BMC Microbiology

RESEARCH

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Effects of *Bacillus subtilis* N24 combined with liquid water-soluble carbon fertilizer on soil chemical properties and microbial community of fresh maize

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Abstract

Recent years have witnessed increasingly extensive application of microbial fertilizers in agriculture. However, the effectiveness of microbial fertilizers remains inconsistent because of the significant effects of soil's physical and chemical properties on microbial colonization. Therefore, exploring the scientific application of microbial fertilizers is of great significance for improving their application effect on crops. This study aimed to investigate the effects of *Bacillus subtilis* combined with liquid water-soluble carbon fertilizer on soil chemical properties and the rhizosphere microbial community of fresh maize. It employed a pot experiment design, incorporating five distinct treatments: T1 (liquid water-soluble carbon fertilizer), T2 (*B. subtilis* N24 fermentation solution), T3 (*B. subtilis* + liquid water-soluble carbon fertilizer), CK0 (clean water), and CK1 (conventional fertilization). Illumina high-throughput sequencing was used to analyze corn potting soil. The results indicated that the fertilization treatments influenced the chemical properties of the rhizosphere soil of fresh maize in the following order: T3 > CK1 > T2 > T1 > CK0. The T3 treatment significantly increased the contents of total nitrogen, available nitrogen, total phosphorus, available phosphorus, potassium, and organic matter ($P < 0.05$). It enhanced nitrogen availability and effectively preserved phosphorus and organic matter within the soil. Furthermore, the treatment enriched the microbial community diversity in the corn rhizosphere, thereby significantly increasing the abundance of *Firmicutes*, *Acidobacteriota*, *Bacteroidota*, *Mortierellomycota*, and *Basidiomycota* ($P < 0.05$), demonstrating superior effects compared with the individual applications. The soil properties were strongly linked to microbial composition, as shown by the redundancy analysis ($P < 0.05$). In summary, the combined application of *B. subtilis* N24 and liquid water-soluble carbon fertilizer enhanced the chemical properties and fertility of the soil for fresh maize while also positively influencing the structure of the microbial community. This study provides a theoretical foundation for developing novel fertilizer application models for corn cultivation.

Keywords *Bacillus subtilis*, liquid water-soluble carbon fertilizer, microbial diversity, soil chemical properties

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Introduction

The rapidly growing global population and the challenges posed by climate change have led to major issues related to food security. Although the application of traditional fertilizers has contributed to short-term increases in crop yields [1], their long-term use has led to a series of environmental problems such as soil degradation, ecological imbalance, water pollution, and biodiversity reduction [2, 3]. Consequently, the pursuit of effective, efficient, and environmentally sustainable alternatives to conventional fertilizers has become a focus of research in contemporary agricultural studies [4].

Liquid water-soluble carbon fertilizers have garnered significant attention from researchers in recent years due to their better nutrient composition and adaptability to environmental conditions [5]. These fertilizers have high amounts of trace elements, amino acids, organic carbon, and high–molecular weight bioactive substances. They effectively supply essential nutrients, such as nitrogen, phosphorus, and potassium [6], and also positively influence the growth of corn and other crops [7]. Furthermore, these fertilizers have been shown to enhance soil organic matter content, improve soil water retention capacity, enhance soil aggregate structure [8], and regulate pH levels, and nutrient availability. Moreover, liquid water-soluble carbon fertilizers stimulate the growth of soil microorganisms, thereby promoting the diversity and abundance of subsurface microbial communities [9, 10]. This, in turn, creates a more favorable growth environment for plant roots, thus supporting healthy crop development, facilitating soil nutrient cycling, and enhancing microbial community stability [11].

Bacillus subtilis has been extensively studied in recent years. It produces various bioactive substances, such as bacteriocins and plant hormones, which promote root growth, improve nutrient uptake, and enhance the disease resistance of plants [12]. Further, *B. subtilis* improves plant resistance under adverse conditions by producing biofilms and enhancing plant resistance to disease, especially when combined with organic fertilizers. Also, it significantly improves the composition and function of soil microbial communities [13–16]. The new *B. subtilis* bio-organic fertilizer not only reduced the nitrogen loss in agricultural soil as a soil amendment but also significantly promoted the growth of strawberry and blood orange and changed the soil microbial community structure [17–19]. The diversity of the microbial community not only enhanced the functionality of the soil but also improved the ecological stability of the soil; consequently, the soil was better able to cope with the changes in the external environment [20–22].

The use of microbial fertilizers in agriculture has increased in recent years. This study systematically examined the effects of five different fertilization treatments through a pot experiment, combined with the target crop (maize) and its growing environment, to explore the scientific application of microbial fertilizers. It revealed the mechanism of *B. subtilis* combined with liquid water-soluble carbon fertilizer in soil improvement and microbial diversity via comparative analysis of the effects of different fertilization methods on soil chemical properties and microbial community structure. The findings of this study may provide a theoretical basis for the practice of scientific management of maize fertilization and a new method for optimizing fertilizer use in agricultural practice.

Materials and methods

Materials

The liquid water-soluble carbon fertilizer used in this study was sourced from Fujian Oasis Biochemical Co., Ltd. The fundamental chemical characteristics of the fertilizer included a pH of 5.8, an organic carbon content of 146 g/L, a total nitrogen (N) content of 22.53 g/L, a phosphorus (P_2O_5) content of 1.19 g/L, and a potassium (K_2O) content of 47.67 g/L. Further, diammonium phosphate and urea were procured from the farmers' market located in Yining City, Xinjiang, China. The corn variety employed in the pot experiment was Fresh Bainuo, a fresh food corn variety sourced from Xinjiang Hewang Seed Industry Co., Ltd.

The strain under investigation was *B. subtilis* N24, which is cataloged as CCTCC No: M2020873. The fundamental chemical characteristics of the potting soil used in this study were as follows: pH, 8.85; an available phosphorus content, 24.23 mg/kg; available nitrogen content, 20.60 mg/kg; available potassium content, 272.51 mg/kg; total phosphorus content, 0.91 g/kg; total nitrogen content, 0.406 g/g; total potassium content, 11.99 g/kg; and organic matter content, 3.76 g/kg.

Experimental design

The greenhouse pot experiment was conducted at the demonstration facility of the Industry-University-Research Institute at Yili Normal University. Planting was conducted on May 30, 2024, following which the soil samples were collected for 1 month latter. Five treatment groups were established in accordance with the principle of fertilization equivalence:

CK0 (Clear water control): 50 mL/plant
CK1 (conventional fertilization): 0.5 g urea/plant + diammonium phosphate 1 g/plant

T1: liquid water-soluble carbon fertilizer, 50 mL/plant

T2: 0.1 mL of bacterial solution (1×10^{10} CFU/g) was added to a mixture of 2.5 mL carbon fertilizer stock solution + 47.5 mL of water. The bacterial solution was diluted 100 times with 1×10^{11} CFU/g bacterial powder provided by Genrido Biotechnology Co., Ltd. It was then fermented in a vibration culture apparatus at a constant temperature of 32℃ and 180 rpm for 12 h.

T3: 2.5 mL of carbon fertilizer solution + 47.5 mL of water + 0.1 g bacterial powder, five times per treatment.

For the CK1 treatment, 0.5 g of urea and 1 g of diammonium phosphate were added to each pot. After crushing, the soil was mixed and filled into the pot. For the T1 treatment, liquid water-soluble carbon fertilizer was used as the raw material, diluted 20 times, and pH adjusted to 7.0. For the T2 treatment, liquid water-soluble carbon fertilizer was used as the raw material, diluted 20 times, and pH adjusted to 7.0. Then, *B. subtilis* N24 was added for fermentation and then used when the viable bacteria count reached 2×10^{8} CFU/mL. For the T3 treatment, diluted liquid water-soluble carbon fertilizer was prepared as earlier. Then, *B. subtilis* N24 (viable bacteria count 2×10^{8} CFU/mL) was directly added without fermentation. Corn seeds of the same size were selected. The red coat on the surface of the seeds was cleaned with water, disinfected with 75% alcohol for 15 s, soaked in 10% sodium hypochlorite solution for 10 min, and finally rinsed with water three times. Potting soil was prepared in the soils and vermiculite perlite ratio of 3:1:1:1. Then, 2 kg from the mixture was added to a pot. Three seeds were sown in each pot to a depth of about 1 cm. A completely random placement was adopted, and regular and quantitative watering was performed. Plants were thinned 3 days after emergence, and one plant of the same size and height in each pot was retained. After thinning, the T1, T2, and T3 treatments were applied for the first time, and thereafter the fertilizer was applied every seventh day for three applications. The roots of corn were gently dug out with a shovel after 30 days, large soil particles were shaken off, and part of the norhizosphere soil was collected for chemical analysis. The rhizosphere soil adhered to the root surface was put into a sterile bag, sealed, immediately placed in a biological sample sampling box, transported to the laboratory at low temperature, and stored in an ultra-low temperature refrigerator at −80℃.

Determination of soil chemical properties

Soil pH was measured with a pH meter. The soil organic matter contents were determined by the potassium dichromate oxidation method. Kjeldahl method was used for determining soil total nitrogen (TN) content. The soil available nitrogen (AN) content was determined by alkaline hydrolysis diffusion. The molybdenum antimony resistance colorimetric method was employed for determining soil available phosphorus (AP) content. Soil total phosphorus (TP) content was determined by the NaOH alkali fusion method. The flame photometer method was used for determining total potassium (TK) content. The soil available potassium (AK) content was determined by flame spectrophotometry after extracting ammonium acetate solution [23–26].

High-throughput sequencing

DNA was extracted using a YH-soil kit following the manufacturer's protocol. Universal 16S V3–V4 region primers used for PCR amplification were as follows: upstream primer 338F: ACTCCTACGGGAGGCAGCAG; downstream primer 806R: GGACTACHVGGGTWTCTAAT; fungi-specific primer for polymerase chain reaction (PCR) amplification: ITS5-1737F (5'-CTTGGTCATTTAGAGGAAGTAA-3') and ITS2-2043R (5'-GCTGCGTTCTTCATCGATGC-3'). Further, 30 μL of Phusion1 Hi-Fidelity PCR Master Mix (New England Biology Laboratory) was used. The PCR reaction system (30 μL) comprised the following: Fusion Master Mix (2×) 15 μL; forward primer (1oul/μL) 1 μL, reverse primer (1oul/μL) 1 μL, DNA (1 ng/μL) 1 g, 10 μL (10 ng) H_2O_2. The reaction procedure was as follows: pre-denaturation at 98℃ for 1 min; 30 cycles at 98 °C for 10 s; annealing at 50 °C for 30 s; and maintaining at 72 °C for 30 s. The reaction was conducted at 72℃ for 5 min and stopped at 10℃. The PCR products were purified by agarose gel electrophoresis and fully mixed with 1×TAE at a concentration of 2% and the target DNA (TianGen, China). The libraries were constructed using an NEBNext Ultra DNA library preparation kit (Illumina, CA, USA). The constructed libraries were detected and quantified by quantitative PCR using an Agilent 5400 fragment analyzer (Agilent Technologies Co., USA). The quality check was performed on the constructed libraries, and the libraries were qualified and then sorted online using the Illumina MiSeq PE300/NovaSeq PE250 platform (Illumina, CA, USA). The sequencing of all samples in this study was performed by Shanghai Meiji Biomedical Technology Co., Ltd.

Data analysis

The α- and β-diversities of microbial communities were analyzed using QIIME2 and R software (version 3.5.1) and visualized by nonmetric multidimensional scale ranking. SPSS 27.0 was used to perform one-way analysis of variance, multifactor comparison, and correlation analysis of the samples. The principal coordinate analysis chart was drawn using the R language tool. The orthogonal partial least squares discriminant analysis was used to detect group differences. The microbial diversity was mapped using R software. The redundancy analysis (RDA) was used to analyze the relationship between fungal communities and environmental factors.

Results and analysis

Analysis of soil chemical properties

As illustrated in Table 1, the comparative efficacy of various fertilization treatments in enhancing the chemical properties of rhizosphere soil was ranked as follows: T3 > CK1 > T2 > T1 > CK0. The T1 and T2 treatments significantly increased the TN content of the rhizosphere soil by 24.22% and 51.14%, respectively, compared with the CK0 treatment. The T3 treatment led to a notable increase in the contents of TN, AN, TP, AP, AK, and organic matter, with increases of 78.26%, 12.1%, 22.90%, 21.88%, 43.35%, and 31.79%, respectively. The effects of the T1, T2, and T3 treatments were significant compared with the CK1 treatment. The T1 treatment significantly reduced TN and TP contents by 65.83% and 6.74%, respectively, while simultaneously increasing the AK content by 11.83%. The T2 treatment was associated with significant decreases in the pH level and TN content by 1.48% and 36.30%, respectively, besides an increase in AK content by 10.49%. Furthermore, the T3 treatment significantly enhanced the contents of AP, TP, AK, and organic matter by 10.48%, 7.37%, 13.18%, and 18.84%, respectively.

Changes in soil microbial community structure

Venn diagrams of the sample communities were generated to determine the overall composition of the microbial community. The number of bacteria-specific OTUs in the sample gradually increased after different fertilization treatments. The specific OTU for the CK0, CK1, T1, T2, and T3 treatments was 318, 349, 417, 400, and 362, respectively (Fig. 1A). This result showed that different fertilization treatments significantly promoted the increase in bacterial diversity. Especially, the number of bacterial OTUs reached 417 under the T1 treatment, reflecting the favorable nonselective promoting effect of this treatment on bacterial diversity. The endemic OTU of CK0, CK1, T1, T2, and T3 treatments was 64, 196, 103, 147, and 90, respectively (Fig. 1B). Among these, the number of fungal OTUs under the CK1 treatment was the highest, reaching 196. This indicated that conventional fertilization treatment provided a relatively suitable habitat for fungal growth. Overall, the variation range of fungal OTU was smaller compared with that of bacterial OTU. However, the difference in the number of endemic OTUs among different treatments still reflected the effects of various fertilization treatments on the composition of soil microbial community.

As illustrated in Figs. 2(f) and 3(f), the coverage across all five treatment groups exceeded 98.5%, thereby accurately representing the soil microbial community composition and affirming the reliability of the sequencing results. The bacterial samples yielded 821,956 valid sequences, with an average sequence length of 415 base pairs. The analysis of bacterial alpha diversity is presented in Fig. 2. The results were as follows: As shown in Fig. 2(a), the Sobs index ranked as T1 > T3 > CK1 > T2 > CK0, with the T1 treatment exhibiting a 6.11% increase compared with the CK0 treatment. As shown in Fig. 2(b, c), the Chao and ACE indices were ordered as T3 > T1 > CK1 > T2 > CK0, with the T3 treatment showing a 5.90% increase compared with the CK0 treatment. As shown in Fig. 2(d), the

Table 1 Soil chemical properties

Chemical index Treatment group	pH	Rapidly available nitrogen (mg/kg)	Total nitrogen (mg/g)	Available phosphorus (mg/kg)	Total phosphorus (g/kg)	Whole potassium (g/kg)	Rapidly available potassium (mg/kg)	Organic matter (g/kg)
CK0	8.81 ± 0.01ab	18.27 ± 0.71b	4.83 ± 0.34e	12.11 ± 1.18c	0.83 ± 0.07b	11.05 ± 0.34a	209.57 ± 1.41c	6.70 ± 0.17c
CK1	8.91 ± 0.01a	19.21 ± 1.25ab	9.95 ± 0.43a	13.36 ± 0.44bc	0.95 ± 0.02a	11.05 ± 0.44a	265.42 ± 26.91b	7.43 ± 0.32b
T1	8.68 ± 0.02c	18.97 ± 1.04ab	6.00 ± 0.35d	12.59 ± 0.56bc	0.89 ± 0.08b	11.02 ± 1.45a	296.83 ± 12.35a	8.73 ± 0.06a
T2	8.78 ± 0.05bc	18.39 ± 0.53b	7.30 ± 0.39c	13.73 ± 0.60ab	0.98 ± 0.02a	11.22 ± 0.60a	293.25 ± 8.66a	7.57 ± 0.25b
T3	8.86 ± 0.12ab	20.48 ± 0.44a	8.61 ± 0.44b	14.76 ± 0.42a	1.02 ± 0.01a	11.42 ± 0.44a	300.41 ± 8.64a	8.83 ± 0.75a

Data are expressed as mean ± standard deviation. Different letters indicate significant differences among mean values ($P < 0.05$)

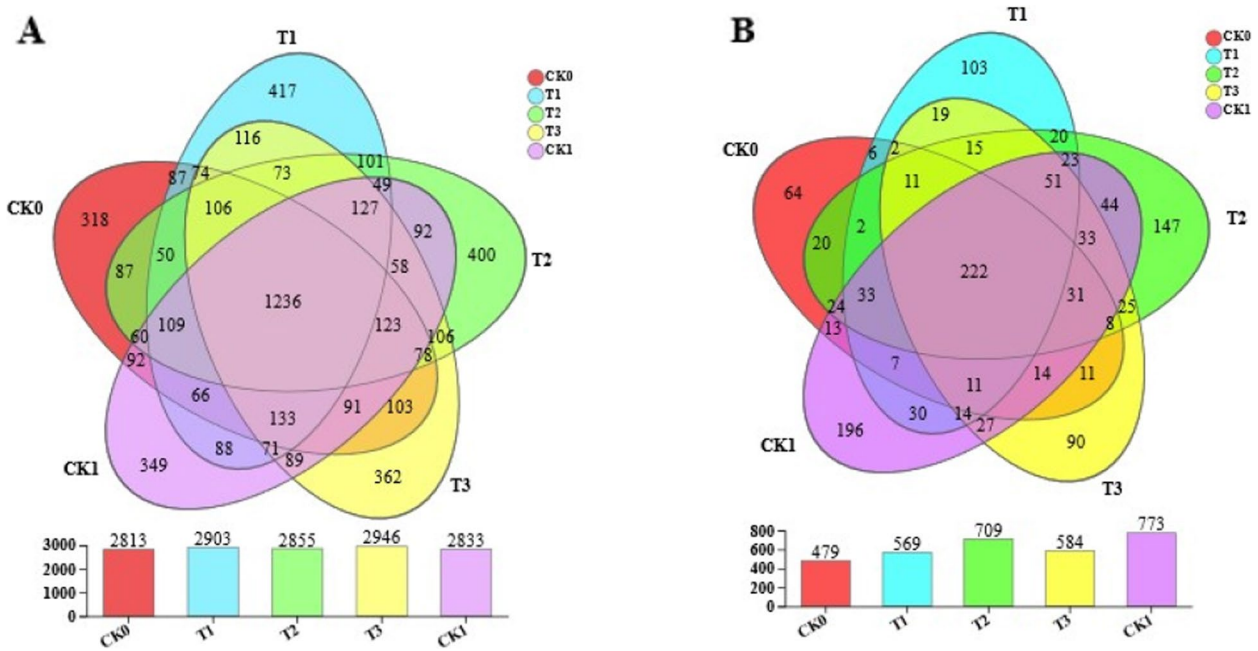

Fig. 1 Venn diagram analysis showing common and endemic species (**A**, bacteria; **B**, fungi) in soil samples

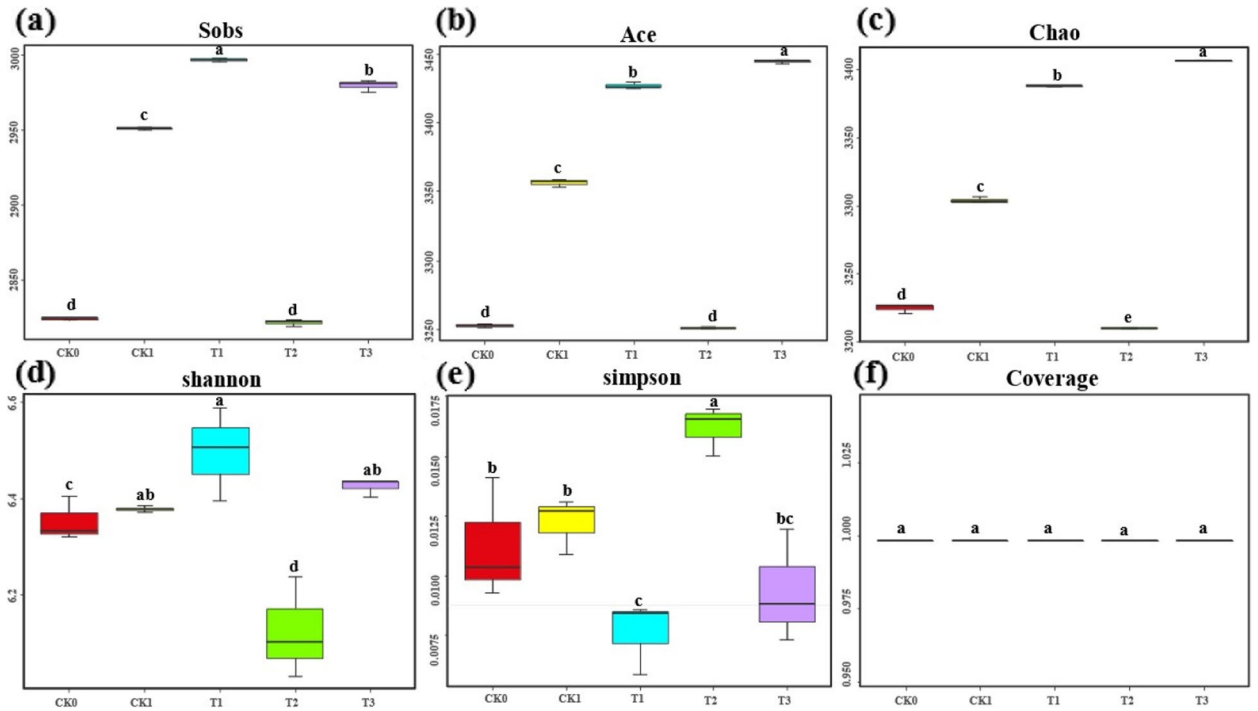

Fig. 2 Analysis of the α-diversity of bacterial communities. Note: The *x*-axis represents the sample name, whereas the *y*-axis represents the content. Duncan's multiple range test was used to compare the significant differences among all groups ($P < 0.05$; $N = 3$). **a** stands for Sobs, **b** stands for ACE, **c** stands for Chao, **d** stands for Shannon, **e** stands for Simpson, and **f** stands for coverage

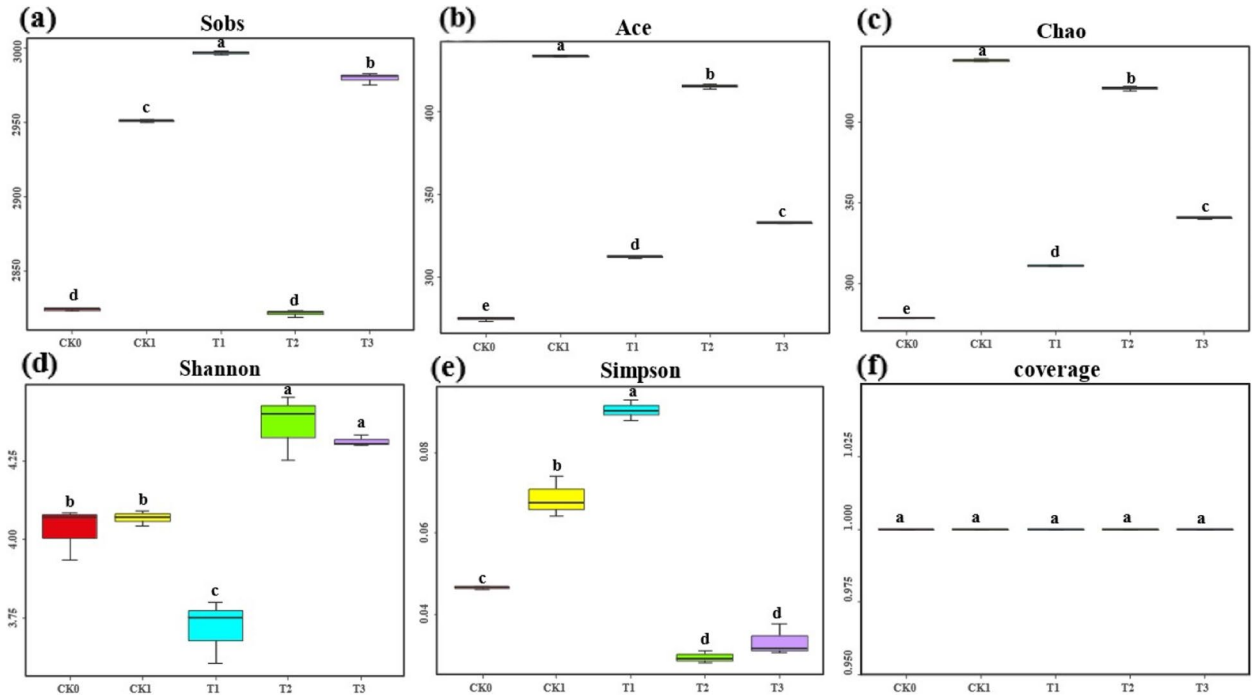

Fig. 3 Analysis of the α-diversity of fungal communities. Note: The *x*-axis represents the sample name, and the *y*-axis represents the content. Duncan's multiple range test was used to compare the significant differences among all groups ($P < 0.05$; $N = 3$). **a** stands for Sobs, **b** stands for ACE, **c** stands for Chao, **d** stands for Shannon, **e** stands for Simpson, and **f** stands for coverage

Shannon index was ordered as T1 > T3 > CK1 > CK0 > T2, As shown in Fig. 2(e), whereas the Simpson index was ranked as T2 > CK1 > CK0 > T3 > T1. The fungal samples produced 1,058,292 valid sequences, with an average sequence length of 248 base pairs. The fungal alpha diversity is depicted in Fig. 3. As shown in Fig. 3(a, b, c) the Sobs, Chao, and ACE indices were ranked as CK1 > T2 > T3 > T1 > CK0, the analysis indicated the CK1 treatment demonstrated a 58.09% increase compared with the CK0 treatment. As shown in Fig. 3(d), the Shannon index was ordered as T3 and T2 > CK0 and CK1 > T1, with the T3 treatment showing a 15.58% increase compared with the T1 treatment. As shown in Fig. 3(e), the Simpson index was ranked as T1 > CK1 > CK0 > T3 and T2.

Changes in bacterial community

Different fertilization treatments had specific effects on the composition and structure of soil bacterial communities, as shown in Fig. 4A. The COMP1 axis explained the difference in samples by 11.39%. The T1, T2, and T3 treatments displayed significant differences on the COMP1 axis. The COMP2 axis explained 8.37% of the sample differences. The microbial community structure of CK0 and CK1 samples on the COMP2 axis was significantly different from that of T1, T2, and T3 samples. The principal component analysis (PCA) of the bacterial community structure in a single sample was performed at the genus level using R language to verify whether bacterial communities were different, as shown in Fig. 5A. The results indicated that the PC1 and PC2 axes explained 21.26% and 13.86% of the variance, respectively. Significant differences were observed between the soil samples and the control group after various fertilization treatments, indicating that the bacterial community structure changed significantly after application. A total of 13 phyla with relative abundance greater than 1% were identified at the phylum level, as shown in Fig. 6A. Among these, the total relative abundance of Actinobacteriota, Proteobacteria, Chloroflexi, Firmicutes, and Acidobacteriota was greater than 80%. Is the dominant bacteria phylum. Significant differences were found in the abundance of Proteobacteria and Firmicutes under each fertilization treatment ($P < 0.05$). The abundance of Proteobacteria under the CK1, T1, T2, and T3 treatments increased by 1.1%, 2.8%, 4.7%, and 1%, respectively. The abundance of Firmicutes under the T1, T2, and T3 treatments increased by 1.6%, 1.5%, and 2.5%, respectively. The T1 treatment decreased the abundance of Actinomyces, Chloroflexi, and Gemmatimonadota, but increased the abundance of Proteobacteria, Firmicutes, Acidobacteriota, and Bacteroidota. The T2 treatment reduced the

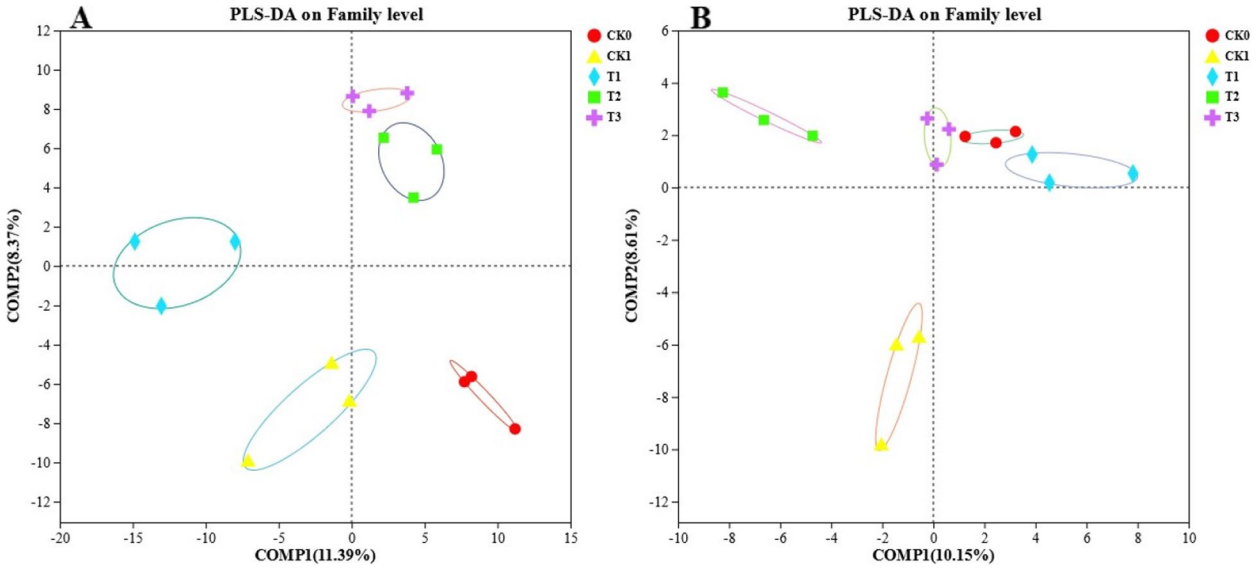

Fig. 4 PLS-DA analysis of sample microbial community. (**A**) Analysis of bacterial and (**B**) fungal communities at the family level

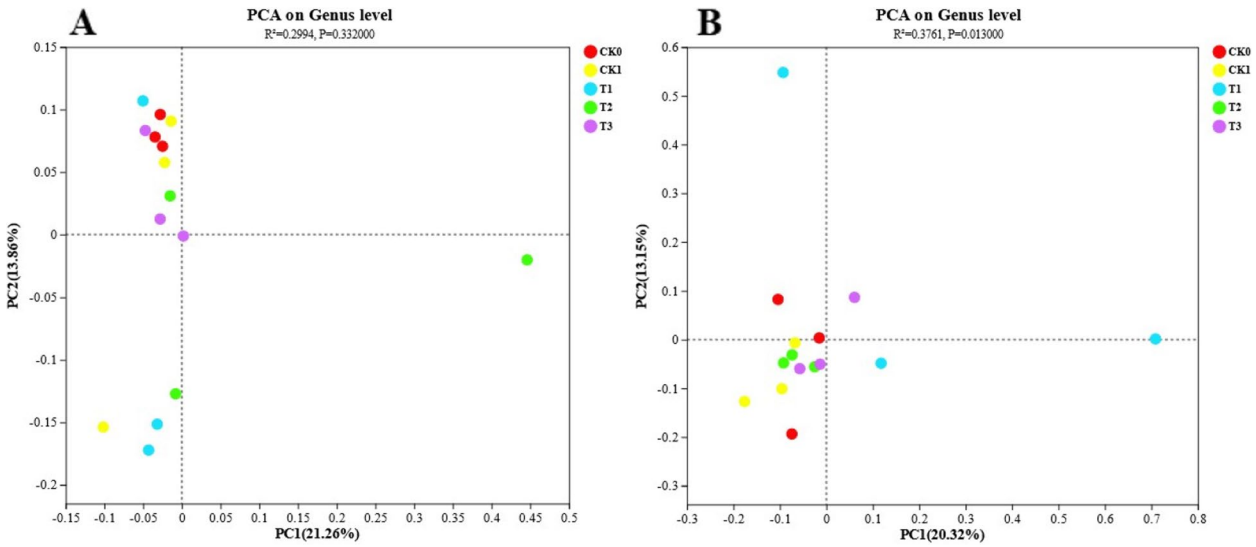

Fig. 5 Principal component analysis (PCA) of β-diversity of soil microbial communities: (**A**) bacteria and (**B**) fungi

abundance of Actinomyces, Chloroflexi, Acidobacteriota, Gemmatimonadota, and Myxomycota, and increased the abundance of Proteobacteria, Firmicutes, Bacteroidetes, and Cyanobacteria. The T3 treatment decreased the abundance of actinomycota, and increased the abundance of Firmicutes, Acidobacteria, and Bacteroidetes.

Changes in the fungal community

Different fertilization treatments also specifically affected the soil fungal community structure, as shown in Fig. 4B. The COMP1 axis explained 10.15% of the sample difference, whereas the COMP2 axis explained 8.61%. The microbial community structure of control soil samples under the CK0 and CK1 treatments significantly differed from that under the T1, T2, and T3 treatments. The difference between the samples fully accounted for the variation in soil microbial community structure between liquid water-soluble carbon fertilizer and conventional fertilizer treatments. The PCA of the soil samples was performed at the fungal genus level, as shown in Fig. 5B. The results showed minimal effect of the treatment with liquid water-soluble carbon fertilizer on the fungal

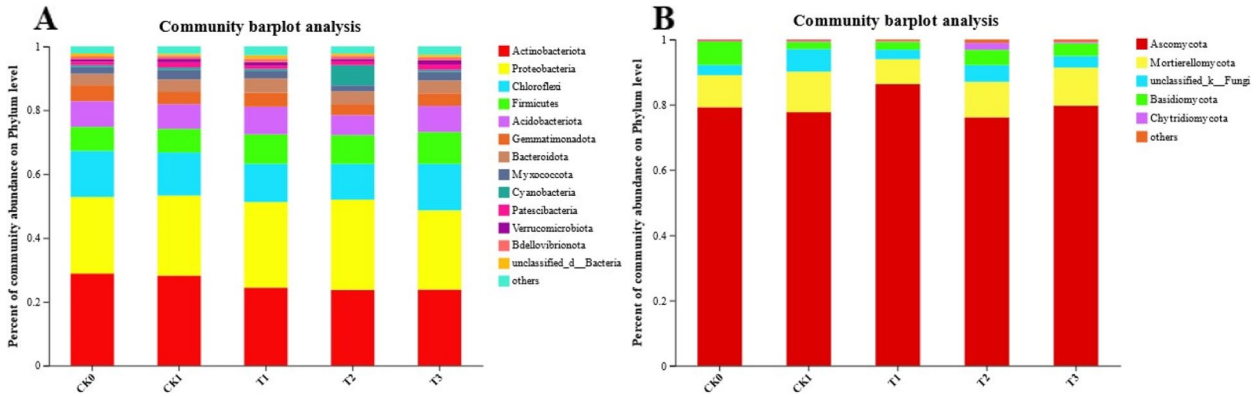

Fig. 6 Composition and structure of soil microbial communities at the phylum level: (**A**) bacteria and (**B**) fungi

community. As shown in Fig. 6B, five phylum had relative abundance greater than 1%: Ascomycota, Mortierellomycota, unclassified_k__Fungi, Basidiomycota, and Chytridiomycota. The total relative abundance of Ascomycota was greater than 50%, indicating that Ascomycota was the dominant phylum. The abundance of Ascomycota was significantly higher under the T1 treatment compared with other treatments ($P<0.05$), with an increase by 7.1%, 8.6%, 10.2%, and 6.6% compared with that under the CK0, CK1, T2, and T3 treatments, respectively. The T1 treatment increased the abundance of Ascomycetes but decreased the abundance of Basidiomycota and Mortierellomycota. The T2 treatment decreased the abundance of Ascomycetes and Basidiomycota and increased the abundance of Mortierellomycota, unclassified_k__Fungi, and Chytridomycota. The T3 treatment increased the abundance of Mortierellomycota and Basidiomycetes.

Correlation analysis between microbial diversity index and soil factors

A certain correlation was detected between the bacterial diversity index and soil chemical properties after different fertilization treatments. As shown in Table 2, the Chao, ACE, and Sobs index of bacteria were significantly positively correlated with AN and AK contents ($P<0.05$), and highly significantly positively correlated with organic matter content ($P<0.01$). As shown in Table 3, the Chao, ACE, and Sobs indices of fungi were positively correlated with TN ($P<0.01$) and AP contents ($P<0.05$). In contrast, the Shannon index was positively correlated with AP content ($P<0.01$). Therefore, the soil factors that significantly impacted microbial diversity and richness were organic matter, AN, AK, TN, AP, and organic matter.

Correlation analysis between soil chemical properties and microbial diversity

The redundancy analysis is shown in Fig. 7, where the blue solid line represents the dominant bacteria and the red solid line represents the eight chemical factors. As shown in Fig. 7A, RDA axis 1 explained 21.72%, whereas axis 2 explained 17.44% of the variance, for a cumulative explanation rate of 39.16%. Among these, TN, TP, AK, and organic matter contents had the most significant effects on bacterial communities. The correlation analysis in Table 4 indicates different correlations between the abundance of each dominant bacterial phyla and

Table 2 Correlation analysis between bacterial diversity index and soil factors

Diversity index	pH	Rapidly available nitrogen	Total nitrogen	Available phosphorus	Total phosphorus	Whole potassium	Rapidly available potassium	Organic matter
Sobs	−0.036	0.560*	0.425	0.263	0.260	0.021	0.531*	0.753**
ACE	−0.058	0.623*	0.373	0.357	0.330	0.073	0.587*	0.825**
Chao	−0.094	0.621*	0.298	0.325	0.286	0.067	0.544*	0.828**
Shannon	−0.177	0.317	−0.029	−0.07	−0.206	0.048	0.003	0.426
Simpson	0.371	−0.238	0.211	0.069	−0.147	−0.147	−0.054	−0.409

Positive numbers indicate a positive correlation, whereas negative numbers indicate a negative correlation

* Significant correlation ($P<0.05$)

** extremely significant correlation ($P<0.01$)

Table 3 Correlation analysis between fungal diversity index and soil factors

Diversity index	pH	Rapidly available nitrogen	Total nitrogen	Available phosphorus	Total phosphorus	Whole potassium	Rapidly available potassium	Organic matter
Sobs	0.368	0.032	0.768**	0.583*	0.016	0.016	0.393	−0.035
ACE	0.380	0.026	0.769**	0.571*	0.015	0.015	0.370	−0.065
Chao	0.405	0.039	0.782**	0.593*	0.027	0.027	0.371	−0.065
Shannon	0.405	0.177	0.377	0.649**	0.082	0.082	0.151	−0.110
Simpson	−0.361	−0.093	−0.084	−0.435	−0.167	−0.167	0.031	0.192

Positive numbers indicate a positive correlation, whereas negative numbers indicate a negative correlation

* Significant correlation ($P < 0.05$)

** extremely significant correlation ($P < 0.01$)

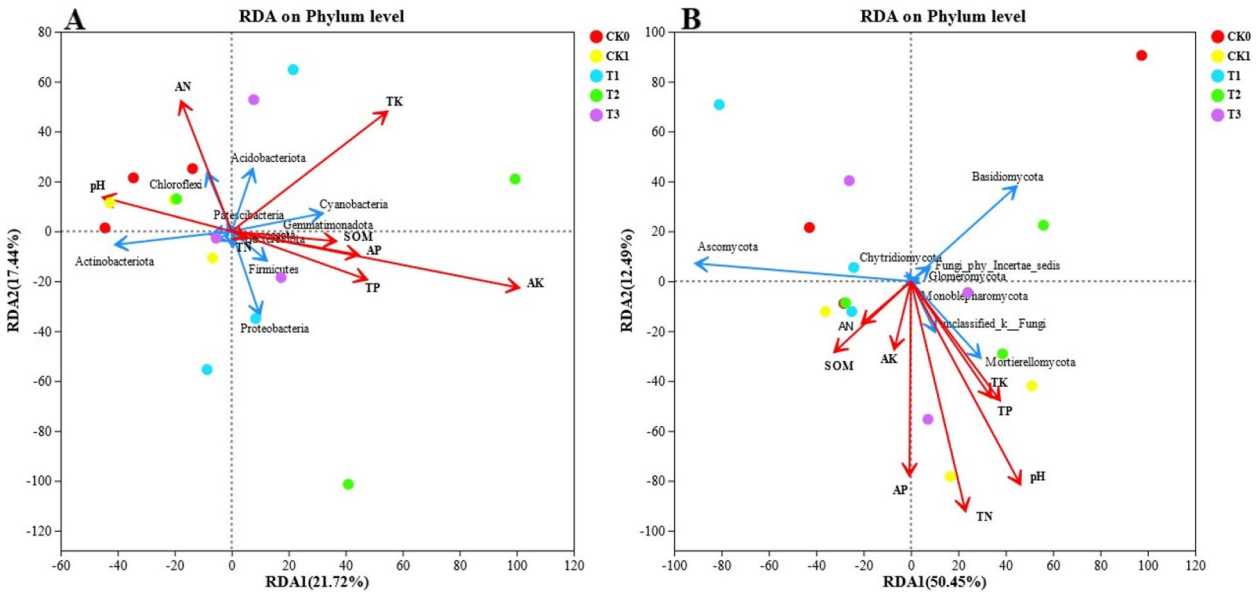

Fig. 7 RDA analysis between microbial communities and environmental factors (**A**, bacteria; **B**, fungi)

Table 4 Correlation analysis between the abundance of dominant bacterial flora and soil chemical properties

Dominant bacteria phylum	pH	Rapidly available nitrogen	Total nitrogen	Available phosphorus	Total phosphorus	Whole potassium	Rapidly available potassium	Organic matter
Actinobacteriota	0.377	−0.286	−0.095	0.521*	0.574*	−0.145	0.840**	0.717**
Proteobacteria	−0.446	−0.202	0.018	0.151	0.307	0.011	0.620*	0.253
Chloroflexi	0.499	0.326	0.061	0.049	−0.115	0.051	0.620*	−0.144
Firmicutes	−0.313	0.444	0.063	0.554*	0.542*	0.172	−0.494	0.801**
Acidobacteriota	−0.155	0.283	−0.186	−0.254	−0.380	−0.061	0.765**	0.346
Gemmatimonadota	−0.259	−0.179	0.750**	0.594*	0.773**	−0.092	−0.122	−0.227
Bacteroidota	0.536*	0.193	0.089	0.076	0.156	−0.024	0.717**	0.731**
Myxococcota	0.484	0.472	0.673**	0.195	0.199	−0.006	0.067	0.232

Positive numbers indicate a positive correlation, whereas negative numbers indicate a negative correlation

* Significant correlation ($P < 0.05$)

** extremely significant correlation ($P < 0.01$)

soil chemical properties. The abundance of Actinomycetes was negatively correlated with AP and TP contents ($P<0.05$) and extremely negatively correlated with AK and organic matter contents ($P<0.01$). The abundance of Proteobacteria was positively correlated with AK content ($P<0.05$). The abundance of Firmicutes was significantly positively correlated with AP and TP contents ($P<0.05$) and highly significantly positively correlated with AK and organic matter contents ($P<0.01$). A significant positive correlation was detected between the abundance of *Blastomonas* and AP content ($P<0.05$), whereas a highly significant negative correlation was observed between TN, TP, and AK contents ($P<0.01$). The abundance of Bacteroidetes was negatively correlated with pH and positively correlated with AK and organic matter contents. A significant positive correlation was noted between the abundance of Myxomycetes and TN content. As shown in Fig. 7B, RDA axis 1 explained 50.45%, whereas axis 2 explained 12.49% of the variance, with a cumulative interpretation rate of 62.94%. The analysis results in Table 5 show that the relationship between dominant phyla and soil chemical properties was equally important, and the abundance of *Mortierella* had a significant positive correlation with pH ($P<0.05$). Further, a significant positive correlation was found between the abundance of unclassified fungi and TN content ($P<0.05$). In conclusion, different treatment modes of liquid water-soluble carbon fertilizer significantly affected the composition of the soil microbial community and its relationship with soil chemical properties.

Discussion

This study found that the T3 treatment had the best effect in terms of improving the chemical properties of maize soil. Compared with the CK0 treatment, the T1 and T2 treatments significantly increased the TN content of rhizosphere soil. This might be attributed to the enhanced soil microbial activity after fertilization, which promoted nitrogen mineralization and transformation, thus improving nitrogen availability [16]. The T3 treatment not only improved the nitrogen supply but also allowed effective retention of phosphorus and organic matter in the soil, thus providing more comprehensive nutritional support for plant growth [27, 28]. The high content of organic matter and soluble nutrients under the T3 treatment maintained soil sustainability and plant health [29, 30]. The findings showed that the combination of *B. subtilis* and liquid water-soluble carbon fertilizer not only enhanced nutrient cycling in the soil but also increased soil nutrients for plant growth, thereby providing a more ideal growth environment for plants due to the improvement in microbial community diversity [12, 31].

The bacterial diversity analysis showed that the Chao and ACE indices of the combination of *B. subtilis* combined with liquid water-soluble carbon fertilizer were the highest, indicating that the T3 treatment had the most significant impact on species abundance and richness. The potential reason was that the addition of *B. subtilis* guided the structure of the soil microbial community and enhanced its potential ecological function [32, 33]. The fungal diversity analysis revealed high fungal diversity under the T3 and T2 treatments, indicating that the combined application of *B. subtilis* and liquid water-soluble carbon fertilizer could improve ecological complexity [34, 35]. Increased diversity of bacterial communities may promote the stability and functionality of soil ecosystems, whereas the changes in fungal communities may be closely related to specific environmental conditions [20].

Soil fertilization management can influence the diversity and community structure of microbial populations. The findings of this study indicated that the application of a liquid water-soluble carbon fertilizer in conjunction

Table 5 Correlation analysis between dominant fungal flora and soil chemical properties

Dominant fungal phylum	pH	Rapidly available nitrogen	Total nitrogen	Available phosphorus	Total phosphorus	Whole potassium	Rapidly available potassium	Organic matter
Ascomycota	−0.327	0.145	−0.206	−0.044	−0.274	−0.237	0.005	0.198
Mortierellomycota	0.556*	0.186	0.373	0.314	0.291	0.404	−0.034	0.106
unclassified_k__Fungi	0.333	−0.143	0.525*	0.204	0.265	−0.007	0.179	−0.195
Basidiomycota	−0.104	−0.188	−0.297	−0.369	−0.043	−0.054	−0.199	−0.280
Chytridiomycota	−0.095	−0.217	−0.018	0.082	0.194	0.235	0.262	−0.108

Positive numbers indicate a positive correlation, whereas negative numbers indicate a negative correlation

* Significant correlation ($P<0.05$)

** extremely significant correlation ($P<0.01$)

with *B. subtilis* led to significant alterations in the composition of the soil microbial community, consistent with previous findings [34]. Actinomycetes decompose organic matter and enhance soil health, and thus are widely acknowledged as crucial contributors to soil ecological functions and nutrient cycling [20, 35]. In this study, the abundance of Actinomycetes was reduced in different treatments (T1, T2, and T3), suggesting that the application of water-soluble fertilizer might have altered the soil environment and inhibited the growth of Actinomycetes [36]. This study found that the T1, T2, and T3 treatments increased the abundance of Proteobacteria, which enhanced soil fertility through nitrogen fixation and denitrification processes [37]. This increase might be related to the abundance of available nutrients in the liquid water-soluble carbon fertilizer, providing a favorable living environment for Proteobacteria [38]. Firmicutes are crucial in soil organic matter decomposition and nutrient release [39]. In this study, the increased relative abundance of Firmicutes under the T1, T2, and T3 treatments enhanced the competitiveness of Firmicutes and promoted the reproduction of their communities [40]. The abundance of Gemmatimonadota decreased significantly under T2 treatment, possibly due to nutrient competition for fertilization and the interaction between the flora. Gemmatimonadota generally predominates in nutrient-poor soils, but this relatively "low-tolerance" flora may be replaced by one more adapted to the dominant environment with nutrient supply [41]. In addition, the abundance of Acidobacteriota is also affected, which may reflect the negative impact of the changes in soil pH on its growth. Acidobacteria usually act as the decomposers of organic matter in soil and help regulate the soil's acidic environment [42]. The fertilizer application could significantly reduce the abundance of specific soil microorganisms, possibly due to nutrient competition, changes in soil pH, or other changes in physical and chemical properties [43]. The abundance of Firmicutes, Acidobacteria, and Bacteroidetes increased under the T3 treatment, further suggesting a synergistic effect of the combination of liquid water-soluble carbon fertilizer and *B. subtilis* [44–46].

Ascomycetes are the most abundant and diverse phyla of soil fungi and usually play a key role in organic decomposition and nutrient cycling [47, 48]. This study showed that the T1 treatment significantly increased the abundance of Ascomycetes. This might be related to the abundant carbon source in liquid water-soluble carbon fertilizer, which provided sufficient nutrients for the growth of Ascomycetes and increased the competitiveness of their community [49, 50]. The decrease in the abundance of Ascomycetes under the T2 treatment also indicated that *B. subtilis* inhibited the propagation of Ascomycetes [51]. The T1 and T2 treatments significantly reduced the abundance of Basidiomycetes. This was possibly because Basidiomycetes preferred substrates rich in lignin and cellulose. However, the application of liquid water-soluble carbon fertilizer changed the nutrient structure and organic composition of the soil, thus impacting the living conditions of Basidiomycetes [52–54]. The abundance of Mortierellomycota decreased under T1 treatment, possibly due to its slightly different water and nutrient requirements compared with Ascomycota [55, 56]. The abundance of Mortierellomycota increased significantly under the T2 treatment. Previous studies have shown that the microbial fermentation liquid can effectively stimulate the activity of soil microorganisms and optimize microbial interactions, thereby improving soil biodiversity and enhancing plant nutrient absorption capacity [57, 58]. The T3 treatment increased the abundance of Ascomycetes, Basidiomycetes, and Mortierellomycota, indicating that the combination of *B. subtilis* and liquid water-soluble carbon fertilizer can provide abundant available carbon sources for soil. Related studies have shown that liquid fertilizer and microbial agents can enhance the interaction of soil microorganisms, increase the proportion of specific fungi and stimulate metabolic activity. This, in turn, increases their abundance and activity in the soil and improves the utilization of microorganisms [59–62].

Conclusions

This study systematically assessed the impact of various fertilization treatment modalities on the chemical characteristics and microbial community composition of corn rhizosphere soil. The findings indicated that implementing diverse fertilization strategies significantly enhanced soil quality. The combination of *B. subtilis* N24 and liquid water-soluble carbon fertilizer (T3) demonstrated the most pronounced effects, thereby improving soil fertility and microbial activity. Furthermore, both species abundance and richness were notably high. Thus, the synergistic application of liquid water-soluble carbon fertilizer and *B. subtilis* is a novel fertilization approach for enhancing soil ecosystems and facilitating the growth of fresh maize, while also serving as an effective strategy for optimizing corn fertilization management.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12866-025-03928-2.

Supplementary Material 1.

Supplementary Material 2.

Acknowledgements
This study was performed by the staff of the Key Laboratory of Xinjiang Lavender Conservation and Utilization of Yili Normal University with the support of the College of Biological Science and Technology of Yili Normal University.

Authors' contributions
Xia Deng wrote, analyzed and mapped the original manuscript, Wenwen Liu sorted out the experimental data, Ziwei Jiao and Shifang Wu examined and revised the original manuscript. Ziwei Jiao, Peng Huang, Yunge Zhang, Yanbin Guo and Sasa Zhang supervised the experiment.

Funding
This study was financially supported by the Key Research and Development Project of Xinjiang Uygur Autonomous Region (2022B02021-2), the YZ2023A05 Science and Technology Plan Project of Yili Prefecture, and the Key Talents Project of Agriculture, Rural Areas of Autonomous Region (2023SNGGGCC015).

Data availability
We have deposited the data into the NCBI database with the accession number PRJNA1215788.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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Received: 24 December 2024 Accepted: 24 March 2025
Published online: 09 April 2025

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