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Effects of zeolite and microbial inoculants on vegetables and soil under continuous cropping in protected cultivation

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Abstract

Background and Aims:

Continuous cropping obstacles threaten the sustainability of protected vegetable production by degrading soil structure, disrupting microbial communities, and reducing yield and quality. Zeolite amendment and functional microbial agents are promising countermeasures, but their combined effects in long-term continuously cropped soils have rarely been verified in the field. This study tested whether zeolite combined with microbial agents could synergistically improve soil quality and increase crop yield and quality.

Methods

A two-factor field experiment was conducted at three long-term (> 10 years) continuous cropping sites in Hebei Province, China: Kuancheng (tomato, *Solanum lycopersicum* L. cv. Yanyu), Luanping (tomato, *S. lycopersicum* L. cv. Xiaohongfan), and Chongli (colored pepper, *Capsicum annuum* L. cv. Meihong No. 1). Zeolite was applied at 0 and 3 t hm^{-2} (F0, F1) in factorial combination with three microbial agent treatments (J0, no agent; J1, *Bacillus amyloliquefaciens*; J2, *Bacillus subtilis* + *Trichoderma harzianum*).

Results

Combined application increased yield by 33.5%–41.3% over the control (F0J0), increased soluble sugar, vitamin C, and soluble solids contents, and decreased titratable acidity. It also reduced bulk density by 8.7%–11.5% and EC by 33.7%–56.7%, increased bacterial and actinomycete counts by 152.4%–360.8% and 105.9%–176.6%, respectively, and raised the bacterial-to-fungal (B/F) ratio by 146.3%–326.6%.

Conclusion

Zeolite at 3 t hm^{-2} combined with *Bacillus subtilis* and *Trichoderma harzianum* (F1J2) performed best at all sites. By improving soil properties and restoring microbial community structure, this combination alleviates continuous cropping obstacles and offers a practical option for protected vegetable production.

1. Introduction

Continuous cropping obstacles are a major constraint on agricultural sustainability worldwide, reducing crop yield and quality (Ma et al. 2023). In intensive protected vegetable production, long-term continuous cropping degrades soil structure, reduces nutrient availability, disrupts soil microbial communities, promotes the accumulation of autotoxic substances, and aggravates soil-borne diseases (Li et al. 2021b; Ma et al. 2024; Ma et al. 2023). These problems arise from interacting changes in soil physicochemical properties, autotoxin accumulation, and microbial communities (Li et al. 2021a; Ma et al. 2024).

Control of continuous cropping obstacles has shifted from broad-spectrum suppression of the entire soil microbial community to ecosystem-based management centered on regulating that community (Ma et al. 2025; Wang et al. 2024). Plant growth-promoting rhizobacteria (PGPR) have emerged as a key tool for enhancing crop stress tolerance and yield (Ren et al. 2025); they promote growth through mechanisms such as nitrogen fixation, phosphate solubilization, potassium mobilization, and phytohormone production (Meena et al. 2017; Vaitiekūnaitė et al. 2021). Under continuous cropping stress, plants may recruit beneficial rhizosphere bacteria through root exudates (Ma et al. 2025; Rolfe et al. 2019), suggesting that introducing functional microorganisms could help regulate the soil microbial community (Ma et al. 2025).

Accordingly, three functionally complementary microorganisms were selected in this study to remediate continuously cropped soils. *Bacillus subtilis* is a widely distributed, stress-tolerant plant growth-promoting bacterium (Hashem et al. 2019). In continuously cropped soils, *B. subtilis* reduces salt accumulation by regulating microbial community structure and metabolic activity, while also enhancing soil enzyme activities, accelerating organic matter transformation, and improving nutrient supply (Bi et al. 2025; Hidri et al. 2019). *Trichoderma harzianum*, a classic biocontrol fungus, suppresses pathogens and promotes growth through mycoparasitism, production of cell wall-degrading enzymes, competition for rhizosphere niches, and induction of systemic resistance (Ghazanfar et al. 2018; Rashid 2025). It can also increase soil available nutrients (N, P, K) and organic matter, modify rhizosphere bacterial community structure, and enhance plant tolerance to abiotic stress (Wei et al. 2023). *Bacillus amyloliquefaciens* inhibits soil-borne pathogens by producing antimicrobial lipopeptides (surfactin, iturin, and fengycin) and activates plant antioxidant enzyme systems that strengthen defense against disease and stress (Zalila-Kolsi et al. 2023; Zhang et al. 2024). These complementary functions suggest that combined use may improve both plant growth and pathogen suppression; however, the efficacy of such combinations in long-term continuously cropped soils has not been verified under field conditions.

Zeolite is a natural aluminosilicate mineral with a porous framework, high cation exchange capacity, and selective adsorption capacity (Ramesh et al. 2011). In soil, zeolite can improve aggregate structure, increase porosity, reduce bulk density, adsorb NH_4^+ and K^+, and mitigate salt accumulation (Cataldo et al. 2021). Zeolite thus acts mainly on soil physicochemical conditions, whereas microbial agents act on the soil microbial community. Whether this complementarity produces synergistic effects in long-term continuously cropped protected vegetable systems, and how the two amendments should be combined to improve soil fertility and microbial community structure, remain insufficiently investigated under field conditions.

A two-factor field experiment was therefore conducted at three typical protected vegetable production sites in Hebei Province, China, each continuously cropped for more than 10 years (Kuancheng tomato, Luanping tomato, and Chongli colored pepper). Six treatments combined two zeolite levels (0 and 3 t hm^{-2}) with three microbial agent levels (no agent; *Bacillus amyloliquefaciens* biocontrol + growth-promoting strains; and *Bacillus subtilis* + *Trichoderma harzianum*). Their effects on soil physicochemical properties, microbial community structure, plant growth, and yield were evaluated to provide a basis for

the combined use of mineral amendments and microbial inoculants in managing continuous cropping problems in protected vegetable systems.

2. Materials and methods

2.1 Experimental sites and materials

The experiment was conducted in 2025–2026 at three typical sites of continuous protected vegetable cropping in Hebei Province: a solar greenhouse at Zirun Sightseeing Park, Kuancheng Manchu Autonomous County, Chengde; a solar greenhouse of Xinshuo Agriculture Co., Ltd., Luanping County, Chengde; and a plastic greenhouse of Wanjiale Agricultural Vegetable Co., Ltd., Chongli District, Zhangjiakou. Tomato had been continuously cropped for 10 years at the first two sites, and colored pepper for 10 years at Chongli. The test crops were tomato (*Solanum lycopersicum* L.) cv. Yanyu (Kuancheng) and cv. Xiaohongfan (Luanping), and colored pepper (*Capsicum annuum* L.) cv. Meihong No. 1 (Chongli). All soils were cinnamon soils sampled from the 0–20 cm plough layer; their basic physicochemical and microbial properties are listed in Table 1.

Table 1
Physicochemical and microbial properties of the continuously cropped soils at the three sites

Location	Continuous cropping years	BD (g/cm³)	pH	EC (μS/cm)	Fungal count (×10⁴ CFU g⁻¹)	Bacterial count (×10⁶ CFU g⁻¹)	Actinomycete count (×10⁶ CFU g⁻¹)
Kuancheng	10 years	1.79 ± 0.10	6.92 ± 0.17	1012 ± 25.2	0.43 ± 0.05	0.75 ± 0.02	1.13 ± 0.03
Luanping	10 years	1.41 ± 0.06	6.96 ± 0.12	504 ± 15.3	4.08 ± 0.08	3.81 ± 0.12	1.48 ± 0.14
Chongli	10 years	1.19 ± 0.04	7.39 ± 0.15	551 ± 15.3	2.19 ± 0.09	3.92 ± 0.16	1.27 ± 0.08

The zeolite used in this study was purchased from Beijing Guotou Shengshi Chengde Technology Co., Ltd. The microbial agents included *Bacillus subtilis* (Ehrenberg 1835) Cohn 1872 (viable cell count ≥ 4.0×10^{10} CFU g^{-1}) and *Trichoderma harzianum* Rifai 1969 (viable cell count ≥ 1.0×10^{9} CFU g^{-1}), both purchased from Hubei Qiming Bioengineering Co., Ltd., and *Bacillus amyloliquefaciens* Priest et al. 1987 (biocontrol and growth-promoting strains, each with a viable cell count ≥ 4.5×10^{9} CFU mL^{-1}), purchased from Hebei Runwo Biotechnology Co., Ltd.

2.2 Experimental design

A two-factor randomized complete block design was used. Factor A was the zeolite application rate (0 t hm^{-2}, F0; 3 t hm^{-2}, F1), and factor B was the microbial agent (no agent, J0; *Bacillus amyloliquefaciens* biocontrol and growth-promoting strains, J1; *Bacillus subtilis* + *Trichoderma harzianum*, J2). The six treatment combinations (F0J0, F0J1, F0J2, F1J0, F1J1, and F1J2) each had three replicates of 30 plants, and the same design was used at all three sites (Table 2). The experiments ran from March to June 2025 at Kuancheng, from November 2025 to March 2026 at Luanping, and from April to October 2025 at Chongli.

Table 2
Experimental treatments combining zeolite and microbial agents

Treatment	Zeolite 0 t hm^{-2} (F0)	Zeolite 3 t hm^{-2} (F1)
Control without microbial agent (J0)	F0J0	F1J0
B. amyloliquefaciens (BC + PG) (J1)	F0J1	F1J1
B. subtilis + *T. harzianum* (J2)	F0J2	F1J2

Before transplanting, zeolite was spread evenly over the ridges at the designated rates and incorporated to a depth of 20 cm by plowing. Microbial agents were diluted according to the manufacturer's instructions and applied as root drenches after transplanting. Irrigation, fertilization, pest control, and all other management practices were identical across treatments. Soil was sampled before transplanting and after the final harvest from 10 points per plot, arranged in an S-shaped pattern, in the 0–20 cm plough layer. The samples from each plot were thoroughly mixed, reduced to approximately 1 kg by quartering, and placed in sterile self-sealing bags, giving three independent samples per treatment. Each sample was divided into two portions: one was air-dried and passed through a 2-mm sieve for physicochemical analysis, and the other was stored at 4°C for microbial enumeration.

2.3 Measured parameters and methods

Soil bulk density was determined by the cutting ring method. Soil pH and electrical conductivity (EC) were measured with a Leici pH meter (PHS-3C) and a conductivity meter (DDS-307A), respectively, at a soil-to-water ratio of 1:5. Bacteria, fungi, and actinomycetes were enumerated by plate counting on beef extract-peptone agar, Rose Bengal agar, and Gause's No. 1 agar, respectively, and the bacterial-to-fungal ratio (B/F) was calculated as the bacterial count divided by the fungal count. Soluble solids, soluble sugar, vitamin C (Vc), and titratable acidity were determined following Cao et al. (2007). Fruits were harvested successively throughout the harvest period, and fruit number and yield per plant were calculated from the cumulative harvest records of each plot. Single fruit weight was determined as the mean of 20 randomly selected fruits per plot (three plots per treatment).

2.4 Statistical analysis

All data were processed in Excel 2025 and analyzed in IBM SPSS Statistics 26. Two-way analysis of variance (ANOVA) was used to test the main effects of zeolite and microbial agent and their interaction on soil physicochemical properties, soil microbial populations, yield, and fruit quality. Where interaction or

main effects were significant, treatments were compared by one-way ANOVA followed by Duncan's multiple range test ($P < 0.05$). Relationships among growth indicators, soil properties, yield, and fruit quality were examined by Spearman rank correlation. Treatments were evaluated comprehensively by principal component analysis (PCA) combined with the membership function method. All indicators were first standardized by Z-score normalization to remove dimensional effects; PCA was then performed, and principal components (PCs) with eigenvalues greater than 1.0 were retained. The membership function method was applied to the retained PCs to calculate a comprehensive score (D value) for each treatment, as follows:

(1) The comprehensive index values of each treatment on the extracted principal components were standardized using the membership function:

$U(Xi) = (Xi - Xmin) / (Xmax - Xmin)$; i = 1, 2, 3, ..., n

where Xmin and Xmax are the minimum and maximum comprehensive index values of all treatments on a given principal component, respectively, and Xi is the comprehensive index value of the i-th treatment.

(2) The weight of each extracted principal component was calculated as:

$$wp = \lambda p / \sum_{p=1}^{p} \lambda p$$

where wp is the weight of the p-th principal component, and λp is its contribution rate.

(3) The comprehensive score D of each treatment was calculated as: $D = \sum_{p=1}^{p} (wp \times U(Xi))$

where D is the comprehensive score of each treatment.

3. Results

3.1 Effects of zeolite and microbial agents on crop yield and fruit quality

The main effects of zeolite and microbial agents on fruit number per plant were not significant at any site, but their interaction was highly significant ($P < 0.01$ in Kuancheng; $P < 0.001$ in Luanping and Chongli). At all three sites, zeolite alone (F1J0) reduced fruit number per plant, and adding microbial agents offset this reduction. Responses to microbial agents differed among sites: at F0, fruit number declined significantly with increasing microbial agent level in Kuancheng and Luanping ($P < 0.05$) but did not differ in Chongli. At F1, J2 consistently performed best, significantly increasing fruit number by 9.6%–12.5% relative to J0. Zeolite significantly reduced fruit number only at J0, not at J1 or J2. Fruit number was markedly lower in Chongli because the crop was colored pepper.

Zeolite and microbial agent both significantly affected single fruit weight at all three sites ($P < 0.001$), as did their interaction ($P < 0.01$ in Kuancheng, $P < 0.001$ in Luanping, and $P < 0.05$ in Chongli). At F0, single

fruit weight increased progressively with microbial agent level (J0 < J1 < J2), whereas at F1, J1 performed best. Because J2 alone significantly increased fruit weight, zeolite added little to J2 (F0J2 and F1J2 did not differ significantly in Kuancheng and Chongli), whereas it significantly increased fruit weight at J0 and J1. F1J1 produced the heaviest fruit at each site, 36.4%–42.4% above F0J0.

Yield per plant was highly significantly affected by zeolite and microbial agents at all three sites ($P < 0.001$). At both zeolite levels, J1 and J2 significantly increased yield relative to J0, and zeolite increased yield at every microbial agent level; the two factors acted additively, with a significant interaction only in Luanping ($P < 0.01$). Yield per plant was highest in F1J1 and F1J2 at all three sites (the two did not differ significantly in Kuancheng and Luanping), and F1J2 exceeded F0J0 by 33.5%–41.3%.

Soluble sugar responded consistently to microbial agents at all three sites: J1 and J2 were significantly higher than J0 at both F0 and F1. The effect of zeolite, however, varied by site: it significantly increased soluble sugar only at J0 and J2 in Kuancheng, only at J1 and J2 in Chongli, and at all microbial agent levels in Luanping. Soluble sugar was highest in the combined treatments (F1J2 in Kuancheng, F1J1 in Luanping, and F1J2 in Chongli), exceeding F0J0 by 29.4%, 55.2%, and 17.9%, respectively.

Zeolite significantly increased Vc content at every microbial agent level at all three sites. Microbial agents significantly increased Vc at F1 (highest in J2), whereas at F0, only J1 and J2 in Luanping were significantly higher than J0; no significant differences were found in Kuancheng or Chongli. F1J2 had the highest Vc content at all three sites, 39.1%–53.9% above F0J0.

Titratable acidity was significantly lower in J1 and J2 than in J0 at both zeolite levels in Kuancheng and Luanping; in Chongli, this reduction occurred at F0 but not at F1. The effect of zeolite varied by site: it reduced titratable acidity at all microbial agent levels in Luanping, only at J0 in Chongli, and at none in Kuancheng. The lowest titratable acidity occurred in F0J1 in Kuancheng (42.5% lower), F1J2 in Luanping, and F0J2 in Chongli, which were significantly lower than F0J0 by 37.5%–71.2%.

In Luanping and Chongli, J1 and J2 significantly increased soluble solids relative to J0 at both zeolite levels. In Kuancheng, J1 and J2 were both significantly higher than J0 at F0, whereas J2 was highest at F1. Zeolite significantly increased soluble solids only at J0 in Kuancheng, but at all microbial agent levels in Luanping and Chongli. Soluble solids were highest in F1J2 at all three sites, significantly exceeding F0J0 by 14.2%–42.6%.

3.2 Effects of zeolite and microbial agents on soil physicochemical properties

Bulk density responded similarly at all three sites. Microbial agents had no significant effect at either zeolite level, whereas zeolite significantly reduced bulk density at every microbial agent level, by 8.7%–11.5% relative to F0J0. Two-way ANOVA confirmed a highly significant zeolite effect at all three sites ($P < 0.001$), no significant microbial agent effect at any site, and a significant interaction only in Kuancheng ($P < 0.01$).

Soil pH ranged from 6.93 to 7.24 across all treatments and sites. No significant differences were observed among treatments in Luanping or Chongli; in Kuancheng, the zeolite × microbial agent interaction was significant ($P < 0.05$), although neither main effect was significant.

EC followed a generally consistent pattern across the three sites. Zeolite significantly reduced EC at every microbial agent level at all sites. Microbial agents had no significant effect in Kuancheng; in Luanping and Chongli, they did not differ at F0, whereas at F1, J1 produced the lowest EC, significantly lower than J0 and J2. EC was lowest in F1J1 at all three sites, 33.7%–56.7% below F0J0, with the largest reduction in Chongli. Two-way ANOVA showed a highly significant zeolite effect at all three sites ($P < 0.001$), no significant microbial agent effect at any site, and a significant interaction at all three sites ($P < 0.05$ in Kuancheng, $P < 0.001$ in Luanping, and $P < 0.05$ in Chongli).

3.3 Effects of zeolite and microbial agents on soil microbial populations

Bacterial counts were significantly or highly significantly affected by zeolite and microbial agents at all three sites, whereas the interaction was significant in Kuancheng and Luanping ($P < 0.05$ or $P < 0.001$) but not in Chongli. At both zeolite levels, J1 and J2 significantly increased bacterial counts compared with J0; zeolite increased bacterial counts at every microbial agent level, more so at J1 and J2 than at J0. The highest counts were recorded in F1J1 or F1J2: 13.62 × 10^6 CFU g^{-1} in Kuancheng (F1J2, 307.8% higher than F0J0), 57.60 × 10^6 CFU g^{-1} in Luanping (F1J1, 360.8% higher), and 8.43 × 10^6 CFU g^{-1} in Chongli (F1J1, 152.4% higher).

Fungal counts responded differently at the three sites. In Kuancheng, the main effects of zeolite and microbial agents and their interaction were all highly significant ($P < 0.001$). In Luanping, only zeolite had a significant effect ($P < 0.001$). In Chongli, the microbial agent effect and the interaction were significant ($P < 0.01$ or < 0.001), but the zeolite effect was not.

In Kuancheng and Chongli, J1 and J2 had significantly lower fungal counts than J0 at F1, whereas differences among microbial agents at F0 were inconsistent. Zeolite significantly increased fungal counts at J0 (by 53.9% in Kuancheng and 62.3% in Chongli) but had no significant effect, or even reduced them, at J1 and J2. Fungal counts at both sites were highest in F1J0 (2.57 × 10^4 CFU g^{-1} in Kuancheng and 2.50 × 10^4 CFU g^{-1} in Chongli) and lowest in F0J1 and F1J2. Luanping showed a different pattern: microbial agents had no significant effect at either zeolite level, whereas zeolite significantly increased fungal counts at every microbial agent level, by 30.6%–63.2%.

The B/F ratio followed a consistent trend at all three sites: J1 and J2 were significantly higher than J0 at both zeolite levels, with larger increases at F1, and zeolite increased the B/F ratio at J1 and J2. The highest B/F ratios occurred in F1J2 (Kuancheng), F1J1 (Luanping), and F1J1 (Chongli), exceeding F0J0 by 326.6%, 161.6%, and 146.3%, respectively.

Actinomycete counts were highly significantly affected by zeolite and microbial agents at all three sites ($P < 0.001$), whereas the interaction was significant only in Luanping. At both zeolite levels, J1 and J2

significantly increased actinomycete counts compared with J0, and zeolite significantly increased them at every microbial agent level, most strongly at J0 (65.7% in Kuancheng, 41.0% in Chongli, and 99.6% in Luanping). Actinomycete counts were highest in F1J2 at all three sites: 93.39 × 10^6 CFU g^{-1} in Kuancheng (105.9% higher than F0J0), 69.70 × 10^6 CFU g^{-1} in Luanping (176.6% higher), and 115.72 × 10^6 CFU g^{-1} in Chongli (107.3% higher).

3.4 Correlation analysis and comprehensive evaluation

Correlations were generally consistent across the three sites (Fig. 4). Yield per plant was significantly positively correlated with single fruit weight and significantly negatively correlated with EC, consistent with fruit weight contributing to yield and with an inverse relationship between EC and yield. Vc, soluble sugar, and soluble solids were positively correlated with one another, suggesting that nutritional and flavor quality can be improved together. Yield was also positively correlated with soluble sugar and Vc, indicating that higher yield did not come at the expense of fruit quality. EC was positively correlated with bulk density, suggesting that soil compaction was accompanied by salt accumulation, and significantly negatively correlated with bacterial counts and the B/F ratio. Bacterial and actinomycete counts were positively, and fungal counts negatively, correlated with the B/F ratio, suggesting that a higher proportion of bacteria and actinomycetes was associated with a healthier soil microecology. Finally, bacterial counts and the B/F ratio were positively correlated with yield per plant, supporting a positive role of an optimized microbial community in yield improvement.

To compare treatments across all indicators simultaneously, PCA was performed on the 14 measured indicators. The negative indicators (bulk density, EC, and titratable acidity) were first multiplied by − 1 so that all indicators followed a "higher-is-better" direction. The first three PCs explained 95.17%, 94.53%, and 97.55% of the total variance in Kuancheng, Luanping, and Chongli, respectively (Fig. 5), capturing most of the information in the original indicators.

Loading patterns were highly consistent across the three sites (Table 4). At all sites, PC1 was dominated by single fruit weight, yield per plant, bacterial count, actinomycete count, B/F ratio, soluble sugar, Vc, soluble solids, the sign-reversed bulk density and EC also loaded positively on it. PC1 therefore represented "high yield, superior quality, abundant beneficial microorganisms, and low soil salinity" and was defined as the "yield–quality–microbe comprehensive factor". PC2 was dominated by fungal count at all sites, whereas PC3 mainly reflected residual variation in fruit number and soil physicochemical properties.

Table 4
Component matrix of principal component analysis (PCA)

Indicator	Kuancheng			Luanping			Chongli		
	PC1	PC2	PC3	PC1	PC2	PC3	PC1	PC2	PC3
Fruit_num	0.061	−0.458	−0.393	0.027	0.393	−0.779	0.055	−0.520	0.008
Fruit_wt	0.324	0.023	0.158	0.289	0.124	0.289	0.321	0.100	0.135
Yield	0.331	−0.132	0.017	0.290	0.245	0.012	0.335	−0.041	0.041
BD	0.266	0.317	−0.216	0.231	−0.402	−0.305	0.244	0.222	−0.470
pH	0.038	−0.400	−0.450	0.109	0.485	0.096	0.112	−0.448	−0.250
EC	0.277	0.240	−0.292	0.264	−0.299	−0.286	0.238	0.195	−0.511
Fungi	−0.018	0.523	−0.266	0.286	−0.262	−0.053	−0.068	0.515	−0.031
Bact	0.344	−0.096	−0.042	0.288	0.226	−0.108	0.309	−0.034	0.324
Act	0.339	0.105	−0.080	0.292	0.107	0.297	0.336	−0.011	−0.024
B/F	0.317	−0.232	0.061	0.260	0.346	0.011	0.298	−0.202	0.209
Sugar	0.313	−0.061	0.167	0.309	−0.082	−0.004	0.332	−0.054	0.062
Vc	0.325	0.026	−0.216	0.308	−0.076	−0.065	0.309	−0.024	−0.310
TA	0.172	−0.225	0.500	0.305	−0.115	0.132	0.202	0.336	0.410
SS	0.258	0.227	0.279	0.309	−0.084	0.024	0.324	0.082	0.134

Notes: Fruit_num, fruit number; Fruit_wt, single fruit weight; Yield, yield per plant; BD, bulk density; pH, soil pH; EC, electrical conductivity; Fungi, fungal count; Bact, bacterial count; Act, actinomycete count; B/F, bacterial-to-fungal ratio; Sugar, soluble sugar; Vc, vitamin C (ascorbic acid); TA, titratable acidity; SS, soluble solids content.

In the PC1–PC2 space (Fig. 5), F1J2 and F1J1 lay at the positive end of PC1 at all three sites and F0J0 at the negative end, clearly separating these treatments. Because the positive direction of PC1 represents overall improvement in yield, quality, and beneficial microbial indicators, the extreme positive position of F1J2 indicates that it performed best across the measured indicators.

The membership function analysis (Table 5) ranked the treatments consistently across the three sites. F1J2 had the highest D value (0.666, 0.912, and 0.700 in Kuancheng, Luanping, and Chongli, respectively), followed by F1J1 (0.622, 0.889, and 0.694), whereas F0J0 ranked last (0.000, 0.126, and 0.002). The D values of F1J2 and F1J1 were substantially higher than those of the single-application treatments, indicating that combining zeolite with microbial agents produced greater overall improvement than either amendment alone. Notably, in Luanping and Chongli, the D value of F1J0 (0.482 and 0.514, respectively) was lower than that of F0J2 (0.607 and 0.555, respectively), indicating that J2 alone outperformed zeolite

alone. Overall, F1J2 was the most effective treatment for alleviating continuous cropping obstacles at all three sites under the tested conditions.

Table 5
Comprehensive scores and rankings based on the membership function method

Treatment	Kuancheng		Luanping		Chongli	
	D value	Rank	D value	Rank	D value	Rank
F0J0	0.000	6	0.126	6	0.002	6
F0J1	0.414	5	0.415	5	0.418	5
F0J2	0.506	4	0.607	3	0.555	3
F1J0	0.580	3	0.482	4	0.514	4
F1J1	0.622	2	0.889	2	0.694	2
F1J2	0.666	1	0.912	1	0.700	1

4. Discussion

4.1 Effects of combined zeolite and microbial agent application on crop yield and quality

The combined application increased yield mainly by enlarging individual fruits rather than by increasing fruit number, indicating that fruit expansion, not fruit set, was the dominant yield pathway under our conditions (Table 3). This agrees with the growth-promoting role of *Bacillus* species, which synthesize indole-3-acetic acid (IAA) via the indole-3-pyruvic acid pathway to stimulate cell division and expansion (Ji et al. 2021); IAA-producing rhizobacteria have been shown to increase tomato fruit weight rather than fruit number (Listanto et al. 2019). Zeolite contributed complementary physical effects: its high cation exchange capacity and porous framework retain water and nutrients in the root zone, sustaining uptake during fruit development and mitigating drought and salinity stress (Cataldo et al. 2021; Prisa 2020). The additive—rather than antagonistic—interaction between these mechanisms likely explains why the combined treatment outperformed either component alone.

Table 3
Effects of zeolite and microbial agents on crop yield and yield components

Location	Treatment	Fruits per plant (No.)	Single fruit weight (kg)	Yield per plant (kg)
Kuancheng	F0J0	18.73 ± 1.10Aa	0.12 ± 0.0036Bc	2.27 ± 0.09Ab
Kuancheng	F0J1	17.93 ± 0.71Aab	0.14 ± 0.009Bb	2.52 ± 0.25Ba
Kuancheng	F0J2	17.30 ± 0.62Bb	0.16 ± 0.002Aa	2.69 ± 0.09Ba
Kuancheng	F1J0	17.07 ± 0.61Bb	0.14 ± 0.005Ab	2.45 ± 0.14Ab
Kuancheng	F1J1	18.40 ± 0.69Aa	0.17 ± 0.004Aa	3.04 ± 0.15Aa
Kuancheng	F1J2	18.83 ± 0.55Aa	0.16 ± 0.003Aa	3.04 ± 0.06Aa
Kuancheng	F	ns	***	***
Kuancheng	J	ns	***	***
Kuancheng	F*J	**	**	ns
Luanping	F0J0	19.10 ± 0.20Aa	0.103 ± 0.002Bc	1.97 ± 0.05Bb
Luanping	F0J1	18.23 ± 0.21Bb	0.125 ± 0.002Bb	2.28 ± 0.02Ba
Luanping	F0J2	17.70 ± 0.36Bc	0.131 ± 0.002Ba	2.31 ± 0.01Ba
Luanping	F1J0	17.43 ± 0.15Bc	0.123 ± 0.002Ab	2.15 ± 0.04Ab
Luanping	F1J1	18.70 ± 0.10Ab	0.141 ± 0.002Aa	2.63 ± 0.06Aa
Luanping	F1J2	19.10 ± 0.10Aa	0.138 ± 0.002Aa	2.63 ± 0.05Aa
Luanping	F	ns	***	***
Luanping	J	ns	***	***
Luanping	F*J	***	***	**
Chongli	F0J0	7.20 ± 0.20Aa	0.17 ± 0.005Bc	1.26 ± 0.03Bb
Chongli	F0J1	6.97 ± 0.15Aa	0.20 ± 0.006Bb	1.47 ± 0.08Ba
Chongli	F0J2	6.90 ± 0.10Aa	0.22 ± 0.007Aa	1.53 ± 0.05Ba
Chongli	F1J0	6.40 ± 0.20Bb	0.21 ± 0.006Ab	1.42 ± 0.05Ab
Chongli	F1J1	7.03 ± 0.15Aa	0.24 ± 0.012Aa	1.71 ± 0.07Aa
Chongli	F1J2	7.20 ± 0.20Aa	0.23 ± 0.008Aa	1.78 ± 0.06Aa

Note: Different lowercase letters indicate significant differences among microbial agent treatments within the same zeolite level ($P < 0.05$); different uppercase letters indicate significant differences among zeolite levels within the same microbial agent treatment ($P < 0.05$). *, **, and *** denote significance at $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively; ns indicates not significant.

Location	Treatment	Fruits per plant (No.)	Single fruit weight (kg)	Yield per plant (kg)
Chongli	F	ns	***	***
Chongli	J	ns	***	***
Chongli	F*J	***	*	ns

Note: Different lowercase letters indicate significant differences among microbial agent treatments within the same zeolite level ($P < 0.05$); different uppercase letters indicate significant differences among zeolite levels within the same microbial agent treatment ($P < 0.05$). *, **, and *** denote significance at $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively; ns indicates not significant.

The improvement in fruit quality was driven mainly by the inoculated microbes and reinforced by zeolite. *Bacillus subtilis* and *Trichoderma harzianum* secrete phytohormones and organic metabolites that enhance nutrient uptake and shift plant metabolism toward the accumulation of sugars, organic acids, and antioxidants (Shoresh et al. 2010); accordingly, pre-harvest application of *B. subtilis* has been shown to raise soluble solids, titratable acidity, and ascorbic acid contents in tomato fruit (da Silva Marques et al. 2025). Zeolite supplies potassium through ion exchange, a cation essential for translocating photosynthates into developing fruit and for activating sugar-metabolizing enzymes; potassium-enriched zeolite substrates have been reported to increase ascorbic acid and soluble solids in tomato (Bernardi 2007). Together, microbial metabolic stimulation and improved mineral nutrition explain the higher soluble sugar, Vc, and soluble solids contents in F1J2 and F1J1 (Fig. 1).

4.2 Improvement of soil physicochemical properties by combined zeolite and microbial agent application

Deterioration of soil physicochemical properties contributes substantially to continuous cropping obstacles (Wang et al. 2017). In the present study, zeolite significantly reduced bulk density and EC at all three sites, whereas pH remained within a narrow neutral range (Fig. 2), consistent with the well-documented capacity of zeolite to improve soil structure and alleviate salinity (Mondal et al. 2021; Venati et al. 2018).

The reduction in bulk density reflects the physical architecture of zeolite: its three-dimensional framework of aluminosilicate tetrahedra contains abundant micro- and mesopores that increase total porosity and aggregate stability once incorporated into soil (Szatanik-Kloc et al. 2021). In parallel, its high cation exchange capacity allows it to adsorb excess salt cations (e.g., Na^+, K^+, Ca^{2+}, Mg^{2+}) from the soil solution and release them selectively through molecular sieving, thereby reducing EC and mitigating secondary salinization (Cataldo et al. 2021; Javaid et al. 2024; Mondal et al. 2021). These two mechanisms reinforce each other: a looser, well-aggregated structure promotes salt leaching, and the removal of dispersive salts in turn stabilizes aggregates.

The limited pH response may partly reflect the initial pH of the study soils (6.93–7.24; Table 1), at which the weakly alkaline buffering capacity of zeolite is barely expressed; its pH-modulating effect is more

evident in strongly acidified or alkalinized soils (Ramesh et al. 2011). The site-dependent EC reduction (Chongli > Luanping > Kuancheng) likely reflects differences in initial salt composition and soil texture. Chongli had the lowest bulk density and a moderate initial EC (Table 1), which may have favored ion exchange by zeolite, whereas the smaller reduction in Kuancheng, despite its highest initial EC, suggests that site-specific soil properties modulated the salt-adsorption efficiency of zeolite; the underlying mechanisms warrant further investigation.

Microbial agents had no significant main effects on bulk density, pH, or EC, indicating that their direct physicochemical amelioration was limited under the present conditions. However, the zeolite × microbial agent interaction on EC was significant at all three sites, with F1J1 achieving the largest salt reduction (Fig. 2C). This synergy may involve a two-step feedback: zeolite first lowers the rhizosphere salt concentration through ion exchange, creating a favorable microenvironment for the colonization and metabolism of functional microorganisms (Bikkinina et al. 2020); the colonizing microbes then help maintain rhizosphere ionic balance—for example, *Bacillus* species secrete extracellular polymeric substances that bind soil particles into aggregates and promote salt leaching—thereby prolonging the salt-reduction effect (Bi et al. 2025). Thus, zeolite acts mainly through physical pathways (improving soil structure and adsorbing salts) and microbial agents through biological pathways (restructuring the microbial community); because the two are complementary rather than interchangeable, their combination outperformed either alone (Lang et al. 2024).

4.3 Regulation of the soil microbial community by combined zeolite and microbial agent application

Microbial community imbalance is a core biological cause of continuous cropping obstacles (Chen et al. 2022). In the present study, inoculation significantly increased bacterial and actinomycete counts relative to the non-inoculated control (Fig. 3A, 3D), which can be attributed to two complementary mechanisms. First, as PGPR, *Bacillus subtilis* and *Bacillus amyloliquefaciens* secrete organic acids and phosphatases that solubilize soil nutrients, supplying readily available substrates for both themselves and indigenous microorganisms (Hnini et al. 2025), while the biocontrol fungus *Trichoderma harzianum* contributes through mycoparasitism and the secretion of cell-wall-degrading enzymes (Ghazanfar et al. 2018). Second, Bacillus species are strong rhizosphere colonizers that occupy favorable ecological niches and indirectly promote beneficial groups such as actinomycetes (Santoyo et al. 2021).

Combined application increased bacterial counts significantly more than either amendment alone (Fig. 3A). This synergy likely arises because the porous framework of zeolite provides a habitat that shelters introduced microorganisms from competition with indigenous microbes and from environmental stress (Prisa 2020), and because the looser, better-aerated soil created by zeolite favors aerobic beneficial bacteria (Bikkinina et al. 2020).

Fungal responses varied by site. In Kuancheng and Chongli, zeolite alone increased fungal counts, but this increase was suppressed when microbial agents (particularly J2) were added, producing a highly significant zeolite × microbial agent interaction at both sites (Fig. 3B). In Luanping, by contrast, zeolite

increased fungal counts regardless of microbial agent, and neither the microbial agent effect nor the interaction was significant. This discrepancy is most plausibly explained by initial fungal abundance, which was substantially higher in Luanping than in Kuancheng and Chongli (Table 1). Although inoculant establishment was not measured here, introduced microorganisms are often outcompeted for colonization sites and nutrients when the indigenous fungal community is already large, and inoculation frequently fails to establish (Kaminsky et al. 2019). A single-season application may therefore be insufficient to reverse fungal dominance in heavily colonized soils. Because long-term continuous cropping progressively depletes beneficial microbes while pathogens accumulate, restoring the balance requires sustained ecological intervention (Ding et al. 2024); sites with severe microbial obstacles may need repeated applications or higher doses of microbial agents.

The marked increase in the B/F ratio reflects an overall improvement in microbial community structure. Long-term continuous cropping typically shifts the soil microbiome from bacterial toward fungal dominance, whereas a higher B/F ratio is associated with a healthier soil ecosystem and greater disease resistance (Chen et al. 2022). The combined treatments increased the B/F ratio by 146.3% to 326.6% relative to the control (Fig. 3C), indicating that they effectively reversed the fungal-dominated imbalance induced by continuous cropping. Moreover, the B/F ratio was significantly positively correlated with yield per plant, suggesting that an optimized microbial community structure is an important biological basis for yield improvement.

5. Conclusions

Across three continuously cropped protected vegetable sites in Hebei Province (Kuancheng tomato, Luanping tomato, and Chongli colored pepper), combined application of zeolite and microbial agents improved soil physicochemical properties by reducing bulk density and EC, optimized microbial community structure by increasing bacterial counts and the B/F ratio, and ultimately enhanced crop yield and quality. The zeolite × microbial agent interaction was significant for several outcomes, although the interaction for yield differed among sites. Membership function scores ranked treatments combining zeolite with microbial agents above zeolite or microbial agents alone at all sites, indicating that the benefit of combined application was consistent across regions. By integrating the soil-ameliorating function of zeolite with the microecological restoration function of microbial agents, this strategy offers a feasible approach for alleviating continuous cropping obstacles in protected vegetable production.

This study has several limitations. The field experiment at each site spanned only a single growing season, whereas the soil-improvement effects of zeolite are cumulative; multi-year trials are needed to verify the persistence of these effects and determine the optimal application frequency. In addition, the three sites were studied in different seasons (spring, winter–spring, and spring–autumn for Kuancheng, Luanping, and Chongli, respectively), which may have introduced confounding effects of temperature and irradiance on soil microbial activity and crop growth; future studies should standardize the experimental period across sites. The efficacy of zeolite is also influenced by soil texture, initial salinity, and other site-specific factors, so its applicability across ecological regions requires further validation. Future work

should optimize the ratio of zeolite rate to microbial agent concentration through multi-factor gradient experiments and investigate the microbiological mechanisms underlying zeolite–microbial agent–soil interactions.

Abbreviations

ANOVA, analysis of variance; B/F, bacterial-to-fungal ratio; BD, bulk density; CFU, colony-forming units; EC, electrical conductivity; IAA, indole-3-acetic acid; PCA, principal component analysis; PGPR, plant growth-promoting rhizobacteria; SS, soluble solids; TA, titratable acidity; Vc, vitamin C.

Declarations

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Data Availability Statement

The original contributions presented in this study are included in the article/supplementary material; further inquiries can be directed to the corresponding authors.

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Figures

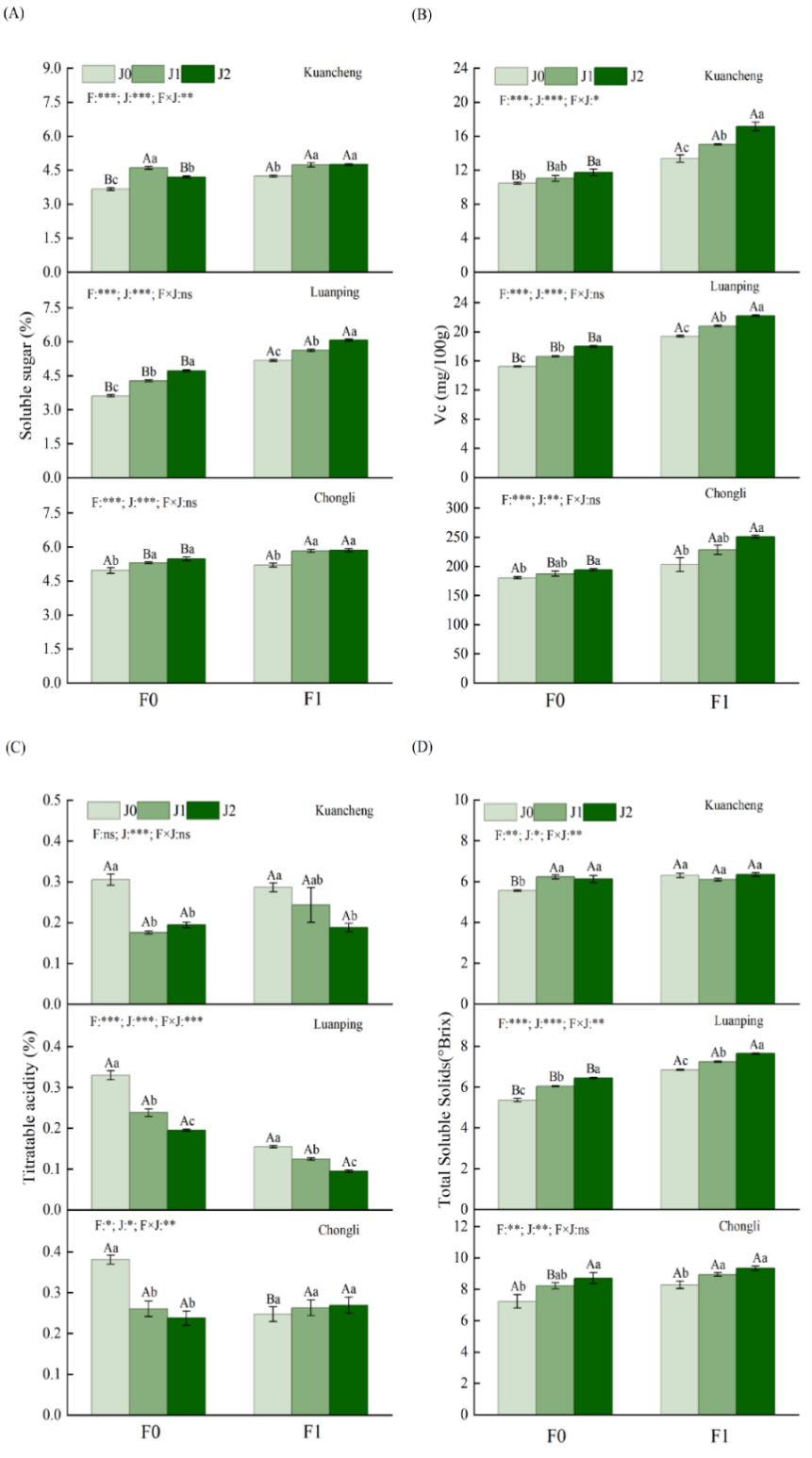


Figure 1

Effects of zeolite and different microbial agents on crop quality. F0, zeolite at 0 t hm⁻²; F1, zeolite at 3 t hm⁻²; J0, control without microbial agent; J1, *Bacillus amyloliquefaciens*(biocontrol + growth-promoting strains); J2, *Bacillus subtilis* + *Trichoderma harzianum*. Different uppercase letters indicate significant differences between zeolite levels within the same microbial agent treatment; different lowercase letters

indicate significant differences among microbial agent treatments within the same zeolite level ($P < 0.05$).

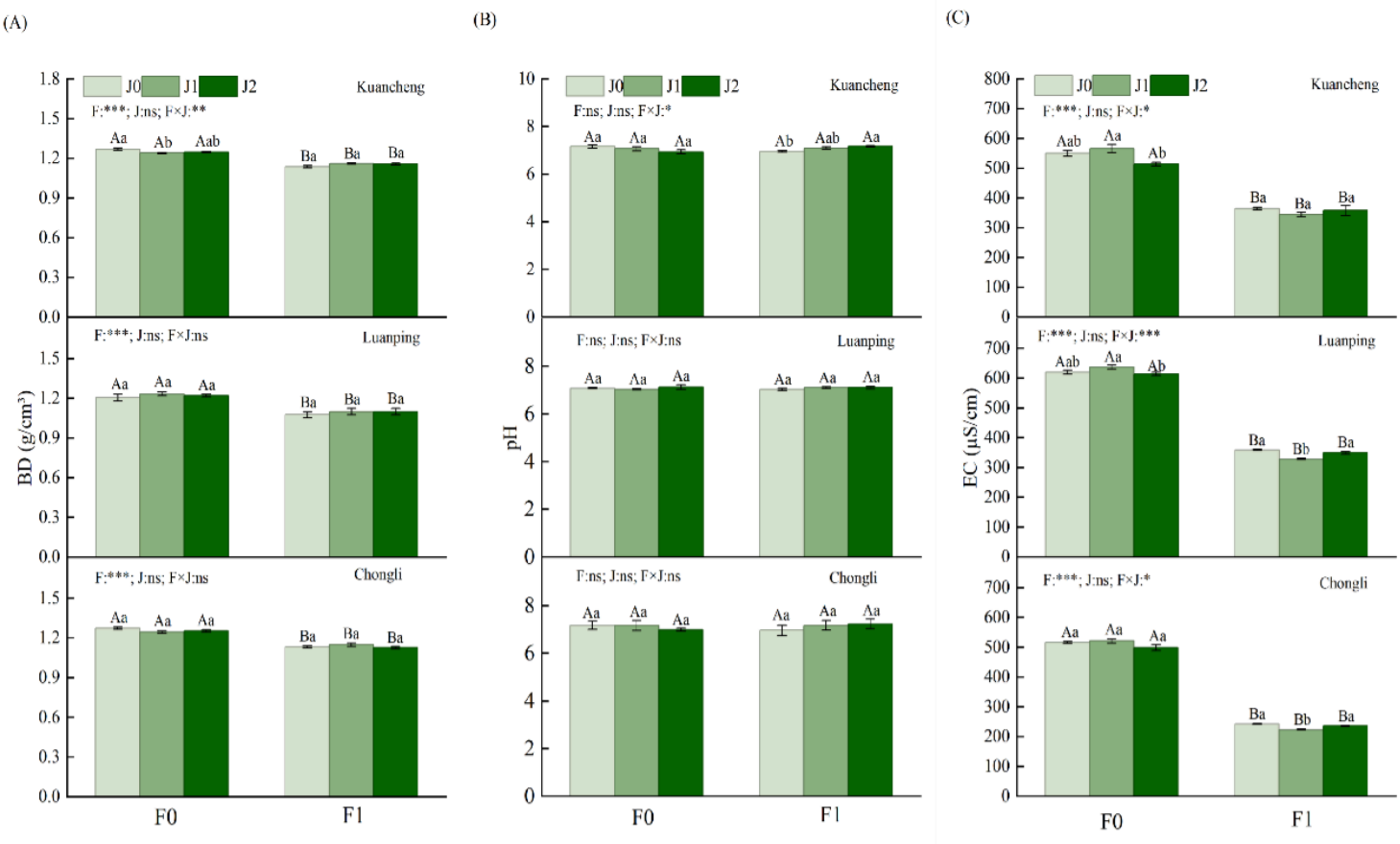


Figure 2

Effects of zeolite and different microbial agents on soil bulk density (A), pH (B), and electrical conductivity (C) across the three sites. F0, zeolite at 0 t hm^{-2}; F1, zeolite at 3 t hm^{-2}; J0, control without microbial agent; J1, *Bacillus amyloliquefaciens* (biocontrol + growth-promoting strains); J2, *Bacillus subtilis* + *Trichoderma harzianum*.

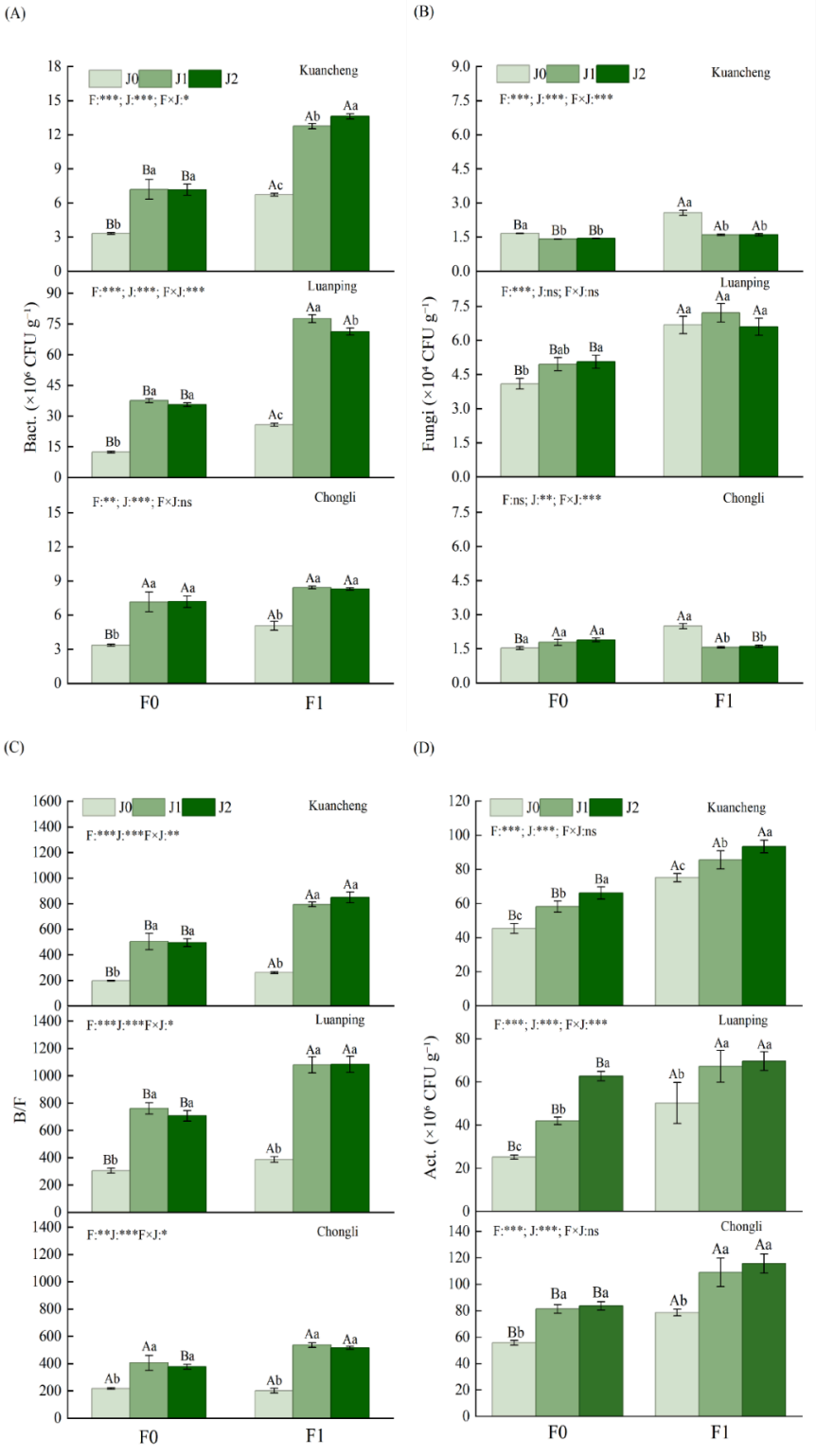


Figure 3

Effects of zeolite and different microbial agents on soil microbial populations across the three sites: (A) bacterial count, (B) fungal count, (C) bacterial-to-fungal (B/F) ratio, and (D) actinomycete count.

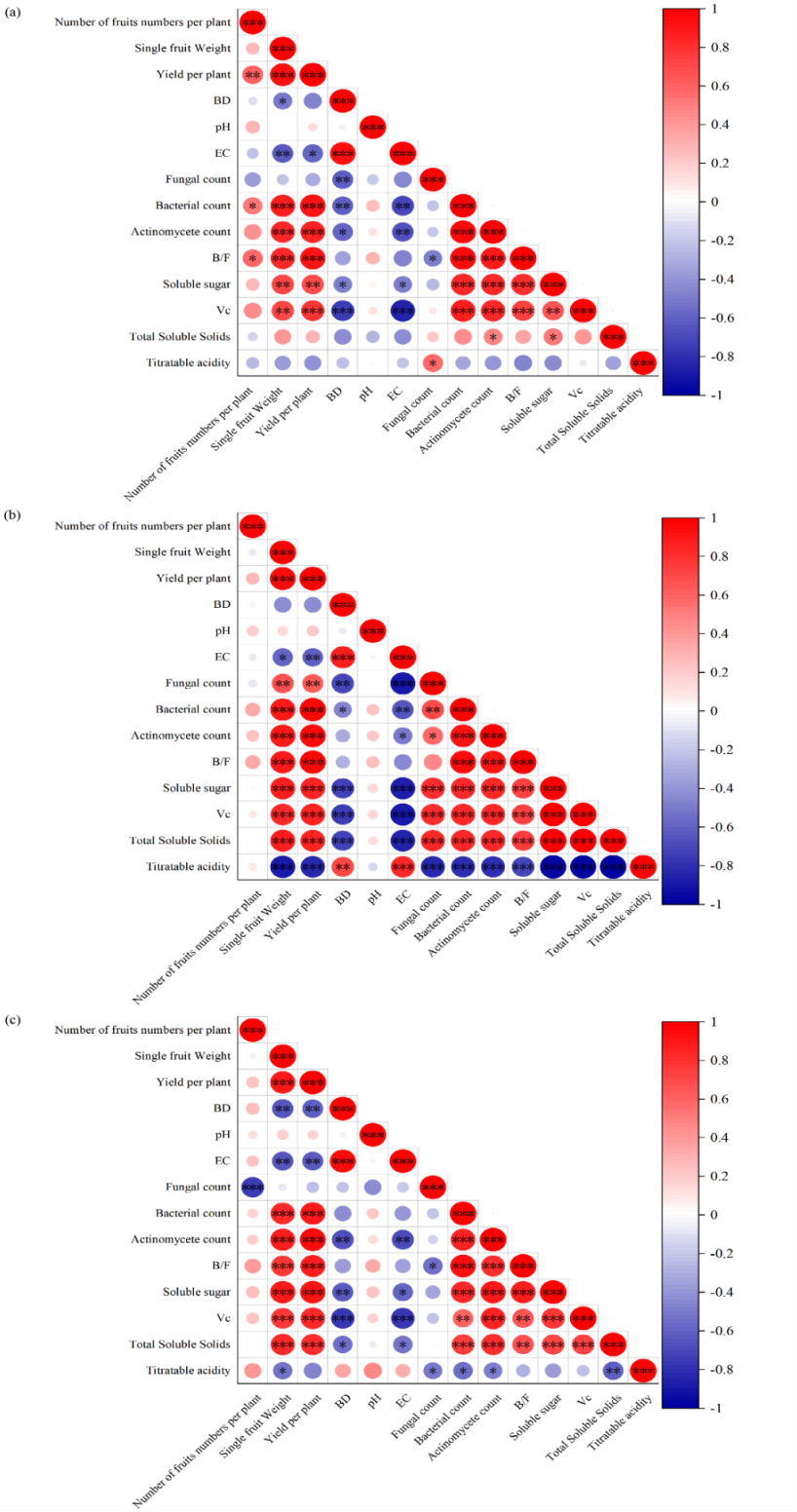


Figure 4

Correlations among crop traits, soil properties, and microbial indicators

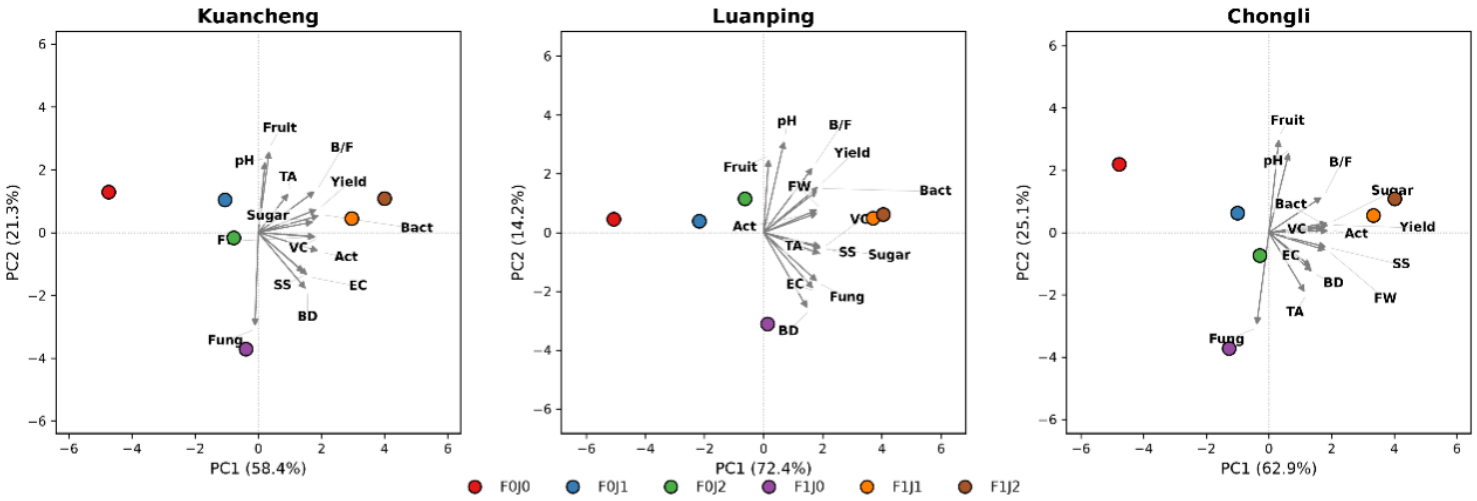


Figure 5

Distribution of different treatments in the PC1–PC2 space across the three sites. F0, zeolite at 0 t hm⁻²; F1, zeolite at 3 t hm⁻²; J0, control without microbial agent; J1, *Bacillus amyloliquefaciens* (biocontrol + growth-promoting strains); J2, *Bacillus subtilis* + *Trichoderma harzianum*. Ellipses indicate 95% confidence intervals for each treatment, and arrows indicate the loading directions of the indicators. Values in parentheses for PC1 and PC2 are the percentages of variance explained by each principal component.