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Effect of *Streptomyces costaricanus* Strain A-m1 as a Bioinoculant on Tea Garden Soil and Tea Quality

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Abstract: Chemical fertilization is usually associated with some unreasonable problems that affect the sustainable production of tea gardens. The micro-organism fertilizer created from plant growth-promoting microbes (PGPM) integrates the beneficial properties of functional micro-organisms and bioinoculants. Application of PGPM can activate soil nutrients, prevent soil-borne diseases, and promote crop growth, thus improving crop quality and yield. In this study, the effects of bioinoculants composed of *Streptomyces costaricanus* strain A-m1 on the properties, enzyme activity, and micro-organisms of soil in a tea garden and on the chemical composition and production of tea were investigated. The present results showed that the application of A-m1 bioinoculant could increase the activities of urease, protease and catalase, the content of alkali-hydrolyzable nitrogen, and the number of bacteria, fungi, and actinomycetes in tea garden soil. After application, the free amino acid content, 100-bud weight, and bud density of spring tea were also elevated. In the year of fertilization, the treatment composed of 70% bioinoculant + 30% chemical fertilizer showed the best effects on soil physical and chemical properties, enzyme activity, culturable microbial counts, and tea quality. A high ratio of organic to chemical fertilizer coapplication can significantly improve the growing conditions for tea plants, reduce the use of chemical fertilizers, improve the efficiency of nutrient utilization, and enhance both the yield and quality of tea. One year after fertilization, the 50% bioinoculant + 50% chemical fertilizer was more conducive to enhancing the quality of tea, while the 30% bioinoculant + 70% chemical fertilizer was more beneficial for improving the production of tea. A high ratio of chemical to bioinoculant coapplication is more favorable for maintaining high yield and quality in tea production, achieving healthy and sustainable tea garden management. The application of A-m1 bioinoculant will reduce the use of chemical fertilizers, improve the utilization efficiency of soil nutrients, and increase the production and quality of tea, contributing to the sustainable production of tea gardens.

Keywords: chemical constituents of tea; fertilizer reduction; plant growth-promoting microbesbioinoculant; soil enzyme activity; soil micro-organisms

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1. Introduction

Tea plants are an important economic crop in China. At present, the fertilization in Chinese tea gardens mainly relies on quick-effect chemical fertilizers. Reasonable fertilization is beneficial to the maintenance and development of the soil ecosystem. However, long-term application of chemical fertilizers has led to problems such as soil compaction,

soil acidification, soil pollution, and a sharp decrease in soil biodiversity [1–3]. Microbial fertilizers created from plant growth-promoting microbes (PGPM) combine the characteristics of functional micro-organisms and bioinoculants. The application of PGPM could contribute to the activation and release of soil nutrients, prevent and control soil-borne diseases, promote crop growth, enhance crop quality, and increase crop yield [4–6]. Among the various plant growth-promoting microbes, *Streptomyces* can produce a variety of secondary metabolites that have antagonistic effects on plant pathogens, as well as components such as indole acetic acid (IAA) and siderophores that have a growth-promoting effect on plants [7–9]. Research has shown that the application of bioinoculants created from *Streptomyces* to the soil can enhance the activity of enzymes related to the cycles of carbon, nitrogen, and phosphorus, activate nitrogen, phosphorus, and potassium nutrients in the soil, improve soil fertility, optimize the composition of soil micro-organisms, reduce the incidence of soil-borne fungal diseases, and increase plant yield and quality, thereby being a candidate for an environmentally friendly alternative to chemical fertilizers [10]. For instance, *S. jingyangensis* 5406 can significantly improve the growth of *Pinellia ternata*, thus enhancing its yield and quality [11]. *S. lydicus* A01 can increase the number of tomato leaves, improve photosynthetic capacity, and enhance the efficiency with which tomatoes utilize nitrogen, phosphorus, and potassium from the soil [12]. Under drought stress, the fermentation liquid of *S. griseus* can significantly increase the activity of urease, catalase, cellulase, and protease in the soil [13].

S. costaricanus is an important microbial resource that has been reported to have the ability to inhibit bacteria, kill insects, promote growth, and degrade cellulose [14,15]. In the previous research, we isolated a strain of *S. costaricanus*, A-m1, whose fermentation filtrate has a broad antibacterial spectrum and high antibacterial activity [16]. It is capable of producing plant growth hormones such as indole acetic acid (IAA) and cytokinins, and it encodes enzymes that have direct or indirect growth-promoting effects, including nitrogenase, 1-aminocyclopropane-1-carboxylate (ACC) deaminase, phosphatase, and phytase. It has demonstrated effective growth promotion on tomatoes [17] and holds potential for further development as a biocontrol agent. Currently, tea garden soils are facing issues such as nutrient deficiency, poor structure, and functional degradation. This study applied the strain A-m1 as a microbial fertilizer in tea gardens to improve the soil, reduce the use of chemical fertilizers, increase the yield and quality of tea leaves, and provide a foundation for sustainable production in tea gardens.

2. Materials and Methods

2.1. Plant Materials and Growth Conditions

The strain A-m1 was isolated by the Plant Protection Microbial Engineering Technology Center in Xinyang City [16] and preserved in the Key Laboratory of Agricultural Microbial Resources Development and Utilization in Xinyang City. The compound fertilizer (Hubei Dimei Paul Fertilizer Co., Ltd., Suizhou, China) had a total nutrient content of 45%, with the ratio of N:P_2O_5:K_2O being 15:15:15. The urea (Anhui Mao Shi New Type Fertilizer Co., Ltd., Hefei, China) contained 46% nitrogen.

Test Media: NA Medium (peptone 10 g/L, beef leaching powder 3 g/L, sodium chloride 5 g/L, agar 20 g/L, pH 7.0–7.4), PDA Medium (potato powder 6 g/L, glucose 20 g/L, agar 20 g/L, pH 7.0–7.4) [18], Gao's No.1 Medium (soluble starch 20 g/L, KNO_3 1 g/L, K_2HPO_4 0.5 g/L, $MgSO_4.7H_2O$ 0.5 g/L, NaCl 0.5 g/L, $FeSO_4.7H_2O$ 0.01 g/L, agar 20 g/L, pH 7.2–7.4) [19], Bengal Rose Medium (peptone 5.0 (g/L), dipotassium hydrogen phosphate 1.0 (g/L), Glucose 10.0 (g/L), Magnesium sulfate 0.5 (g/L), Bengal Red 0.0333 (g/L), Ager 20.0 (g/L), chloromycetin 0.1 (g/L), pH 7.0–7.4) [20]. The test media here means the media used in the following paragraph of the paper.

The experiment was conducted in Dashimen, Dongshuanghe Township, Shihe District, Xinyang City, Henan Province (32°04′ N, 114°05′ E). The tea garden is situated on low rolling hills with a gentle slope, featuring a uniform growth condition of the Xinyang tea variety, which is 20 years old. The row spacing is 1.3 m, and the soil is classified as loess soil.

The basic physicochemical properties of the topsoil (3–15 cm) before the experiment were as follows: pH value of 3.77, organic matter content of 24.95 $g \cdot kg^{-1}$, available nitrogen content of 74.68 $mg \cdot kg^{-1}$, available phosphorus content of 2.89 $mg \cdot kg^{-1}$, and available potassium content of 71.26 $mg \cdot kg^{-1}$.

2.2. Experimental Design

Optimized fermentation conditions for strain A-m1 [16] were followed, and fresh fermentation liquid was obtained after 3 days of liquid shaking culture for later use. The fermentation conditions of strain A-ml were set as 4% inoculation amount, 100 mL/500 mL liquid volume, pH value between 6 and 8, fermentation temperature 23~32 °C and fermentation culture for 4 days. The microbial titer after liquid-phase cultivation was 10^9 $cfu \cdot g^{-1}$ (Colony Forming Unit, CFU). The solid fermentation substrate consists of rice: wheat bran: wheat in a ratio of 2:2:1, supplemented with 0.8% soybean powder, 0.1% calcium carbonate, and 2% peptone, with an initial water-to-substrate mass ratio of 1.2:1. In each 4 L round black-bottomed meal box (inner diameter at the top 29 cm, at the bottom 25 cm, height 9 cm), 1000 g of solid substrate (including water weight) was added, and sterilized at 115 °C for 30 min in a high-pressure sterilizer. Each meal box was inoculated with 55 mL of A-m1 fermentation liquid and cultured in a dark chamber at 26 °C for 28 days, yielding a spore count of 10^8 $cfu \cdot g^{-1}$, resulting in A-m1 bioinoculant. After the solid biofertilizer fermentation, the dry weight nutrient content is as follows: organic matter 30%, nitrogen content 3.31%, P_2O_5 1.33%, and K_2O 0.56%.

The experiment was set up with six treatments: (1) CK: sterile solid substrate; (2) CF: 100% chemical fertilizer; (3) T3: 100% bioinoculant; (4) T4: 70% bioinoculant +30% chemical fertilizer; (5) T5: 50% bioinoculant +50% chemical fertilizer; (6) T6: 30% bioinoculant +70% chemical fertilizer. Each treatment was arranged in three subplots randomly, with each subplot covering an area of 20 square meters and a 2-m interval between subplots. The bioinoculant and chemical fertilizer were applied twice a year, as a winter base fertilizer (6 November 2021) and a spring top dressing (6 March 2022), with the application rates for each treatment detailed in Table 1 [21,22]. The winter base fertilizer and spring top dressing were applied through spot application and broadcasting, respectively, with the spot application points located on the upper side of the gentle slope where the tea plants were planted. Apart from the different fertilization practices, all other management measures were consistent with those of other tea gardens.

Table 1. Fertilization amount of different fertilization treatments.

Treatment	Fertilization Amount of Base Fertilizer/($kg \cdot hm^{-2}$)		Fertilization Amount of Dressing Fertilizer/($kg \cdot hm^{-2}$)	
	A-m1 Bioinoculant	Compound Fertilizer	A-m1 Bioinoculant	Urea
CK	3000 (No A-m1 strain)	0	3000 (No A-m1 strain)	0
CF	0	450	0	180
T3	3000	0	3000	0
T4	2100	135	2100	54
T5	1500	225	1500	90
T6	900	315	900	126

2.3. Sample Collection and Analysis

A total of 2 soil samples were collected on 6 November 2021 and 23 September 2022. Within each plot, five sampling points were established, and soil was extracted using an auger at a distance of 25 cm from the main root of the tea plant, opposite to the fertilization point. The top 3 cm of soil was removed, and the soil from these five points was mixed to form a single sample. After homogenizing the soil samples from the same plot, roots and rocks were removed, and the samples were evenly divided into three parts. One part

was quickly placed into an insulated foam box with an ice pack and brought back to the laboratory for the determination of cultivable soil micro-organisms, stored at 4 °C in a refrigerator. Another part was lightly air-dried and sieved for the determination of soil enzyme activity. The remaining part was air-dried and sieved for the determination of basic soil properties.

Autumn and spring teas were harvested on 10 September 2022, and 3 April 2023, respectively. The density of tea buds in each experimental plot was surveyed, and tea samples were collected according to the standard of one bud and one leaf. After collection, the weight of one hundred buds was immediately measured using an electronic balance. The samples were fixed using the heat fixation method [23] within 24 h, heated in an oven at 105 °C for 30 min, and then dried at 75 °C until a constant weight was achieved. Each treatment was repeated three times. The dried tea leaves were ground into powder using a crusher, passed through a 100-mesh sieve, and stored at −20 °C in a refrigerator for analysis.

The determination of soil physicochemical properties was based on the published method [24]. The determination of organic matter, alkali-hydrolysable nitrogen, available phosphorus, and potassium were conducted using the potassium dichromate volumetric method, alkali diffusion method, HCl-H_2SO_4 extraction-molybdenum antimony anticolorimetric method, and 1 mol·L^{-1} ammonium acetate-flame photometry method, respectively. The activities of soil catalase, urease, and sucrase were determined using the potassium permanganate volumetric method, indophenol blue colorimetric method, and 3,5-dinitrosalicylic acid colorimetric method, respectively [25]. The activities of soil protease and phosphatase were determined using the ninhydrin colorimetric method and disodium phenyl phosphate colorimetric method, respectively [26]. The water-soluble substances, free amino acids, and tea polyphenol content of the tea samples were determined in accordance with GB/T 8305−2013 "Tea-determination of water extracts content", GB/T 8314−2013 "Tea-determination of free amino acids content", and GB/T 8313−2018 "Determination of total polyphenols and catechins content tea", respectively.

The cultivable micro-organism count in soil was determined using the plate gradient dilution method [18]. Bacteria, fungi, and actinomycetes were isolated using NA medium, Martin's Bengal Rose medium (containing 100 mg·L^{-1} chloramphenicol and 30 mg·L^{-1} streptomycin), and Gao's No.1 medium (containing 100 mg·L^{-1} potassium dichromate solution), respectively. Appropriate dilution gradients were selected, and 200 μL of the soil dilution was spread onto the corresponding plates. In this appropriate dilution gradient, the dilution concentration is approximately 10–20 colonies per culture dish, with five replicates per dilution. The NA medium plates were incubated for 2 days, the Martin's Bengal Rose medium plates for 4 days, and Gao's No. 1 medium plates for 7 days at a constant temperature of 28 °C in an incubator. The colonies were counted. The number of colonies formed per gram (colony forming unit, cfu) of fresh soil is expressed as cfu·g^{-1}.

2.4. Data Processing

The experimental data were entered, organized, and calculated using Microsoft Excel 2016 (Microsoft, Redmond, WA, USA)). All measured values were presented as mean ± standard deviation of the means. An analysis of variance (ANOVA) and Duncan's multiple-range test were performed by SPSS 26.0 (IBM Corp., Chicago, IL, USA), and a least significant range (LSR) analysis at 5% significant differences was shown. Graphs were created using Microsoft Excel 2016.

3. Results

3.1. The Impact of Different Fertilization Treatments on the Physicochemical Properties of Tea Garden Soil

The effects of different fertilization treatments on the physicochemical properties of soil showed significant differences. The soil organic matter and available phosphorus content in the CK group generally showed an increasing trend, while the alkali-hydrolysable

nitrogen and available potassium content decreased. In the A-m1 bioinoculant group and the CF group, the soil organic matter (Figure 1a), alkali-hydrolysable nitrogen (Figure 1b), available phosphorus (Figure 1c), and available potassium (Figure 1d) contents all showed an increasing trend. The effects of different proportions of A-m1 bioinoculant on the physicochemical properties of tea garden soil were also different. The treatment of 70% bioinoculant +30% chemical fertilizer (T4) compared to the CK group increased the soil organic matter, alkali-hydrolysable nitrogen, available phosphorus, and available potassium contents by 14.60 g·kg^{-1}, 35.89 mg·kg^{-1}, 3.07 mg·kg^{-1}, and 49.95 mg·kg^{-1}, respectively, and increased the soil organic matter, alkali-hydrolysable nitrogen, and available phosphorus contents by 10.61 g·kg^{-1}, 11.97 mg·kg^{-1}, and 2.71 mg·kg^{-1}, respectively. The treatment of 50% bioinoculant +50% chemical fertilizer (T5) compared to the CK group increased the soil organic matter, alkali-hydrolysable nitrogen, available phosphorus, and available potassium contents by 7.40 g·kg^{-1}, 34.02 mg·kg^{-1}, 2.62 mg·kg^{-1}, and 48.45 mg·kg^{-1}, respectively, and increased the soil organic matter, alkali-hydrolysable nitrogen, and available phosphorus contents by 3.41 g·kg^{-1}, 10.10 mg·kg^{-1}, and 2.26 mg·kg^{-1}, respectively, compared to the CF group ($p < 0.05$); the 100% bioinoculant group (T3) significantly increased the soil organic matter and alkali-hydrolysable nitrogen contents; the 30% bioinoculant +70% chemical fertilizer group (T6) significantly increased the alkali-hydrolysable nitrogen content in the tea garden soil ($p < 0.05$). Specifically, the 70% bioinoculant +30% chemical fertilizer and the 50% bioinoculant +50% chemical fertilizer groups are characterized by a significant enhancement of soil organic matter, alkali-hydrolysable nitrogen, and available phosphorus content, with available potassium content on par with the 100% chemical fertilizer group.

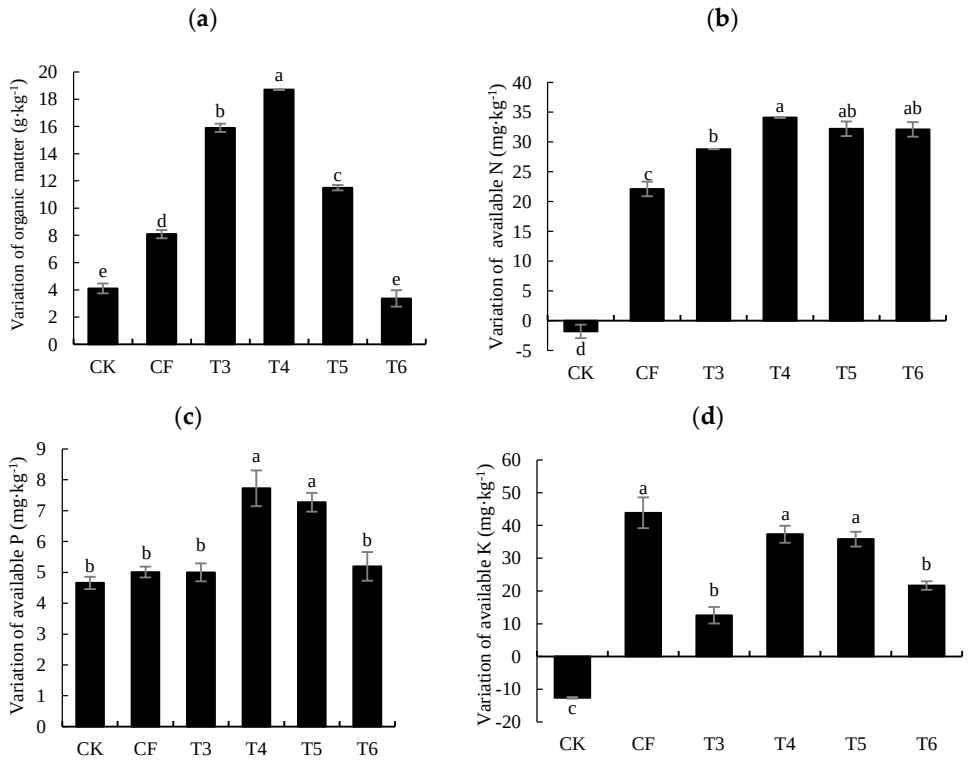


Figure 1. Effects of different treatments on soil properties. CK: sterile solid substrate; CF: 100% chemical fertilizer; T3: 100% bioinoculant; T4: 70% bioinoculant +30% chemical fertilizer; T5: 50% bioinoculant +50% chemical fertilizer; T6: 30% bioinoculant +70% chemical fertilizer. The values on the vertical axis represented the variations in organic matter (**a**), available N (**b**), available P (**c**), and available K (**d**) as measured on 23 September 2022, compared to those measured on 6 November 2021, before the treatment. Lowercase letters in the figure indicated significant differences ($p < 0.05$).

3.2. The Impact of Different Fertilization Treatments on the Activity of Soil Enzymes in Tea Gardens

The A-m1 bioinoculant had a significant effect on the soil enzyme activity in the tea garden. In the CK and CF groups, only the activity of soil protease (Figure 2d) increased, while the activities of catalase (Figure 2a), urease (Figure 2b), phosphatase (Figure 2c), and sucrase (Figure 2e) decreased. In contrast, the activity of soil catalase (T4), urease (T4, T5, T6), acid phosphatase (T3, T4), and protease all increased in the A-m1 bioinoculant group. Compared to the CK and CF groups, the treatment with A-m1 bioinoculant significantly enhanced the activities of catalase, urease, and protease in the tea garden soil (Figure 2) ($p < 0.05$). Among them, T4 showed the best soil improvement effect ($p < 0.05$). Compared to the CK group, the contents of catalase, urease, acid phosphatase, protease, and sucrase in the tea garden soil were increased by 0.45 mg·g^{-1}, 3.57 mg·g^{-1}, 0.17 mg·g^{-1}, 7.71 mg·g^{-1}, and 2.07 mg·g^{-1}, respectively, and compared to the CF group, the activities of catalase, urease, acid phosphatase, and protease were increased by 0.49 mg·g^{-1}, 3.26 mg·g^{-1}, 0.10 mg·g^{-1}, and 2.81 mg·g^{-1}, respectively. The A-m1 bioinoculant treatment significantly increased the content of alkali-hydrolysable nitrogen, and the activity of sucrase, urease, protease, and catalase in the soil of the tea garden.

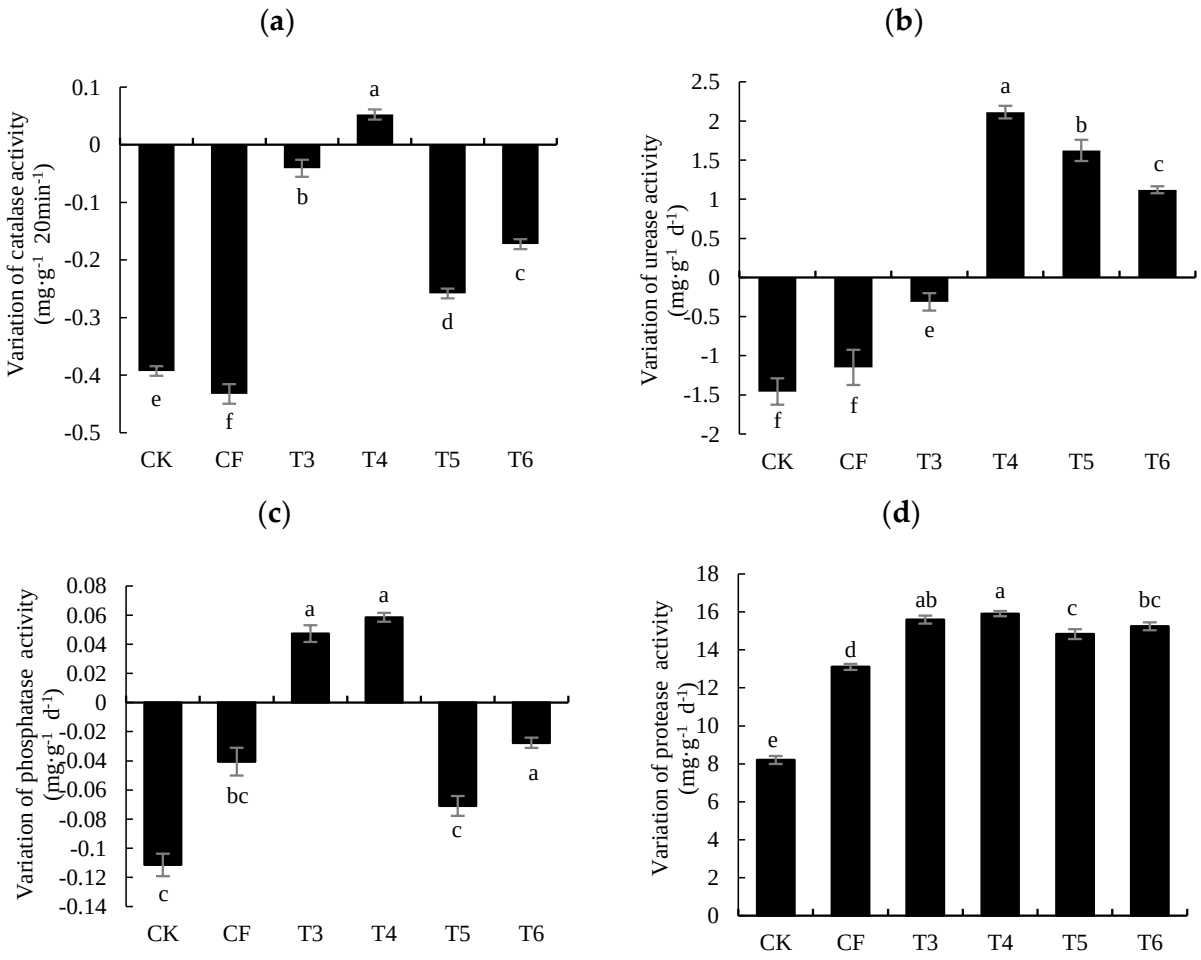


Figure 2. *Cont.*

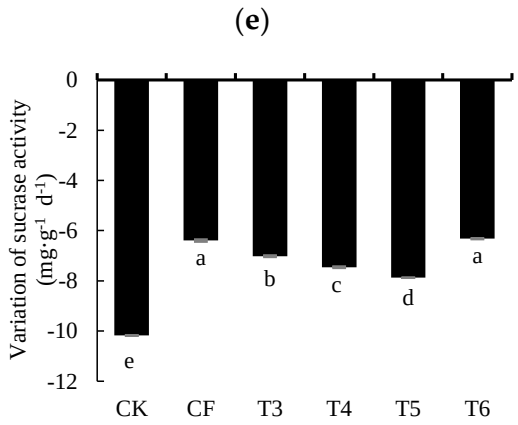


Figure 2. Effects of different fertilization treatments on the activities of enzymes in tea garden soil. The treatments on the horizontal axis were the same as in Figure 1. The values on the vertical axis represented the variations in activities of catalase (**a**), urease (**b**), phosphatase (**c**), protease (**d**), and sucrose (**e**) after treatment. Positive values represented the amount of increase, and negative values represented the amount of decrease. Lowercase letters in the figure indicated significant differences ($p < 0.05$).

3.3. The Impact of Different Fertilization Treatments on the Soil Micro-Organisms in Tea Gardens

After selecting appropriate dilution gradients for the separation of bacteria (Figure 3a), fungi (Figure 3b), and actinomycetes (Figure 3c), the impact of different fertilization treatments on the cultivable microbial (Figure 3d) counts in the tea plant root system was analyzed. In the CK and CF groups, the cultivable counts of bacteria, fungi, and actinomycetes in the soil from the tea garden decreased from the first to the second sampling. However, the addition of A-m1 bioinoculant significantly reduced the decline in the cultivable counts of bacteria and fungi ($p < 0.05$), and the cultivable count of actinomycetes increased, as shown in Figure 3. The comprehensive comparison of the results showed that the addition of A-m1 bioinoculant significantly increased the cultivable counts of bacteria, fungi, and actinomycetes in the soil around the tea plant roots. Among them, the T4 and T5 groups showed the most significant increase in the microbial counts in the soil around the tea plant roots, indicating that this fertilization pattern maintained a high abundance of soil micro-organisms. The A-m1 bioinoculant treatment significantly increased the quantity of culturable bacteria, fungi, and actinomycetes.

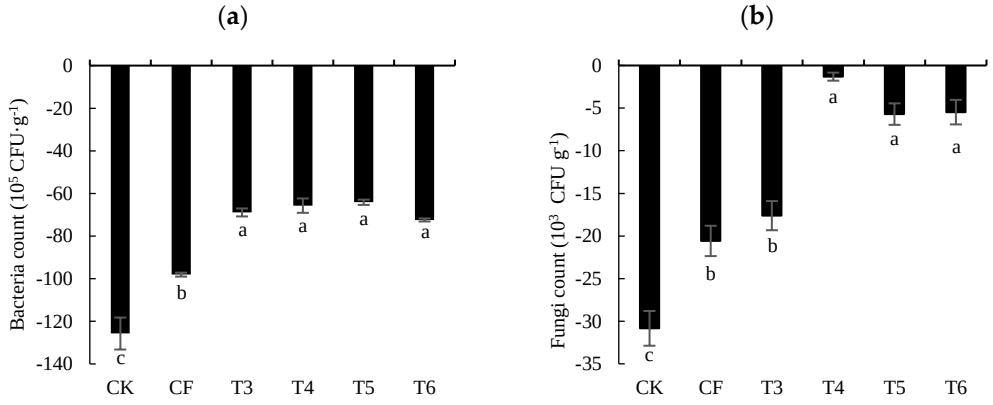


Figure 3. *Cont.*

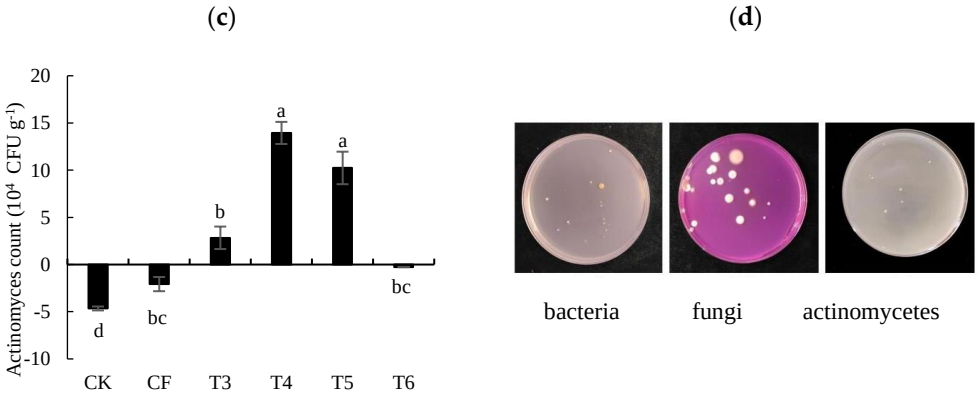


Figure 3. Effects of different fertilization treatments on the number of cultivable micro-organisms in tea roots. The treatments on the horizontal axis were the same as in Figure 1. The values on the vertical axis represented variations in the number of cultivable bacteria (**a**), fungi (**b**), and actinomyces (**c**) after treatment. (**d**): The colonies of bacteria, fungi, and actinomyces. Positive values represented the amount of increase, and negative values represented the amount of decrease. Lowercase letters in the figure indicated significant differences ($p < 0.05$).

3.4. The Impact of Different Fertilization Treatments on the Growth Condition of Tea Plants

The coapplication of A-m1 bioinoculant with reduced chemical fertilizer significantly increased the weight of one hundred buds of tea plants in the year of fertilization. In the second year after application, the weight of one hundred buds in all treatments continued to increase, with the 30% bioinoculant +70% chemical fertilizer group showing the most significant increase ($p < 0.05$), as shown in Table 2. In the year of fertilization, the average weight of one hundred buds in the T4, T5, and T6 groups increased by 8.18%, 19.32%, and 10.36% compared to the CK group, respectively, with the T5 group showing a 6.67% increase compared to the CF group. In the second year after the application of fertilizer, the average weight of one hundred buds in the T4, T5, and T6 groups increased by 12.98%, 20.93%, and 32.87% compared to the CF group, respectively.

Table 2. Effects of different fertilization treatments on tea production.

Treatment	One-Hundred-Bud Weight/(g FW)		Bud Density/(g·m^{-2} FW)	
	Summer Tea	Spring Tea	Summer Tea	Spring Tea
CK	4.50 ± 0.09 c	7.04 ± 0.04 cd	36.81 ± 0.32 f	110.00 ± 4.73 c
CF	5.03 ± 0.13 b	6.77 ± 0.10 d	41.38 ± 0.53 e	110.67 ± 2.33 c
T3	4.19 ± 0.12 c	7.58 ± 0.05 b	78.77 ± 1.05 c	124.00 ± 3.21 b
T4	4.87 ± 0.14 b	7.26 ± 0.09 c	90.73 ± 1.24 a	122.00 ± 4.16 b
T5	5.37 ± 0.12 a	8.79 ± 0.12 b	64.43 ± 0.77 d	117.67 ± 1.45 bc
T6	4.96 ± 0.07 b	9.42 ± 0.10 a	86.09 ± 0.38 b	138.33 ± 4.06 a

Note: Lowercase letters in the table indicated significant differences ($p < 0.05$).

Except for the 50% bioinoculant +50% chemical fertilizer group, the application of A-m1 bioinoculant can increase the bud density of tea plants both in the year of fertilization and one year after the cessation of fertilizer application ($p < 0.05$). Compared with the summer tea of the fertilization year, the bud density of spring tea in the second year after fertilizer application generally increased, as shown in Table 2. In the year of fertilization, the bud density of tea plants in the T3, T4, T5, and T6 groups increased by an average of 114.01%, 146.52%, 75.05%, and 133.90% compared to the CK group, respectively, and increased by 90.36%, 119.28%, 55.71%, and 108.06% compared to the CF group, respectively. In the second year after the application of fertilizer, the bud density of tea plants in the T3, T4, and T6 groups increased by an average of 12.73%, 10.91%, and 25.76% compared to the CK group, respectively, and increased by an average of 12.05%, 10.24%, and 25.00% compared to the CF group, respectively.

3.5. The Impact of Different Fertilization Treatments on the Quality Components of Tea

Different treatments have varying effects on the content of tea polyphenols in tea leaves in the year of fertilization and the year following fertilization. In the year following the application of fertilizer, the content of tea polyphenols in the tea leaves of the 50% bioinoculant +50% chemical fertilizer group and the 30% bioinoculant +70% chemical fertilizer group significantly decreased, as shown in Table 3. Compared to the summer tea of the fertilization year, the content of tea polyphenols decreased in the spring tea of the T5 and T6 groups, while it increased in CK, CF, T3, and T4 groups, in the year after fertilization. In the year following the application of fertilizer, the content of tea polyphenols in the tea of the T5 and T6 groups decreased by 8.58% and 7.92%, respectively, compared to the CK group, and decreased by 10.41% and 9.76%, respectively, compared to the CF group.

Table 3. Effects of different fertilization treatments on tea quality components.

	Tea Polyphenol/(g·kg^{-1})		Amino Acid/(g·kg^{-1})		TP/AA Ratio		Water Extracts/(g·kg^{-1})	
	Summer Tea	**Spring Tea**	**Summer Tea**	**Spring Tea**	**Summer Tea**	**Spring Tea**	**Summer Tea**	**Spring Tea**
CK	202.32 ± 0.90 c	217.34 ± 2.72 c	11.96 ± 0.15 c	28.28 ± 0.62 d	16.92 ± 0.27 c	7.69 ± 0.10 e	522.30 ± 3.09 b	494.01 ± 7.07 c
CF	211.42 ± 0.34 b	221.78 ± 1.76 bc	13.32 ± 0.04 b	33.57 ± 0.25 b	15.88 ± 0.02 b	6.61 ± 0.05 c	522.93 ± 1.44 b	507.53 ± 5.56 ab
T3	215.62 ± 0.90 a	227.10 ± 0.33 b	12.91 ± 0.23 b	32.43 ± 0.26 b	16.72 ± 0.35 c	7.00 ± 0.01 d	533.67 ± 3.24 a	502.58 ± 3.37 c
T4	215.96 ± 1.56 a	237.43 ± 1.33 a	14.42 ± 0.11 a	30.14 ± 0.33 c	14.98 ± 0.12 a	7.88 ± 0.04 f	535.86 ± 0.51 a	509.11 ± 5.18 ab
T5	214.94 ± 1.56 a	198.70 ± 2.64 d	14.26 ± 0.23 a	36.08 ± 0.52 a	15.08 ± 0.25 a	5.51 ± 0.07 a	536.37 ± 5.39 a	523.83 ± 6.39 a
T6	210.50 ± 0.90 b	200.13 ± 1.33 d	14.09 ± 0.31 a	32.43 ± 0.29 b	14.95 ± 0.31 a	6.17 ± 0.04 b	529.29 ± 1.34 ab	508.70 ± 5.63 ab

Note: Lowercase letters in the table indicate significant differences ($p < 0.05$).

The application of A-m1 bioinoculant can increase the content of free amino acids in tea leaves. In the second year after fertilization, the content of free amino acids in the spring tea leaves of all treatments generally increased. Compared with 100% chemical fertilizer, the coapplication of A-m1 bioinoculant with reduced chemical fertilizer significantly increased the content of free amino acids in tea leaves in the year of fertilization. The 50% bioinoculant +50% chemical fertilizer group still had a significant enhancing effect on the content of free amino acids in tea leaves in the second year after fertilization ($p < 0.05$), as shown in Table 3. In the year of fertilization, the content of free amino acids in tea leaves of the T3, T4, T5, and T6 groups increased by 7.30%, 20.55%, 19.18%, and 17.81%, respectively, compared to the CK group, and increased by 8.31%, 7.08%, and 5.85%, respectively, compared to the CF group. One year after fertilizer application, compared to the CK group, the content of free amino acids in tea leaves of the T3, T4, T5, and T6 groups increased by an average of 14.68%, 6.58%, 27.58%, and 14.68%, respectively. The T5 group increased by an average of 7.46% compared to the CF group.

The application of A-m1 bioinoculant in combination with reduced chemical fertilizer can significantly reduce the phenol-ammonia ratio in tea leaves in the year of fertilization. Among them, the 50% bioinoculant +50% chemical fertilizer and the 30% bioinoculant +70% chemical fertilizer groups still have a significant reducing effect on the phenol-ammonia ratio in tea leaves in the second year after fertilization, as shown in Table 3. Compared with the summer tea of the fertilization year, the phenol-ammonia ratio in spring tea leaves generally decreased after one year of cessation of fertilization, leading to an improvement in tea quality. In the year of fertilization, the phenol-ammonia ratio in tea leaves of the T4, T5, and T6 groups decreased by 11.48%, 10.85%, and 11.64%, respectively, compared to the CK group, and decreased by 5.68%, 5.01%, and 5.85%, respectively, compared to the CF group. After one year of cessation of fertilizer application, the phenol-ammonia ratio in tea leaves of the T3, T5, and T6 groups decreased by 8.88%, 28.34%, and 19.70%, respectively, compared to the CK group, and the T5 and T6 groups decreased by 16.62% and 6.57%, respectively, compared to the CF group.

The coapplication of A-m1 bioinoculant with reduced chemical fertilizer can increase the water-soluble extract content in tea leaves both in the year of fertilization and one year after the cessation of fertilizer application. One year after the fertilizer was stopped, the water-soluble extract content in tea leaves of all treatments generally showed a downward

trend, as shown in Table 3. In the year of fertilization, the water-soluble extract content in the summer tea of the T3, T4, and T5 groups increased by 2.18%, 2.60%, and 2.70%, respectively, compared to the CK group, and increased by 2.05%, 2.47%, and 2.57%, respectively, compared to the CF group. After one year of cessation of fertilizer application, the water-soluble extract content in tea leaves of the T4, T5, and T6 groups increased by 3.06%, 6.04%, and 2.97%, respectively, compared to the CK group.

In the year of fertilization, the 70% bioinoculant +30% chemical fertilizer provided the best improvement effects on soil physicochemical properties, enzyme activity, culturable microbial counts, and tea quality. One year after the cessation of fertilization, the 50% bioinoculant +50% chemical fertilizer was more conducive to enhancing the quality components of tea, while the 30% bioinoculant +70% chemical fertilizer was more beneficial for improving the production status of tea.

3.6. Correlation between Soil Physicochemical Properties, Enzyme Activity, Culturable Microbial Counts, Tea Production Status, and Quality Components

The correlation analysis between soil physicochemical indicators and enzyme activity with the number of culturable micro-organisms in the soil indicated that there was a close relationship between soil enzyme activity, physicochemical indicators, and the number of culturable micro-organisms in the soil, as shown in Table 4. The number of culturable bacteria in the soil was significantly and positively correlated with soil organic matter (OM) content, alkali-hydrolysable nitrogen (AN) content, catalase (Cat) activity, acid phosphatase (ACP) activity, protease (Pro) activity, and sucrase (Suc) activity. The number of culturable fungi in the soil was significantly and positively correlated with soil AN content, Cat activity, and Pro activity, showed a significant positive correlation with soil available phosphorus (AP) content and available potassium (AK) content, and exhibits a significant negative correlation with soil urease (Ure) activity. The number of culturable actinomycetes in the soil was significantly and positively correlated with soil OM content, AN content, AP content, Cat activity, and Pro activity, and showed a significant positive correlation with soil AK content.

Table 4. The correlation between soil physical and chemical properties, enzyme activity, and the number of soil culturable micro-organisms.

	OM	AN	AP	AK	Cat	Ure	ACP	Pro	Suc
Bacteria	0.59 **	0.86 **	0.38	0.40	0.79 **	−0.45	0.72 **	0.91 **	0.68 **
Fungi	0.43	0.81 **	0.56 *	0.57 *	0.62 **	−0.54 *	0.38	0.74 **	0.45
Actinomycete	0.71 **	0.66 **	0.84 **	0.55 *	0.61 **	−0.12	0.39	0.63 **	0.16

Note: OM, Organic matter; AN, Alkaline N; AP, Available P; AK, Available K; Cat, Catalase; Ure, Urease; ACP, Acid phosphatase; Pro, Protease; Suc, Sucrase. * indicated significant correlation at a 0.05 level; ** indicated significant correlation at a 0.01 level.

In the year of fertilization, there was a significant positive correlation between the number of culturable bacteria in the soil and the BD, TP, WE, and AA content of summer tea leaves. The number of culturable fungi in the soil showed a highly significant positive correlation with the BD, TP, AA, and WE content of summer tea leaves, but a highly significant negative correlation with the TP/AA ratio. The number of culturable actinomycetes in the soil had a highly significant positive correlation with the TP, AA, BD, and WE content of summer tea leaves, but a significant negative correlation with the TP/AA ratio. Additionally, the number of culturable bacteria in the soil had a highly significant positive correlation with the BD of spring tea leaves, and the number of culturable fungi in the soil showed a significant positive correlation with the BD and WE content of spring tea leaves. This indicates that in the year of fertilization, a high ratio of organic to chemical fertilizer coapplication can significantly improve the growing conditions for tea plants, reduce the use of chemical fertilizers, improve nutrient use efficiency, and enhance both the yield and quality of tea. One year after the cessation of fertilization, a high ratio of chemical

to bioinoculant coapplication is more favorable for maintaining high yield and quality in tea production, achieving healthy, and sustainable tea garden management (Table 5).

Table 5. The correlation between the yield and quality of tea and the number of soil culturable micro-organisms.

	Summer Tea						Spring Tea					
	BD	**OW**	**TP**	**AA**	**TP/AA**	**WE**	**BD**	**OW**	**TP**	**AA**	**TP/AA**	**WE**
Bacteria	0.84 **	−0.07	0.75 **	0.55 *	−0.37	0.65 **	0.64 **	−0.18	0.11	0.41	−0.21	0.25
Fungi	0.76 **	0.44	0.62 **	0.82 **	−0.75 **	0.58 *	0.58 *	0.24	−0.12	0.34	−0.28	0.53 *
Actinomycete	0.58 *	0.31	0.64 **	0.68 **	−0.57 *	0.76 **	0.15	0.00	0.16	0.3	−0.08	0.41

Note: BD, Bud density; OW, One-hundred-bud weight; TP, Tea polyphenols; AA, Amino acids; TP/AA, Amino acid-tea polyphenol ratio; WE, Water extracts. * indicated significant correlation at a 0.05 level; ** indicated significant correlation at a 0.01 level.

4. Discussion

4.1. Application of A-m1 Bioinoculant Affected the Physicochemical Properties of Tea Garden Soil

Studies have indicated that the coapplication of organic fertilizers and organic fertilizers with inorganic fertilizers can markedly improve the basic physicochemical properties of soil [5,27], increase soil microbial counts and diversity, fix atmospheric nitrogen, decompose soil nutrients that are not readily absorbed by crops into absorbable forms, and enhance the rate of nutrient supply [28], promoting the absorption of nutritional elements N, P, and K by plants, thereby promoting plant growth and increasing yield [29,30]. For example, the optimal ratio of 60% wood mold bioinoculant +40% chemical fertilizer can significantly increase soil nitrate nitrogen and available phosphorus content [31]. The use of bioinoculants created from *Bacillus subtilis* and *Bacillus mucilaginosus*, compared to conventional chemical fertilizers, can significantly increase soil available phosphorus and potassium content and the grain yield of wheat [32], and the application of Guter organic fertilizer can significantly enhance the levels of organic matter, alkali-hydrolysable nitrogen, and available phosphorus in the rhizospheric soil of barley at different growth stages [33]. Different ratios of A-m1 bioinoculant coapplied with reduced chemical fertilizer lead to varying changes in soil physicochemical properties. Specifically, the 70% bioinoculant +30% chemical fertilizer and the 50% bioinoculant +50% chemical fertilizer groups are characterized by a significant enhancement of soil organic matter, alkali-hydrolysable nitrogen, and available phosphorus content, with available potassium content on par with the 100% chemical fertilizer group (Figure 1).

The 70% bioinoculant +30% chemical fertilizer group had the highest organic matter content compared to the other five treatments, which may be related to the C:N:P ratio in the soil. A-m1 bioinoculant can provide the soil with abundant organic matter (including high organic carbon and some inorganic salts), which is the foundation for the growth of soil micro-organisms. The application of a small amount of chemical fertilizer is beneficial for maintaining the ratio of N and P elements required for the life activities of micro-organisms, thereby increasing the amount of soil micro-organisms, creating a good root environment, and improving soil organic matter content [34,35]. The 70% bioinoculant +30% chemical fertilizer group, as well as the 50% bioinoculant +50% chemical fertilizer and 30% bioinoculant +70% chemical fertilizer groups, had higher alkali-hydrolysable nitrogen content. This result can be attributed to the synergistic effect of A-m1 bioinoculant-chemical fertilizer coapplication, which increases the biomass and residue of tea plant roots. On the one hand, it enhances nitrogen input (including fertilizer application, microbial nitrogen fixation, dissolution of minerals, etc.), and on the other hand, it reduces nitrate-nitrogen leaching [36,37].

The 70% bioinoculant +30% chemical fertilizer group and the 50% bioinoculant +50% chemical fertilizer group also had higher available phosphorus and potassium content, indicating that these two treatments were more effective in activating nutrients, especially phosphorus. This may be due to the fact that strain A-m1 contains genes that can encode

phosphatase [17]. Through the regulation of phosphorus cycle genes and the expression of phosphatase, strain A-m1 can dissolve inorganic phosphorus and mineralize organic phosphorus, thereby improving the availability of soil phosphorus [35,38]. Studies have shown that the absorption of nitrogen, phosphorus, potassium, and other elements by plant individuals increases with yield. Compared with the CK group without any organic material application, the straw return and the combined application of straw and woody peat significantly increased the yield of corn kernels and straw. At the same time, more nutrients in the soil were absorbed and taken away by the plants, leading to a decrease in the available potassium content in the soil [39]. This study also reached a similar conclusion. Compared with the single application of chemical fertilizer (CF), the application of A-m1 bioinoculant significantly increased tea yield, while the available potassium content in the soil was at a lower level. Compared with the CF group, only the alkali-hydrolysable nitrogen content in T6 was significantly increased, which may be related to the lower content of A-m1 bioinoculant in the T6 group. Similar studies on the impact of reduced chemical fertilizer combined with bioinoculant on soil physicochemical properties [40] are basically consistent with the results of this study, which is related to the relatively long time-effectiveness of microbial activity [41,42].

4.2. Application of A-m1 Bioinoculant Influenced the Activity of Soil Enzymes and Culturable Microbial Population

Soil enzymes are the primary drivers of nutrient cycling and metabolism in soil ecosystems and, are important factors affecting the microecological environment. Urease, acid phosphatase, sucrase, and protease are closely related to plant nutrition and are important indicators for evaluating soil fertility levels and soil health [43]; catalase can decompose hydrogen peroxide within micro-organisms, reducing its toxic effects on plants, and is closely related to the health of the plant root environment [44]. This study found that the coapplication of different ratios of A-m1 bioinoculant and reduced chemical fertilizer led to different changes in soil enzyme activity. The main feature of the 70% bioinoculant +30% chemical fertilizer and 100% bioinoculant group was a significant increase in soil catalase, phosphatase, and protease activity; the 70% bioinoculant +30% chemical fertilizer and 50% bioinoculant +50% chemical fertilizer groups had a better effect on enhancing soil urease activity; and the 30% bioinoculant +70% chemical fertilizer and 100% chemical fertilizer groups had the best effect on enhancing soil sucrase activity (Figure 2). Studies on enzyme activity in tea gardens have also reported that the coapplication of organic and chemical fertilizers has the best effect on enhancing the activity of urease, phosphatase, and protease in tea garden soil. The increase in sucrase activity is also significant when chemical fertilizer is applied alone or in combination; thus, the application of chemical fertilizer is more conducive to enhancing sucrase activity, while the presence of organic manure helps to increase the activity of protease, urease, and phosphatase [45]. This study found that the soil catalase, urease, and protease activity in the A-m1 bioinoculant-chemical fertilizer coapplication group and the A-m1 bioinoculant alone group were all higher than that in the chemical fertilizer alone group, with the 70% bioinoculant + 30% chemical fertilizer group (nitrogen application rate of 184.11 $kg \cdot hm^{-2}$) being the highest, while the enzyme activity in the 100% bioinoculant group was slightly lower. This may be due to the C/N ratio of the carrier matrix in the bioinoculant being generally higher than the suitable ratio range for microbial activity in the soil. After supplementing with an appropriate amount of chemical fertilizer, it can better meet the nutrients required for microbial activity, allowing more enzymes to enter the soil with vigorous root activity and the life activities of soil animals and micro-organisms, which is more conducive to the enhancement of soil catalase, urease, and protease activity [46,47]. The effect of chemical fertilizer application alone and high-ratio chemical fertilizer coapplication on enhancing acid phosphatase activity is not significant, which may be attributed to the easy availability of phosphorus elements due to the application of high-dose phosphate fertilizer, making the activity of phosphorus-dissolving bacteria and the synthesis and secretion of acid phosphatase less active. The

application of a high ratio of A-m1 bioinoculant and a small amount of chemical fertilizer not only provides rich nutrients for the growth and reproduction of micro-organisms but also supplements a large number of A-m1 microbial bodies with phosphorus-dissolving and nitrogen-fixing functions, as well as a rich amount of enzymes, to the tea garden soil, promoting the absorption and utilization of phosphorus nutrients by tea plants, thereby enhancing soil acid phosphatase activity [48].

Soil micro-organisms primarily participate in the decomposition of organic matter, degradation of xenobiotics, soil carbon fixation, and prevention and control of crop diseases, playing a crucial role in soil energy flow and nutrient cycling [49]. The coapplication of bioinoculants and chemical fertilizer not only replenishes the soil with a large number of beneficial micro-organisms but also provides ample sources of organic carbon, nitrogen, and phosphorus, thereby increasing the quantities of bacteria, fungi, and actinomycetes in the soil and enriching the structure of the soil microbial community [50]. Studies have reported that Bacillus subtilis strain SQR9, *Trichoderma harzianum* NJAU4742, and composite functional bacterial fertilizers can increase the quantities of culturable bacteria, fungi, and actinomycetes in the soil and improve the soil microbial flora during the crop growth period [51,52]. This study also shows a significant enhancement effect on the quantity of culturable micro-organisms in the soil. Among them, the 70% bioinoculant +30% chemical fertilizer and 50% bioinoculant +50% chemical fertilizer groups had the most pronounced effect on increasing the quantities of culturable bacteria, fungi, and actinomycetes in the soil. This may be due to the application of bioinoculants, which can quickly occupy the main ecological niches in the plant root environment, creating additional ecological niches for beneficial microbial communities in the soil, thereby regulating the quantity of culturable micro-organisms in the rhizosphere soil [53]. In addition, this study shows that, compared with early November 2021, the activities of catalase, urease, phosphatase, sucrase, and the quantities of culturable bacteria and fungi in the soil in late September 2022 generally showed a downward trend. This may be due to the drought in Xinyang from July to September, which affected the soil root environment, leading to a decrease in the quantity of soil micro-organisms, metabolic activity, and enzyme activity.

4.3. Impact of Application of A-m1 Bioinoculant on Tea Plant Growth and Mineral Nutrient Content

Bioinoculants can increase the supply of plant nutrients, secrete plant growth hormones, promote the absorption and utilization of nutrients by plants, antagonize some pathogenic micro-organisms, reduce pests and diseases of crops, and improve crop yield and quality [54]. The coapplication of organic and chemical fertilizers throughout the tea growth cycle can supply the soil with a moderate amount of nutrients, ensuring that the tea plants are always in a good state of nutrient supply with strong sustainability, thereby improving tea yield and quality. For instance, when the application of chemical fertilizers is reduced by 25% N (urea) and 50% P (phosphate rock powder), and co-applied with indigenous nitrogen-fixing and phosphorus-solubilizing bacteria, it can increase the yield of tea shoots compared to the sole application of chemical fertilizers [55]. Research into the impact of reduced chemical fertilizer combined with bioinoculant on tea production and quality has shown that treatments combining 25–50% bioinoculant with 75–50% chemical fertilizer are superior [56]. The substitution of bioinoculant for chemical fertilizer can significantly increase the content of organic matter, available phosphorus, and readily available potassium in the tea garden soil, and promote the accumulation of free amino acids in tea, reducing the phenol-ammonia ratio, with the treatment of 30% bioinoculant substitution showing the most significant effect on quality and yield [57]. This study indicates that the 50% bioinoculant +50% chemical fertilizer group showed the most significant effect on increasing the weight of one hundred buds of tea in the year of fertilization, the 70% bioinoculant +30% chemical fertilizer group was the most effective in increasing the bud density of tea in the year of fertilization, and the 30% bioinoculant +70% chemical fertilizer group showed the most significant effect on increasing both the weight of one hundred

buds and the bud density of tea one year after cessation of fertilization. The reason for these differences may be that the content of available nutrients in the bioinoculant is relatively low. A high ratio of organic to chemical fertilizer can meet the demand for N and P by tea plant growth and microbial activity in the short term. However, due to the drought in the summer and autumn seasons, the soil microbial numbers and enzyme activities decreased, thus, the coapplication of a high ratio of chemical fertilizer with bioinoculant is best for a continuous and stable increase in tea yield [58,59].

The study also found that in the year of fertilization, the 70% bioinoculant +30% chemical fertilizer and 50% bioinoculant +50% chemical fertilizer groups significantly increased the free amino acid and water extract content of tea leaves and reduced the phenol-ammonia ratio compared to the CK group. In the second year after fertilization, the 50% bioinoculant +50% chemical fertilizer and 30% bioinoculant +70% chemical fertilizer groups significantly increased the free amino acid and water extract content and reduced the phenol-ammonia ratio and tea polyphenol content in tea leaves compared to the CK group. Among them, the tea quality of the 50% bioinoculant +50% chemical fertilizer group was superior to the 100% chemical fertilizer group both in the year of fertilization and one year after fertilization. The reason for this may be that although strain A-m1 can produce a large number of growth-promoting hormones and enzymes, its activity has the disadvantage of having a relatively long-term effect and being easily affected by the environment. The application of a small amount of bioinoculant and inorganic chemical fertilizer cannot only supplement beneficial micro-organisms in the soil but also change and improve the activity of soil micro-organisms, promote an increase in tea yield and the accumulation of internal components, and improve the quality of tea. This is consistent with the nutritional effect research results of biofertilizers by [60].

4.4. Correlation Analysis between Changes in Garden Soil and Tea Production and Mineral Nutrient Content

The quantity of culturable micro-organisms in the soil is closely linked to soil physico-chemical properties, enzyme activity, and the production and mineral nutrient content of tea leaves [45]. Soil enzymes, primarily secreted by soil micro-organisms, play a key role in catalyzing the decomposition of organic matter and the cycling of nutrients, breaking down complex macromolecular organic compounds in the soil into smaller molecules such as sugars, amino acids, NH^{4+}, etc., which can be absorbed and utilized by soil micro-organisms, thereby increasing the number of soil micro-organisms. Soil micro-organisms are the main drivers of the nutrient cycles of carbon, nitrogen, phosphorus, and other elements in the soil. An increase in microbial numbers can accelerate the transformation of soil nutrients, providing ample nourishment for crop growth and promoting the improvement of crop yield and quality [61]. It is known that strain A-m1 can encode enzymes that have direct or indirect growth-promoting effects, which can directly enhance soil enzyme activity. Studies have found that soil nutrient content and soil enzyme activity are positively correlated with microbial numbers, indicating a close correlation between soil microbial numbers and soil nutrients and enzyme activity [62].

This study obtained results consistent with previous research, indicating that there is a significant positive correlation between the number of culturable bacteria, fungi, and actinomycetes in the soil and the content of soil organic matter, alkali-hydrolyzable nitrogen, available phosphorus, and available potassium, as well as the activity of enzymes such as catalase, protease, sucrase, and acid phosphatase. This suggests that the application of microbial fertilizers can increase the number of beneficial micro-organisms in the soil, accelerate microbial growth and metabolism, enhance soil enzyme activity, promote the mobilization of soil trace elements from the solid phase to the soil solution, and increase their availability [63]. Soil micro-organisms can affect the nutrient status of plants by altering soil nutrients, thereby improving the quality and yield of tea leaves [64]. Yang Y. et al. used soil fungal diversity as a biological indicator of tea quality and found a significant correlation between tea quality parameters and soil fungal diversity indices [65].

This study found that the quantities of culturable bacteria, fungi, and actinomycetes in the soil were significantly or highly significantly correlated with the quality of summer tea in the year of fertilization. Even one year after the cessation of fertilization, the quantity of culturable fungi in the soil still had a certain correlation with the quality of spring tea. Therefore, the application of bioinoculants, by inputting nutrients, organic matter, and functional micro-organisms into the soil, plays a role in activating nutrients, improving nutrient use efficiency, increasing yield, and enhancing quality, thereby reducing the use of chemical fertilizers and effectively amending agricultural soil.

5. Conclusions

Our research team previously isolated a strain of *S. costaricanus*, A-m1, whose fermentation filtrate has a broad antibacterial spectrum and high antibacterial activity. It is capable of producing plant growth hormones such as indole acetic acid (IAA) and cytokinins, and it encodes enzymes that have direct or indirect growth-promoting effects, including nitrogenase, 1-aminocyclopropane-1-carboxylate (ACC) deaminase, phosphatase, and phytase. It has demonstrated effective growth promotion on tomatoes and holds potential for further development as a biocontrol agent. Currently, tea garden soils are facing issues such as nutrient deficiency, poor structure, and functional degradation. The A-m1 bioinoculant treatment significantly increased the content of alkali-hydrolysable nitrogen and the activity of sucrase, urease, protease, and catalase in the soil of the tea garden, as well as the quantity of culturable bacteria, fungi, and actinomycetes. It also had a positive impact on the bud density, weight of one hundred buds, water extract content, free amino acid content, and phenol-ammonia ratio of tea leaves. In the year of fertilization, the 70% bioinoculant +30% chemical fertilizer provided the best improvement effects on soil physicochemical properties, enzyme activity, culturable microbial counts, and tea quality. One year after the cessation of fertilization, the 50% bioinoculant +50% chemical fertilizer was more conducive to enhancing the quality components of tea, while the 30% bioinoculant +70% chemical fertilizer was more beneficial for improving the production status of tea. This indicates that in the year of fertilization, a high ratio of organic to chemical fertilizer coapplication can significantly improve the growing conditions for tea plants, reduce the use of chemical fertilizers, improve nutrient use efficiency, and enhance both the yield and quality of tea. One year after the cessation of fertilization, a high ratio of chemical to bioinoculant coapplication is more favorable for maintaining high yield and quality in tea production, achieving healthy and sustainable tea garden management. This experiment applies the strain A-m1 bioinoculant to the improvement of tea garden soils, in conjunction with the reduced application of chemical fertilizers, to explore the optimal ratio for coapplication. It is beneficial to enhance the yield and quality of tea leaves while reducing the use of chemical fertilizers, thereby providing technical support for sustainable production in tea gardens.

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References

1. Wang, Z.T.; Geng, Y.B.; Liang, T. Optimization of reduced chemical fertilizer use in tea gardens based on the assessment of related environmental and economic benefits. *Sci. Total Environ.* **2019**, *713*, 136439. [CrossRef] [PubMed]
2. Sun, R.B.; Guo, X.S.; Wang, D.Z.; Chu, H.Y. Effects of long-term application of chemical and organic fertilizers on the abundance of microbial communities involved in the nitrogen cycle. *Appl. Soil Ecol.* **2015**, *95*, 171–178. [CrossRef]
3. Chen, Y.T.; Hu, S.Y.; Guo, Z.G.; Cui, T.H.; Zhang, L.P.; Lu, C.R.; Yu, Y.Q.; Luo, Z.B.; Fu, H.; Jin, Y. Effect of balanced nutrient fertilizer: A case study in Pinggu District, Beijing China. *Sci. Total Environ.* **2021**, *754*, 142069. [CrossRef] [PubMed]
4. Cai, F.; Chen, W.; Wei, Z.; Pang, G.; Li, R.X.; Ran, W.; Shen, Q.R. Colonization of *Trichoderma harzianum* strain SQR-T037 on tomato roots and its relationship to plant growth, nutrient availability and soil microflora. *Plant Soil* **2015**, *388*, 337–350. [CrossRef]
5. Graziano, S.; Caldara, M.; Gullì, M.; Bevivino, A.; Maestri, E.; Marmiroli, N. A metagenomic and gene expression analysis in wheat (*T. durum*) and maize (*Z. mays*) biofertilized with PGPM and biochar. *Int. J. Mol. Sci.* **2022**, *23*, 10376. [CrossRef]
6. Nader, A.A.; Hauka, F.I.A.; Afify, A.H.; E1-Sawah, A.M. Drought-tolerant bacteria and arbuscular mycorrhizal fungi mitigate the detrimental effects of drought stress induced by withholding irrigation at critical growth stages of soybean (*Glycine max*, L.). *Microorganisms* **2024**, *12*, 1123. [CrossRef]
7. Alam, K.; Mazumder, A.; Sikdar, S.; Zhao, Y.M.; Hao, J.F.; Song, C.Y.; Wang, Y.Y.; Sarkar, R.; Islam, S.; Zhang, Y.M.; et al. *Streptomyces*: The biofactory of secondary metabolites. *Front. Microbiol.* **2022**, *13*, 968053. [CrossRef]
8. Khamna, S.; Yokota, A.; Peberdy, J.F.; Lumyong, S. Indole3-acetic acid production by *Streptomyces* sp. isolated from some Thai medicinal plant rhizosphere soils. *EurAsian J. Biosci.* **2010**, *4*, 23–32. [CrossRef]
9. Challis, G.L.; Hopwood, D.A. Synergy and contingency as driving forces for the evolution of multiple secondary metabolite production by Streptomyces species. *Proc. Natl. Acad. Sci. USA* **2003**, *100* (Suppl. S2), 14555–14561. [CrossRef]
10. Chouyia, F.E.; Ventorino, V.; Pepe, O. Diversity, mechanisms and beneficial features of phosphate-solubilizing *Streptomyces* in sustainable agriculture: A review. *Front. Plant Sci.* **2022**, *13*, 1035358. [CrossRef]
11. Tang, H.M. Effects of Microbial Fertilizer and Biochar Applications on the Yield and Quality of *Pinellia ternata* and Soil Microecology. Master's Thesis, College of Resources & Environment of Huazhong Agricultural University, Wuhan, China, 2019. (In Chinese).
12. Wu, Q.; Lu, C.G.; Ni, M.; Wang, H.L.; Liu, W.C.; Chen, J. *Streptomyces lydicus* A01 affects soil microbial diversity, improving growth and resilience in tomato. *J. Integr. Plant Biol.* **2019**, *61*, 182–196. [CrossRef] [PubMed]
13. Li, D.S.; He, N.W.; Ji, Y.L. Effects of *Streptomyces griseus* on soil enzyme activity, nutrients and microorganism of the seedlings of *Platycodon grandiflorum* rhizosphere under drought stress. *Agric. Res. Arid. Areas* **2017**, *35*, 173–180. (In Chinese)
14. Zhang, Q.M.; Yong, D.J.; Zhang, Y.; Shi, X.P.; Li, B.H.; Li, G.F.; Liang, W.X.; Wang, C.X. *Streptomyces rochei* A-1 induces resistance and defense-related responses against *Botryosphaeria dothidea* in apple fruit during storage. *Postharvest Biol. Technol.* **2015**, *115*, 30–37. [CrossRef]
15. Arima, J.; Uesugi, Y.; Iwabuchi, M.; Hatanaka, T. *Streptomyces* aminopeptidase P: Biochemical characterization and insight into the roles of its N-terminal domain. *Protein Eng. Des. Sel. Peds* **2008**, *21*, 45–53. [CrossRef]
16. Li, Y.L.; Wang, Y.H.; Zhou, Z.; Qu, L.J. Identification and optimization of fermentation conditions and antibacterial activity of endophytic *Streptomyces rochei* A-m1 from the branch of *Malus sieversii*. *Sci. Silvae Sin.* **2020**, *56*, 70–81. (In Chinese)
17. Shi, J.; Li, Y.L.; Chang, L.; Zheng, W.F.; Zhou, Z.; Qu, L.J. Analysis of the growth promoting effect and mechanism of *Streptomyces costaricanus* A-m1. *Acta Agric. Boreali-Sin.* **2021**, *36*, 205–211. (In Chinese)
18. Fang, Z.D. *Methods of Plant Disease Research*, 3rd ed.; China Agricultural Publishing House: Beijing, China, 1998. (In Chinese)
19. Actinomycetes Taxonomy Group; Institute of Microbiology; Chinese Academy of Sciences. *Streptomycetes Identification Manual*; Science Press: Beijing, China, 1975. (In Chinese)
20. Wang, Y.; Wang, W.L.; Liu, C.Y.; Hao, Y.J. Studies on identification and cultural characterization of *Trichothecium roseum*. *Acta Agric. Boreali-Sin.* **2008**, *23*, 97–100. (In Chinese)
21. Ren, H.L.; Xiao, B.; Yu, Y.B.; Wang, F.Q.; Yao, L.J.; Li, D.H. Application effect of bio-organic fertilizer on spring tea. *J. Northwest AF Univ. Nat. Sci. Ed.* **2009**, *37*, 105–109, 116. (In Chinese)
22. Quan, Y.T.; Li, J.; Zhang, H.N.; Fan, Y.; Jing, B.N. Effects of reduction of chemical fertilizer and organic manure supplement on soil nutrients in Xinyang tea garden and the quality of spring tea. *Henan Sci.* **2020**, *38*, 1781–1785. (In Chinese)
23. Yan, P.; Shen, C.; Zou, Z.H.; Fu, J.Y.; Li, X.; Zhang, L.P.; Zhang, L.; Han, W.Y.; Fan, L.C. Biochar stimulates tea growth by improving nutrients in acidic soil. *Sci. Hortic.* **2021**, *283*, 110078. [CrossRef]
24. Bao, S.D. *Soil and Agricultural Chemistry Analysis*; China Agriculture Press: Beijing, China, 2000. (In Chinese)
25. Guan, S.Y. *Soil Enzyme and Its Research Method*; Agricultural Press: Beijing, China, 1986; pp. 221–240, 327. (In Chinese)
26. Zhou, L.K. *Soil Enzymology*; Science Press: Beijing, China, 1987. (In Chinese)
27. Fang, H.; Liu, K.L.; Li, D.M.; Peng, X.H.; Zhang, W.J.; Zhou, H. Long-term effects of inorganic fertilizers and organic manures on the structure of a paddy soil. *Soil Tillage Res.* **2021**, *213*, 105137. [CrossRef]
28. Yuan, J.; Ruan, Y.Z.; Wang, B.B.; Zhang, J.; Waseem, R.; Huang, Q.W.; Shen, Q.R. Plant growth-promoting rhizobacteria strain *Bacillus amyloliquefaciens* NJN-6-enriched bio-organic fertilizer suppressed Fusarium wilt and promoted the growth of banana plants. *J. Agric. Food Chem.* **2013**, *61*, 3774–3780. [CrossRef] [PubMed]
29. Mehmood, U.; Inam-ul-Haq, M.; Saeed, M.; Altaf, A.; Azam, F.; Hayat, S. A brief review on plant growth promoting rhizobacteria (PGPR): A key role in plant growth promotion. *Plant Prot.* **2018**, *2*, 77–82.

30. Stamford, N.P.; Felix, F.; Oliveira, W.; Silva, E.; Carolina, S.; Arnaud, T.; Freitas, A.D. Interactive effectiveness of microbial fertilizer enriched in N on lettuce growth and on characteristics of an Ultisol of the rainforest region. *Sci. Hortic.* **2019**, *247*, 242–246. [CrossRef]
31. Yu, Y.N.; Wu, H.Y.; Wang, P.X.; Ding, M.Y.; Ma, X.C.; Jiang, S.Q.; Cai, F.; Shen, Q.R.; Chen, W. Biological effect of *Trichoderma*-enriched biofertilizers on cabbage cultivation in coastal saline soil. *Acta Pedol. Sin.* **2022**, *59*, 1112–1124. (In Chinese)
32. Yang, W.L.; Gong, T.; Wang, J.W.; Li, G.J.; Liu, Y.Y.; Zhen, J.; Ning, M.; Yue, D.D.; Du, Z.M.; Chen, G.C. Effects of compound microbial fertilizer on soil characteristics and yield of wheat (*Triticum aestivum* L.). *J. Soil Sci. Plant Nutr.* **2020**, *20*, 2740–2748. [CrossRef]
33. Zhu, D.; Zhang, L.; Wei, Z.X.; Liu, X.Y.; Zhou, Z.F.; Dai, X.Z.; Wang, X.F.; Xia, Z.Q.; Wu, X.Q.; Wei, J.J.; et al. Effects of bacterial manure on soil physicochemical properties and microbial community diversity in rhizosphere of highland barley. *Acta Pedol. Sin.* **2014**, *51*, 627–637. (In Chinese)
34. Ren, C.J.; Zhao, F.Z.; Kang, D.; Yang, G.H.; Han, X.H.; Tong, X.G.; Feng, Y.Z.; Ren, G.X. Linkages of C: N: P stoichiometry and bacterial community in soil following afforestation of former farmland. *For. Ecol. Manag.* **2016**, *376*, 59–66. [CrossRef]
35. Liu, C.J.; Gong, X.W.; Dang, K.; Li, J.; Yang, P.; Gao, X.L.; Deng, X.P.; Feng, B.L. Linkages between nutrient ratio and the microbial community in rhizosphere soil following fertilizer management. *Environ. Res.* **2020**, *184*, 109261. [CrossRef]
36. Cheng, Y.; Luo, M.; Zhang, T.G.; Yan, S.H.; Wang, C.; Dong, Q.G.; Feng, H.; Zhang, T.B.; Kisekka, I. Organic substitution improves soil structure and water and nitrogen status to promote sunflower (*Helianthus annuus* L.) growth in an arid saline area. *Agric. Water Manag.* **2023**, *283*, 108320. [CrossRef]
37. Zhang, B.B.; Su, S.S.; Duan, C.X.; Feng, H.; Chau, H.W.; He, J.Q.; Li, Y.; Hill, R.L.; Wu, S.F.; Zou, Y.F. Effects of partial organic fertilizer replacement combined with rainwater collection system on soil water, nitrate-nitrogen and apple yield of rainfed apple orchard in the Loess Plateau of China: A 3-year field experiment. *Agric. Water Manag.* **2022**, *260*, 107295. [CrossRef]
38. Yuste, J.C.; Baldocchi, D.D.; Gershenson, A.; Goldstein, A.; Misson, L.; Wong, S. Microbial soil respiration and its dependency on carbon inputs, soil temperature and moisture. *Glob. Chang. Biol.* **2007**, *13*, 2018–2035. [CrossRef]
39. Li, H.M.; Tian, S.Y.; Li, D.D.; Li, Z.Q.; Tan, J.; Zhao, B.Z. Effect of application of organic materials on content of labile organic carbon and composition of microbial community in fluvio-aquatic soil. *Acta Pedol. Sin.* **2021**, *58*, 777–787. (In Chinese)
40. Ye, S.J.; Zheng, C.M.; Zhang, Y.; Liu, X. Effects of reduced chemical nitrogen input combined with organic fertilizer application on the productivity of winter wheat and summer maize rotation and soil properties in central Henan Province. *Chin. J. Eco-Agric.* **2022**, *30*, 900–912. (In Chinese)
41. Zhang, R.; Huang, J.X.; Duan, Y.N.; Wang, H.Y.; Wang, M.; Chen, X.S.; Shen, X.; Yin, C.M.; Mao, Z.Q. Application of fermentation products of *Penicillium commune* D12 and *Lactobacillus reuteri* to stimulate the growth of *Malus hupehensis* Rehd. seedling and improve soil biological environment in a continuous cropping. *J. Plant Nutr. Fertil.* **2022**, *28*, 344–356. (In Chinese)
42. Li, X.Q.; Li, D.Y.; Jiang, Y.G.; Xu, J.; Ren, X.X.; Zhang, Y.; Wang, H.; Lu, Q.J.; Yan, J.L.; Ahmed, T.; et al. The effects of microbial fertilizer based *Aspergillus brunneoviolaceus* HZ23 on pakchoi growth, soil properties, rhizosphere bacterial community structure, and metabolites in newly reclaimed land. *Front. Microbiol.* **2023**, *14*, 1091380. [CrossRef]
43. You, Y.M.; Wang, J.; Huang, X.M.; Tang, Z.X.; Liu, S.R.; Sun, O.J. Relating microbial community structure to functioning in forest soil organic carbon transformation and turnover. *Ecol. Evol.* **2014**, *4*, 633–647. [CrossRef]
44. Liu, G.M.; Zhang, X.C.; Wang, X.P.; Shao, H.B.; Yang, J.S.; Wang, X.P. Soil enzymes as indicators of saline soil fertility under various soil amendments. *Agric. Ecosyst. Environ.* **2017**, *237*, 274–279.
45. Wang, L.M.; Li, W.H.; Fan, P.; Qiu, X.X.; Huang, D.F.; Lin, X.J. Variation in soil enzyme activities under long-term fertilization of tea garden in red-yellow soil area. *J. Tea Sci.* **2012**, *32*, 347–352. (In Chinese)
46. Liang, L.; Ma, C.; Zhang, R.; Zhai, B.N.; Li, Z.Y.; Wang, C.H. Improvement of soil nutrient availability and enzyme activities in rainfed wheat field by combined application of organic and inorganic fertilizers. *J. Plant Nutr. Fertil.* **2019**, *25*, 544–554. (In Chinese)
47. Shen, W.S.; Lin, X.G.; Shi, W.M.; Min, J.; Gao, N.; Zhang, H.Y.; Yin, R.; He, X.H. Higher rates of nitrogen fertilization decrease soil enzyme activities, microbial functional diversity and nitrification capacity in a Chinese polytunnel greenhouse vegetable land. *Plant Soil* **2010**, *337*, 137–150. [CrossRef]
48. Liu, H.; Li, S.S.; Qiang, R.W.; Lu, E.J.; Li, C.L.; Zhang, J.J.; Gao, Q. Response of soil microbial community structure to phosphate fertilizer reduction and combinations of microbial fertilizer. *Front. Environ. Sci.* **2022**, *10*, 899727. [CrossRef]
49. Jin, N.; Jin, L.; Wang, S.Y.; Li, J.W.; Liu, F.H.; Liu, Z.C.; Luo, S.L.; Wu, Y.; Lyu, J.; Yu, J.H. Reduced chemical fertilizer combined with bio-organic fertilizer affects the soil microbial community and yield and quality of lettuce. *Front. Microbiol.* **2022**, *13*, 863325. [CrossRef] [PubMed]
50. Pu, R.F.; Wang, P.P.; Guo, L.P.; Li, M.H.; Cui, X.M.; Wang, C.X.; Liu, Y.; Yang, Y. The remediation effects of microbial organic fertilizer on soil microorganisms after chloropicrin fumigation. *Ecotoxicol. Environ. Saf.* **2022**, *231*, 113188. [CrossRef] [PubMed]
51. Yuan, Y.Y.; Li, M.Q.; Hu, W.; Zhang, J.; Zhao, L.F.; Li, H.X. Effect of biological organic fertilizer on tomato bacterial wilt and soil microorganism. *J. Agro-Environ. Sci.* **2011**, *30*, 1344–1350. (In Chinese)
52. Mai, Y.Y. Development of Organic Manure from Sheep Manure and Its Influence on the Growth of Pepper. Master's thesis, Nanjing Agricultural University, Nanjing, China, 2020. (In Chinese).
53. Li, Y.Q.; Zhao, B.Q.; Li, Z. Research progress of organic-inorganic fertilizer combined application system. *J. Agric.* **2017**, *7*, 22–30. (In Chinese)

54. Wang, M.; Bian, Z.L.; Shi, J.; Wu, Y.X.; Yu, X.; Yang, Y.; Ni, H.P.; Chen, H.N.; Bian, X.Y.; Li, T.; et al. Effect of the nitrogen-fixing bacterium *Pseudomonas protegens* CHA0-Delta retS-nif on garlic growth under different field conditions. *Ind. Crop. Prod.* **2020**, *145*, 111982. [CrossRef]
55. Saikia, D.N.; Sarma, J.; Dutta, P.K.; Baruah, D.K. Sustainable productivity of tea through conservation of bio-mass, addition of bio-fertilizers and reduction of in-organic fertilizer. *Two Bud.* **2011**, *58*, 109–117.
56. Wu, Z.D.; You, Z.M.; Jiang, F.Y.; Wang, F.; Weng, B.Q. Effects of combined application of organic manure and chemical fertilizer on properties of tea garden soil and the yield and quality of tea. *Soils* **2015**, *47*, 874–879. (In Chinese)
57. Shu, Z.F.; Ji, Q.Y.; Shao, J.N.; Zheng, S.H.; Zhou, H.J.; He, W.Z. Effects of organic fertilizer replacement on soil nutrients in tea garden and yield-quality of tea plant. *Acta Hortic. Sin.* **2023**, *50*, 2207–2219. (In Chinese)
58. Yang, X.D.; Ni, K.; Shi, Y.Z.; Yi, X.Y.; Zhang, Q.F.; Fang, L.; Ma, L.F.; Ruan, J.Y. Effects of long-term nitrogen application on soil acidification and solution chemistry of a tea plantation in China. *Agric. Ecosyst. Environ.* **2018**, *252*, 74–82. [CrossRef]
59. Ji, L.F.; Wu, Z.D.; You, Z.M.; Yi, X.Y.; Ni, K.; Guo, S.W.; Ruan, J.Y. Effects of organic substitution for synthetic N fertilizer on soil bacterial diversity and community composition: A 10-year field trial in a tea plantation. *Agric. Ecosyst. Environ.* **2018**, *268*, 124–132. [CrossRef]
60. Nadeem, S.M.; Ahmad, M.; Zahir, Z.A.; Javaid, A.; Ashraf, M. The role of mycorrhizae and plant growth promoting rhizobacteria (PGPR) in improving crop productivity under stressful environments. *Biotechnol. Adv.* **2014**, *32*, 429–448. [CrossRef] [PubMed]
61. Caldwell, B.A. Enzyme activities as a component of soil biodiversity: A review. *Pedobiologia* **2005**, *49*, 637–644. [CrossRef]
62. Peng, Y.N.; Qi, H.S.; Pei, Y.L.; Xi, P.; Ji, B. The variation characteristics of soil nutrient, enzyme activity and microbial count under different vegetation types of Heihe wetland. *Hubei Agric. Sci.* **2023**, *62*, 44–50. (In Chinese)
63. Lazcano, C.; Gómez-Brandón, M.; Revilla, P.; Domínguez, J. Short-term effects of organic and inorganic fertilizers on soil microbial community structure and function. *Biol. Fertil. Soils* **2013**, *49*, 723–733. [CrossRef]
64. Wallenius, K.; Rita, H.; Mikkonen, A.; Lappi, K.; Lindström, K.; Hartikainen, H.; Raateland, A.; Niemi, R.M. Effects of land use on the level, variation and spatial structure of soil enzyme activities and bacterial communities. *Soil Biol. Biochem.* **2011**, *43*, 1464–1473. [CrossRef]
65. Yang, Y.R.; Kim, J.; Chung, J.O.; Cho, D.; Roh, J.H.; Hong, Y.D.; Kim, W.G.; Kang, H. Variations in the composition of tea leaves and soil microbial community. *Biol. Fertil. Soils* **2022**, *58*, 167–179. [CrossRef]

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