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Study on the effect of microbial agent M44 on straw decay promoting in cold soils

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Compound microbial agents are an important means to optimize soil quality and maintain soil microbial activity. When supplemented with microbial agents, straw returned to a field shows improved degradation efficiency and hence better nutrient release. However, due to the low temperature in the northern winter climate and the complex chemical composition of corn straw, the resultant low decomposition efficiency of straw returning to the field hinders the application of this process. In this study, the low-temperature-degradation microbial agent M44 of corn straw was used as the test material, and the effects of adding the pro-rot microbial agent on straw decomposition, nutrient release, enzyme activity, and the regulation of soil microorganisms were analyzed through an indoor pot straw degradation test. After 16 weeks of degradation under indoor pot conditions, the application of the microbial agent M44 promoted the shedding of the waxy layer on the surface of the straw, the average degradation efficiency of the straw increased by 8.9%, and the average nutrient-release rate of the straw carbon, nitrogen, phosphorus, and potassium increased by 6.7%, 12.8%, 7.4%, and 9.6%, respectively. The average enzyme activities of soil β-glucosidase (BG), β-xylosidase (BX), laccase (EC), acetyl glucosaminidase (NAG), and leucine aminopeptidase (LAP) increased by 9.82, 4.13, 9.46, 2.73, and 5.55 [nmol/(g·h)], respectively, which promoted the degradation of methoxyl carbon and alkoxy carbon, increased the relative content of alkyl carbon, anomeric carbon, aromatic carbon, and carbonyl carbon, and decreased the O-alkyl C/alkyl C value by 2.52; the composition and structure of soil bacterial and fungal communities were significantly changed, and *Pseudomonas*, *Stenotrophomonas*, *Microbacterium*, *Penicillium*, and *Gibberella* were significantly enriched, which increased the overall microbial activity through the production of degrading enzymes such as cellulase, thereby promoting the rapid degradation of straw. The present results thus provide theoretical support for the efficient decomposition of corn stalks in cold and arid regions.

Crop straw, which is rich in lignocellulose and contains a large amount of organic matter and nitrogen, phosphorous, potassium, and other nutrients, is a renewable biomass resource[1]. Returning straw to the field is considered an effective measure to improve soil fertility and increase soil biological activity[2,3]. However, due to the lignocellulosic composition of straw and cold and dry climatic conditions in some areas, the returned straw does not degrade completely to release the nutrients contained in its natural state[4] for use as a fertilizer source for crops in the current season, thereby affecting the sowing quality and becoming unfavorable for the growth of the next crop in rotation[5]. Especially in the cold and arid regions in the north, the synergistic effect of low temperature and drought in the fall and winter seasons significantly slows down the process of straw degradation, and this bottleneck seriously restricts the ecological benefits of straw return to the field and generates environmental risks.

Microbial-driven processes are the primary force behind lignocellulose degradation and nutrient transformation. Although cellulose-degrading microorganisms are prevalent in agricultural soils, studies have shown that their degradation efficiency varies depending on location and environmental conditions[6,7]. Previous research has demonstrated that efficient lignocellulose degradation requires the synergistic action of multiple microorganisms, as no single strain is capable of synthesizing all the enzymatic systems necessary to break down

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cellulose, hemicellulose, and lignin. Moreover, a single bacterial or fungal strain often struggles to independently degrade all straw components, particularly under harsh conditions where its metabolic capacity is limited and its sensitivity to environmental stress is heightened[8,9]. In contrast, microbial communities can degrade complex substrates synergistically through cooperative metabolism and functional complementarity[10–12]. Recent studies by Jiménez et al.[13] and Carlos et al.[14] have shown that microbial consortia exhibit superior efficiency and adaptability in lignocellulose degradation.

Studies have shown that natural synthetic flora are an excellent choice for straw degradation due to their stability, synergistic effects, and broad adaptability[15]. In contrast, single strains have limited functionality, and artificially complex bacteria often lack stability and synergistic effects. Natural synthetic flora, derived from natural environments, exhibit strong resistance to stress, minimal antagonism with indigenous microorganisms, and high efficiency in lignocellulose degradation. Through acclimatization, target microorganisms can be selectively enriched, enhancing their pollutant degradation capabilities and improving the stability of the community structure[16]. This, in turn, boosts their practicality and persistence in agricultural production.

As a new type of microbial product, a microbial agent has been widely used in recent straw-returning practices. There are several types of functional microorganisms in decay-promoting agents with high metabolic and proliferative abilities and strong environmental adaptability[17]. Straw returning when combined with microbial agents can improve the utilization efficiency of straw[18,19], improve soil enzyme activity[20], increase fertilizer and crop yield[21,22], and balance the soil microflora[23]. Wang et al.[24] studied different straw-returning methods in combination with a microbial agent and found that the straw-degradation efficiency of returning shallow rotary straw when combined with a microbial agent was better than that of returning no-tillage and subsoiling straw when combined with a microbial agent. Yang et al.[25] showed that the combination of returning straw with a decomposing agent and whole-film double-ridge tillage accelerated the straw decomposition and nutrient-release process. However, some studies have demonstrated that the application of microbial agents in the alpine regions did not significantly increase the straw decomposition rate and soil nutrients[26,27]. Existing microbial agents often exhibit limited efficacy in straw degradation under unfavorable environmental conditions. Therefore, it is essential to investigate functional microbial communities capable of efficiently degrading straw under field conditions, particularly in cold and arid regions.

Building on this, the present study focuses on the natural synthetic flora M44, which was enriched and screened from soil under low-temperature conditions. By evaluating its straw degradation efficiency in simulated cold and arid environments, the study aims to elucidate the mechanisms of microbial interactions. The goal is to provide new insights into the application of local microbial communities for sustainable straw management and soil improvement in extreme environmental conditions.

Materials and methods
Experimental design
The experiment was set up as follows: T1 - sterilized soil + microbial agent, T2 - nonsterilized soil + microbial agent, and T3-nonsterilized soil + no microbial agent. The microbial agent dosage was 0.1 g. The sterilized soil was sterilized by gamma radiation. Each treatment was set up in 35 POTS, and each culture bottle was filled with an appropriate amount of soil. Nylon mesh bags with 4 g of straw segment were washed, dried (at 60 ℃) to a constant weight, cut to a length of 3–5 cm, and placed in the middle of the soil. Each treatment was combined with ammonium phosphate and urea with the soil water content maintained at 20.0% and incubated in the dark at 15 ℃; this process was repeated 7 times for each treatment.

Experimental materials
The microbial agent M44[28,29] was mainly composed of dominant bacteria such as *Pseudomonas*, *Brevundimonas*, and *Flavobacterium*(It was stored in the microbial strain resource bank of Maize Center, Inner Mongolia Agricultural University, numbered IMAU-MCGFM44). The preparation process was as detailed elsewhere[16], and the number of viable bacteria was 1×10^9 CFU. The soil and straw tested in the culture bottle were taken from the 5–20 cm soil collected from the experimental field and harvested corn straw (Xianyu 696) sourced from China's Modern Agriculture Expo Park in Chile, Inner Mongolia Agricultural University. The soil type was sandy loam, and the basic fertility characteristics are shown in Table S1. The lignocellulosic content in the corn stalks is shown in Table S2.

Straw and soil sample collection
The straw samples were removed destructively at week 1 (W1), week 2 (W2), week 4 (W4), week 8 (W8), and week 16 (W16) to determine the relevant indexes of straw degradation, while the soil on the surface of the straw and the background soil around the nylon mesh bag were collected to determine the relevant indexes of microorganisms and soil enzyme activities.

Degradation ratio and nutrient content of the straw
The straw-degradation rate (DSR) was determined by using the weight-difference subtraction method. The degraded straw was dried and crushed, sieved through the 1-mm mesh, and tested for the content of cellulose, hemicellulose, and lignin as per the method of cellulose analyzer (Model ANKOM220, U.S.A.), followed by the calculation of the change in the degradation rate of lignocellulose. The content of soluble polysaccharides in the straw was determined by using the anthrone colorimetric method. Briefly, a small amount of dry straw sample was coated on a double-sided conductive adhesive. After gold spraying, images were obtained by scanning electron microscopy (SEM) at 10-kV acceleration voltage and 400X magnification. The organic carbon content was determined by using the potassium dichromate volumetric method with external heating, the total

nitrogen content by H_2SO_4-H_2O_2 cooking and distillation method, the total phosphorus content by vanadium-molybdenum-yellow colorimetry, and the total potassium content by the flame photometric method, and the nutrient-release rates were also calculated.

Organic carbon structure of the straw

The chemical structure of the organic carbonization in the straw residues was determined based on CPMAS solid-state nuclear magnetic resonance (13 C-NMR) technology using a superconducting high-resolution NMR spectrometer (Bruker Ascend) with a rotating speed of 12 kHz and a preorganic carbonization frequency of 13 C of 500 MHz. The chemical shift distribution of the straw NMR ranged from 0 to 220. As per a previous study[30], the NMR map can be divided into six main resonance regions: 0–44 ppm (Alkyl C, Alkyl Carbon), 44–68 ppm (O-CH3/NCH C, Methoxy Carbon), 68–94 ppm (O-Alkyl C, Alkoxy Carbon), 94–113 ppm (O-C-O anomeric C, Anomeric Carbon), 113–162 ppm (Aromatic C, Aromatic Carbon), and 162–220 ppm (Carbonyl C, Carbonyl Carbon).

Soil enzyme activity and microbial diversity

The enzyme activities of β-glucosidase (BG), β-xylosidase (BX), laccase (EC), acetyl glucosidase (NAG), and leucine aminopeptidase (LAP) were determined using a fluorescent microplate enzyme-labeled kit. Microbial biomass carbon (MBC) was determined by fumigation-extraction-volumetric analysis[31], while microbial biomass nitrogen (MBN) was determined by fumigation-extraction-Kjelberg nitrogen determination[31]. The Illumina MiSeq sequencing platform was used to detect the soil microbial community structure and diversity.

Statistical analysis

SPSS 26.0 statistical software was used to conduct the variance analysis of the data, principal coordinate analysis (PCoA) was performed based on the Binary_hamming distance, and data analysis of the straw carbon functional groups was performed by MestReNova software. Plot and equation fitting were performed with SigmaPlot 12.5 and Origin 2021, respectively. To reduce the network complexity and improve the calculation accuracy, bacteria and fungi ($P < 0.05$, $r > 0.06$, Spearman-related) were screened, and the above screening results were imported into Gephi.0.10, after which bacterial and fungal networks were constructed in soil treated without and with microbial agents.

Results and analysis

Degradation rate of the straw and lignocellulosic components

The degradation rate of each of the treatments increased with the continuous culture time showing a "fast-slow" trend during the culture cycle. The fitting of the relationship between the DSR and the degradation time of corn straw was found to be in line with the logarithmic equation (Fig. 1a), indicating that the treatment with the microbial agents (T1, T2) was significantly higher than that without the microbial agent (T3) ($P < 0.05$). The change of corn straw residue rate with time accords with the double exponential Eqs.[32,33] $y = CR \exp^{(-KRt)} + CS \exp^{(-KSt)}$, where CR is the decomposed component in the fast decomposition stage with decay rate KR (weeks), CS is the decomposed component in the slow decomposition stage with decay rate KS (weeks). T1 = $100.96e^{-2.19x} + 78.80e^{-0.02x}$, $R^2 = 0.9992$; T2 = $96.38e^{-2.04x} + 78.67e^{-0.02x}$, $R^2 = 0.9866$; T3 = $82.44e^{-2.19x} + 80.30e^{-0.02x}$, $R^2 = 0.9990$. When compared with the T3 treatment, the fast decay constants of the T1 and T2 treatments were significantly increased by 18.52 and 13.94, respectively. The slow decay constant displayed no significant difference among the T1, T2, and T3 treatments (Fig. 1b).

During the period of rapid straw decomposition in weeks 1st to 2nd, the degradation rates of cellulose, hemicellulose, and lignin treated by T1 and T2 were significantly higher than those of T3 treatment ($P < 0.05$) by
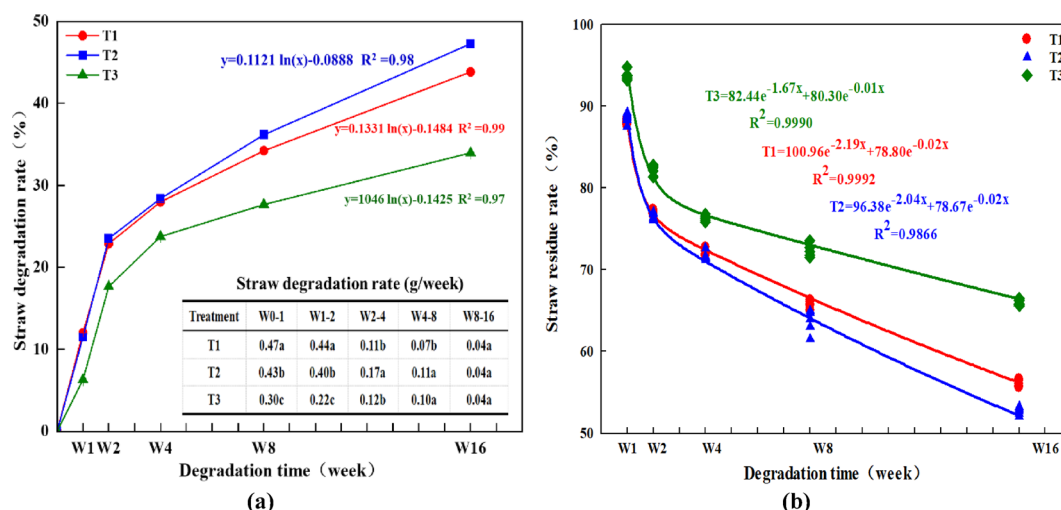

Fig. 1. Straw-degradation rate (**a**) and residual rate (**b**) under different treatments.

7.5%, 2.7%, 15.4%, 11.5%, and 4.8%, 5.0%, respectively (Fig. S1). At the 4th to 16th week, the degradation rates of cellulose, hemicellulose, and lignin treated by T1 and T2 were significantly higher than those by T3 treatment ($P < 0.05$), specifically by 6.3%, 5.5%, 9.8%, 12.8%, and 7.1%, 4.5%, respectively. The soluble sugar content of the straw showed the opposite trend; the T1 and T2 treatments showed significantly lower outputs compared to the T3 treatment ($P < 0.05$), and the straw soluble sugar content of the microbial agents (T1, T2) applied treatments was close to zero at week 16.

At the initial stage of degradation (W1–2), a large number of colonies were attached to the surface of the straw treated with microbial agents M44 (T1 and T2), and the surface structure changed from dense to sparse, with large uneven cracks appearing on the surface, while the waxy layer of the surface structure of the stalks treated with T3 was relatively smooth, regular, flat, and dense (Fig. S2). In the middle and later stages of degradation (W4–16), straw treated with microbial agents M44 (T1 and T2) exhibited uneven pores, exposed fiber structure, and microorganisms embedded in the straw for degradation, while a small number of small pores appeared on the straw surface under the T3 treatment. The overall structure of the straw under the T2 treatment was severely disrupted among the different treatment regimens, and the effect on straw degradation was especially evident.

Nutrient-release rate of the straw

The nutrient-release rates of the straw under each treatment increased with the cultivation time (Fig. 2). During the straw rapid decomposition period (W1–2) and the slow decomposition period of the straw (W4–16), the release rates of carbon, nitrogen, phosphorus, and potassium under the T1 and T2 treatments were significantly higher than those under the T3 treatment ($P < 0.05$), by 3.5%, 2.2%, 11.6%, 14.3%, 5.2%, 10.5%, 8.9%, 8.7% and 7.9%, 9.7%, 9.5%, 11.8%, 3.4%, 6.5%, 10.6%, 10.5%, respectively. In general, the release efficiency of the straw nutrient elements in W1–2 weeks was higher than that in W4–16, and the nonsterilized soil + combined microbial agent (T2) treatment showed the best results(Fig. 2).

The NMR spectrum

The straw NMR-absorption peaks were identified as detailed elsewhere[30]. The straw alkoxy carbon (O-alkyl C) and carbonyl carbon (Carbonyl C) were the easily degradable components, while the alkyl carbon (Alkyl C)
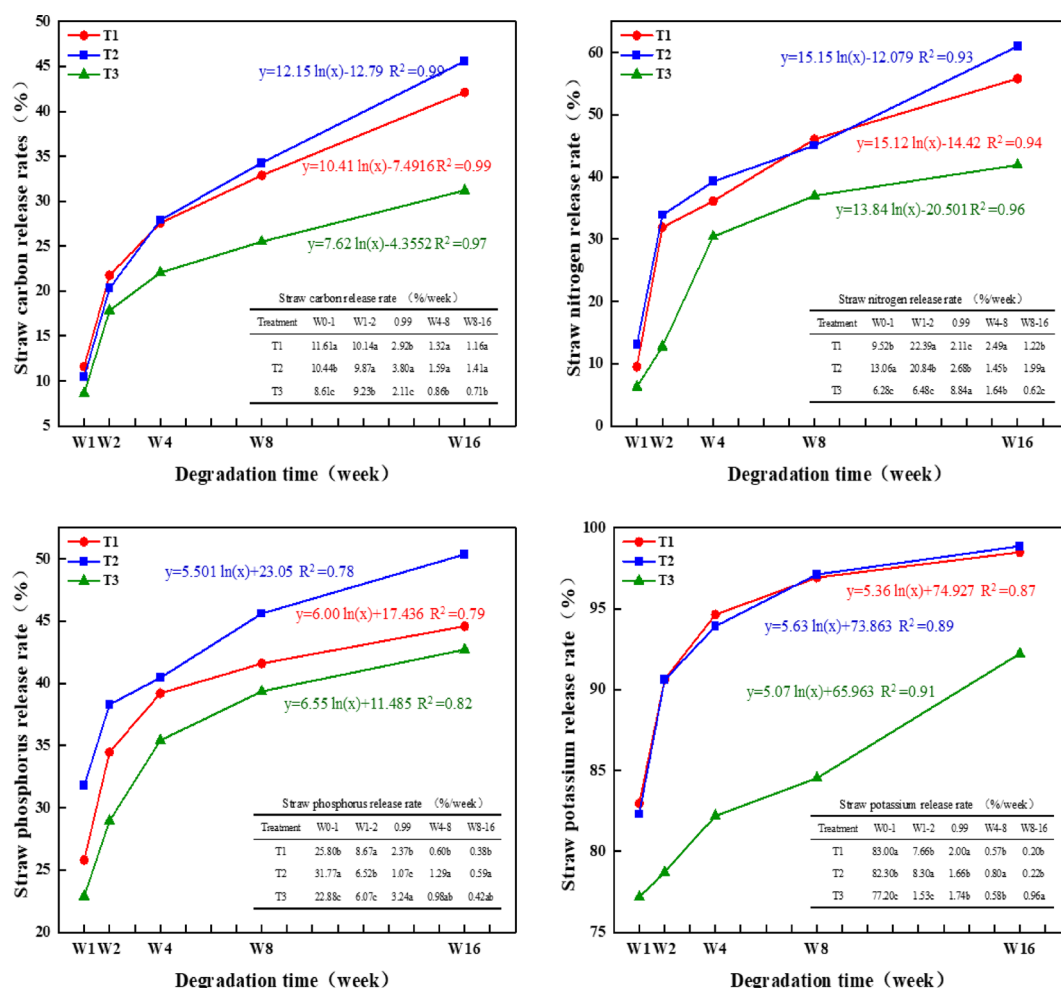

Fig. 2. Nutrient-release rate of raw maize stalk under different treatments.

Degrading time(week)	O-alkyl C/Alky1 C		
	T1	T2	T3
W1	13.14c	14.33b	19.91a
W2	7.47b	6.15c	12.65a
W4	5.62b	4.85c	10.26a
W8	4.86c	5.29b	7.29a
W16	3.55b	3.56b	6.08a

Table 1. Changes in the alkoxy carbon/alkyl carbon of the straw carbon functional groups under different degradation stages of each treatment.
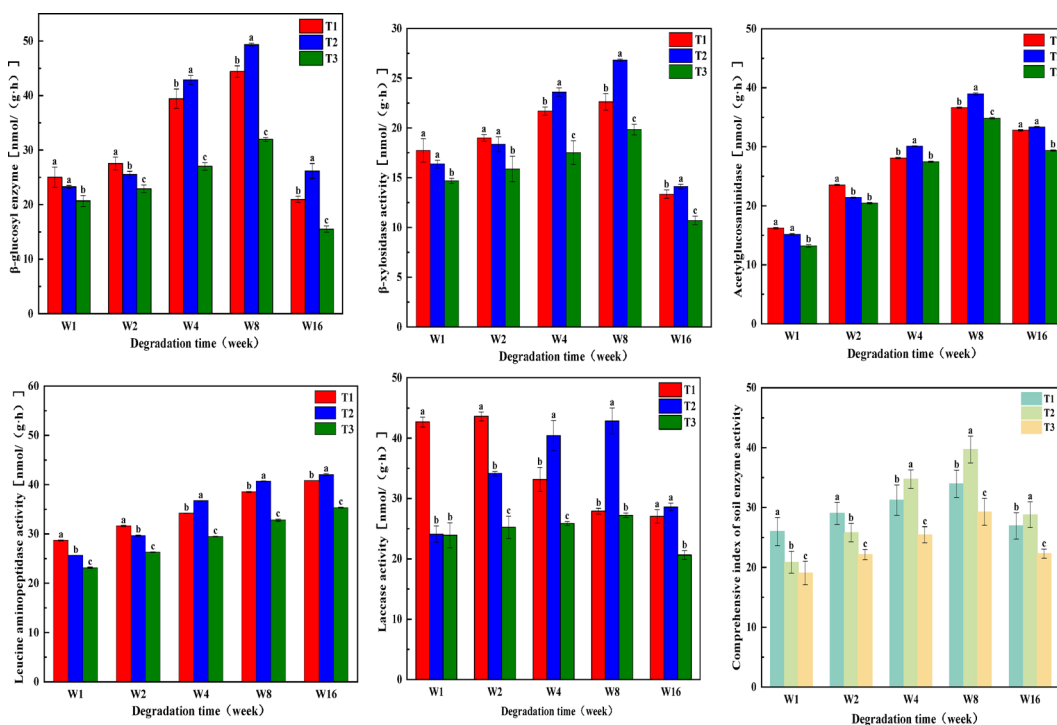

Fig. 3. Soil enzyme activities in different treatments.

was difficult to degrade. The relative contents of the easily degradable components O-CH3/NCH Cand O-alkyl C decreased continuously under different treatments, while the degradation efficiency of the straw under the microbial agents M44 (T1, T2) treatment was significantly higher than that under the non-microbial agent (T3) treatment. The results indicated that the addition of agent M44 mainly decomposed cellulose, hemicellulose, and other easily decomposed carbon in straw at the early stage of decomposition. Alkyl C, O-C-O anomeric C, aromatic carbon, and carbonyl C are on the rise, and the latter mainly degrade lignin, tannin, and other difficult-to-decompose carbon (Fig. S3a-c). O-alkyl C/alkyl C was used to indicate the degree of rot of the straw, as shown in Table 1, with no significant difference among the O-alkyl C/alkyl C values between the T1 and T2 treatments ($P > 0.05$), which was significantly lower than that for the T3 treatment.

The correlation analysis between the DSR and the functional groups (Fig. S4) indicated a significant negative correlation between the DSR and the straw alkyl carbon and methoxy carbon content. In other words, the lower the DSR, the greater the proportion of alkyl carbon and methoxy carbon content. Among them, the correlation coefficients between the proportions of the relative contents of alkyl carbon and methoxy carbon and the DSR were larger under the T2 treatment, suggesting that it was more sensitive to the distribution of the alkyl carbon and methoxy carbon contents under the fungus application (T2) treatment.

The soil enzyme activity

As shown in Fig. 3, soil BG, BX, and NAG activities gradually increased with the process of straw degradation, reaching the peak value in the 8th week, while the LAP activity showed a continuous upward trend, and the enzymatic activity of T1 and T2 treatments was significantly higher than that of T3 treatment. At the early stage of decomposition (W1–2), the EC activity for T1 treatment was significantly higher than that for T2 and T3 treatments, and, from weeks 4–8, the activity was significantly higher for T2 treatment than for T1 and T3 treatments. The comprehensive index of soil enzyme activity showed that the microbial agents (T1, T2)

treatment was significantly higher than that of the non-microbial agent (T3) treatment ($P < 0.05$), which was 4.28 and 10.18-higher, respectively.

Soil microbiota carbon and nitrogen

The soil microbial biomass carbon and nitrogen content under each treatment exhibited a single-peak curve over the cultivation period (Table S3). At the W8 stage, the soil microbiomass carbon and nitrogen contents were significantly higher ($P < 0.05$) in the microbial agent treatments (T1 and T2) compared to the non- microbial agent treatment (T3). Specifically, soil microbiomass carbon content increased by 509.13 and 231.01 mg·kg^{-1}, while soil microbiomass nitrogen content increased by 114.41 and 27.39 mg·kg^{-1}, respectively. These results indicate that the application of the microbial agent M44 significantly enhanced soil microbiomass carbon and nitrogen content, which favors microbial reproduction and provides essential nutrients for straw degradation. Consequently, this improvement promotes microbial efficiency in degrading straw.

Analysis of the soil microbial alpha diversity and community structure

Significant differences were noted in the Chao index, Ace index, Shannon index, and Simpson index of soil bacteria and fungi under different treatments ($P < 0.05$) (Fig. 4, Fig. S5). The Chao, Ace, and Shannon indices of the T3 treatment were significantly higher than those of the T1 and T2 treatments ($P < 0.05$). The Simpson index of the T3 treatment was significantly lower than that of the T1 and T2 treatment ($P < 0.05$), which were lower by 0.19, 0.11, 0.16, and 0.32, respectively. The Chao and Ace indices under the T2 treatment were significantly higher than those under the T1 treatment, indicating that the application of the microbial agent M44 significantly changed the diversity and richness of the soil bacterial and fungal communities. Based on the composition of the amplicon sequence variant (ASV) of soil bacteria and fungi treated with different treatments, PCoA analysis was performed using the Binary_hamming distance matrix (Fig. 4cf), and the species composition and structure of different degradation stages were significantly clustered under treatments with microbial agents (T1, T2). The absence of microbial agent (T3) was relatively discrete, and the application of the microbial agent was the main driving factor of soil β diversity.

Based on the classification level of bacteria and fungi (**Fig. S6**a-l), the dominant soil microbial under different treatments were Proteobacteria, *Actinobacteriota*, *Firmicutes*, *Bacteroidota*, Ascomycotam, and *Basidiomycota*. The application of microbial agent M44 can change the composition of the soil indigenous flora, and although T1 and T2 treatments significantly reduce the soil microbial richness and diversity (Fig. 4), the difference analysis results of T2 with microbial agent M44 and T3 without non-microbial agent M44 treatment showed that, during the degradation time, T2 treatment significantly enriched *Pseudomonas*, *Stenotrophomonas*, *Enterococcus*, *Achromobacter*, *Massilia*, *Microbacterium*, *Penicillium*, *Caphalotrichum*, and *Gibberella* ($P < 0.05$) (Fig. 5a-j).
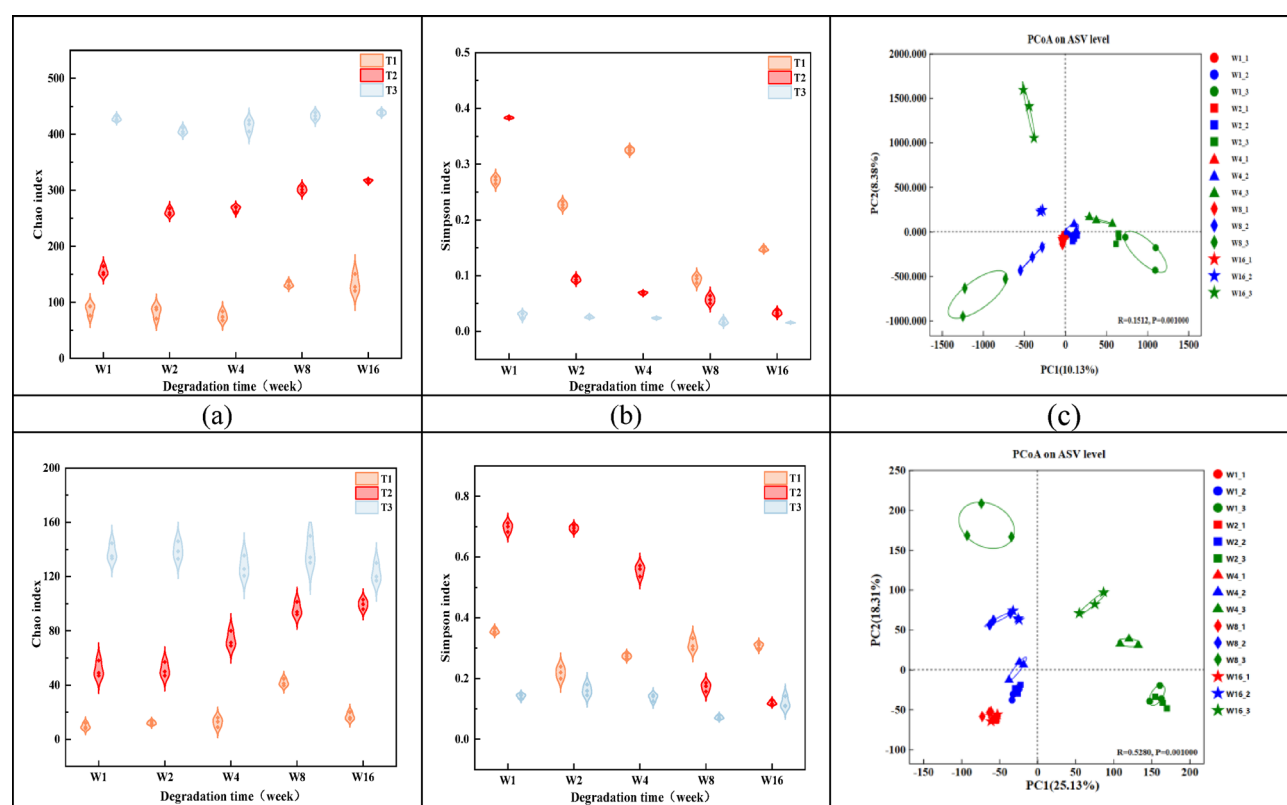

Fig. 4. Alpha diversity analysis and principal component analysis of soil bacteria and fungi under different treatments.
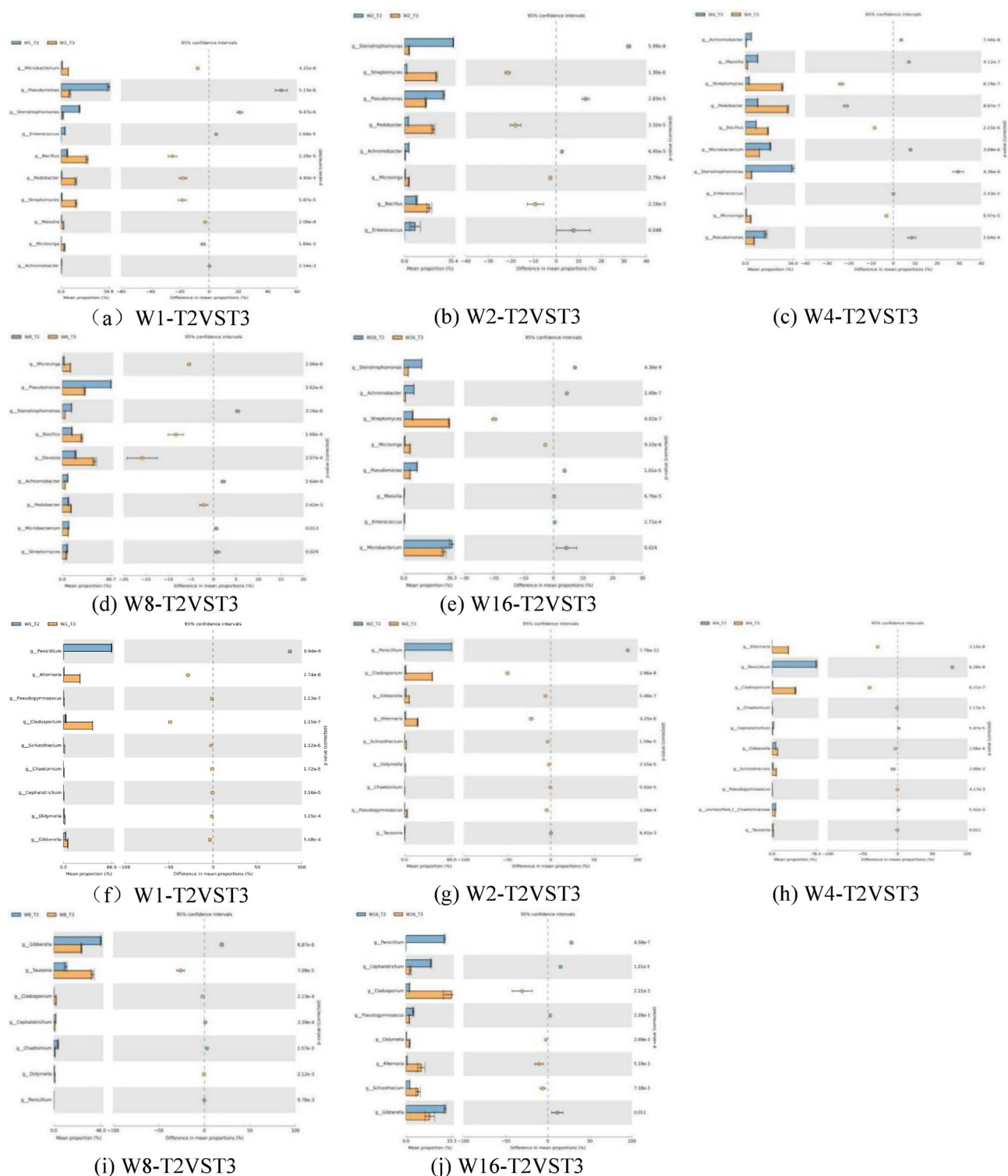
(a) W1-T2VST3   (b) W2-T2VST3   (c) W4-T2VST3

(d) W8-T2VST3   (e) W16-T2VST3

(f) W1-T2VST3   (g) W2-T2VST3   (h) W4-T2VST3

(i) W8-T2VST3   (j) W16-T2VST3

Fig. 5. Bacterial (**a**-**e**) and fungi (**f**-**j**) abundance at the genus level between the T2 and T3 treatments.

Soil bacterial and fungal network analysis and Zi-Pi module analysis

To further explore the response of indigenous microorganisms to the application of microbial agents under different treatments, a symbiotic network analysis of the soil flora was conducted in this study (Fig. 6). The network connectivity was 18.9% and 16.7% higher in the T3 treatment compared to that in the T1 and T2 treatments, respectively. Modular analysis indicated that the main dominant genera in Modules 1 and 2 were *Streptomyces*, *Microvirga*, *Enterococcus*, *Penicillium*, *Alternaria*, and *Pseudogymnoascus* in the T1 treatment and *Pseudomonas*, *Stenotrophomonas*, *Microbacterium*, *Penicillium*, *Gibberella*, and *Cephalotrichum* in the T2 treatment. *Streptomyces*, *Pedobacter*, *Bacillus*, *Cladosporium*, *Alternaria*, and *Gibberella* were the main dominant
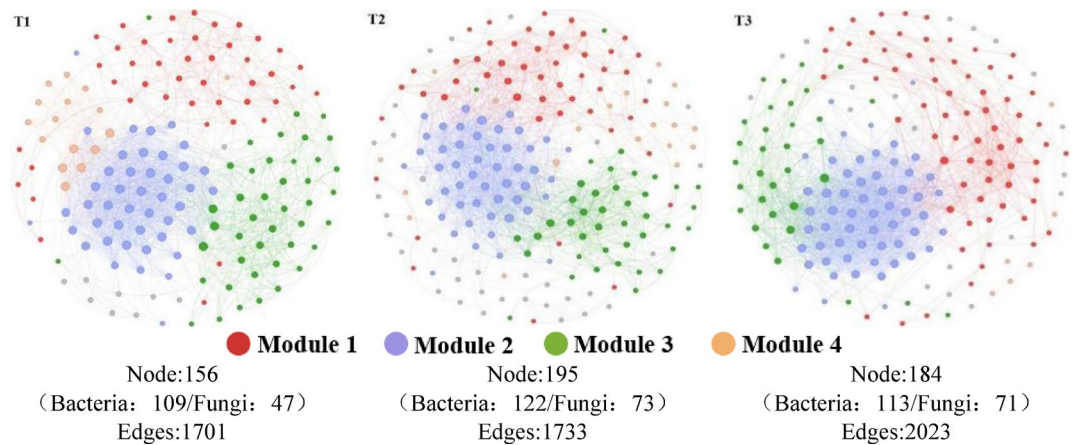
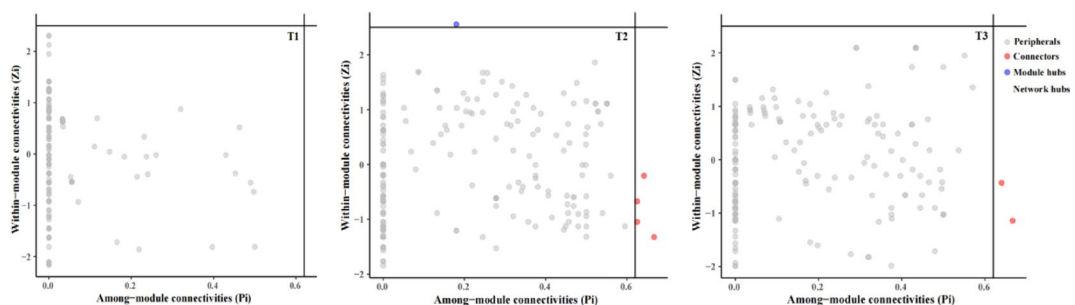**Fig. 6**. Network of soil bacteria and fungi community structure under different treatments.

Fig. 7. Zi-pi module analysis of the soil microorganisms.

genera in the T3 treatment. In conclusion, the application of the microbial agent M44 in the treatment could regulate the soil microbial community and the soil microbial synergistic relationship, which significantly increased the abundance of *Pseudomonas*, *Stenotrophomonas*, *Microbacterium*, *Penicillium*, and *Gibberella* in the soil (Fig. 6 and Fig. S7).

According to Zi (intra-module connectivity) and Pi (inter-module connectivity), most ASVs within these cooccurring networks could be classified as peripherals (Fig. 7). The key bacterial dominant species between the different treatments were defined as 1 Modulehubs and 6 Connectors, wherein *Proteobacteria* was the main module hub and ASV135 of *Proteobacteria* was *Pantoea*. The connectors of bacteria included *Actinobacteriota*, *Adhaeribacter*, and *Proteobacteria*. The fungal bacterial dominant species connectors include *Basidiomycota* and *Ascomycota*. Meanwhile, no network hubs were detected in bacteria and fungi in different treatments. These results proved that different application treatments mainly induced changes in the core flora of *Proteobacteria* (*Pantoea*), *Bacteroidota*, *Actinobacteriota*, and *Ascomycota*.

Structural equation modeling (SEM) of the soil microbial network modules and indicators related to straw degradation

To quantify the causal relationship between the soil microbial network modularity and environmental factors, SEM was used, which showed that the application of the microbial agent M44 (T2 treatment) could significantly increase the negative correlation effects of the soil bacterial network Module 1 on β-glucosidase, LAP, laccase, and β-glucosidase on the nitrogen-release rate and phosphorus-release rate, with a positive correlation effect of laccase on the nitrogen-release rate (Fig. 8). Moreover, the explained DSR was higher than that of the T3 treatment, which was 0.054, indicating that the application of the microbial agent M44 could increase the explained rate of the maize stover-related indexes on the explanation rate of the stover-degradation rate (Fig. 8b). This suggests that the application of the microbial agent M44 could improve the soil microbial activity by altering microbial interactions, reducing competition, or promoting cooperative relationships between certain bacterial groups, which, in turn, could improve the efficiency of straw degradation (Fig. 8, Fig. S8).

Correlational analysis between the DSR and environmental factors

Correlational analysis between soil microbial diversity and environmental factors (Fig. 9) revealed that DCR, DBCR, C, P, LAP, Anomeric-C, Aromatic-C, and MBC were highly significantly and positively correlated with the microbial agent (T2) treatment and that C, BG, BX, LAP, Alkyl-C, and Methoxyl-C were positively correlated with the fungal community composition. Moreover, DSR, DCR, DBCR, DLR, C, P, BX, LAP, Allkyl-C,
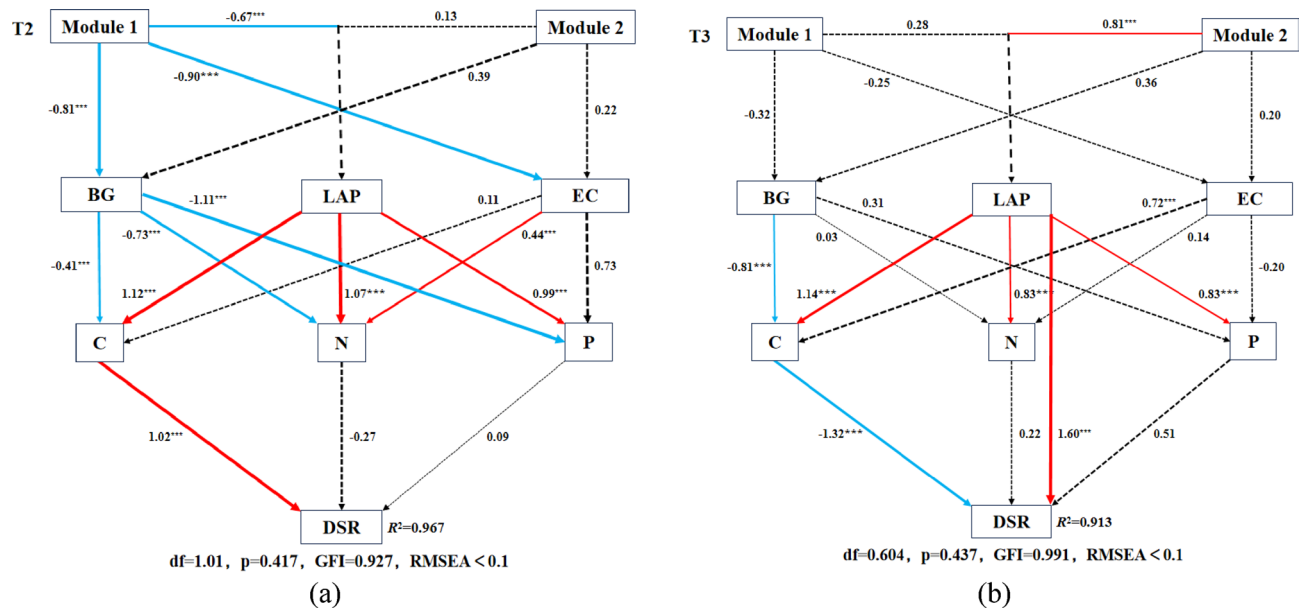
Fig. 8. Structural equation modeling (SEM) of soil bacterial network modules and indicators related to straw degradation. (1) The arrows indicate the standardized path coefficients (SPCs); *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. (2) Red color represents positive correlation, blue color represents negative correlation, solid lines represent significance, black dashed lines represent non-significance, and the thickness of the lines indicates the size of the path coefficients. (3) DSR: Straw-degradation rate; Module 1: Soil bacterial network module 1; Module 2: Soil bacterial network module 2; BG: β-glucosidase; LAP: Leucine aminopeptidase; EC: Laccase; C: Straw carbon-release rate; N: Straw nitrogen-release rate; P: Straw phosphorus-release rate.

Fig. 9. Correlational analysis of soil bacteria, fungi, and straw degradation-related indexes. DSR: Straw-degradation rate; DCR: Cellulose-degradation ratio; DHR: Hemicellulose-degradation ratio; DLR: Lignin-degradation ratio; LAP: Leucine aminopeptidase; BX: β-xylosidase; BG: β-glucosidase; NAG: Acetylglucosaminidase; EC: Laccase.

Anomeric-C, and Carbonyl-C were highly significantly and positively correlated with the bacterial community composition in the no-microbial agent (T3) treatment, and DCR, DBCR, DLR, P, BX, LAP, NAG, O-allkyl-C, Anomeric-C, and Carbonyl-C were highly significantly and positively correlated with the fungal community composition. In conclusion, it was shown that soil MBC, straw alkyl carbon (Alkyl-C), methoxyl carbon (Methoxyl-C), and fast-acting nitrogen and phosphorus were the main factors driving changes in the microbial communities. Thus, the application of microbial agents not only directly affected the composition of microbial communities but also indirectly enhanced microbial interactions and functional efficiency by modifying the soil environment.

Discussion and conclusion

Decomposition and nutrient release of returning straw are the keys to soil fertility. Straw return to the field provides a sufficient carbon source for soil microorganisms, promotes the growth and reproduction of microorganisms, and improves soil biological activity. The degradation of straw and the transformation of organic matter are mainly controlled by microorganisms. Straw microbial degradation is a new way to effectively promote the crop straw decomposition process, and the stability of the microbial flora is an important prerequisite to ensure its effectiveness.

In this study, we demonstrated that the microbial agent M44 significantly increased the maize straw degradation rate under low-temperature conditions, showing a 12.2% higher degradation rate compared to the control group. This indicates that microbial agent M44 possesses high adaptability and degradation capacity in cold environments. The major functional bacteria in the microbial agent M44, such as *Pseudomonas* and *Microbacterium*, secrete enzymes involved in cellulose degradation, thereby significantly promoting cellulose hydrolysis[34–36]. High-throughput sequencing results revealed that the application of the microbial agent M44 enriched core microbial communities with lignocellulose degradation capabilities and accelerated the decomposition of easily degradable carbon components, in line with previous studies[37]. In this study, microbial co-occurrence networks and Zi-Pi analysis identified *Pseudomonas*, *Stenotrophomonas*, and *Penicillium* as core species in the degradation modules, collectively forming an efficient synergistic degradation system. The abundance of these core species was significantly correlated with key enzyme activities and straw degradation indicators, confirming the positive effect of optimized network structures on degradation efficiency. Furthermore, the application of the microbial agent M44 significantly increased soil microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN) content, thereby enhancing the soil carbon pool[38]. It also accelerated straw decomposition through a multi-layered mechanism, including microscopic structural damage, enhanced enzymatic activity, and community reconstruction. Although the application of the microbial agent M44 reduced soil microbial diversity and richness, it enriched dominant degrading microbial groups such as *Proteobacteria* and *Enterococcus*. Under microbial agent-driven conditions, certain microbes exhibited selective advantages, consistent with previous research findings[39].

In this experiment, under indoor sealed conditions, the microbial agent M44 significantly enhanced straw degradation efficiency by regulating the soil microbial community structure and optimizing microbial interactions. SEM analysis revealed that the microbial agent M44 promoted negative correlations between microbial network module 1 and enzymes involved in nitrogen and phosphorus release, while also enhancing microbial synergy. Network analysis further showed that the microbial agent M44 improved the connectivity of the microbial community by increasing the abundance of beneficial genera such as *Pseudomonas* and *Stenotrophomonas*. Correlation analysis indicated that the microbial agent M44 indirectly promoted microbial activity and straw degradation by altering the soil environment, including the availability of nitrogen, phosphorus, and organic carbon. In conclusion, the microbial agent M44 significantly enhanced straw degradation efficiency under indoor sealed conditions by modulating microbial community composition, strengthening synergistic effects, and optimizing the soil environment.

Unlike previous studies, which have primarily focused on temperate and humid environments, this study is the first to validate the degradation effect of microbial consortia under cold and arid conditions. The results demonstrate that, even under such restrictive conditions, the microbial agent M44 significantly promotes the degradation of cellulose, hemicellulose, and lignin, overcoming the degradation bottleneck of straw in cold and arid regions, with promising application prospects. However, it is important to note that this experiment was conducted under controlled indoor conditions, which did not account for the complex environmental factors present in field conditions. Therefore, future field trials are necessary to evaluate the environmental adaptability, sustained degradation capacity, and potential impact on crop growth.

In summary, the microbial agent M44 promotes straw degradation by regulating microbial community structure, enhancing enzymatic activity, and reshaping the abundance and interactions of key genera in modules 1 and 2 (such as *Streptomyces*, *Enterococcus*, *Pseudomonas*, and *Gibberella*). This process leads to the decomposition of easily degradable components (O-alkyl C, Carbonyl C) and the accumulation of more resistant components, contributing to the establishment of a more stable and efficient degradation system[40]. The study highlights the potential application of the microbial agent M44 in regulating the straw decomposition process in restrictive environments, such as cold and arid conditions. This research provides theoretical support and practical pathways for rapid straw return and sustainable soil management in cold and arid regions, demonstrating the potential of microbial community activity to drive straw decomposition under unfavorable environmental conditions.

Data availability

The original contributions presented in this study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author(s). The microbial raw sequence data were deposited in the NCBI and are available under accession numbers PRJNA 1205916.

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Author contributions

WLJ wrote the first and revised drafts. WLJ and ZWS performed the primary data analysis. QGE conceived project idea, and design, provided suggestions for revising the paper. LRZ, JHR recorded and measured the test data. YXF, GJL, HSP made suggestions for revising the paper. All authors read and approved the final manuscript.

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Declarations

Competing interests

The authors declare no competing interests.

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