organic carbon; AN, available nitrogen; AP, available phosphorus; C:N, C:P and N:P represent DOC:AN, DOC:AP, and AN:AP, respectively; MBC: soil microbial biomass carbon; MBN: soil microbial nitrogen; MBP: soil microbial phosphorus; BG, β-1,4-glucosidase; CBH, β-D-cellobiohydrolase; NAG, β-1,4-N-acetylglucosaminidase; ACP, acid phosphatase; VL, vector length; VA, vector angle. The red color represents soil ecoenzymatic activities and microbial nutrient limitation, and the black color represents soil properties and microbial biomass. **, $p < 0.01$; *, $p < 0.05$.

3.5. Relationship between Soil Microbial Nutrient Limitation and Tea Quality

The contents of TPP and SS exhibited noteworthy variations across the five tea cultivars, as illustrated in Figure 5. Significantly lower TPP content was observed in HJC2, while HJC1 had the significantly higher SS content ($p < 0.05$). Conversely, the FAA content did not display any significant differences among the five tea cultivars ($p > 0.05$). It was found that ZYQ had lower SS content but higher TPP content, which may suggest that the tea quality of ZYQ could be considered inferior to other cultivars.

The PLS-SEM analysis determined that soil microbial nutrient limitation, soil EEA and EES, and soil available nutrients had direct and indirect effects on tea quality (Figure 6). Generally speaking, microbial C (−0.045, $p < 0.05$) and P (−0.435, $p < 0.05$) limitation had negative total effects on tea quality (Figure 6b).
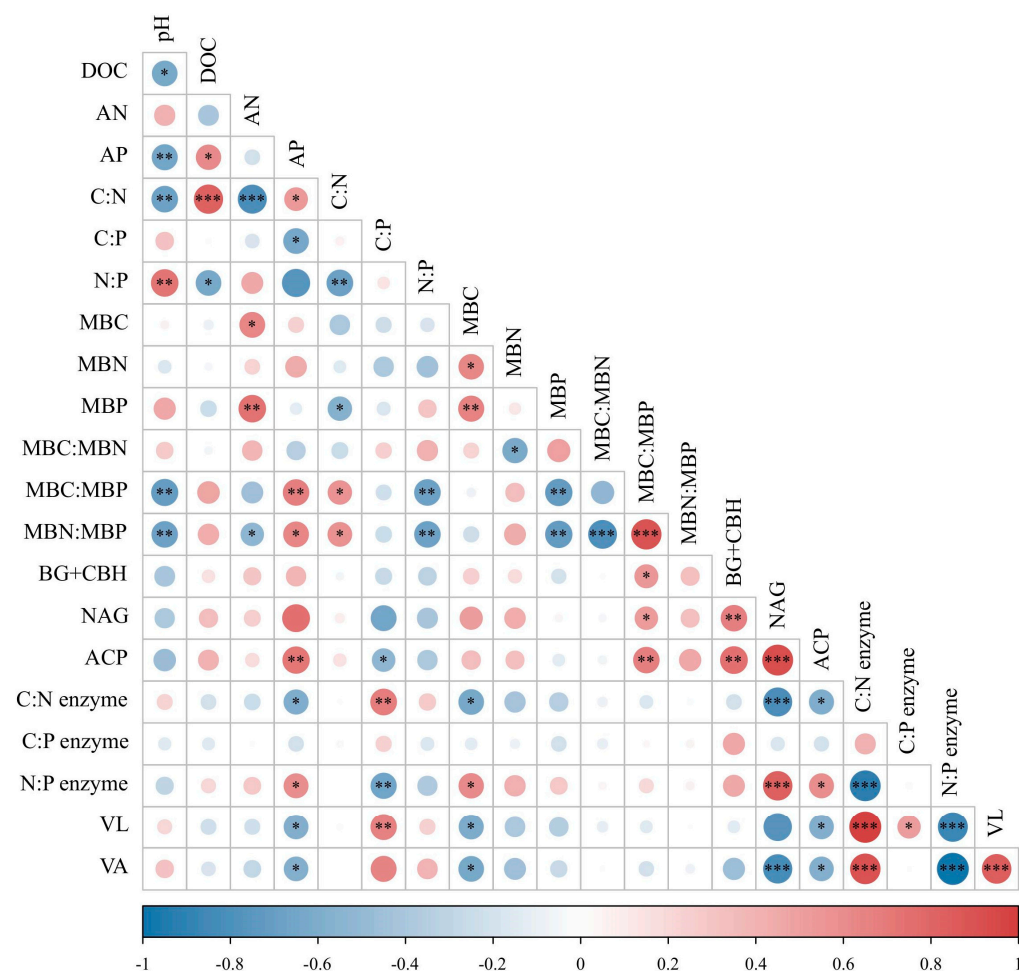
Figure 4. Pearson's correlations between soil properties, microbial biomass, ecoenzymatic activities and their stoichiometry. DOC, dissolved organic carbon; AN, available nitrogen; AP, available phosphorus; C:N, C:P and N:P represent DOC:AN, DOC:AP, and AN:AP; MBC: soil microbial biomass carbon; MBN: soil microbial nitrogen; MBP: soil microbial phosphorus; BG, β-1,4-glucosidase; CBH, β-D-cellobiohydrolase; NAG, β-1,4-N-acetylglucosaminidase; ACP, acid phosphatase; C:N enzyme, ln(BG + CBH):ln(NAG); C:P enzyme, ln(BG + CBH):ln(ACP); N:P enzyme, ln(NAG):ln (ACP). Red and blue circles represent the positive and negative correlations. The larger the circles, the more significant the correlation. ***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$.
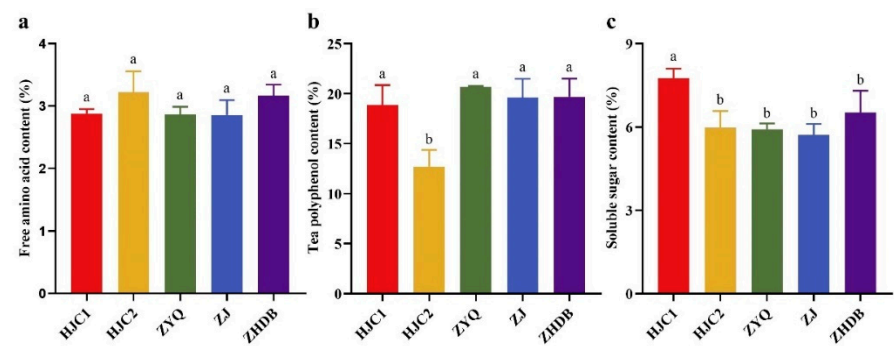

Figure 5. Tea quality of different tea cultivars. (**a**–**c**): FAA, free amino acid; TPP, tea polyphenol; SS, soluble sugar. Different letters indicate significant variation among the tea cultivars according to one-way ANOVA (LSD, $p < 0.05$). HJC1: Baojing Huangjincha NO. 1; HJC2: Huangjincha NO. 2; ZYQ: Zhuyeqi; ZJ: Zijuan; ZHDB: Zhenghedabai.
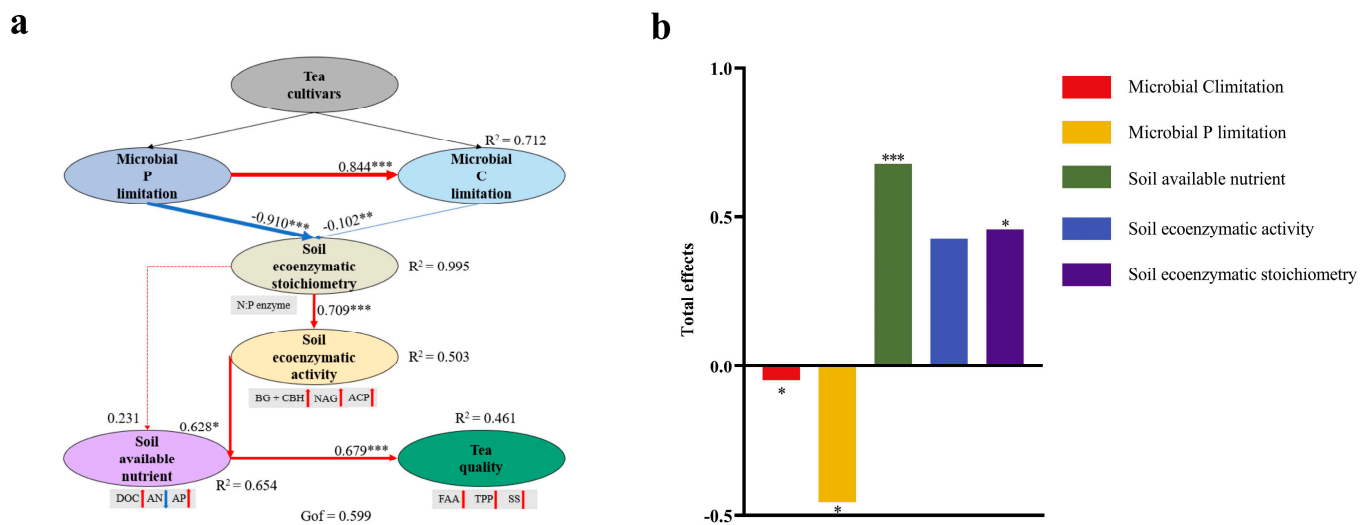
Figure 6. The partial least squares structural equation modeling (PLS-SEM) was used to identify the effect of soil microbial nutrient limitation, soil ecoenzymatic activity and stoichiometry, and soil available nutrients on tea quality. Red and blue arrows indicate positive and negative relationships, respectively. Solid lines indicate significant correlations ($p < 0.05$), while dashed lines indicate insignificance (**a**), the total effect of microbial C and P limitation, soil ecoenzymatic activity and stoichiometry, and soil available nutrient on tea quality (**b**). Numbers on the arrow indicate significant standardized path coefficients. Gof represents goodness of fit. ***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$.

4. Discussion

4.1. Differences in Soil Ecoenzymatic Activity in Different Tea Cultivars

The present study supported the first hypothesis by revealing significant differences in soil EEAs of BG + CBH, NAG, and ACP among different tea cultivars. ZHDB exhibited the highest soil EEAs of C, N, and P, while ZYQ and ZJ had the lowest. It was observed that ZYQ had the highest soil microbial nutrient limitation and lowest soil EEAs simultaneously. This can be attributed to the extremely low availability of nutrients in the soil of ZYQ, which may not provide enough energy for microorganisms to secrete enzymes as the enzymatic secretion process is energy-consuming [44] and thus results in the lowest EEAs. Another possible explanation may be the resource allocation theory, which suggests that soil EEAs are closely related to nutrient availability until microbial responses to disturbances or fluctuations in nutrient availability reach the limitations [45,46]. Under such circumstances, microorganisms tend to invest more energy in their own community growth instead of enzyme secretion. Additionally, when plants are confronted with limiting resources, they tend to prioritize biological processes and restrict others [47]. In various eco-systems, many soil factors can affect the characteristics of soil extracellular enzymes [48]. In this study, it was found that soil AP predominantly influenced soil extracellular enzymes, indicating that changes in soil EEAs could be elucidated by variations in soil AP levels. Additionally, a positive correlation between soil AP and soil EEAs implies that soil P availability tightly regulates microbial enzyme processes in tea plantations [35]. However, this finding contradicts a previous study that found no association between enzymatic potential and soil AP in tea plantations [29]. This discrepancy can be caused by different soil N and P content in various tea plantations. It is pertinent to note that previous studies have found that soil pH decline can inhibit enzyme activities [49,50], though no significant direct association between soil pH and soil ecoenzymatic activities was observed in this study. Consequently, further investigations are warranted to explore how soil pH influences soil enzyme activities, considering the differences in plant cultivars.

Sinsabaugh et al. (2008) [8] conducted a global meta-analysis, which suggested that soil microorganisms typically maintain a balanced ratio of C, N, and P in various ecosystems. Notably, the ratio of C:N:P acquisition enzymes in the soil approximated 1:1:1, indicating

this homeostasis. Nevertheless, it was found that the average ratio of C:N:P acquisition enzyme stood at 1:0.78:1.28, deviating from the excepted 1:1:1 ratio, pointing to relatively higher P than C or N-acquiring enzyme activities. This indicates that changes in ecosystem types and environmental conditions can disrupt the ratio of C:N:P acquisition enzymes [29]. Notably, the low activity of N-acquiring enzymes in this study may be attributed to the introduction of fertilizer N, which has been reported to suppress NAG enzyme activity in a previous study [51]. Although soil AP content was at a high level, the utilization of P is low in the studied region, indicating that soil microorganisms produce more P-acquiring enzymes to support their growth. Nonetheless, these results indicate that different tea cultivars have a crucial effect on soil EEA, and future studies should examine more tea cultivars to strengthen these findings.

4.2. Microbial Nutrient Limitation in Different Tea Cultivars

Previous studies have demonstrated that in agricultural systems, carbon is a significant limiting element for soil microbes [13,52,53]. This study revealed that all studied tea cultivars were limited by microbial C, as indicated via the C:N enzyme ratio and vector length. The C:N enzyme ratio exceeding 1 indicates that microorganisms have higher C requirements than N requirements. The analysis of both C:N enzyme and vector length showed that ZYQ had the highest C limitation. Soil microbial C limitation could be attributed to its low soil AP content, which had a significantly negative impact on vector length. Previous studies have also demonstrated that low soil nutrient contents such as DOC and AP may aggravate microbial C limitation [54]. However, this study found no significant difference in microbial C limitation among tea cultivars, possibly due to a multifaceted trade-off in microbial nutrient limitation [55,56].

Phosphorus, an essential nutrient element for microbial growth, exhibited significant limitation as indicated via vector angles greater than 45° across all studied tea cultivars, and there was a substantial disparity in P limitation among the different cultivars, confirming the second hypothesis. This finding is substantiated by the scatter plot of the stoichiometry of soil ecoenzymatic activity, which indicated P limitation in soil microorganisms. Additionally, ZYQ aggravated microbial P limitation, as evidenced by greater vector angles and lower N:P enzyme [12,57], emphasizing the importance of considering soil nutrients in the management of tea plantations according to the nutrient demand of different cultivars [58]. Notably, previous research has shown that a lower N:P enzyme in other ecosystems indicated higher P limitation [57]. The increase in microbial P limitation may be due to the decrease in soil AP content, which can provide more available P for microorganisms. This finding aligns with previous research indicating that microbial P limitation decreases with increasing soil AP in agricultural systems [59]. Furthermore, the increase in microbial P limitation can be attributed to relative nutrient limitation, which is not only dependent on the availability of the specific nutrient but also on other nutrients [60]. In this study, soil DOC and AN content decreased, while the soil C:P and N:P increased, resulting in decreased soil P availability and an increase in microbial P limitation. This explanation can be supported by previous studies that found a larger increase in soil C content than soil N content, leading to an elevated soil C:N and aggravating N-limitation in microorganisms [61]. Another potential factor contributing to the microbial P limitation is the presence of highly crystalline iron-phosphate (Fe-P) and aluminum-phosphate (Al-P) in the studied soil, as it is generally believed that the original loosely bound phosphates are gradually converted into highly crystalline Fe–P and Al–P in acidic soil [62,63]. Moreover, the positive relationship between soil C:P and N:P and microbial P limitation indicates that in the case of elemental stoichiometric changes [64], microorganisms have a tendency to increase their acquisition of the most limiting P to maintain stoichiometric homeostasis [65,66]. In light of these findings, it is advisable for tea plantation managers to consider the influence of cultivars and formulate specialized cultivar fertilizers in fertilization management practices.

4.3. Effects of Soil Microbial Nutrient Limitation on Tea Quality in Different Tea Cultivars

The total effects of soil microbial nutrient limitation on tea quality, as indicated using PLS-SEM analysis, were found to be significantly negative, supporting the third hypotheses. This negative correlation between microbial nutrient limitation and tea quality may arise from the negative impact of microbial nutrient limitation on soil ecoenzymatic activity, which further decreases soil available nutrients, resulting in a decline in tea quality. Previous studies have similarly highlighted the negative association between soil microbial nutrient limitation and ecoenzymatic activity. For instance, Yi et al. (2022) [29] reported a reduction in soil ACP activity in tea plantations due to increased soil microbial C limitation, depending on pH levels. Likewise, Auwal et al. (2023) [24] found that soil microbial C limitation is negatively correlated with soil NAG and ACP activities in forest soils. Chen et al. (2021) [67] demonstrated that long-term mineral fertilizer addition in continuous crop rotations of soybeans, wheat, and maize led to decreased soil ecoenzymatic activities and increased soil microbial nutrient limitation. In this study, the low availability of soil nutrients may lead to the negative impact of soil microbial nutrient limitation on soil EEA, as microorganisms typically prioritize community growth over investing in enzyme production. For example, Jiang et al. (2022) [68] demonstrated that combining organic fertilizer with rotation cropping management can alleviate soil microbial nutrient limitation and enhance crop production. Yang et al. (2023) [69] discovered that intercropping can regulate the stoichiometric soil C-N-P and mitigate soil microbial P limitation, thus contributing to increased maize productivity. It is known that plant-associated microorganisms can improve plant quality, thus, future studies should focus on investigating the changes of plant-associated microorganisms under nutrient limitation. Enhancing the ability of tea plants to interact and cooperate with microorganisms should be considered a fundamental aim in the management of tea plantations or the breeding programs of tea cultivars to improve tea quality.

4.4. Limitation of Current Research

This study revealed that the status of soil microbial P limitation is related to tea cultivars. However, this study has several limitations that need to be addressed. Firstly, the sample size of examined cultivars was limited, indicating the need for future research to include a broader array of cultivars on a larger scale to strengthen the conclusions drawn. Secondly, in order to enhance the validity of the conclusions, it is crucial to utilize high-throughput sequencing for analyzing the composition and structure of soil microorganisms.

5. Conclusions

In this study, ecoenzymatic stoichiometry was used to investigate soil microbial nutrient limitation in a tea plantation with five representative tea cultivars. It was found that soil microbial nutrient metabolism with varying tea cultivars was limited by both carbon and phosphorus. In particular, the strength of microbial P limitation may be associated with tea cultivars, with ZYQ exhibiting more heavily. Conversely, microbial C limitation showed no significant difference among tea cultivars. Furthermore, this finding suggests that soil microbial nutrient limitation adversely impacts tea quality via reducing the secretion of enzymes. It can provide a deeper understanding of the nutrient cycling mechanism in tea plantations via establishing connections between soil properties, soil ecoenzymatic activity, soil microbial nutrient limitation, and tea quality. These results have crucial implications for the sustainable development and enhancement of tea quality in tea plantations.

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