

Article

Improvement of Yield and Quality Properties of Radish by the Organic Fertilizer Application Combined with the Reduction of Chemical Fertilizer

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Abstract: Chemical fertilizers can improve crop productivity, but irrational fertilization often results in low crop quality and yield, poor soil fertility, and severe environmental pollution. Nevertheless, little research has been conducted with a close focus on the cultivation of radish in high mountain regions, a widely cultivated root vegetable known for its nutritional value and economic importance. Here, a method of reducing chemical fertilizers combined with the application of organic fertilizers is proposed upon studying four different ratios of chemical and organic fertilizers, including control (375 kg·ha⁻¹ chemical fertilizer + 4500 kg·ha⁻¹ organic fertilizer) and combinations (T₁: 12% reduction in chemical fertilizer + 4500 kg·ha⁻¹ organic fertilizer; T₂: 20% reduction in chemical fertilizer + 4500 kg·ha⁻¹ organic fertilizer; T₃: 28% reduction in chemical fertilizer + 4500 kg·ha⁻¹ organic fertilizer). Their effects on radish quality, yield, and soil environment were investigated. Compared with the control group, T₂ significantly increased radish yield by 12.92% and improved the contents of vitamin C, soluble sugars, sulforaphane soluble solids, and titratable acidity in the radish roots by 10.62%, 2.15%, 50.00%, 26.90%, and 43.90%, respectively. The soil nutrient content was increased by the T₂ treatment, with a 7.69% and 14.29% increase in total nitrogen and total phosphorus content, respectively, compared with the control. Moreover, soil urease activity, sucrose activity, alkaline phosphatase activity, and catalase activity were significantly enhanced by the T₂ treatment, showing an improvement of 11.13%, 44.30%, 26.41%, and 9.33% compared with the control, respectively ($p < 0.05$). The relative abundance of beneficial bacterial phyla such as Proteobacteria and Actinobacteria was increased in the T₂ treatment, potentially helping to maintain better soil health and long-term fertility. In summary, a promising fertilizer management strategy is herein unveiled through the reduction of chemical fertilizers and the application of organic fertilizer that not only improves radish yield and quality but also optimizes the soil environment, providing an effective means for sustainable crop production.



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1. Introduction

Radish (*Raphanus sativus* L.), also known as water radish, is a herbaceous vegetable crop belonging to the Brassicaceae family. The common edible part of radish is the modified root, rich in various nutrients, such as vitamin C, dietary fiber, and amino acids. It is also a good source of calcium but lacks oxalic acid. Radish has significant beneficial effects on human health due to its anticancer, antimicrobial, blood pressure-lowering, and immune-boosting properties [1]. Over the past decade, the area used for radish cultivation in China

has remained steady at approximately 1.2 million hectares. This represents 9% of the total vegetable production area, demonstrating the consistent development of this sector. The total production of radish has reached 26.8 million tons, making it an important economic crop in China [2]. However, the large-scale cultivation of radish has led to an excessive use of chemical fertilizers and pesticides. While these practices have significantly increased yield, they have also resulted in serious soil environmental issues. These include soil compaction, salinization, a reduction in microbial populations, decreased soil enzyme activity, disruption of soil aggregates, and a weakened capacity for water and nutrient retention [3]. These issues ultimately lead to imbalanced soil nutrients and environmental pollution [4], which consequently have adverse effects on yield and quality, contradicting the requirements of sustainable agriculture [5].

Previous studies have indicated that balanced fertilization plays a pivotal role in ensuring optimal and sustainable crop production [6]. Proper supply of the necessary nutrients is crucial for effectively enhancing both the yield and quality of vegetables. Therefore, balanced fertilization is considered an indispensable agronomic management strategy for maximizing agricultural productivity. Notably, the type and quantity of fertilizer significantly influence the quality of radishes. Particularly, optimal fertilization based on soil fertility and the specific nutrient requirements of radish not only ensures yield but also significantly improves economic and ecological benefits [7]. Furthermore, studies have highlighted the substantial contribution of organic fertilizers in preserving and enhancing soil biological properties [8]. In comparison to conventional fertilization methods, reducing chemical fertilizer usage while incorporating organic alternatives can improve overall porosity, decrease soil bulk density, enhance water retention capacity, and increase soil organic matter content [9]. This approach also enhances soil microbial population and structure while modifying rhizosphere soil enzyme activity [10], thereby promoting nutrient uptake efficiency in crops and ultimately leading to increased yields [11]. Additionally, reducing chemical fertilizer usage while utilizing organic alternatives not only substantially increases crop yield but also revitalizes the soil ecosystem while mitigating risks of environmental pollution associated with excessive fertilizer application [12]. Ultimately, minimizing chemical fertilizer usage while adopting organic fertilizers represents an effective strategy for achieving high crop yields, optimizing fertilizer resource utilization efficiency, as well as safeguarding farmland environments [13,14].

Organic fertilizer substitution technology is a fertilization method that combines chemical fertilizers with organic fertilizers, which has been widely proven to have the ability to improve soil environment, promote crop root growth, and enhance crop quality and yield [15,16]. Despite the slow release of nutrients in organic fertilizers, using organic fertilizers alone cannot meet the rapid growth needs of crops. Therefore, the application of organic fertilizer substitution technology can fully utilize the advantages of chemical fertilizers and organic fertilizers to achieve better crop growth results [17]. Previous studies have found that the application of organic fertilizers has a significant positive effect on improving the yield and compositional content of major grain crops such as wheat, rice, and corn [18–20]. Currently, there is a substantial body of research focusing on the growth, development, quality traits [21,22], and nutrient requirements [23] of radish. However, there is limited research on the effects of reducing chemical fertilizer usage and applying organic fertilizers on radish nutritional quality and soil microbial structure. Therefore, this experiment was designed to investigate the impacts of a gradient reduction in chemical fertilizer application combined with a fixed amount of organic fertilizer on various aspects of radish growth, development, yield, soil fertility, and soil microbial communities. The study aims to systematically elucidate the advantages of replacing chemical fertilizers with organic fertilizers and explore appropriate combinations of reduced chemical fertilization combined with organic fertilizer application to achieve the optimal fertilization combination that reduces chemical fertilizer usage and increases radish yield. The ultimate goal is to contribute to the healthy, green, and sustainable development of the radish cultivation industry.

2. Materials and Methods

2.1. Experimental Site Description

The field experiment was conducted from 15 June 2023 to 15 August 2023 in Guangyuan City, Sichuan Province, China. The longitude and latitude of the experimental site are 105.52° E and 32.26° N; the climate type is Subtropical Humid Monsoon Climate; the average temperature in 2023 was 16.1 °C. The experimental site is located at an altitude of 1800 m, with an average annual precipitation of 1600 mm. The predominant soil type is mountainous yellow soil, characterized by a clay loam texture, with a dry and compact nature and relatively low organic matter content. Prior to the start of the experiment, the basic physicochemical properties of the topsoil (0–20 cm) were determined. The results are shown in the Table 1:

Table 1. Basic Physical and Chemical Properties of Original Soil Sample.

Property	TN g/kg	HN mg/kg	TP g/kg	AP mg/kg	pH kg	OM %
Original soil sample	1.22	96.0	0.016	102	5.77	12.98

Note: TN: total nitrogen; HN: hydrolyzable nitrogen; TP: total phosphorus; AP: available phosphorus; OM: organic matter. Element content is expressed on dry soil basis.

2.2. Experimental Materials

2.2.1. Radish Variety

The radish variety used in the experiment was “Jie Rumei”. This variety has the following main characteristics: The leaves are semi-erect, the flowering period is short, and the maturity time is about 55–60 days. The root length is about 26 cm, and the diameter is between 5.5–6 cm. The root surface is smooth, clean, and white, with the plant exhibiting a rapid growth rate, high-temperature resistance, and excellent disease resistance. This special variety was provided by Hebei Jieerumei Agricultural Science and Technology Development Co., Ltd., China (Hebei, China).

2.2.2. Fertilizer

The chemical fertilizer used in the experiment was a ternary compound fertilizer (15-15-15); the notation “15-15-15” indicates that the fertilizer contains 15% nitrogen (N), 15% phosphorus (P₂O₅), and 15% potassium (K₂O). This fertilizer provided balanced amounts of nitrogen (N), phosphorus (P), and potassium (K) to support the growth and development of radish plants. Specific products from JiaShiLi Chemical Fertilizer Co., Ltd. (Hubei, China), such as Bai Xing compound fertilizer, were utilized. The organic fertilizer used in the experiment was a type of organic fertilizer containing humic acid and amino acids. It had an organic matter content of at least 40%. The organic fertilizer also had an effective microbial count of at least 0.2 billion per gram and a total content of nitrogen (N), phosphate pentoxide (P₂O₅), and potassium oxide (K₂O) of at least 6%.

2.3. Experimental Design

The experiment was designed based on the local farming practices, with the aim of comparing different fertilizer reduction treatments. Four treatments were set up, including a control treatment based on the conventional fertilizer application by local farmers and three reduced fertilizer treatments. Irrigation, pest control, and other field management practices were carried out as usual [24]. The details of the treatments are presented in Table 2.

The experiment was conducted using open-field, wide-row, double-ridge planting, with a row spacing of 30 cm, plant spacing of 40 cm, and furrow depth of 35 cm. Each plot had dimensions of 7 m in length and 1 m in width, resulting in a plot area of 7 m². Five plots of land (five biological repeats) were randomly assigned under each treatment.

Table 2. Fertilization under different fertilizer treatments.

Treatments	Organic Fertilizer (kg·ha ⁻¹)	Compound Chemical Fertilizer (kg·ha ⁻¹)	Reduction of Chemical Fertilizer (%)
Control	4500	375	0%
T ₁	4500	330	12%
T ₂	4500	300	20%
T ₃	4500	270	28%

2.4. Measurement Indices and Methods

2.4.1. Measurement of Plant Growth Parameters

Plant growth parameters were measured at different stages of radish growth, such as the seedling stage (28 June 2023), vigorous leaf growth stage (5 July 2023 and 11 July 2023), fleshy root enlargement stage (19 July 2023 and 26 July 2023), and harvest stage (2 August 2023). Particularly, the maximum leaf length and width were measured using a precision of 0.1 cm using a measuring tape during the seedling stage, vigorous leaf growth stage, fleshy root enlargement stage, and harvest stage. These measurements were conducted six times. Fifteen plants with uniform and representative growth were selected from each plot. The fleshy root diameter and thick skin were measured using a vernier caliper with a precision of 0.02 mm during the seedling stage, vigorous leaf growth stage, fleshy root enlargement stage, and harvest stage.

For the measurement of dry matter accumulation, five intact plants were randomly selected from each treatment plot, resulting in a total of 25 plants. The above-ground and below-ground parts of the radish plants were weighed using an electronic balance with a precision of 0.001 g. Afterward, both above-ground and below-ground plant parts were oven-dried at 105 °C for 30 min and then at 80 °C until a constant weight before recording the dry weight of the plants.

2.4.2. Measurement of Radish Quality Indices

The soluble protein content of the radish samples was determined using the Coomassie Brilliant Blue G-250 assay. This colorimetric method involves the binding of the Coomassie Brilliant Blue G-250 dye to proteins, resulting in a blue-colored complex that can be quantified spectrophotometrically. The absorbance of the dye–protein complex was measured at a specific wavelength, and the soluble protein concentration was then calculated by comparing the sample absorbance to a standard curve prepared using known concentrations of a protein reference standard [25]. The soluble sugar content of the radish samples was measured using the sulfuric acid–anthrone colorimetric method on an ultraviolet–visible spectrophotometer. In this analytical procedure, the radish extracts were first treated with concentrated sulfuric acid, which hydrolyzed the soluble carbohydrates to monosaccharides. The addition of the anthrone reagent then resulted in the formation of a green-colored complex, the intensity of which was proportional to the total soluble sugar concentration. The absorbance of this colored complex was measured using the UV–vis spectrophotometer at a specific wavelength, typically around 620–640 nm (UV 8000 spectrophotometer, Shanghai Yuan analysis Instrument Co., Ltd., Shanghai, China) [26]. The cellulose content of the radish samples was determined using the anthrone–sulfuric acid colorimetric method, utilizing the same ultraviolet–visible spectrophotometer mentioned earlier. In this analytical technique, the radish samples were first treated with sulfuric acid to hydrolyze the cellulose molecules into glucose monomers. The addition of the anthrone reagent, which is a cyclic ketone, then resulted in the formation of a green-colored complex when reacted with the glucose. The intensity of this colored complex, as measured by the absorbance at a specific wavelength (typically 620–640 nm) using the UV–vis spectrophotometer, was directly proportional to the cellulose content in the radish samples [26]. The vitamin C content was determined using the 2,6-dichloroindophenol titration method. This titri-

metric technique relies on the redox reaction between ascorbic acid (vitamin C) and the 2,6-dichloroindophenol indicator. In this method, the radish samples were first extracted, and the ascorbic acid was oxidized by the 2,6-dichloroindophenol, which is blue-colored in its oxidized form. As the titration progressed, the blue color persisted until all the ascorbic acid was oxidized, at which point the solution turned pink, indicating the endpoint of the titration. The volume of the 2,6-dichloroindophenol titrant required to reach the endpoint was then used to calculate the ascorbic acid (vitamin C) content of the radish samples [27]. In this analysis, the radish samples were first extracted to isolate the isothiocyanate compounds. The extracted samples were then injected into the UHPLC system, which uses a high-pressure liquid mobile phase to carry the sample components through a specialized stationary phase column. As the compounds elute from the column, they are detected by a sensitive detector, such as a UV-vis or mass spectrometry detector. The retention times and peak areas of the isothiocyanate compounds in the radish samples were then compared with those of known isothiocyanate standards to quantify the levels of different isothiocyanate species present (UHPLC-Agilent 1290, Agilent Technologies, GA, USA) [28].

2.4.3. Yield Determination

Yield determination was conducted on 15 August 2023 during the radish harvest period. In each plot, a consecutive selection of 25 radish plants was harvested and weighed to measure the total yield, which was then adjusted to yield per plant and further converted to yield per hectare (ha). The total biomass yield (weight of the whole radish plant) and economic yield (weight of the fleshy roots) were recorded.

2.4.4. Determination of Physical and Chemical Properties of Soil

Soil physicochemical properties were determined using the five-point sampling method for soil samples collected from the 0–20 cm depth. Each treatment was replicated five times. The soil samples were air-dried and then ground and sieved. The fraction that passed through a 0.25 mm sieve was saved for the determination of soil available nutrients and organic matter content. Another portion was immediately sieved through a 2 mm sieve for the determination of soil microbial biomass and the three microbial groups. These samples were immediately transferred back to the lab in an ice box and stored in a freezer at $-80\text{ }^{\circ}\text{C}$ for DNA extraction and sequencing. A third portion was sieved through a 1 mm sieve and saved for the determination of soil enzyme activity.

Soil pH value was determined by the potentiometric method using a pH meter (PHS-3C pH meter, Shanghai Yi Electrical Scientific Instruments Co., Ltd., Shanghai, China). The organic matter content of the soil was determined using the potassium dichromate oxidation method. The total nitrogen content of the soil was determined using acid digestion and the Kjeldahl method with an ATN-300 Fully Automatic Kjeldahl Nitrogen Determinator, Shanghai Hongji Instrument Equipment Co., Ltd., Shanghai, China. The soil hydrolyzable nitrogen content was determined using alkaline extraction–cultivation and volumetric methods with a Constant Temperature and Humidity Incubator, model: LHS-100CL, Shanghai Yiheng Scientific Instrument Co., Ltd., Shanghai, China. The total phosphorus content of the soil was determined using alkaline fusion and molybdenum antimony anti-colorimetry with an Ultraviolet Spectrophotometer (UV 8000, Shanghai Yuan analysis Instrument Co., Ltd., Shanghai, China). The available phosphorus content of the soil was determined using ammonium fluoride–hydrochloric acid extraction and molybdenum antimony anti-colorimetry [29].

Soil urease activity (S-UE activity) was determined by the colorimetric method using indophenol blue [30], with 1 mg $\text{NH}_3\text{-N}$ produced per g of soil sample per d at $37\text{ }^{\circ}\text{C}$ as one unit of enzyme activity (U/g soil sample); soil sucrase activity (S-SC activity) was determined by the colorimetric method using 3,5-dinitrosalicylic acid [31], with 1 mg reduced sugar produced per g of soil sample per d at $37\text{ }^{\circ}\text{C}$ as one unit of S-SC activity (U/g soil sample); soil catalase activity (S-CAT activity) was determined by UV spectrophotometry [32], with 1 mg reduced sugar produced per g of air-dried soil sample per day. S-CAT

activity was determined by UV spectrophotometry, and 1 mmol of degradation catalyzed H_2O_2 per g of air-dried soil sample per day was defined as a unit of enzyme activity. Soil alkaline phosphatase activity (S-AKP activity) was determined by the colorimetric method of disodium phosphate [33], and 1 μmol of phenol was defined as a unit of enzyme activity per g of soil sample released per d under 37 °C incubation. One unit of enzyme activity (U/g soil sample) was defined as 1 μmol of phenol per d of soil sample incubated at 37 °C.

2.4.5. Soil DNA Extraction and 16s rDNA Sequencing

Total microbial genomic DNA was extracted from 12 samples using the E.Z.N.A.[®] soil DNA Kit (Omega Bio-tek, Norcross, GA, USA) according to the manufacturer's instructions. The quality and concentration of DNA were determined by 1.0% agarose gel electrophoresis and a NanoDrop2000 spectrophotometer (Thermo Scientific, GA, USA) and kept at −80 °C prior to further use. The hypervariable region V3–V4 of the bacterial 16S rRNA gene was amplified with primer pairs 338F (5'-ACTCCTACGGGAGGCAGCAG-3') and 806R(5'-GGACTACHVGGGTWTCTAAT-3') [34] by T100 Thermal Cycler PCR thermocycler (BIORAD, GA, USA). The PCR reaction mixture included 4 μL 5 × Fast Pfu buffer, 2 μL 2.5 mM dNTPs, 0.8 μL each primer (5 μM), 0.4 μL Fast Pfu polymerase, 10 ng of template DNA, and ddH₂O to a final volume of 20 μL . PCR amplification cycling conditions were as follows: initial denaturation at 95 °C for 3 min, followed by 27 cycles of denaturing at 95 °C for 30 s, annealing at 55 °C for 30 s, extension at 72 °C for 45 s, single extension at 72 °C for 10 min, and end at 4 °C [35]. The PCR product was extracted from 2% agarose gel and purified using the PCR Clean-Up Kit (YuHua, Shanghai, China) according to the manufacturer's instructions and quantified using Qubit 4.0 (Thermo Fisher Scientific, GA, USA).

2.4.6. Soil Microbial Community Analysis

After demultiplexing, the resulting sequences were quality-filtered with fastp (0.19.6) [36] and merged with FLASH (v1.2.11) [37]. Then, the high-quality sequences were de-noised using DADA2(ver1.8)) [38] pipeline with recommended parameters, which obtains single-nucleotide resolution based on error profiles within samples. QIIME2's visualization tools, such as alpha-group-significance, were used to visualize the calculated alpha diversity index. The relationships between soil environmental factor and soil microbial communities were analyzed using redundancy analysis (RDA).

2.4.7. Statistical Analysis

The experiment was laid out under a randomized complete block design with four treatments, and each treatment was repeated five times. The data preprocessing and graph preparation were performed using Microsoft Excel 2007 (Microsoft Corporation, Redmond, WA, USA). The statistical analysis, including one-way analysis of variance (ANOVA) and post hoc multiple comparisons using the Duncan method ($\alpha = 0.05$), was conducted using the Statistical Package for the Social Sciences (SPSS) version 20.0 software (IBM Corporation, Armonk, NY, USA). The data presented in the figures and tables are expressed as the mean \pm standard error.

3. Results

3.1. Effect of Chemical Fertilizer Reduction with Organic Fertilizer Application on Agronomic Traits of Radish

The experimental results of growth parameters throughout the entire growth period of radish are presented in Table 3. Results revealed that compared with the control, the T₂ treatment significantly influenced the maximum functional leaf width, root diameter, and root length at the harvest stage, showing an increase of 47.62%, 9.78%, and 7.23%, respectively ($p < 0.05$). However, the T₂ treatment resulted in a significant reduction of 23.04% in the thick skin of radish fleshy roots compared with the control ($p < 0.05$). The effects of different fertilizer treatments on the biomass yield of radish fleshy roots and leaves varied. The accumulation trend of dry matter in radish was similar among different

treatments, with an increasing trend as the growth period progressed. Compared with the control, all other treatments significantly increased the dry weight of fleshy roots. The T₂ treatment showed an increase of 44.12% in dry matter accumulation compared with the control ($p < 0.05$). These findings indicate that an appropriate reduction in chemical fertilizer application combined with the application of organic fertilizers can promote the growth of maximum functional leaf width, root diameter, root length, and dry weight of radish fleshy roots.

Table 3. Effect of different treatments on the growth of radish.

Treatments	Maximum Functional Leaf Width (cm)	Root Diameter at Harvest Time (mm)	Long Root Length at Harvest Time (cm)	Leaf Dry Weight (kg plant ⁻¹)	Dry Weight of Fleshy Roots (kg plant ⁻¹)	Thick Skin (mm)
Control	15.75 ± 0.75 c	56.67 ± 4.05 b	41.50 ± 0.58 c	0.18 ± 0.19 bc	0.34 ± 0.01 c	3.82 ± 0.23 a
T ₁	18.50 ± 1.45 b	56.45 ± 1.56 b	43.00 ± 0.82 b	0.22 ± 0.20 a	0.50 ± 0.01 a	3.23 ± 0.14 b
T ₂	23.25 ± 1.48 a	62.21 ± 2.74 a	44.50 ± 0.58 a	0.18 ± 0.18 b	0.49 ± 0.01 a	2.94 ± 0.07 c
T ₃	19.08 ± 0.79 b	56.30 ± 1.17 b	41.75 ± 0.50 c	0.15 ± 0.17 c	0.45 ± 0.01 b	3.00 ± 0.05 c

Different lowercase letters in the same column indicate significant differences among treatments ($n = 3, p < 0.05$).

3.2. The Effect of Chemical Fertilizer Reduction with Organic Fertilizer on the Nutrient Content and Radish Quality

As shown in Table 4, the results of the radish quality test indicated that compared with the control, different percentages of reductions in fertilizer dose were able to improve the flesh quality of the radish roots, with the T₂ treatment showing the most significant effects. Compared with the control, the T₂ treatment increased the contents of vitamin C, soluble sugars, sulforaphane soluble solids, and titratable acidity in the radish roots by 10.62%, 2.15%, 50.00%, 26.90%, and 43.90%, respectively, while the cellulose content decreased by 10.14% ($p < 0.05$). This indicates that reducing chemical fertilizers combined with the application of organic biofertilizers can effectively improve the nutrient content and quality of radishes.

Table 4. Effect of different fertilizer treatments on the concentrations of nutritional quality components of radish fleshy roots.

Treatments	Vitamin C (mg/g. FW)	Soluble Sugar (mg/g. FW)	Sulforaphane (mg/g. FW)	Soluble Solids (%)	Titratable Acid (%)	Cellulose (mg/g. FW)
Control	56.38 ± 1.14 b	52.93 ± 0.07 c	0.06 ± 0.00 c	3.94 ± 0.06 c	0.41 ± 0.01 c	164.33 ± 3.22 b
T ₁	61.97 ± 1.75 a	54.13 ± 0.09 a	0.08 ± 0.00 ab	5.04 ± 0.08 a	0.60 ± 0.01 a	193.00 ± 5.29 a
T ₂	62.37 ± 1.00 a	54.07 ± 0.08 ab	0.09 ± 0.00 a	5.00 ± 0.13 a	0.59 ± 0.01 a	147.67 ± 6.66 c
T ₃	57.75 ± 1.26 b	53.63 ± 0.43 b	0.08 ± 0.00 b	4.71 ± 0.40 b	0.54 ± 0.02 b	132.33 ± 7.23 d

Different lowercase letters in the same column indicate significant differences among treatments ($n = 3, p < 0.05$).

3.3. Effect of Chemical Fertilizer Reduction and Organic Fertilizer Application on Radish Yield

From our study, it is evident that reducing chemical fertilizer application significantly increases radish yield (Table 5). Both per-plant yield and total yield were increased by reducing chemical fertilizer dose compared with the control, which was briefly in the following order: T₂ > T₁ > T₃ > control. This indicates that reducing chemical fertilizer application and incorporating organic fertilizer is beneficial for the translocation of photosynthates from the aboveground parts to the belowground fleshy roots of radish. Crucially, the treatment T₂ exhibited the highest increase in total radish yield, being 1.06 and 1.07 times higher than the T₁ and T₃ treatments, respectively. This demonstrates that an appropriate reduction in chemical fertilizer application combined with the application of organic biofertilizers can enhance radish yield.

Table 5. Effect of different fertilizer treatments on radish yield.

Treatments	Per Plant Yield (g)	Equivalent Total Yield (kg h ⁻¹)	Yield Increase Rate (%)
Control	1493.80 ± 67.59 b	26,189.24 ± 1185.01 b	-
T ₁	1586.68 ± 61.23 b	27,817.67 ± 1073.49 b	6.22
T ₂	1778.42 ± 99.43 a	29,572.10 ± 1516.26 a	12.92
T ₃	1573.58 ± 54.20 b	27,588.06 ± 950.23 b	5.34

Different lowercase letters in the same column indicate significant differences among treatments (n = 3, p < 0.05).

3.4. Effect of Chemical Fertilizer Reduction with Organic Fertilizer Application on Soil Nutrient Content

Table 6 shows that there were no significant differences in soil hydrolyzable nitrogen content among the treatments. The T₂ treatment significantly increased the total nitrogen content in the soil by 7.69% compared to the control. The total phosphorus content in the soil was 14.29% higher in the T₂ treatment compared to the control treatments. The available phosphorus content did not differ significantly between the T₂ treatment and the control. These results indicate that appropriate reduction of the amount of chemical fertilizer application has a significant effect on increasing soil nutrient content.

Table 6. Effect of different fertilizer treatments on soil nutrient content.

Treatments	Organic Matter g/kg	TN %	TP %	HN mg/kg	AP mg/kg
Control	19.47 ± 0.23 b	1.04 ± 0.05 b	0.07 ± 0.00 b	91.03 ± 0.25 a	70.27 ± 0.49 a
T ₁	19.67 ± 0.25 ab	1.10 ± 0.04 ab	0.07 ± 0.00 b	90.90 ± 3.82 a	63.60 ± 2.10 b
T ₂	19.93 ± 0.25 ab	1.12 ± 0.02 a	0.08 ± 0.00 a	88.27 ± 3.59 a	68.03 ± 1.10 a
T ₃	20.37 ± 0.76 a	1.05 ± 0.02 b	0.07 ± 0.00 a	89.93 ± 2.29 a	70.43 ± 0.67 a

Different lowercase letters in the same column indicate significant differences among treatments (n = 3, p < 0.05). TN: total nitrogen; HN: hydrolyzable nitrogen; TP: total phosphorus; AP: available phosphorus; OM: organic matter.

3.5. Effect of Chemical Fertilizer Reduction with Organic Fertilizer Application on Soil Enzyme Activity

According to Table 7, different fertilizer application rates had a significant impact on soil enzyme activities. Compared with the control, the activities of urease, sucrase, alkaline phosphatase, and catalase in soil were increased by 11.13%, 44.30%, 26.41%, and 9.33% (p < 0.05). These results suggest that an appropriate reduction in chemical fertilizer application combined with the application of organic biofertilizers enhances the activities of urease, sucrase, alkaline phosphatase, and catalase enzymes in the soil.

Table 7. Effect of different fertilizer treatments on soil enzyme activities.

Treatments	S-UE μg/d/g	S-SC mg/d/g	S-AKP μmol/d/g	S-CAT mmol/d/g
Control	455.33 ± 27.50 ab	4.56 ± 0.35 c	1.06 ± 0.09 b	15.00 ± 0.50 b
T ₁	416.00 ± 28.00 c	5.28 ± 0.35 b	1.26 ± 0.03 a	14.57 ± 0.35 b
T ₂	506.00 ± 40.45 a	6.58 ± 0.18 a	1.34 ± 0.12 a	16.40 ± 0.82 a
T ₃	490.67 ± 27.65 a	6.39 ± 0.19 a	0.87 ± 0.06 c	12.97 ± 0.55 c

Different lowercase letters in the same column indicate significant differences among treatments (n = 3, p < 0.05).

3.6. Effect of Chemical Fertilizer Reduction Combined with Organic Fertilizer Application on the Soil Bacterial Community

At a 97% similarity level, the analysis of OTU sequences detected 41 bacterial phyla. As shown in Figure 1a, the dominant bacterial phyla in the soil were *Proteobacteria* (26.25–30.70%), *Acidobacteria* (15.74–22.49%), *Actinobacteria* (14.73–18.84%), *Chloroflexi* (9.53–10.47%), *Firmicutes* (5.37–6.44%), and *Gemmatimonadetes* (4.71–6.41%). Compared with the control

treatment, the relative abundance of *Proteobacteria* and *Actinobacteria* increased by 12.25% and 10.43% in the T₂ treatment, while the relative abundance of *Acidobacteria* and *Chloroflexi* decreased by 25.30% and 8.98%, respectively.

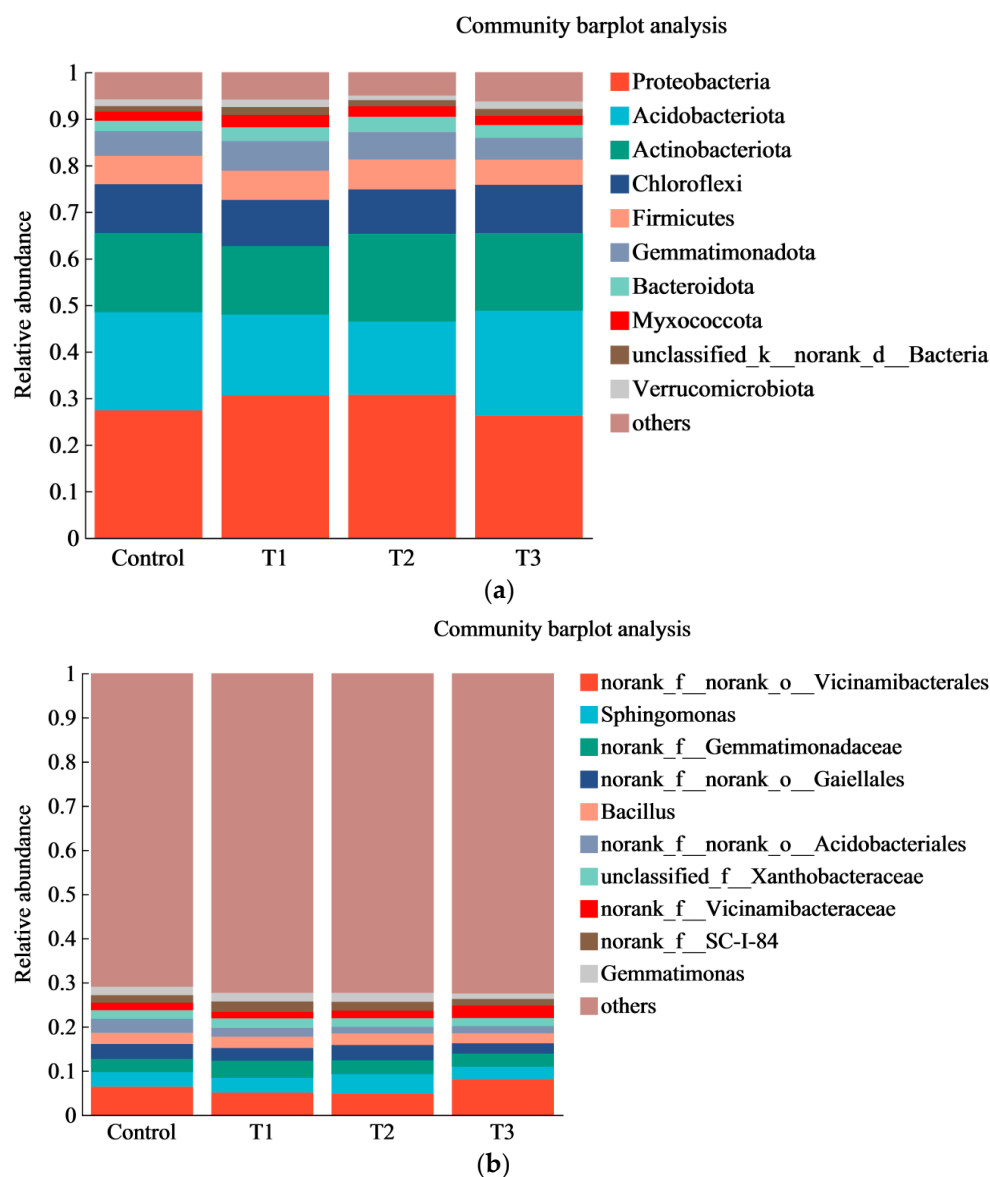


Figure 1. Relative abundance of soil bacteria at the phylum and genus level: (a) for phylum level; (b) for genus level. CK: 375 kg/ha chemical fertilizer + 4500 kg/ha organic fertilizer; T₁: 12% reduction in chemical fertilizer + 4500 kg/ha organic fertilizer; T₂: 20% reduction in chemical fertilizer + 4500 kg/ha organic fertilizer; T₃: 28% reduction in chemical fertilizer + 4500 kg/ha organic fertilizer.

In the sequence analysis, a total of 1007 bacterial genera were detected. As shown in Figure 1b, the top 10 bacterial genera accounted for approximately 29.01% of the total genera [36]. Excluding undefined bacterial genera, the dominant bacterial genera in the soil were *Vicinamibacterales* (4.77–8.10%), *Sphingomonas* (2.80–4.45%), *Gemmatimonadaceae* (2.99–3.88%), *Gaiellales* (2.29–3.6%), *Bacillus* (2.25–2.61%), and *Acidobacteriales* (1.50–3.22%). Compared with the control treatment, the relative abundance of *Sphingomonas* in the T₂ treatment increased by 34.44%.

3.7. RDA Analysis of Soil Physicochemical Properties and Environmental Factors

The correlation between the community composition of bacterial phyla at the soil level and environmental factors through redundancy analysis (RDA) (Figure 2) indicates significant influence of environmental factors on bacterial communities. The first and second axes explain 17.17% and 5.23% of the bacterial community variation, respectively. Using Canoco5 software-constrained ordination, four environmental factors contributed the most to the composition of bacterial communities, namely organic matter (OM, 4.05%), total nitrogen (TN, 14.46%), total phosphorus (TP, 33.27%), and available phosphorus (AP, 42.66%). The dominant phyla *Acidobacteriota* and *Chloroflexi* show a positive correlation with TN, TP, and AP while exhibiting a negative correlation with OM. The phylum *Proteobacteria* shows a positive correlation with TN but a negative correlation with OM, TP, and AP. These results suggest that organic matter ($p = 0.845$), total nitrogen ($p = 0.551$), total phosphorus ($p = 0.144$), and available phosphorus ($p = 0.096$) are the major driving factors shaping the composition of soil bacterial communities.

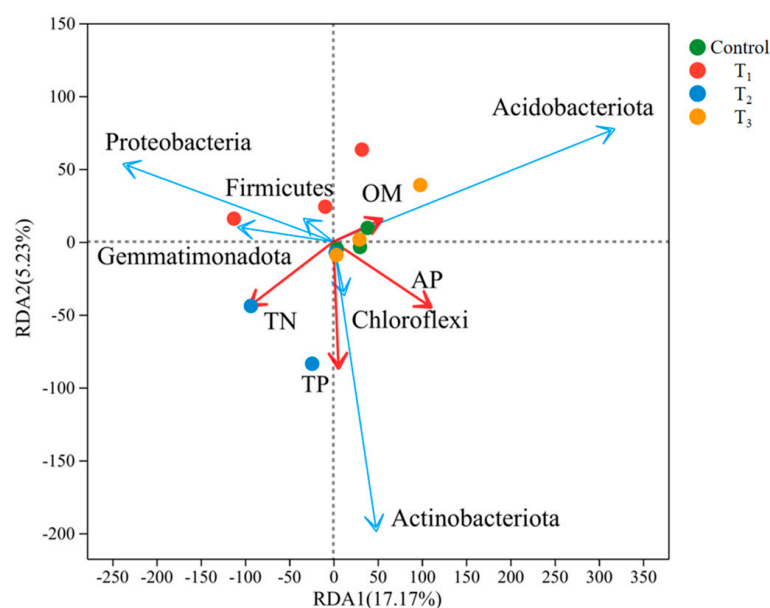


Figure 2. Redundancy analysis (RDA) of the soil bacterial community of field soil and environmental factors.

4. Discussion

Numerous studies have demonstrated that the appropriate combination of organic and inorganic fertilizers is beneficial for crop growth. With balanced fertilization, soil fertility was preserved by increasing product yield and soil nutrient content, and the use of chemical fertilizers in cabbage cultivation was reduced [39]. Likewise, tomato growth was promoted by appropriate application of organic fertilizers, increasing fruit yield and quality [40]. Shi et al. also showed that reducing chemical fertilizers combined with the application of organic fertilizers significantly improved the stem thickness and seed yield of cotton [41]. Table 4 indicates that a 20% reduction in chemical fertilizers combined with organic fertilizer application significantly increases the content of vitamin C, soluble sugars, soluble solids, soluble proteins, titratable acidity, and glucosinolates in radish fleshy roots. These findings are consistent with the research results of Zhang et al. [42] and Tang et al. [43] on the effects of organic/inorganic fertilizer ratios on vegetable quality. Qiu et al. [44] found that under conventional fertilization conditions, reducing chemical fertilizer application by 50% and applying organic fertilizer significantly improved the fresh cob and fresh grain yield as well as dry matter accumulation in sweet corn. Zhou et al. [45] also reported that compared with conventional fertilization, optimizing fertilization and replacing 30% of chemical fertilizers with organic fertilizers increased radish yield. In our study, the radish

plants treated with reduced doses of chemical fertilizers combined with organic fertilizers exhibited greater functional leaf width and fleshy root dry weight than the control group (control) receiving conventional fertilization (Table 3). Crop biomass is closely related to nutrient accumulation, which serves as the foundation for biomass accumulation and yield formation [46]. Under the same application rate of organic fertilizers, the radish plants treated with a 20% reduced level of chemical fertilizers showed the maximum functional leaf width, root length, root diameter, and fleshy root dry weight during the harvest period. As shown in Table 5, the T₂ treatment (20% reduction in chemical fertilizers + 4500 kg ha⁻¹ of organic fertilizers) achieved the highest individual and total radish yield, with increases of 19.05% and 12.92%, respectively, compared with the control group. As shown in Figure 1, the abundance of beneficial microorganisms such as *Actinomyces*, *Proteus*, *Burkholderia*, and *Bacillus* in soil increased significantly when chemical fertilizer and organic fertilizer were applied together, possibly due to the improved efficiency of plant nutrient acquisition [47]. Furthermore, beneficial microorganisms improve the biological properties of the soil and enhance nutrient release to meet the nutritional requirements for crop growth [48–50], thereby promoting radish growth, improving nutritional quality, and increasing yield.

The application of chemical fertilizer combined with organic fertilizer has a comprehensive effect on improving crop yield, which is related to soil nutrient content, soil enzyme activity, and the improvement of soil microbial community [51]. They collectively promote the formation of crop yield. Therefore, reducing the use of chemical fertilizer and properly applying organic fertilizer can effectively regulate the structure of soil microbial communities, enhance various enzyme activities, and improve soil fertility to achieve the highest yield and realize efficient nutrient utilization. Consistent with our results (Tables 6 and 7), a significant correlation between soil nutrient content and the activity of soil enzymes such as peroxidase, urease, alkaline phosphatase, and sucrose was revealed in previous study, indicating an inseparable relationship between soil enzyme activity and soil nutrient content [52]. During the radish harvest period, soil total nitrogen content, total phosphorus content, and available phosphorus content were higher in the T₂ treatment receiving a 20% reduced level of chemical fertilizer than in the control treatment, with soil total phosphorus content and available phosphorus content significantly higher than the T₁ treatment. Proper reduction of chemical fertilizer combined with the application of bioorganic fertilizer can increase the content of available nutrients in the soil. The reason may be that bioorganic fertilizer contains a large amount of organic matter and various active bacteria, and microorganisms continuously promote the release of nitrogen, phosphorus, and potassium from the slow-release state of the soil during their life activities, thereby improving the soil microecological environment and increasing the content of available nutrients in the soil [53]. This is also consistent with the research results of Zhou et al. [54]. Soil enzyme activity can be widely used to evaluate soil fertility and the effectiveness of various agricultural measures and fertilization practices. Fertilization treatments primarily alter the composition, biomass, and metabolic processes of soil microbial communities, thereby changing soil enzyme activity [51]. Studies have shown that the combined application of bioorganic fertilizer can significantly enhance the activity of soil sucrase, alkaline phosphatase, peroxidase, and urease [55,56]. In this study, compared with the control treatment, the 20% reduction in chemical fertilizer combined with the application of organic fertilizer significantly increased the activity of soil urease, peroxidase, sucrase, and alkaline phosphatase, which is consistent with the research results of Wang et al. [51] and Qu et al. [57]. On the one hand, organic fertilizer can increase soil organic matter content, providing the necessary carbon source for soil microorganisms and synergistically improving the stability and biological properties of soil aggregates [58], thereby providing a favorable environment for microorganisms and increasing the substrates required by soil enzymes [59]. On the other hand, bioorganic fertilizer increases soil microbial biomass and enhances microbial activity, and the combined application of reduced chemical fertilizer and application of organic fertilizer has a better effect.

In this study, the dominant bacterial phyla in the four different fertilization treatments were similar and included *Proteobacteria*, *Actinobacteria*, *Acidobacteria*, *Chloroflexi*, and *Firmicutes*, which are consistent with the dominant bacterial groups found in previous studies [60,61]. However, there were certain differences in the relative abundance of dominant groups among the treatments. The T₂ treatment, which involved a reduction in chemical fertilizer combined with organic fertilizer application, enriched soil nutrient resources, promoted the growth of *Proteobacteria* and *Actinobacteria*, and inhibited the growth of oligotrophic bacteria such as *Chloroflexi* and *Acidobacteria* [62,63]. Studies have shown that the increased abundance of *Actinobacteria* under the T₂ treatment may play an important role in improving agricultural soil quality, as it has been found to degrade organic matter in fertilizers in field practices [64,65]. In the control treatment, the relative abundance of *Acidobacteria* and *Chloroflexi* was higher than in the other treatments, as *Acidobacteria* and *Chloroflexi* tend to be enriched in low-fertility soils [66]. Chemoheterotrophy and aerobic chemoorganotrophy are important ecological functions related to carbon cycling [67]. Compared with the control treatment, the T₂ treatment significantly increased the abundance of chemoheterotrophic functional populations, indicating a significant increase in bacterial population abundance in the T₂ treatment. This is beneficial for the release of more inorganic nutrients that are available for crop utilization, which may be one of the reasons for the increase in crop yield under the T₂ treatment. The main bacterial groups in soil microbial communities are *Acidobacteria* and *Proteobacteria* [68], and they play crucial roles in soil nutrient cycling and organic matter decomposition processes [69]. In this study, we observed that *Proteobacteria*, *Acidobacteria*, and *Actinobacteria* were the most abundant phyla. Compared with the T₂ treatment, the control treatment significantly reduced the relative abundance of *Proteobacteria* and *Actinobacteria* in the soil, indicating that excessive fertilizer application may lead to soil fertility decline. Bacteria belonging to the phylum *Acidobacteria* may have numerical dominance and metabolic activity in soil samples, suggesting their important role in the biogeochemical cycling of rhizosphere soil [70]. According to the research by Li et al., soil organic matter, total phosphorus, available phosphorus, and total nitrogen content are the main environmental factors influencing the structure of rhizosphere soil bacterial communities during the rice maturation stage [71]. Additionally, fertilization can significantly alter soil pH and nutrient cycling [72,73]. Furthermore, Zhang et al. indicated that pH value, soil moisture content, and water-soluble organic carbon are the major factors influencing the structure of soil bacterial communities, with soil moisture content being considered the most critical environmental factor [74,75]. Therefore, based on the results presented in Figure 2, the contents of ammonia nitrogen (AN), total nitrogen (TN), total phosphorus (TP), and pH value in the soil are the main factors determining the characteristics of soil bacterial communities, while other factors such as soil organic matter and available phosphorus (AP) have relatively minor effects. Chai et al. showed a positive correlation between ammonia nitrogen content in the soil and the relative abundance of *Proteobacteria* and *Bacteroidetes*, while ammonia nitrogen content was negatively correlated with the relative abundance of *Acidobacteria* [76]. In this study, we found that the relative abundance of *Acidobacteria* and *Chloroflexi*, two dominant phyla, was positively correlated with TN, TP, and AP in the soil while negatively correlated with organic matter content. This suggests that the relative abundance of these phyla is influenced by nutrient content, with nitrogen and phosphorus elements positively regulating their relative abundance, while organic matter may have a negative impact. These results are partially consistent with previous studies [77].

5. Conclusions

In conclusion, the T₂ treatment (20% reduction in chemical fertilizer + 4500 kg·ha⁻¹ of organic fertilizer) was found to be the optimal combination for reducing chemical fertilizer together with the application of organic fertilizer in local radish cultivation. This approach can reduce the use of chemical fertilizers, increase yield, promote radish growth, improve nutrient quality, enhance soil enzyme activity, increase soil microbial population, and

increase the content of readily available nutrients in the soil. The improvements in soil health are likely to have long-term benefits for soil fertility and sustainability. Our work highlights the potential of integrating organic fertilizers into conventional farming practices as a promising approach for enhancing crop yield and quality while preserving soil health. Moreover, a balanced use of chemical and organic fertilizers offers a viable solution to the challenges posed by over-reliance on chemical fertilizers, paving the way for sustainable crop production and future research in this area.

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