

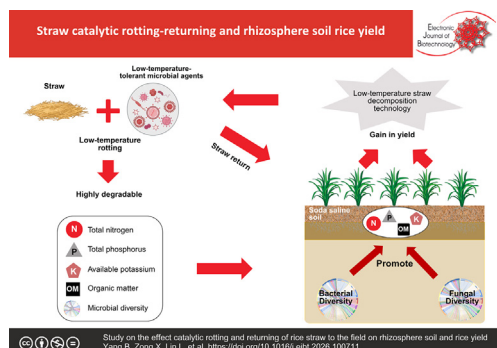


## Research article

Study on the effect of catalytic rotting and returning of rice straw to the field on rhizosphere soil and rice yield <sup>☆</sup>Bing Yang <sup>a,b,c</sup>, Xianchun Zong <sup>a</sup>, Lin Lin <sup>b,c</sup>, Junyou Shi <sup>b,c</sup>, Chao Wang <sup>b,c,\*</sup>, Fachun Guan <sup>b,c,d,\*</sup><sup>a</sup> College of Biological Science and Technology, Mudanjiang Normal University, Mudanjiang city, Heilongjiang Province, China<sup>b</sup> School of Agricultural Engineering and Food Science, Shandong University of Technology, Zibo city, Shandong Province, China<sup>c</sup> Shandong Key Laboratory of Biomass Efficient Conversion and Utilization, Zibo city, Shandong Province, China<sup>d</sup> Jilin Academy of Agricultural Sciences, Northeast Agricultural Innovation Center, Changchun city, Jilin Province, China

## GRAPHICAL ABSTRACT

Study on the effect of catalytic rotting and returning of rice straw to the field on rhizosphere soil and rice yield.



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## ABSTRACT

**Background:** *In-situ* straw return is an effective agronomic practice for improving soil quality and increasing crop yields. However, in Northeast China, prolonged seasonal freezing lasting more than 6 months markedly restricts straw decomposition, thereby limiting the benefits of straw return in saline-alkali soils. To overcome this constraint, cold-tolerant bacterial agents were applied to promote the decomposition and *in-situ* return of rice straw under frozen and low-temperature conditions.

**Results:** The results demonstrated that under freezing and low-temperature conditions, the application of the microbial agent (CF) increased the rice straw decomposition rate to 51.22%, which was significantly higher than that observed in the control treatment (34.64%). The germination index of seeds exposed to the decomposed straw reached 102.87%, indicating that the decomposition products met safety standards. CF treatment reduced rhizosphere soil pH and salinity, while significantly increasing the diversity and abundance of rhizosphere microorganisms. In particular, it promoted the enrichment of bacterial genera associated with nitrogen fixation and straw decomposition. At the same time, the CF application enhanced rhizosphere soil nutrient levels, which in turn significantly improved rice growth parameters,

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Saline-alkali soil  
Straw return

including tiller number, plant height, and dry weight. Consequently, rice yield increased by 5.84% compared with the control treatment.

**Conclusions:** In summary, the application of cold-tolerant bacterial agents enables efficient *in-situ* decomposition and return of rice straw under freezing and low-temperature conditions. This approach effectively enhances rice productivity in saline-alkali farmland and provides a simple, practical, and scalable strategy for overcoming straw decomposition limitations in cold regions worldwide.

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## 1. Introduction

Soil salinization is a pervasive global environmental problem that severely constrains agricultural development and food production. The western Songnen Plain contains approximately  $3.0 \times 10^6$  ha of saline-alkali land, representing one of the world's three major concentrated distribution areas of soda saline-alkali soils. These soils are characterized by a pH range of 8.5–10.5 and high concentrations of soluble salts, primarily  $\text{NaHCO}_3$  and  $\text{Na}_2\text{CO}_3$  [1,2]. Excessive salinity adversely affects plant growth and development mainly through osmotic stress, ion toxicity, and salt-induced physiological disorders, which together limit water and nutrient uptake and ultimately lead to substantial yield reductions [3].

Microbial remediation of saline-alkali soils has become a major research focus because of its economic advantages and potential for sustainable land utilization, largely through the enhancement of soil microbial diversity and richness [4]. Numerous studies have shown that the application of microbial fertilizers or inoculants to saline-alkali soils can significantly increase organic carbon and  $\text{NH}_4^+$  contents, promote nutrient uptake by plants, and enhance soil microbial biomass, enzymatic activity, and overall fertility, thereby contributing to the improvement of saline-alkali land [5]. Organic acids generated during microbial metabolism can directly influence soil alkalinity [6]. In addition, microbial enzymes accelerate the decomposition and transformation of soil organic matter, reduce the fixation of phosphorus and potassium, and facilitate the conversion of insoluble P and K into available forms [7]. Owing to these critical functions, microorganisms are now recognized as indispensable agents in the restoration and management of saline-alkali ecosystems. Straw returning is an important approach for the sustainable utilization of crop residues. As a widely adopted farmland management practice, it promotes the recycling of soil organic matter and has increasingly been regarded as an effective strategy for improving saline-alkali soils and advancing sustainable soil management [8]. Straw is rich in essential nutrients such as nitrogen, phosphorus, and potassium, and its incorporation into soil can significantly improve soil structure, increase soil organic matter content, inhibit salt accumulation, and reduce soil electrical conductivity (EC) and pH [9]. Furthermore, straw return enhances soil porosity and aeration in saline soils, thereby facilitating plant nutrient uptake. It can also regulate topsoil temperature [10,11] and increase microbial abundance, activity, and biodiversity [12]. Despite these advantages, straw returning is constrained by several limitations and potential negative effects. First, owing to its compact and tightly bound structure, straw exhibits inherent resistance to biodegradation, which complicates the decomposition process and severely limits degradation efficiency [13,14]. Second, in Northeast China, prolonged and persistent winter freezing further exacerbates the difficulty of straw decomposition [15,16]. At present, no mature low-temperature straw decomposition technology is available either domestically or internationally. Consequently, the *Outline for the Protection of Black Soil in Northeast*

*China (2017–2030)* explicitly emphasizes the need to “focus on overcoming technical barriers in low-temperature straw decomposition” [17]. Third, when straw is directly returned to fields but fails to decompose in a timely manner, phenolic compounds and organic acids may be released, negatively affecting the establishment and early growth of subsequent crops [18]. Alleviating these adverse effects often requires repeated field flooding to leach toxic substances and substantial labor input to remove undecomposed residues, resulting in markedly increased production costs. Fourth, straw returning may reintroduce pathogens and insect eggs into the soil, thereby increasing the incidence of seedling pests, diseases, and soil-borne pathogens [19]. Finally, there is a potential risk that heavy metals accumulated in straw could be released during decomposition, increasing the concentrations of certain metals, such as Cd, Hg, and As, in crops and raising food safety concerns [20].

Therefore, in this study, a previously developed cold-tolerant decomposer inoculant was applied to accelerate the decomposition of whole rice straw directly incorporated into soda saline-alkali paddy fields. We systematically evaluated straw decomposition efficiency, rhizosphere soil salinity status, nutrient availability, microbial community characteristics, rice growth parameters, and grain yield. The objectives were to verify the feasibility of whole-straw decomposition and *in-situ* incorporation under freezing and low-temperature conditions, and to assess the effects of straw returning on saline-alkali soil properties and rice productivity. By integrating low-temperature straw decomposition technology with *in-situ* straw incorporation, this study aims to establish a synergistic microbe–plant–soil interaction framework to support the sustainable development of agriculture in cold saline-alkali regions.

## 2. Materials and methods

### 2.1. Overview of the test site

The experimental site was located in Zhenlai County, Jilin Province, China (122°47′ 124°04′ E, 45°28′ 46°18′ N). It lies within the transition zone between the Songnen Plain and the Horqin Grassland and is characterized by a temperate continental monsoon climate. The region experiences four distinct seasons, with an average annual precipitation of 402 mm and a mean annual air temperature of 4.9°C. During winter, the average daily minimum temperature is  $-18^\circ\text{C}$ , and the extreme minimum temperature can reach  $-31.7^\circ\text{C}$ . The soil at the experimental site is classified as soda saline-alkali soil, and its basic physicochemical properties are summarized in Table 1.

### 2.2. Test materials

The rice variety used in this experiment was ‘Ji-Japonica 816’, a locally cultivated high-quality rice cultivar in Jilin Province that exhibits strong stress resistance and high yield potential.

**Table 1**  
Physical and chemical properties of initial soil.

pH	Electrical conductivity ( $\mu\text{S}/\text{cm}$ )	Organic matter ( $\text{g}/\text{kg}$ )	Total nitrogen ( $\text{mg}/\text{kg}$ )	Total phosphorus ( $\text{mg}/\text{kg}$ )	Available potassium ( $\text{g}/\text{kg}$ )
8.54	773.12	13.36	98.73	100.25	0.05

The low-temperature-resistant composite microbial agent (LTA) was self-developed in the laboratory and prepared in powder form. It mainly consists of a consortium of microorganisms, including *Bacillus subtilis*, *Bacillus licheniformis*, *Lactiplantibacillus plantarum*, *Pediococcus acidilactici*, *Hyphopichia burtonii*, *Saccharomyces cerevisiae*, and *Trichoderma harzianum*. All strains were isolated by the laboratory from frozen soil, naturally decomposed cow dung, and straw, and are considered safe for humans and animals. Glucose, rice bran powder, and wheat bran were used as carrier materials. The total number of effective viable microorganisms was  $\geq 1 \times 10^{10}$  CFU/g, and the moisture content was  $\leq 9.0\%$ . The microbial agent LTA was applied by evenly spreading it over the surface of the straw, where it effectively promotes straw decomposition and improves soil microbial community structure under low-temperature conditions.

### 2.3. Experimental design

The experiment consisted of two treatments. In the CF treatment, the microbial agent LTA was added to accelerate decomposition after the full amount of rice straw was directly incorporated into the soil. This treatment was implemented in mid-November 2023, with a straw return rate of 5 tons per hectare. The inoculant was applied at a rate of 25 kg/hm<sup>2</sup> and uniformly mixed with 25 kg brown sugar and 25 kg urea as carbon and nitrogen sources to stimulate microbial proliferation [21]. The mixture was spread evenly over the straw surface, followed by soil incorporation to a depth of 15–20 cm.

The control treatment (CK) involved returning straw to the field without any additional treatment, with the straw subsequently burned in the following spring. The total experimental area for each treatment was 3000 m<sup>2</sup>, and each treatment included three sub-plots of 1000 m<sup>2</sup>. Each sub-plot received 500 kg of straw. Rice cultivation began in the spring of 2024. Both the CF and CK treatments followed local conventional cultivation practices, and identical management measures were applied throughout the experiment to ensure comparability between treatments.

### 2.4. Measurement items and methods

#### 2.4.1. Straw decomposition degree and seed germination index

Undecomposed straw was uniformly collected during winter, placed into nylon mesh bags, labeled, and weighed. The bags were then reburied in the field. In the spring of the following year, the straw samples were retrieved and weighed again to calculate the decomposition rate, with five replicates for each treatment.

The decay rate of rice straw after being returned to the field for time  $t$  was calculated using the [Equation 1] [22]:

$$\text{Straw decay rate}(\%) = \frac{(M_0 - M_t)}{M_0} \times 100\% \quad (1)$$

where  $M_t$  represents the mass of straw at time  $t$  after returning to the field (g), and  $M_0$  represents the mass of straw on the day it was returned to the field (g).

Before the application of transplanting fertilizer after spring land preparation, residual straw at the base of rice stems was collected from the field and cleaned to determine the seed germination index (GI), following the method described by Jiang et al. [23].

Seed germination and root length assays were conducted using water extracts. Fresh straw samples were mechanically shaken with distilled water for 1 h at a solid:liquid ratio of 1:10 (w/v, based on dry weight). Approximately 5.0 mL of each extract was transferred into a sterilized plastic Petri dish lined with filter paper. Ten cucumber seeds were evenly placed on the filter paper and incubated at 25°C in the dark for 48 h.

The germination index (GI) was calculated according to [Equation 2]:

$$GI(\%) = \frac{\text{Germination rate}_{\text{treatment}} \times \text{Mean root length}_{\text{treatment}}}{\text{Germination rate}_{\text{control}} \times \text{Mean root length}_{\text{control}}} \times 100\% \quad (2)$$

#### 2.4.2. Sequencing of soil bacteria and fungi

Rhizosphere soil samples were collected in mid-August during the rice irrigation period and used to determine the relative abundance of bacterial and fungal communities.

Total microbial community DNA was extracted following the instructions of the E.Z.N.A.<sup>®</sup> soil DNA kit. DNA concentration and purity were assessed using a NanoDrop2000 spectrophotometer. For bacteria, the V3–V4 region of the 16S rRNA gene was amplified by PCR using primers 338F (5'-ACTCCTACGGGAGGCAGCAG-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3'). For fungi, PCR amplification was performed using primers ITS1F (5'-CTTGGTTCATTA GAGGAAGTAA-3') and ITS2R (5'-GCTGCGTTCATCGATGC-3').

PCR products from the same samples were pooled and recovered from a 2% agarose gel. The recovered fragments were purified using the AxyPrep DNA Gel Extraction Kit, verified by electrophoresis on a 2% agarose gel, and quantified using a Quantus™ Fluorometer. Library construction was carried out using the NEXTFLEX Rapid DNA-Seq Kit. Sequencing was performed on the Illumina MiSeq platform by Shanghai Majorbio Bio-pharm Technology Co., Ltd.

#### 2.4.3. Basic soil physicochemical properties

Rhizosphere soil samples were collected from each experimental plot at both the seedling and harvesting stages. Five soil cores were randomly collected and thoroughly mixed to minimize spatial variability within each plot.

For the determination of soil pH and EC, 20 g of air-dried and sieved soil was placed into a beaker, and 100 mL of distilled water was added to achieve a soil:water ratio of 1:5. The suspension was stirred for 5 min to ensure complete mixing and then allowed to stand for 2 h [24]. Soil pH and EC were subsequently measured using a PB-10 pH meter (Sabolis, Germany) and a CON-10 EC meter (Thermo Feiyoute, America), respectively. Each sample was measured three times.

Additional rhizosphere soil samples were collected in mid-August during the rice filling stage. Five randomly selected samples from each plot were combined, air-dried at room temperature, ground, and sieved prior to analysis. The following soil parameters were determined according to the methods described by Huang et al. [24]: organic matter (OM) by the potassium dichromate oxidation method with external heating; total nitrogen (TN) by the semi-micro Kjeldahl method; total phosphorus (TP) by the sulfuric acid–perchloric acid digestion and molybdenum–antimony colorimetric method; and available potassium (AK) by the ammonium acetate extraction method.

#### 2.4.4. Rice growth and yield

Rice plants were sampled at the filling and maturity stages. Plant height, tiller number, and dry weight (after natural drying followed by air-drying) were measured, with five replicates for each treatment.

Yield determination was conducted in mid-October using the sampling method. Eighteen sampling quadrats were established for each treatment, each covering an area of 0.5 m<sup>2</sup>. One representative plant from each quadrat was selected for yield component analysis, while the remaining grains were harvested, dried, and weighed to calculate grain yield.

### 2.5. Data analysis

Data were organized using Excel 2021. Statistical analyses were performed using SPSS 26.0 software, including one-way ANOVA followed by Duncan's multiple range test ( $\alpha = 0.05$ ). Graphs were generated using Origin 2024 and GraphPad Prism 10.

## 3. Results

### 3.1. Effects of different treatments on straw decay rate and seed germination index of rice straw

The seed germination index (GI) is one of the most important indicators for evaluating the degree of straw decomposition after field return as well as its potential biotoxic effects on seedlings. Under the CF treatment, the GI of spring rice straw residues reached 102.87%, which was 60.72% higher than that observed under the CK treatment (Fig. 1). Moreover, the differences among treatments were statistically significant ( $p < 0.05$ ).

Similarly, the straw decomposition rate under the CF treatment was 51.22%, representing an increase of 34.64% compared with CK, and this difference was also significant ( $p < 0.05$ ). These results indicate that the application of low-temperature-resistant fungicides effectively promotes straw decomposition under low-temperature conditions, facilitates complete straw rotting and return to the field, and alleviates the adverse effects of incomplete straw decomposition on seedling growth.

### 3.2. Effects of different treatments on soil salinity indexes of rice at different growth stages

EC is an important indicator of the content of water-soluble salts in soil. For both CF and CK treatments, rhizosphere soil EC values were higher at the seedling stage than at the harvest stage (Table 2). At the seedling and harvest stages, the rhizosphere soil EC under the CF treatment was 8.62% and 9.29% lower than that under the CK treatment, respectively, with significant differences

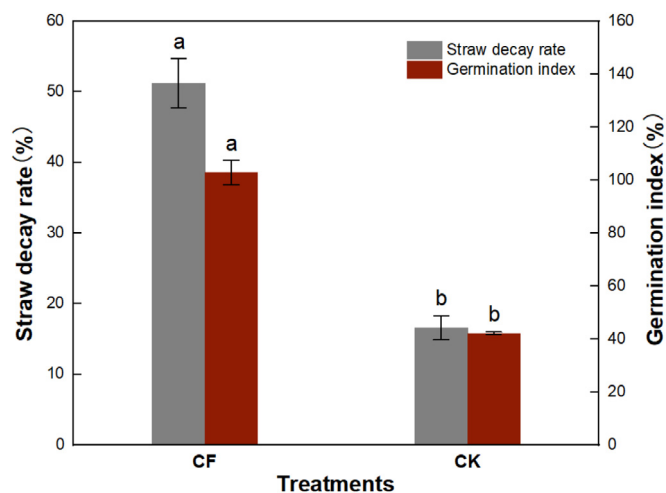


Fig. 1. Straw decay rate and seed germination index under different treatments. Note: Different lower-case letters on the bar graphs indicate significant differences ( $p < 0.05$ ).

Table 2  
Soil EC and pH at different stages.

Treatments	EC ( $\mu\text{S}/\text{cm}$ )		pH	
	Seeding stage	Harvesting stage	Seeding stage	Harvesting stage
CF	545.41 $\pm$ 11.75b	524.9 $\pm$ 10.15b	8.48 $\pm$ 0.02a	8.70 $\pm$ 0.03a
CK	592.46 $\pm$ 22.90a	573.65 $\pm$ 12.42a	8.54 $\pm$ 0.04a	8.75 $\pm$ 0.01a

Note: Data in the same column followed by a lowercase letter indicate significance ( $p < 0.05$ ), respectively.

between treatments ( $p < 0.05$ ). These results indicate that the CF treatment effectively reduced soil salinity in the rice rhizosphere, thereby mitigating soil salinization stress.

Soil pH is another key indicator reflecting soil salinity conditions. Under different treatments, the pH of rice rhizosphere soil generally showed higher values at the harvest stage than at the seedling stage. However, the differences in pH between CF and CK were minimal (Table 2), and no significant differences were detected between treatments ( $p > 0.05$ ). This suggests that the application of low-temperature-tolerant fungi for straw decomposition had a limited effect on soil pH regulation. The slight increase in pH observed during the harvest stage may be associated with the soil anti-salt phenomenon, namely the accumulation and redistribution of salts during the rice growing season.

In summary, straw decomposition induced by low-temperature-tolerant fungicides not only reduced the salt content in the rhizosphere soil but also improved the soil environment and alleviated the negative effects of salinization on rice growth. Although the change in soil pH was not significant, the reduction in soil salinity contributed to improved plant growth conditions and enhanced yield potential.

### 3.3. Effect of different treatments on soil nutrient content

Changes in soil physicochemical properties and microbial community structure can markedly influence soil nutrient availability. As shown in Fig. 2, the average total phosphorus (TP) content under the CF treatment was 1.27% higher than that under CK, and the difference between treatments was significant ( $p < 0.05$ ) (Fig. 2A). The average total nitrogen (TN) content was slightly higher in CF than in CK; however, this difference was not statistically significant ( $p > 0.05$ ) (Fig. 2B).

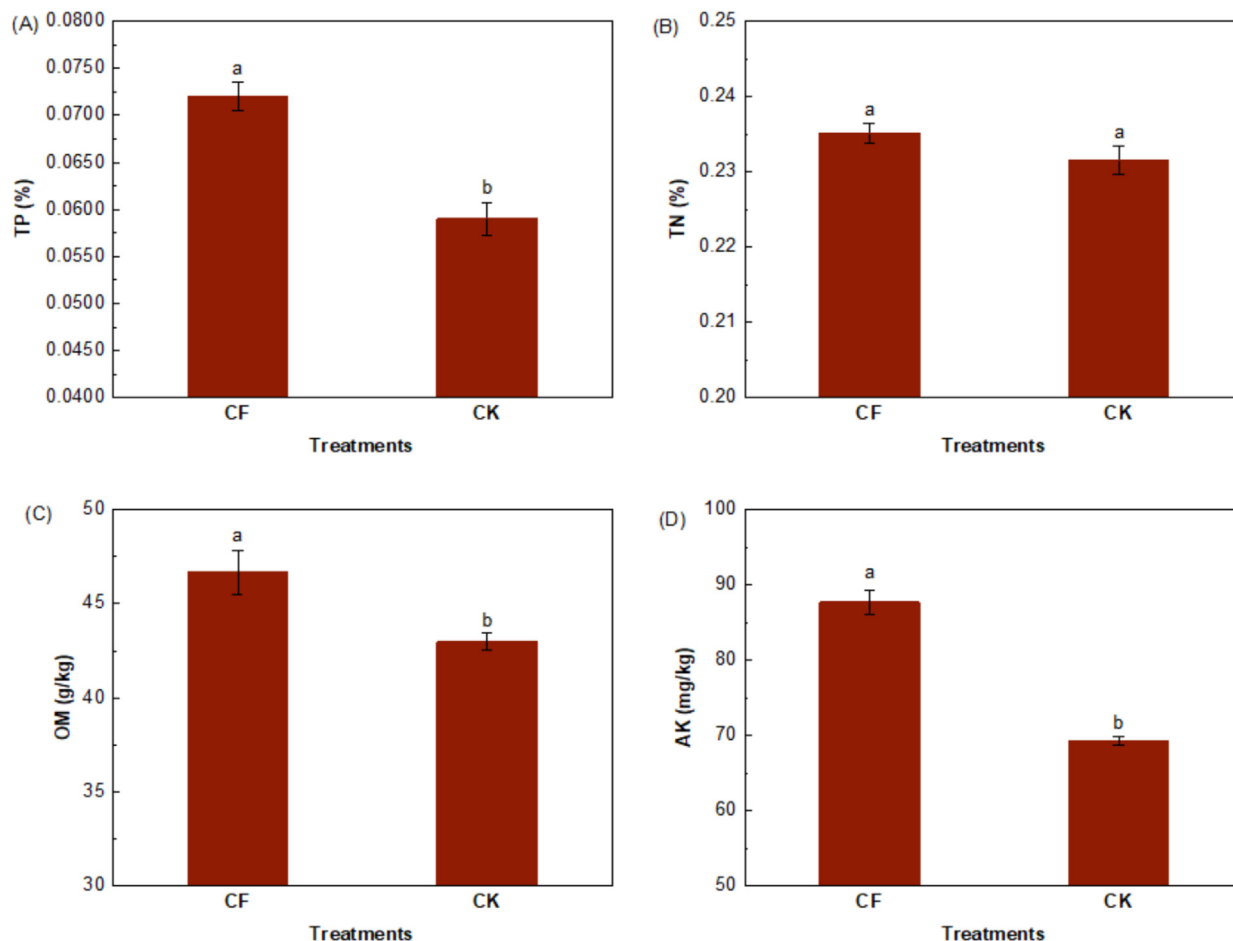
The average organic matter (OM) content in the CF treatment exceeded that in CK by 3.76 g/kg, showing a significant difference ( $p < 0.05$ ) (Fig. 2C). In addition, the average available potassium (AK) content in CF was 18.34 mg/kg higher than that in CK, with a significant difference between treatments ( $p < 0.05$ ) (Fig. 2D).

These results indicate that straw return combined with the microbial agent LTA promotes straw decomposition, increases soil nutrient content, enhances soil fertility, and is beneficial to the growth and development of rice.

### 3.4. Structural composition of bacterial flora under different treatments

The composition of the soil bacterial community serves as a comprehensive indicator of soil fertility, environmental health, nutrient cycling, and the effectiveness of agricultural management practices. As shown in Fig. 3, the relative abundance of *SBR1031* differed markedly between rhizosphere soils under the CF (4.38%) and CK (6.80%) treatments. Significant differences were also observed in the relative abundances of the top 10 dominant bacterial taxa.

*Anaerolineaceae* was a common dominant group in both treatments, with relative abundances of 3.48% in CF and 4.81% in CK.



**Fig. 2.** Soil nutrient contents under different treatments. (A) TP content, (B) TN content, (C) OM content, (D) AK content. Note: Different lower-case letters on the bar graphs indicate significant differences ( $p < 0.05$ ).

Notably, *Thermodesulfovibrionia* was identified as a unique dominant genus in the CF treatment, accounting for 1.57% of the bacterial community. In addition, the relative abundance of *Thiobacillus* in CF was 0.82% higher than that in CK.

Previous studies have demonstrated that *Thiobacillus* can directly or indirectly oxidize insoluble metal sulfides into soluble metal sulfates, while *Thermodesulfovibrionia* exhibits strong nitrogen-fixing capacity. These functional traits promote carbon and nitrogen metabolism during straw decomposition and accelerate the rotting process.

Overall, these results indicate that the CF treatment significantly altered the soil bacterial community structure, particularly by enriching nitrogen-fixing microorganisms. Such changes are likely to enhance soil nitrogen supply, facilitate straw decomposition, improve the soil micro-ecological environment, and ultimately create more favorable conditions for rice growth.

### 3.5. Structural composition of fungal flora under different treatments

The composition of the soil fungal community provides important information regarding soil health, plant diversity, environmental responses, soil quality, and ecosystem functioning. At the genus level, the top 10 fungal taxa differed between CF and CK soil samples (Fig. 4). Unclassified *fungi* exhibited the highest relative abundance in both treatments, accounting for 28.74% in CF and 48.91% in CK. *Pseudombrophila* was identified as a uniquely dominant genus in the CF treatment, with a relative abundance of 11.82%.

Furthermore, the relative abundances of *Mortierella*, *Glaciozyma*, and *Mrakia* in the CF treatment were 1.54%, 3.24%, and 2.19% higher than those in CK, respectively. These results suggest that straw decomposition promoted by the CF treatment not only increased overall fungal abundance but also enriched fungal taxa associated with cold-adapted fiber degradation, such as *Mortierella*, *Glaciozyma*, and *Mrakia*. This enrichment likely accelerated straw decomposition under low-temperature freezing conditions.

### 3.6. Effect of different treatments on rice growth parameters at the filling and maturity stages

Rice growth parameters are important indicators of plant growth and development. As shown in Table 3, during the filling stage, most morphological traits of rice were similar between the CF and CK treatments. The average number of tillers under the CF treatment was slightly higher than that under CK; however, the difference between treatments was not significant ( $p > 0.05$ ).

At maturity, rice plant height, tiller number, and dry weight under the CF treatment were all higher than those under CK, with average increases of 5.07%, 19.15%, and 5.42%, respectively. All of these differences were statistically significant ( $p < 0.05$ ).

These results indicate that the effect of straw return combined with low-temperature-resistant fungicide on rice growth was not pronounced at the filling stage. However, this treatment significantly promoted rice growth and development at maturity, effectively increasing plant height and tillering, and exerting a positive influence on plant dry weight accumulation.

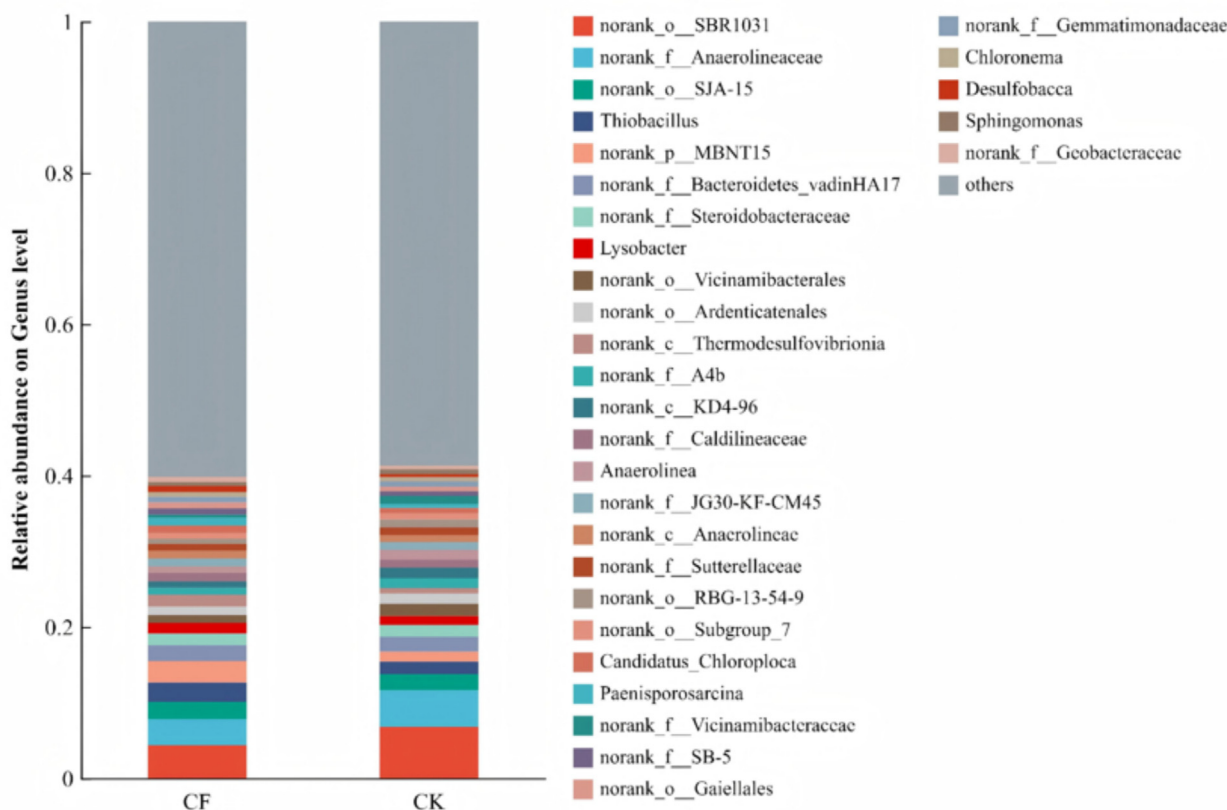


Fig. 3. Composition of bacterial community structure at the genus level.

### 3.7. Effect of different treatments on rice yield and yield components

Rice yield and its components reflect the effectiveness of straw return and decomposition, as well as the overall production potential and efficiency of rice cultivation. As shown in Table 4, under the CF treatment, the average number of grains per panicle, seed-setting rate, and 1,000-grain weight were slightly lower than those under CK, and no significant differences were observed between treatments ( $p > 0.05$ ). The average number of panicles per unit area under the CF treatment was 25.89 panicles/m<sup>2</sup> higher than that under CK, although this difference was also not statistically significant ( $p > 0.05$ ).

These results suggest that straw decomposition combined with the microbial agent LTA increased the number of panicles per unit area; however, yield components exhibited compensatory effects, whereby the increase in panicle number was accompanied by slight reductions in grain number and grain weight.

Overall, the average rice yield under the CF treatment was 483.82 kg/ha higher than that under CK, corresponding to a 5.84% increase. Although this difference was not statistically significant ( $p > 0.05$ ), the application of microbial agent LTA in combination with accelerated straw decomposition and return appears to be conducive to increasing rice yield.

## 4. Discussion

Reasonable straw incorporation has been widely reported to reduce soil salinity, improve soil structure, increase soil nutrient availability, enhance microbial diversity, and stimulate the activity

of soil microorganisms and related enzymes, thereby contributing to increased rice yield [25,26,27].

In the cold regions of Northeast China, freezing temperatures severely restrict the natural decomposition of returned straw, often resulting in decomposition rates of less than 30%. Incompletely decomposed straw can release phenolic compounds and organic acids that inhibit seed germination, damage plant root systems, and ultimately affect the emergence and growth of subsequent crops [17,28]. Therefore, accelerating straw decomposition under low-temperature conditions is critical for sustainable rice production in these regions.

The decomposition of rice straw in this study was mediated by an independently developed cold-tolerant microbial inoculant, which promotes nutrient release, organic carbon mineralization, and soil organic carbon balance [29,30]. Through these processes, soil fertility is enhanced, soil microbial community composition is altered, and plant growth is regulated [31,32].

### 4.1. Straw in-situ low-temperature decomposition technology improves the soil microenvironment by increasing soil nutrients and regulating salinity

The results of this experiment demonstrate that under extremely low-temperature conditions, with minimum temperatures reaching -31.7°C, the straw decomposition rate still reached 51.22%, which was 34.64% higher than that of CK. Yang et al. [33] reported a straw decomposition rate of 76.94% after 120 d of straw return combined with rotary tillage and crushing in a subtropical region. Although the decomposition rate observed in this study

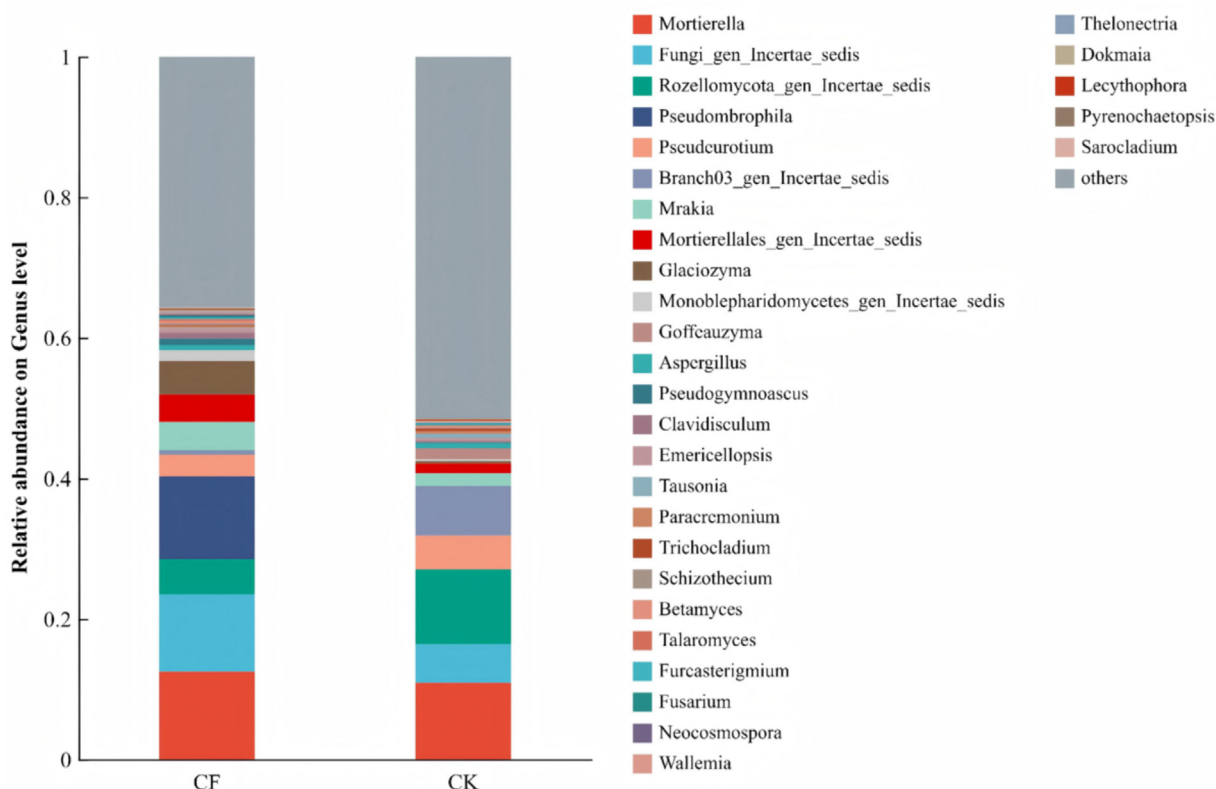


Fig. 4. Composition of fungal community structure at the genus level.

Table 3  
Morphological indicators of rice at different periods.

Treatments	Pustulation period			Maturation period		
	Plant height (cm)	Tillering (piece)	Dry weight (g)	Plant height (cm)	Tillering (piece)	Dry weight (g)
CF	104.44 ± 2.37a	15.75 ± 2.38a	63.16 ± 7.90a	99.94 ± 3.14a	14.56 ± 3.45a	70.61 ± 11.13a
CK	104.94 ± 1.90a	13.75 ± 2.49a	64.61 ± 13.70a	95.12 ± 3.73b	12.22 ± 2.24b	66.98 ± 11.40a

Note: Data in the same column followed by a lowercase letter indicate significance ( $p < 0.05$ ), respectively.

Table 4  
The effect of straw return on yield and yield components of rice.

Treatments	Number of grains per ear (grain/ear)	Setting percentage (%)	Thousand kerner weight (g)	Number of ears per unit area (ear/m <sup>2</sup> )	Production rate (kg/hm <sup>2</sup> )
CF	122.44 ± 27.83a	88.97 ± 4.67a	23.64 ± 1.18b	324.11 ± 76.34a	8761.46 ± 1009.95a
CK	124.01 ± 13.41a	90.01 ± 4.08a	24.32 ± 0.49a	298.22 ± 29.94a	8277.64 ± 934.22a

Note: Data in the same column followed by a lowercase letter indicate significance ( $p < 0.05$ ), respectively.

was lower than that reported for warmer regions, it nevertheless highlights the strong adaptability and degradation capacity of the cold-tolerant microbial inoculant under freezing conditions. Key functional microorganisms contained in the inoculant, such as *Trichoderma harzianum*, *Bacillus subtilis*, and *Bacillus licheniformis*, are known to secrete enzymes involved in cellulose degradation, thereby significantly enhancing cellulose hydrolysis [34,35,36]. This efficient decomposition process is closely associated with the selective enrichment of specific rhizosphere microbial communities induced by the CF treatment. Under the low-temperature conditions of this experiment, CF significantly promoted microbial taxa closely associated with straw decomposition, including *Ther-*

*modesulfovibrionia*, *Thiobacillus*, *Mortierella*, and *Glaciozyma*. Similar dominant microbial groups, particularly *Mortierella*, were also reported by Ma et al. [37]. These microorganisms not only participate directly in organic matter degradation but also accelerate straw decomposition through functional pathways such as nitrogen fixation and cold-adapted fiber degradation. From the perspective of microbial community structure, CF established a more favorable soil micro-ecological environment. Straw decomposition increased fungal abundance and microbial community diversity, thereby enhancing the soil's capacity for organic matter turnover and nutrient cycling and ultimately providing a healthier growth environment for rice. Consistently, Wang et al. [38] reported that

straw decomposition rapidly increases bacterial abundance and community diversity within the straw matrix. In contrast, traditional straw-burning practices, although capable of rapidly removing surface residues, exert negative impacts on soil microbial communities and may reduce beneficial microorganisms, thereby hindering long-term soil fertility recovery [39]. In addition to its superior degradation efficiency, CF exhibited clear ecological safety. Analysis of residual straw after decomposition showed that the seed germination index reached 102.87%, which was significantly higher than that under CK ( $p < 0.05$ ). This result indicates that phytotoxic effects associated with incomplete straw decomposition were effectively eliminated, confirming the safety of applying the cold-tolerant microbial inoculant.

Straw decomposition mediated by cold-tolerant microbial inoculants has also been shown to reduce soil pH and total salt content [40]. In the present study, accelerated low-temperature straw decomposition and return significantly reduced soil EC and slightly decreased soil pH, thereby alleviating soil salinity stress. This improvement can be explained by several mechanisms.

First, straw incorporation improves soil structure and permeability, facilitating the downward leaching of surface salts by natural precipitation and thus reducing salt accumulation in the root zone [37]. Second, straw decomposition generates organic acids [41]. The cold-tolerant inoculant used in this study contains *Weissella* and *Pediococcus*, which have been reported to produce lactic acid capable of lowering environmental pH, increasing  $\text{Ca}^{2+}$  concentration in soil solution, and displacing  $\text{Na}^+$  from soil colloids; the released  $\text{Na}^+$  can then be removed through leaching [42,43]. Finally, straw decomposition produces colloidal substances with negatively charged surfaces that strongly adsorb  $\text{Na}^+$ , thereby reducing  $\text{Na}^+$  concentration in the soil [43].

In parallel, Li et al. [8] reported that combining straw with biochar at appropriate incorporation ratios effectively enhances nitrogen retention and phosphorus availability while reducing nitrogen and phosphorus losses. Qin et al. [44] also demonstrated that straw incorporation significantly increased soil organic matter, nitrate nitrogen, ammonium nitrogen, and available potassium content before cotton sowing and throughout various growth stages, with average increases of 13.45%, 18.57%, 22.80%, and 22.57%, respectively, compared with the control.

In the present study, cold-tolerant microbial inoculation enabled effective straw decomposition and incorporation under low-temperature conditions. The CF treatment resulted in significantly higher soil nutrient contents than CK (Fig. 2), with the most pronounced difference observed in available potassium (AK). AK plays a critical role in plant growth and development and contributes to enhanced stress resistance.

The increase in soil nutrient content can be attributed to several factors. First, rice straw itself is rich in nutrients such as N, P, and K, which replenish soil nutrient pools upon decomposition [9]. Second, straw incorporation increases the soil carbon-to-nitrogen (C/N) ratio, stimulating microbial activity in the rhizosphere and accelerating straw decomposition and soil organic carbon formation [45]. Finally, as straw decomposes, large numbers of microorganisms proliferate and aggregate, forming an active microbial layer that accelerates nutrient release from straw residues and further enhances soil nutrient availability [46].

#### 4.2. Effects of low-temperature straw decomposition technology on plant growth indices and yield

Improvements in soil physicochemical properties, nutrient status, and microbial environments inevitably influence plant growth and development [47]. The grain-filling stage of rice is a critical period for nutrient accumulation and is strongly affected by

environmental factors such as photosynthetic efficiency, temperature, precipitation, and soil nutrient supply [48].

In this study, straw incorporation exhibited a relatively limited stimulatory effect on rice growth during the grain-filling stage. However, by the maturity stage, rice plants under the CF treatment showed significant advantages in morphological traits, including plant height, tiller number, and dry weight (Table 3).

These findings are consistent with those of Ning et al. [49], who reported that straw incorporation slightly reduced early rice yield but promoted increases in panicle number, filled grain number, and 1000-grain weight in late rice. This response may be attributed to the delayed release of nutrients from decomposing straw, which benefits rice growth during later developmental stages and ultimately influences final yield formation.

The CF treatment exhibited its most pronounced effects during the later growth stages, particularly at maturity. Although the stimulatory effects were not evident during the grain-filling stage, the long-term benefits of CF on rice growth and development were substantial, owing to sustained improvements in soil properties and enhanced microbial diversity.

This experiment demonstrated the comprehensive positive effects of low-temperature straw decomposition on rice grown in saline-alkali soil. Due to the compensatory and constraining relationships among yield components, the overall yield increase was not statistically significant; nevertheless, a 5.84% increase in rice yield was achieved.

In summary, in-situ low-temperature straw decomposition technology enables the efficient and safe decomposition of straw even under freezing conditions. Its application improves soil structure and physicochemical properties, increases soil nutrient availability, and provides a solid foundation for rice growth and development. As straw decomposition progresses, the soil environment is progressively optimized, accompanied by significant restructuring and enrichment of the microbial community. Numerous microorganisms capable of nitrogen fixation and cellulose degradation emerge, forming a novel tripartite interaction model centered on “microbe–soil–plant,” in which rice straw serves as the material basis and microorganisms act as the key driving factor.

## 5. Conclusions

Under the extremely low-temperature winter conditions of northeastern China, the CF treatment—characterized by the direct incorporation of all rice straw into the soil combined with the application of the microbial agent LTA to promote decomposition—significantly increased the seed germination index of decomposed straw to 102.87% in soda-alkali soils. The straw decomposition rate reached 51.22%, which was significantly higher than that of the CK treatment ( $p < 0.05$ ).

In addition, the CF treatment effectively reduced the electrical conductivity (EC) and pH of the rhizosphere soil, thereby alleviating soil salinity and alkalinity stress. This treatment also enhanced the diversity and abundance of rhizosphere microorganisms and promoted the enrichment of nitrogen-fixing and straw-degrading microbial taxa, including *Thermodesulfovibronia*, *Mortierella*, *Glaciozyma*, and *Mrakia*, which are closely associated with low-temperature fiber degradation.

Straw decomposition further contributed to improvements in soil nutrient status. The contents of total phosphorus, organic matter, and available potassium under the CF treatment were significantly higher than those under CK ( $p < 0.05$ ). These enhancements significantly influenced rice growth during the maturation stage and resulted in a 5.84% increase in rice yield.

Overall, the application of a low-temperature-tolerant microbial agent effectively overcomes the limitations of *in-situ* straw decomposition under freezing conditions. This approach provides a simple, efficient, and scalable solution for the improvement of saline-alkali paddy soils and the implementation of full-scale *in-situ* straw return during winter in cold regions worldwide.

### CRedit authorship contribution statement

**Bing Yang:** Writing – original draft, Investigation, Funding acquisition, Formal analysis, Data curation. **Xianchun Zong:** Writing – review & editing, Investigation. **Lin Lin:** Writing – review & editing, Investigation. **Junyou Shi:** Writing – review & editing, Conceptualization. **Chao Wang:** Writing – original draft, Investigation, Formal analysis. **Fachun Guan:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization.

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### Declaration of competing 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.

### Supplementary material

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### Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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