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ORIGINAL PAPER

Effects of Bio-organic Fertilizer on Soil Fertility, Yield, and Quality of Tea

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Abstract
Bio-organic fertilizers are gaining an increasing attention, but research studies on their effects on soil fertility, tea yield, and tea quality are still limited. Here, we developed three types of bio-organic fertilizers to assess their effects on soil fertility and enzyme activity and yield and quality of spring and autumn green tea. We carried out a field experiment over a 2-year period with 5 treatments: no fertilizer, *Bacillus megaterium*-based bio-organic fertilizer, *Bacillus colloid*-based bio-organic fertilizer, *Bacillus subtilis*-based bio-organic fertilizer, and conventional chemical fertilizer. Compared with conventional chemical fertilizers, three bio-organic fertilizers reduced soil bulk density but significantly ($p < 0.05$) increased soil pH, available nitrogen, available phosphorus and potassium, as well as the contents of total nitrogen and phosphorus in soils. In particular, the bio-organic fertilizers had the most significant increase in tea yield and quality ($p < 0.02$) and produced the greatest increase relative to the control. *Bacillus megaterium*-, *Bacillus colloid*-, and *Bacillus subtilis*-based bio-organic fertilizers increased the contents of tea polyphenols, amino acids and caffeine by 17.71, 33.05, and 22.20%, respectively, compared with conventional chemical fertilizers. Moreover, three bio-organic fertilizers significantly ($p < 0.05$) increased the activities of soil leucine aminopeptidase, β-glucosidase, and β-N-acetylglucosamine enzymes and soil acid phosphatase, β-cellobiosidase, and β-xylanase. Redundancy analysis further confirmed that tea yield and quality were significantly correlated with the soil nutrients and enzyme activities induced by bio-organic fertilizers, respectively. Our findings suggest that the application of bio-organic fertilizers can significantly improve soil fertility, enhancing the yield and quality of tea compared with chemical fertilizers.

Keywords Bio-organic fertilizer · Tea yield · Quality · Soil fertility · Enzyme activity

1 Introduction

China is the largest tea grower and exporter and accounts for 50% of the world's production (Xu et al. 2021). Yet how to continuously improve the yield and quality of tea is the primary problem, which still needs to be solved in the process of tea production (You et al. 2011). Indeed, fertilization is regarded as the most effective way to address this problem, as the application of chemical fertilizers has brought the continuous increase of yield and income of farmers. According to statistics, the annual nitrogen application rate for tea plantation in China is in the range of 0–1200 kg ha^{-1}, with an average of 533 kg ha^{-1}(Wu et al. 2016). Zhejiang province is an important area of tea production in China, with an average annual nitrogen application of 521 kg ha^{-1} (Yan et al. 2018). However, this value exceeds the upper limit of nitrogen application, which is 450 kg ha^{-1} (Yang et al. 2018). This excessive fertilization does not only increase the

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production costs but also induce soil acidification (Youssef and Farag 2021), soil compaction (Lin et al. 2019), non-point source pollution (Sun et al. 2012), and greenhouse gas emissions (Kahrl et al. 2010). The decline of soil quality can inhibit the root growth, ultimately influencing the yield and quality of tea. Tea is a leafy plant that has a higher nitrogen demand than most other crops. Thus, the long-term application of nitrogen fertilizer, without organic fertilizer, and ignoring the balanced application of additional nutrients can lead to serious soil acidification, decreasing the yield and quality of tea (Xie et al. 2020). The addition of organic fertilizers has some beneficial effects on soil quality, such as soil porosity and organic carbon content (Domingo Olivé et al. 2016). Microorganisms are also an important component of soil micro-ecological environment as they are directly related to soil fertility and health by controlling soil nutrient cycling and plant growth (Hayat et al. 2010). Bio-organic fertilizer is a new type of fertilizer formed by inoculation of nitrogen fixation, phosphorus dissolution, potassium dissolution, and disease-resistant microbial fermentation with animal manure and agricultural by-product residues as raw materials. After the application, functional microorganisms can multiply in the soil, affecting the soil microbial community structure to provide a healthy environment for plant growth. Recently, Ye et al. (2020) have studied the effects of *Trichoderma*-based bio-organic fertilizer on tomato yield and quality and showed that its application with an appropriate proportion of chemical fertilizer had the maximum increase in the yield and quality, with the added benefit of reduced fertilizer application. Ling et al. (2014) also used PCR-DGGE technology to analyze the effect of bio-organic fertilizer continuous application on soil bacterial diversity, suggesting that they can regulate soil microbial communities to maintain plant health. Thus, maintaining the diversity of microbial communities by regulating the composition and community structure of tea rhizosphere microorganisms could improve the quality and yield of tea. Tea bio-organic fertilizer is prepared by mixing beneficial microorganisms such as *Trichoderma*, nitrogen-fixing, phosphorus, and potassium bacteria with an appropriate ratio of organic matter. Cakmakci et al. (2021) made biofertilizers using three microorganisms (nitrogen fixation, phosphorus solubilization, and containing ACC deaminase) to assess their effects on the enzyme activity, growth, and yield of tea. They found that all bio-organic fertilizers stimulated the overall growth of tea by increasing the area, yield, chlorophyll content, and enzyme activity in tea leaves. Bai et al. (2014) also explored the effect of *Bacillus amyloliquefaciens* bio-organic fertilizer fermented with potato starch wastewater on the yield and quality of tea. They further showed that under the optimum concentration of 1.6×10^8 cfu ml^{-1}, the bio-organic fertilizer treatment increased the weight of hundred buds by 22.3%, water soluble matter by 21.9%, the amino acid content by 8.83%, and the tea polyphenols content by 9.76%, but the caffeine content decreased by 8.32%.

The objective of the study was to develop three new types of bio-organic fertilizers composed of *Bacillus megaterium*, *Bacillus colloid*, and *Bacillus subtilis*, which have been frequently developed as soil amendments using peat and cow dung as carriers. Indeed, *Bacillus megaterium* is a functional bacterium with efficient phosphate solubilization. As confirmed by recent study (Zhao et al. 2021), the application of *Bacillus megaterium* increased soil phosphorus and potassium bioavailability by increasing the richness of their bacterial and fungal communities and increasing the yield of cucumber. After application of *bacillus mucilaginosus*, potassium feldspar and apatite can be decomposed by acidolysis, alkaline hydrolysis, ligand degradation, enzymatic hydrolysis, capsule adsorption, extracellular polysaccharide oxidation–reduction, and comprehensive action, thus increasing phosphorus and potassium content in soil (Chen et al. 2020). *Bacillus subtilis* also has a significant effect on preventing plant diseases and insect pests, promoting plant nutrient absorption, and improving the yield and quality of the crop (Güneş et al. 2014). At present, these three microorganisms are used in agricultural production and have excellent effects. In this study, we prepared three different strains of bio-organic fertilizers in the tea garden for a 2-year field experiment. The aim of this study was (1) to study the effect of bio-organic fertilizers on the growth, yield, and quality of tea, (2) to compare the effects of bio-organic fertilizers and conventional chemical fertilizer on soil fertility and enzyme activity in tea garden, and (3) to explore and examine the relationship between soil fertility, enzyme activity, and tea yield and quality. The study can provide critical insights into the effectiveness of *Bacillus megaterium*, *Bacillus mucilaginosus,* and *Bacillus subtilis* as bio-organic fertilizers for sustainable tea production.

2 Material and Methods

2.1 Overview of the Study Area

The experimental site was located in Songyang County, Lishui City, Zhejiang Province, China (119° 22′ 34.06″ E, 28° 31′ 3.57″ N). It has a subtropical monsoon climate, with warm and humid, sufficient rainfall, long frost-free period, and obvious vertical climate difference. The annual average temperature was 17.7 ℃, and the precipitation was 1500–1800 mm. The rainfall was concentrated in spring and summer, and the average relative humidity was 79%, which was conducive to the growth of tea trees and the germination of spring tea. We selected the experimental tea garden where the local tea trees grew naturally for more than 5 years without any artificial fertilization treatment. This selection ensured the experimental reliability in eliminating the influence of external factors. Tea variety was "Longjing 43" with a tree age of 7 years, which was characterized by double row planting, line of spacing 1.5 m, and management methods for artificial picking tea garden. The soil type was entisols, characterized by soil bulk density of 1.65 $g \cdot cm^{-3}$, soil pH of 3.60, available nitrogen of 260.19 $mg \cdot kg^{-1}$, total nitrogen of 2.83 $g \cdot kg^{-1}$, available phosphorus of 90.44 $mg \cdot kg^{-1}$, total phosphorus of 1.74 $g \cdot kg^{-1}$, available potassium 100.26 of $mg \cdot kg^{-1}$, and organic matter of 38.32 $g \cdot kg^{-1}$.

2.2 Bio-organic Fertilizer Production

Bacillus megaterium, *Bacillus mucilaginosus*, and *Bacillus subtilis* were provided by Wuhan Keno Biotechnology Co., Ltd. We used beef extract peptone liquid medium to culture and inoculated 10% into the solid medium. The solid medium was a mixture of wheat bran, rice bran, and husk (mass ratio 6:3:1, material-water ratio 1.0:1.2). After stirring evenly, it was cultured at 28 ℃ for 7 days so the effective number of viable bacteria in the inoculum reached 2×10^8 $CFU \cdot g^{-1}$. According to the mass percentage, the bio-organic fertilizer contains 30% microbial agent, 60% peat, and 40% cow dung. These three materials were weighed quantitatively and mixed evenly to obtain bio-organic fertilizers. The peat was collected from Tonghua, Jilin, China, and the cow dung was collected from Jinhua, Zhejiang, China. The two materials were air-dried at room temperature for 3–5 days, crushed, and sieved through a 2-mm sieve. Particles (< 2 mm) were collected and autoclaved at 121 ℃ for 30 min for further experiments. The bio-organic fertilizer was brown powder and odorless; it was characterized by organic carbon of 270.44 $g \cdot kg^{-1}$, pH of 6.74, moisture content of 17.64%, total nitrogen of 23.84 $g \cdot kg^{-1}$, total phosphorus of 10.26 $g \cdot kg^{-1}$, total potassium of 16.31 $g \cdot kg^{-1}$, total As (arsenic) of 3.21 $mg \cdot kg^{-1}$, total Hg (hydrargyrum) of 0.42 $mg \cdot kg^{-1}$, total Pb (plumbum) of 14.52 $mg \cdot kg^{-1}$, total Cd (cadmium) of 0.36 $mg \cdot kg^{-1}$, and total Cr (chromium) of 22.46 $mg \cdot kg^{-1}$. These properties were in line with the standards of China's agricultural industry organic fertilizer.

2.3 Experimental Design

A total of 5 treatments were set up in the experiment, namely, *Bacillus megaterium* bio-organic fertilizer (BCF1), *Bacillus colloid* bio-organic fertilizer (BCF2), *Bacillus subtilis* bi-organic fertilizer (BCF3), conventional chemical fertilizer (TF), and no fertilization control (CK). A randomized block design was used in the experiment. Each treatment was repeated three times. Area of each repeated plot was 40 m^2 (20 m long and 2 m wide), and a protective row with an interval of 1.5 m was set between different plots.

Based on a questionnaire survey of local tea farmers and tea production companies, the application ratio of N: P_2O_5: K_2O in tea gardens was 5: 2: 3 ($N = 450$ $kg \cdot ha^{-1}$, $P_2O_5 = 180$ $kg \cdot ha^{-1}$, and $K_2O = 270$ $kg \cdot ha^{-1}$). In order to be same with the traditional nitrogen fertilizer substitution method, the amount of bio-organic fertilizer was estimated according to the nitrogen requirement. The conventional chemical fertilizer was compound fertilizer ($N = 200$ $g \cdot kg^{-1}$, $P_2O_5 = 80$ $g \cdot kg^{-1}$, and $K_2O = 120$ $g \cdot kg^{-1}$). Table 1 presents the amounts of various fertilizers applied. The field experiment began in 2020, and the fertilizer application time was in March, July, and November of each year. According to the growth of tea plants, the nitrogen required was applied in three splits: 60% fertilizer was applied in November and 20% was applied in March and July.

2.4 Sampling

Soil samples were collected in April 2022 (spring) and August 2022 (autumn). Five-point sampling method was used for each experimental plot. The surface soil (0–20 cm) was collected from the root of tea plant (10 cm) using stainless steel shovel, and each sample was 1 kg. The collected soil was brought back to the laboratory with low-temperature preservation, and the debris was removed. The collected soil was removed from the debris, passed through a 2-mm sieve, and fully mixed before dividing into two parts. One part was stored in a 4 ℃ refrigerator for the determination of soil enzyme activity, and the other part was naturally dried for the determination of soil physical and chemical properties. The method of handpicking tea was adopted, and the sampling standard was one bud and two leaves of fresh tea. After harvest, they were taken back to the laboratory to put into the oven that had been preheated at 105 ℃ for 10–15 min and then dried at 80 ℃ for 24 h. The prepared samples were dried and stored in a refrigerator at low temperature.

Table 1 Total fertilizer application for each treatment ($kg \cdot ha^{-1}$)

Treatment	Bio-organic fertilizer	Chemical fertilizer
BCF1	18,873	0
BCF2	18,873	0
BCF3	18,873	0
TF	0	2250
CK	0	0

BCF1, *Bacillus megaterium* bio-organic fertilizer; BCF2, *Bacillus colloid* bio-organic fertilizer; BCF3, *Bacillus subtilis* bio-organic fertilizer

2.5 Analysis of Soil Samples

2.5.1 Determination of Soil Physical and Chemical Properties

Soil bulk density (SBD) of soil samples was determined using a 50-cm^3 stainless steel cutting ring (Abu and Nidal 2003). Soil pH was determined using a pH meter (Orion 3 Star; Thermo Ltd., USA) (soil:water = 1:2.5). Available nitrogen (AN) was determined by alkaline diffusion method (Kwon et al. 2009). Total nitrogen (TN) was determined by Kjeldahl nitrogen method (Bremner 1960). Soil available phosphorus (AP) was determined by Olsen method (Recena et al. 2015). Total phosphorus (TP) was determined by molybdenum blue colorimetric method after digestion with perchloric acid-concentrated sulfuric acid (Walker and Adams 1959). Available potassium (AK) was determined using ammonium acetate extraction-flame photometer method (Lu et al. 1952). Soil organic matter (SOM) and soil organic carbon (SOC) were determined by potassium dichromate oxidation-external heating method, and soil organic matter was determined as follows: soil organic matter = soil organic carbon × 1.724 (Nóbrega et al. 2015).

2.5.2 Determination of Soil Enzyme Activity

This study determined six soil enzyme activities, including *β*-glucosidase (BG), *β*-cellobiose mannase (CB), *β*-xylanase (XYL), *β*-N-acetylglucosaminidase (NAG), leucine aminopeptidase (LAP), and acid phosphatase (ACP), in soils. Soil enzyme activity was determined by fluorescence microplate detection technology. Fresh soil 2 g was weighed in a centrifuge tube and added 100 mL of ammonium acetate buffer with pH 5.0 to be shaken at 25 ℃ 180 $r \cdot min^{-1}$ for 30 min. Soil suspension 200 μL was taken into 96-well plates, and 50 μL of reaction substrate was immediately added and cultured in an incubator at 25 ℃ for 3 h. Microplate reader (Synergy TM H1; Biotek, USA) was used to detect the absorbance at 365 nm excitation wavelength and 450 nm emission wavelength and calculate soil enzyme activity. The unit of enzyme activity was expressed in $nmol \cdot g^{-1} \cdot h^{-1}$.

2.6 Determination of Tea Yield and Quality

2.6.1 Determination of Tea Yield and Nutrients

During the germination of tea trees, 5 sites were randomly selected from each plot using a 33 cm × 33 cm plank frame to pick tea. At each picking, the bud density, 100-bud fresh weight, and tea yield were recorded (Yang et al. 2021). The tea samples were digested by H_2SO_4–H_2O_2 combined digestion method. The nitrogen content in the samples was determined by Kjeldahl nitrogen analyzer (Watanabe 1995). The phosphorus content in the samples was determined by vanadium molybdenum colorimetry (Zhu et al. 2019). The potassium content in the samples was determined by flame photometer (Zhang and Kong 2014). The SPAD (chlorophyll meter model SPAD-502; Konica Minolta Inc., Japan) value of the same part of each treated tea (the third tea under the new bud) (Liu et al. 2012).

2.6.2 Determination of Tea Quality

Tea polyphenols were determined by Folin–Ciocalteu colorimetry (Nibir et al. 2017); amino acids were determined by ninhydrin colorimetry (Ma et al. 2022); caffeine was determined by ultraviolet spectrophotometry (Showkat et al. 2015); phenol ammonia ratio = tea polyphenols/amino acids.

2.7 Statistical Analysis

IBM SPSS Statistics 23 software was used for data statistical analysis. One-way analysis of variance (one-way ANOVA) and two-way analysis of variance (two-way ANOVA) were used to test the difference significance of the data. Duncan's method was used to make multiple comparisons of the experimental data. The difference significance standard was $p < 0.05$ level. Origin 2021 was used for plotting the data. In Canoco 5.0, RDA was performed with tea yield and quality as response variables and soil enzyme activity as explanatory variables ($p < 0.05$). Experimental data were expressed as mean ± standard deviation (SD) (Table 2).

3 Results

3.1 Soil Physical and Chemical Properties

Soil bulk density (SBD) was decreased with increase in bio-organic fertilizer application time. In spring, compared with TF treatment, soil bulk density of BCF1 and BCF3 treatments decreased significantly ($p < 0.05$), by 0.21 and 0.24 units, respectively (Table 3). In autumn, the three bio-organic fertilizer treatments changed little, and BCF2 treatment decreased by 0.21 units compared with spring. The content of soil pH, SOM, TN, TP, AN, AP, and AK increased with the extension of bio-organic fertilizer application time. Compared with the TF treatment, bio-organic fertilizer treatment increased significantly the soil pH; the increase rates of which were 5.77–12.64% (spring) and 11.45–20.95% (autumn). AP significantly increased by 18.77–31.79% (spring) and 24.84–37.80% (autumn). There was no

Table 2 Two-way analysis of variance (*F*-value) for effects of season and fertilizer on soil physicochemical properties, enzyme activities, and tea yield and quality

Sources of variability	Season	Treatment	Season × Treatment
Soil bulk density (SBD)	3.176	3.187*	0.299
Soil pH (pH)	3.573	32.971***	1.448
Available nitrogen (AN)	1.898	73.470***	2.488
Total phosphorus (TP)	0.752	6.659***	0.761
Available potassium (AK)	0.665	22.694***	1.932
Soil organic matter (SOM)	0.802	27.093***	0.373
Leucine aminopeptidase (LAP)	56.574***	74.454***	10.633***
β-Glucosidase (BG)	28.217***	25.384***	1.049
β-Cellobiose glycase (CB)	11.421**	32.789***	5.935**
β-N-acetylglucosaminidase (NAG)	6.335*	25.248***	0.874
Acid phosphatase (ACP)	99.102***	52.336***	11.742***
β-Xylosidase (XYL)	112.285***	67.094***	7.239***
Tea polyphenol	1.003	27.672***	3.001*
Amino acid	5.430*	45.207***	0.956
Phenol ammonia ratio	5.630*	17.820***	0.623
Caffeine	6.843*	39.483***	1.522

* Represents the significant difference at the $p < 0.05$ level; ** indicates a significant difference at the $p < 0.01$ level; *** indicates a significant difference at the $p < 0.001$ level

Table 3 Effects of bio-organic fertilizer on soil physical and chemical properties

Factors	Season	Treatments				
		CK	BCF1	BCF2	BCF3	TF
SBD (g•cm^{-3})	Spring	1.68 ± 0.05a	1.41 ± 0.09b	1.58 ± 0.14ab	1.38 ± 0.06b	1.62 ± 0.14a
	Autumn	1.56 ± 0.16a	1.37 ± 0.22a	1.37 ± 0.26a	1.34 ± 0.14a	1.52 ± 0.08a
pH	Spring	3.56 ± 0.03c	3.85 ± 0.08b	3.94 ± 0.07b	4.10 ± 0.06a	3.64 ± 0.09c
	Autumn	3.55 ± 0.03b	3.99 ± 0.05a	4.05 ± 0.12a	4.33 ± 0.13a	3.58 ± 0.26b
SOM (g•kg^{-1})	Spring	36.57 ± 4.09a	38.19 ± 3.00a	39.32 ± 4.82a	40.40 ± 2.29a	36.82 ± 1.25a
	Autumn	36.32 ± 3.19b	39.19 ± 2.98ab	41.21 ± 2.68a	40.64 ± 1.73ab	36.60 ± 3.44ab
TN (g•kg^{-1})	Spring	2.63 ± 0.27c	3.21 ± 0.39ab	3.36 ± 0.03ab	3.50 ± 0.29a	2.98 ± 0.17bc
	Autumn	2.46 ± 0.15c	3.42 ± 0.31ab	3.54 ± 0.23ab	3.76 ± 0.17a	3.34 ± 0.15b
TP (g•kg^{-1})	Spring	1.62 ± 0.14a	2.31 ± 0.76a	2.04 ± 0.34a	2.00 ± 0.14a	1.89 ± 0.22a
	Autumn	1.39 ± 0.17c	2.47 ± 0.20a	2.22 ± 0.11ab	2.39 ± 0.42a	1.92 ± 0.04b
AN (mg•kg^{-1})	Spring	246.17 ± 7.71c	333.91 ± 17.20a	343.83 ± 13.02a	357.07 ± 21.79a	298.43 ± 5.70b
	Autumn	222.42 ± 8.97d	344.35 ± 10.54b	356.78 ± 14.39b	388.54 ± 18.28a	306.24 ± 25.08c
AP (mg•kg^{-1})	Spring	80.06 ± 2.74c	129.48 ± 9.96a	116.69 ± 8.12a	119.48 ± 9.47a	98.25 ± 15.35b
	Autumn	68.29 ± 6.86c	183.71 ± 18.55a	136.37 ± 7.39b	130.20 ± 22.4b	114.29 ± 23.26b
AK (mg•kg^{-1})	Spring	90.67 ± 5.51b	96.67 ± 12.06b	114.00 ± 4.36a	98.01 ± 4.63b	97.42 ± 9.17b
	Autumn	79.75 ± 10.10d	113.33 ± 9.07b	132.62 ± 11.71a	112.00 ± 7.00bc	96.42 ± 2.78c

Data represents means ± standard deviation. Different lowercase letters in the table indicate significant differences between different treatments in the same season ($p < 0.05$). BCF1, *Bacillus megaterium* bio-organic fertilizer; BCF2, *Bacillus colloid* bio-organic fertilizer; BCF3, *Bacillus subtilis* bio-organic fertilizer; soil bulk density (SBD); soil pH (pH); soil organic matter (SOM), soil organic carbon (SOC), total nitrogen (TN), total phosphorus (TP), available nitrogen (AN), available phosphorus (AP), available potassium (AK)

significant difference in TP content among treatments. AN content was significantly increased by 11.89–19.65% (spring) and 12.44–26.87% (autumn). The TN content of BCF3 treatment increased significantly ($p < 0.05$), with an increase of 17.45% (spring) and 12.57% (autumn). AK content in BCF2 treatment increased significantly ($p < 0.05$) by 22.72% (spring) and 37.54% (autumn). The SOM content of three bio-organic fertilizer treatments increased significantly by 3.71–9.73% (spring) and 7.07–12.59% (autumn).

3.2 Soil Enzyme Activity

Season and bio-organic fertilizer treatments significantly affected soil enzyme activities, and the interaction between the two significantly affected LAP, CB, ACP and XYL enzyme activities (Fig. 1). In the three bio-organic fertilizer treatments, soil enzyme activity increased with the application time, showing the regularity of spring < autumn. Compared with TF treatment, the activities of LAP, BG, and NAG enzymes were significantly increased in bio-organic fertilizer treatments of both spring and autumn, with an increase rate of 55.86–97.51%, 35.25–56.95%, 23.30–39.17% (spring), and 36.98–36.98%, respectively, and 90.61, 28.13–74.87, and 37.01–48.52% (autumn). Bio-organic fertilizer treatment significantly affected the activity of CB and XYL enzymes in autumn, with an increase of 32.80–54.11% and 46.32–93.24%, respectively. In spring, the ACP enzyme activity of BCF1 treatment increased significantly by 41.99%, while in autumn, BCF1 and BCF2 treatments increased significantly by 33.12 and 19.15%, respectively.

3.3 Growth and Nutrient Accumulation of Tea Tree

With the increase in application time of bio-organic fertilizer, the chlorophyll SPAD and nitrogen accumulation in the leaves of tea shoots increased, showing a trend of autumn > spring (Fig. 2). Compared with TF treatment, the SPAD value and nitrogen accumulation of the three bio-organic fertilizers in spring were significantly increased ($p < 0.05$), with an increase of 18.42–35.05 and 6.33–12.03%, respectively. Among them, BCF3 treatment had the highest SPAD and nitrogen accumulation in spring and autumn. It could be clearly seen in Fig. 2 that the phosphorus accumulation in tea shoots in spring and autumn was in the order of BCF1 > BCF2 > BCF3 > TF > CK. Compared with TF treatment, the phosphorus accumulation of BCF1 and BCF2 treatments increased significantly ($p < 0.05$), with an increase of 16.46 and 13.63% (spring), and 19.43 and 16.08% (autumn), respectively. There was no significant

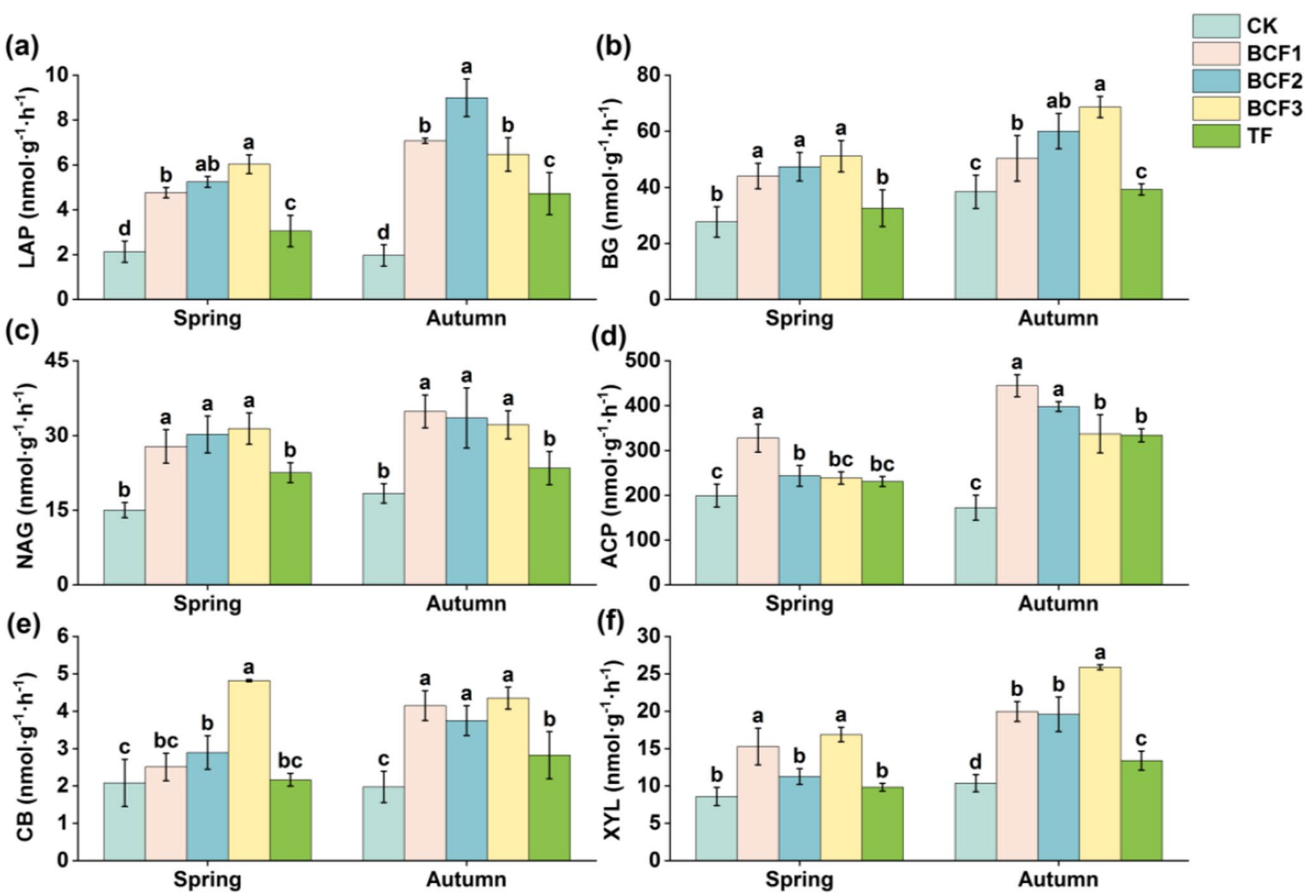


Fig. 1 Effects of different bio-organic fertilizer treatments on leucine aminopeptidase (LAP) (**a**), *β*-glucosidase (BG) (**b**), *β*-N-acetylglucosaminidase (NAG) (**c**), acid phosphatase (ACP) (**d**), *β*-cellobiose glycase (CB) (**e**), and *β*-xylosidase (XYL) (**f**) soil hydrolase activity in tea garden with different seasons. Error bars are means ± standard deviation. BCF1, *Bacillus megaterium* bio-organic fertilizer; BCF2, *Bacillus colloid* bio-organic fertilizer; BCF3, *Bacillus subtilis* bio-organic fertilizer; TF, conventional chemical fertilizer. Different lowercase letters in the figure indicate significant differences between different treatments in the same season ($p < 0.05$)

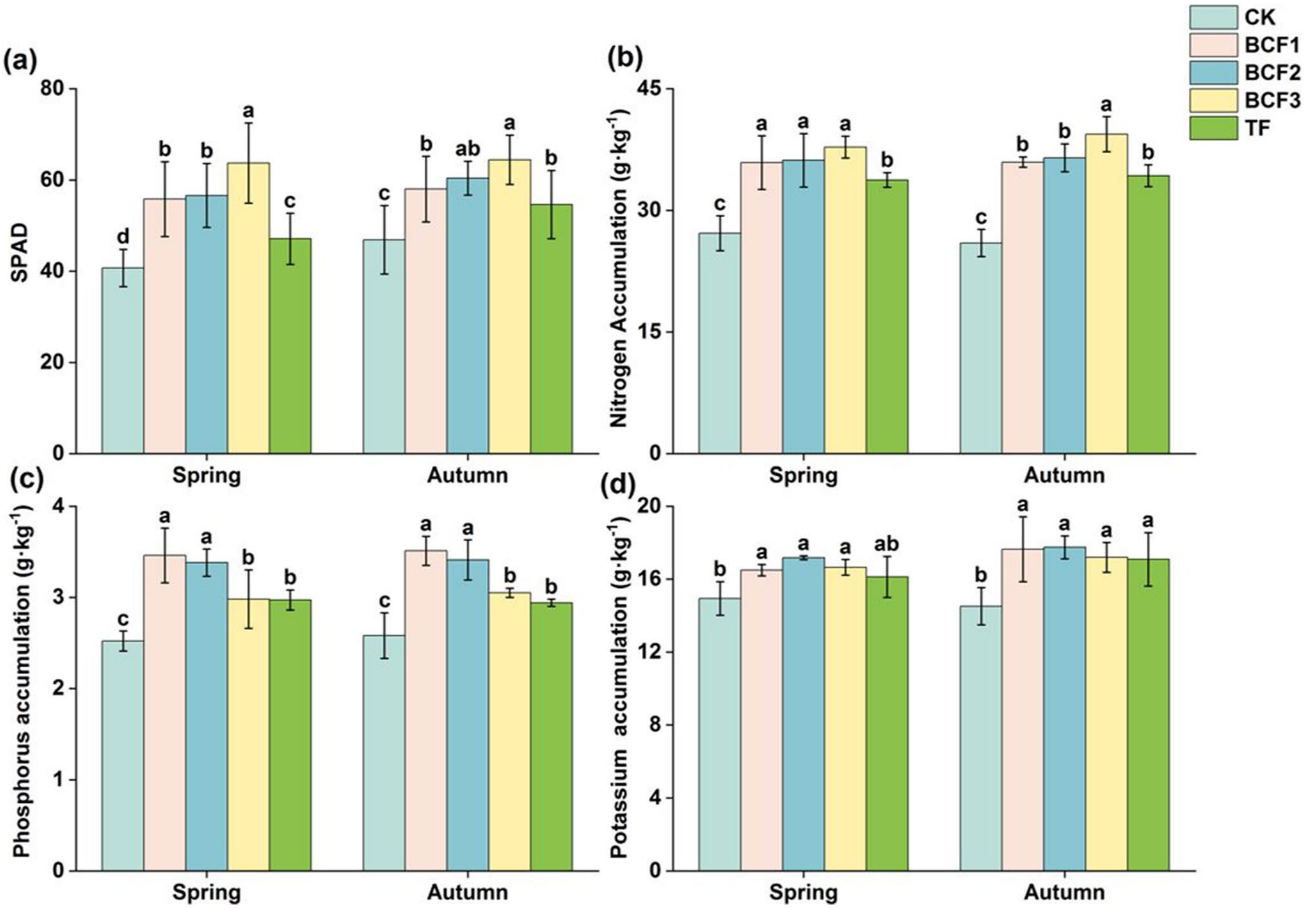


Fig. 2 Effects of bio-organic fertilizer on tea SPAD value (**a**), nitrogen accumulation (**b**), phosphorus accumulation (**c**), and potassium accumulation (**d**). Error bars are means ± standard deviation. BCF1, *Bacillus megaterium* bio-organic fertilizer; BCF2, *Bacillus colloid* bio-organic fertilizer; BCF3, *Bacillus subtilis* bio-organic fertilizer; TF, conventional chemical fertilizer. Different lowercase letters in the figure indicate significant differences between different treatments in the same season ($p < 0.05$)

difference in potassium accumulation between the three bio-organic fertilizers and TF treatment ($p > 0.05$). The potassium accumulation of BCF2 treatment was the highest in spring and autumn.

3.4 Tea Yield

Compared with TF treatment, the 100-bud weight, flushing density, and fresh leaf yield of tea treated with three bio-organic fertilizers increased by 8.14–22.23, 10.14–30.43, and 24.06–59.56%, respectively (Table 4). In autumn, the flushing density and fresh leaf yield of tea trees were significantly increased by the three kinds of bio-organic fertilizer treatments, with an increase rate of 27.17–41.79 and 37.50– 74.25, respectively; the BCF3 treatment had the most significant difference.

3.5 Tea Quality

As shown in Table 2, different seasons significantly affected tea amino acids, phenol-ammonia ratio, and caffeine content. Yet different bio-organic fertilizer treatments significantly affected tea quality indicators, while the interaction between them significantly affected tea polyphenol content. Compared with TF treatment, the contents of tea polyphenols and amino acids in spring tea of BCF1 and BCF3 treatments increased significantly by 12–24.29% (tea polyphenols) and 47.53–73.87% (caffeine), respectively (Fig. 3). The phenol ammonia ratio decreased significantly by 28.05 and 41.11%, respectively. The content of tea polyphenols in BCF1 treatment significantly increased by 14.98% in autumn. The contents of amino acids in BCF1 and BCF3 increased significantly by 31.81 and 37.39%, respectively. The ratio of phenol

Table 4 Effects of different bio-organic fertilizer treatments on tea yield

Factors	Season	Treatments				
		CK	BCF1	BCF2	BCF3	TF
100-bud weight (g)	Spring	18.47 ± 0.57d	26.46 ± 2.27ab	25.10 ± 0.52bc	28.37 ± 0.22a	23.21 ± 0.65c
	Autumn	16.94 ± 0.56c	23.64 ± 1.03b	24.13 ± 2.07b	27.46 ± 0.79a	22.37 ± 1.24b
Flushing density (m^{-2})	Spring	153.33 ± 25.17c	253.33 ± 23.09ab	263.33 ± 11.55ab	300.00 ± 36.06a	230.00 ± 43.59b
	Autumn	140.00 ± 17.32d	292.66 ± 15.14ab	284.00 ± 6.56b	316.67 ± 25.17a	223.33 ± 15.28c
Fresh leaf yield (kg•ha^{-1})	Spring	282.81 ± 42.27c	671.84 ± 98.40b	661.27 ± 40.21b	850.49 ± 96.35a	533.03 ± 95.14b
	Autumn	237.70 ± 36.58d	692.81 ± 65.08b	686.08 ± 72.16b	869.47 ± 70.92a	498.97 ± 33.01c

Data represents means ± standard deviation. Different lowercase letters in the table indicate significant differences between different treatments in the same season ($p < 0.05$). BCF1, *Bacillus megaterium* bio-organic fertilizer; BCF2, *Bacillus colloid* bio-organic fertilizer; BCF3, *Bacillus subtilis* bio-organic fertilizer

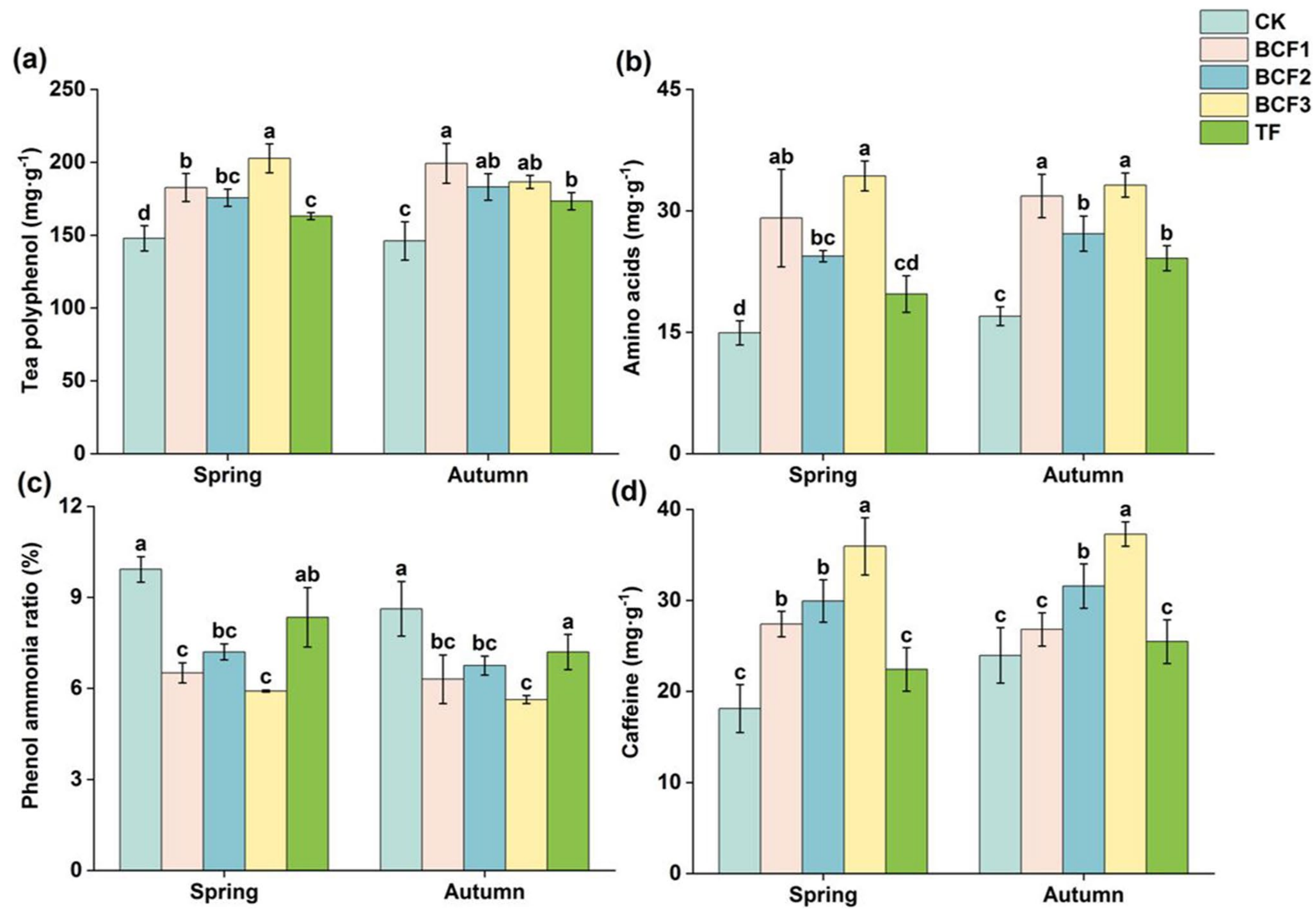


Fig. 3 Effects of bio-organic fertilizer on tea polyphenols (**a**), amino acids (**b**), phenol-ammonia ratio (**c**), and caffeine (**d**). Error bars are means ± standard deviation. BCF1, *Bacillus megaterium* bio-organic fertilizer; BCF2, *Bacillus colloid* bio-organic fertilizer; BCF3, *Bacillus subtilis* bio-organic fertilizer; TF, conventional chemical fertilizer. Different lowercase letters in the figure indicate significant differences between different treatments in the same season ($p < 0.05$)

to ammonia in BCF3 treatment decreased significantly by 27.94%. The caffeine content in spring and autumn tea treated with BCF2 and BCF3 increased significantly by 33.50–60.37% (spring) and 23.95–46.41% (autumn), respectively.

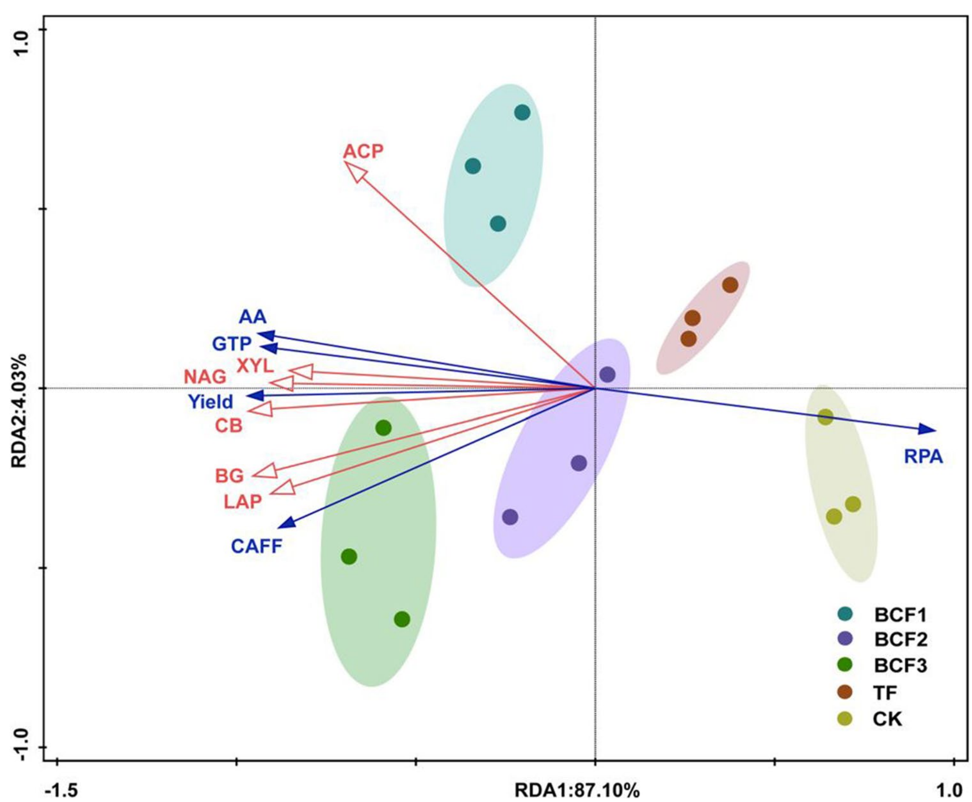
3.6 Redundancy Analysis of Tea Quality and Soil Enzyme Activity

The results of soil enzyme activity and tea quality factor RDA showed that there were significant differences in soil enzyme activities under bio-organic fertilizer treatments (Fig. 4). The first axis (RDA1) and the second axis (RDA2) explained 87.10 and 4.03% of the variation in tea quality, respectively. Among them, soil ACP, CB, XYL, BG, NAG, CB, and LAP enzyme activities were the driving factors of tea quality differences. BG, CB, and LAP significantly affected the caffeine and tea yield in BCF3 treatment.

4 Discussion

In different seasons, soil properties and nutrient contents are significantly different due to fertilizer application, thus affecting tea yield and quality (Wang et al. 2020). This study found that soil bulk density decreased with the continuous application of bio-organic fertilizers. Since the bio-organic fertilizer carrier used in this experiment was cow dung, it could not only promote the formation of aggregate structure but also reduce soil bulk density and increase soil porosity and water conductivity (Meng et al. 2019). In addition, microbial propagation improves soil physical structure. For example, *Bacillus subtilis* is widely distributed in soil, decaying organic matter. It accelerates the decomposition and conversion of organic matter in soil into humus, thus improving soil structure and nutrients and reducing soil bulk density (Tao et al. 2019). The soil pH suitable for the growth of tea trees is 4.5–5.5. However, due to the influence of chemical fertilizer application and the influence of root exudates, soil pH of tea gardens continues to decrease, seriously affecting the growth of tea trees and the yield and quality of tea (Yan et al. 2020). After 2 years of bio-organic fertilizer application in the tea garden, soil pH was continuously increased. This is because the bio-organic fertilizer used in the experiment contains peat as an organic amendment, significantly increasing the pH of acid soils (Chen et al. 2016). The three bio-organic fertilizers also significantly improved soil nutrients. The existing research results show that *Bacillus* has a good effect on phosphorus activation through the secretion of organic acid, phosphatase and extracellular polysaccharides, and other chelating substances, as well as interaction with other microbial communities in the soil (Zhang et al. 2019). Moreover, *Bacillus* accelerates the decomposition of organic materials and plant residues after being applied to the soil. During the decomposition process, humic acid and fulvic acid are produced. These two acids can be combined with Ca^{2+}, Fe^{3+}, Al^{3+}, and other metals, so insoluble phosphorus can be converted into soluble phosphorus, thereby increasing the phosphorus content in soil (Zhu et al. 2018). The analytical results showed that the content of soil AP and TP increased significantly after the application of *Bacillus megaterium* bio-organic fertilizer. In addition, *Bacillus* also increases nitrogen content in soil by decomposing organic materials and nitrogen fixation. For example, *Bacillus subtilis* can transform atmospheric ammonia into ammonia

Fig. 4 RDA analysis describes the correlation between tea yield and quality and soil enzyme activity. Note: CK, no fertilization control; BCF1, *Bacillus megaterium* bio-organic fertilizer; BCF2, *Bacillus colloid* bio-organic fertilizer; BCF3, *Bacillus subtilis* bio-organic fertilizer; TF, conventional chemical fertilizer; AA, amino acids; GTP, green tea polyphenols; Yield, tea yield; CAFF, caffeine; RPA, phenol ammonia ratio, ratio of tea polyphenols and amino acids; ACP, acid phosphatase; NAG, *β*-N-acetylglucosaminidase; CB, *β*-cellobiose glycase; XYL, *β*-xylosidase; LAP, leucine aminopeptidase; BG, *β*-glucosidase



through nitrogen fixation, thereby increasing soil nitrogen content (Sun et al. 2020). This study showed that AN and TN content of BCF3 (*Bacillus subtilis* bio-organic fertilizer) increased significantly, consistent with previous research (Sun et al. 2020). As a potassium-solubilizing bacterium, the main mechanism of potassium-solubilizing *Bacillus* colloid is that soil minerals stimulate the potassium-solubilizing bacteria living freely around it to produce decomposing enzymes, damaging its crystal structure (Annum et al. 2019). There is also the possibility that the enzymes secreted by potassium-solubilizing bacteria react directly with soil minerals (Muthuraja and Muthukumar 2022). Our findings showed that BCF2 (*Bacillus mucilaginosus*) treatment had the highest potassium content, confirming that the application of *Bacillus mucilaginosus* increases soil available potassium content.

Soil enzyme plays an important role in energy transformation, metabolic performance, and nutrient cycle in soil. It is one of the most active organic components in soil and often used as an important index to evaluate soil fertility (Karaca et al. 2011). After the input of bio-organic fertilizer for 2 years, it was found that the activities of LAP, BG, NAG, ACP, XYL, and CB in soil were significantly higher than those in chemical fertilizer treatment, and the effect of *Bacillus subtilis* bio-organic fertilizer was the most significant. The enzymes in the soil mainly came from the decomposition products of microorganisms, plant roots, and animal and plant residues. The formation and development of soil humus and the morphological characteristics and physical structure of the soil, as well as the storage and release of elements such as carbon, nitrogen, and phosphorus, are closely related to the activity of soil enzymes (Yang et al. 2019). For example, BG (β-glucosidase) is involved in soil carbon cycling (Wu et al. 2023); LAP (leucine aminopeptidase) and NAG (β-N-acetylglucosamines) are involved in soil nitrogen cycling (Cenini et al. 2016); ACP (acid phosphatase) is involved in the transformation of phosphorus in soil and improves the dephosphorization efficiency of organic phosphorus (Annum et al. 2019). Because the application of bio-organic fertilizer improves the microbial community structure and the content of beneficial microorganisms in soils, microorganisms can secrete a large number of enzymes during the propagation process to enhance soil enzyme activity (Yang and Zhang 2022).

The results showed that after application of bio-organic fertilizer, the accumulation of nitrogen, phosphorus, and potassium in tea shoots was significantly higher than that of chemical fertilizer. Nitrogen is an important component of tea polyphenols and amino acids, and is of great significance to the formation of tea taste (Wang et al. 2021). However, most nitrogen elements in nature cannot be absorbed and utilized by plants, and plants can only directly absorb nitrogen elements in the form of NO^{3-}, NH^{4+}, and a small number of amino acids, oligopeptides, etc. (Ma et al. 2021). Due to the complex nitrogenase system in plant growth-promoting rhizobacteria, free nitrogen can be converted into nitrogenous compounds that can be directly absorbed and utilized by plants through biological nitrogen fixation (Bellenger et al. 2020). Phosphorus is involved in a series of metabolic processes in plants, affecting plant growth and development, disease resistance, and root development (Chan et al. 2021). Plant growth-promoting rhizobacteria can activate soil phosphorus, thereby promoting plant phosphorus uptake. Previous studies have confirmed that the application of *Bacillus megaterium* into soil can improve the bioavailability of phosphorus and potassium in soil by regulating the structure of soil bacterial community, promoting the growth of pepper (Pérez-Montaño et al. 2014). This is consistent with the results of this study. The role of bio-organic fertilizer does not only promote the absorption of nitrogen, phosphorus, and potassium nutrients by tea trees but also activate iron in the soil. It is an important component of plant chlorophyll and photosynthetic product transport (Moradzadeh et al. 2021). However, the vast majority of iron in soil exists in the form of Fe^{3+}, which cannot be absorbed and utilized by plants. Microorganisms in bio-organic fertilizers can transform Fe^{3+} into Fe^{2+} that can be absorbed and utilized by roots by secreting siderophores (Bhattacharyya et al. 2020). Therefore, it can significantly increase plant chlorophyll content and improve photosynthesis to improve the quality of tea. As one of the most influential beverages in the world, the yield and quality of tea are considered as its important evaluation indexes. At present, due to the acidification of tea garden soil and the reduction of organic matter, the economic benefits of tea are seriously affected. The input of bio-organic fertilizer may be an important measurement to solve this problem. From our findings, the yield of spring and autumn tea was increased after the application of bio-organic fertilizer, whereas the yield increase was the most significant after the application of *Bacilluscolloid* and *Bacillus subtilis* fertilizers. *Bacillus*, as a highly adaptable and spore-producing microorganism, occupies an important part of soil microbial community. It can produce a large number of growth hormones (IAA, gibberellin, etc.), promoting the number of tea germination to improve the yield. Previous study (Dönmez et al. 2010) has also confirmed that *Bacillus* strains isolated from the rhizosphere soil of tea plants have the ability to convert insoluble phosphorus in the soil into available soluble phosphorus, increasing the tea yield. Tea polyphenols and amino acids are important indicators for evaluating tea quality. After the application of *Bacillus subtilis* fertilizer, the spring and autumn teas were significantly higher than that of long-term application of chemical fertilizer, and the amino acid content was significantly increased. *Bacillus megaterium* fertilizer also showed a significant effect. However, the mechanism of microbial effects on tea quality is still unclear. On the one hand, it may be due to increase in the content of available nutrients in soil and enzyme activity, thereby increasing the accumulation of secondary metabolites in tea and achieving optimal tea quality. On the other hand, the formation of quality components such as amino acids, caffeine, and tea polyphenols are mainly secondary metabolites of tea photosynthesis, and bio-organic fertilizers can promote tea tree photosynthesis to improve tea quality.

Redundancy analysis confirmed that the quality and yield of tea were significantly correlated with soil nutrients and enzyme activities in both spring and autumn. Previous studies have also shown that soil pH plays an important role in the growth of tea plants by affecting soil nitrogen and organic matter to increase the synthesis of amino acids and caffeine in tea plants (Xiao et al. 2018). Soil enzyme, as one of the most active organic components in soil, plays an active role in energy transformation and nutrient cycling, as illustrated by the determined soil enzymes in this study. Their increase has a significant positive correlation with the increase in tea quality and yield, corresponding with previous studies (Duan et al. 2019).

5 Conclusions

This study assessed the effects of different bio-organic fertilizers on tea yield, quality, soil fertility, and enzyme activity. Our results showed that soil enzyme and fertility were important factors affecting the tea yield and quality. Compared with conventional chemical fertilizer, the application of bio-organic fertilizer improved the soil fertility, physical structure, and enzyme activity but reduced soil bulk density. In addition, it could also promote the growth of tea and increase the tea yield and quality. *Bacillus subtilis* bio-organic fertilizer had the most significant effect on tea. This study reveals the effect of bio-organic fertilizer on the yield and quality of tea. In order to improve its efficiency and durability, further research is needed to explore the mechanism of soil microorganisms in tea.

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Declarations

Conflict of Interest The authors declare no competing interests.

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