THE EFFECT OF IMMOBILIZED *BACILLUS AMYLOLIQUEFACIENS* JF-1 MODULATE DYNAMICS ON SOIL MICROBIAL COMMUNITIES AND DISEASE SUPPRESSION CAUSED BY *FUSARIUM GRAMINEARUM*

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Abstract. Stalk rot in maize, which is mainly caused by *Fusarium graminearum*, is a common soilborne disease in maize-producing areas of the world. Here we show the potential of *Bacillus amyloliquefaciens* JF-1 as a biological control against *Fusarium graminearum*. The inhibition rate of *F. graminearum* mycelial growth by JF-1 was 83.95%. Scanning electron microscopy revealed that mycelia treated with JF-1 had swelling and deformation. Moreover, the antagonistic mechanism of JF-1 crude extract decreased the intracellular ATP content of pathogens and led to significant nucleic acid leak. To evaluate the potential use of JF-1 in agriculture, a pot experiment revealed that the control efficiency of JF-1 cells immobilized with maize straw biochar against maize stalk rot at seedling stage reached 65.36%. The immobilized microorganisms of maize biochar improved the physical and chemical properties and soil fertility, as well as the microbial community structure, increased the relative abundance of *Proteobacteria*, *Actinobacteria*, *Mortierellomycota* and *Glomeromycota*, and reduced the relative abundance of *Ascomycota*, which includes *F. graminearum*, with pathogenic potential, transforming the soil microbial community from 'pathological' to 'healthy'. This discovery confirms that JF-1 can be used as a biological control agent to effectively reduce the incidence of maize disease.

Keywords: *agricultural wastes, soil nutrients, maize disease, soil remediation, biological control*

Introduction

Maize (*Zea mays* L.) is not only an important food and forage crop but also a major industrial raw material and energy source (Deutsch et al., 2018; Savary et al., 2019). With an increase in the degree of intensive crop planting, continuous cropping obstacles, such as deterioration of maize growth and decline in yield and quality, have been observed. The occurrence of soil-borne diseases is an important factor causing continuous cropping obstacles (Ren et al., 2008). Stalk rot, a soil-borne disease caused by *Fusarium graminearum*, is widely distributed in many maize growing areas of the world. *F. graminearum* is a soil-borne fungus, which mainly infects maize roots and can cause damage throughout the growth period of the plant. *F. graminearum* is considered as a species complex that can produce not only deoxynivalenol in the form of 3-acetyldeoxynivalenol (3ADON chemotype) or 15-acetyldeoxynivalenol (15ADON chemotype) but NIV and acetylated derivatives (NIV chemotype) (Chen et al., 2022). It can also inhibit the synthesis and transformation of proteins in animal cells, leading to apoptosis of animal cells; it thus threatens the health of humans and

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animals in addition to that of infected crops (Pestka, 2010; Rocha et al., 2005). In recent years, due to reasons, such as straw carrying pathogenic fungi returned to the soil and long-term continuous cropping, the continuous accumulation of pathogenic fungi in soil has become an important cause of maize stalk rot infection, posing a serious threat to the soil microecological environment and agricultural economy.

As a plant disease control technology with broad application potential, biological control can control soil-borne diseases through antagonism of microorganisms toward pathogenic fungi, improvement of the physical and chemical properties of soil, and induction of plant resistance gene expression. Recently, biological control of plant diseases has been achieved in many crops (Qi et al., 2020; Liu et al., 2021). Studies have shown that the genera *Bacillus* and *Pseudomonas* play a key role in the control of various plant pathogenic fungi, such as *Eutypa lata*, *Gaeumannomyces graminis*, and *Magnaporthe oryzae* (Wakelin et al., 2002; Shan et al., 2013; Abo-Elyousr et al., 2009). *Paenibacillus polymyxa* can inhibit pathogenic fungi by secreting chitinase, cellulase, protease, and other enzymes that can destroy the cell wall structure of pathogenic fungi (Jensen et al., 2002). *Pseudomonas monteilli* not only enhances the ability of plant roots to absorb nutrients but also enhances the resistance of plants to pesticides and heavy metal pollution stress (Ramesh et al., 2009; Rani et al., 2009). Jain et al. (2019) reported that the encapsulation of pea seeds by biocontrol bacteria can induce systemic resistance to *Sclerotinia sclerotiorum* and improve the activities of catalase and guaiacol.

Many researchers have isolated antagonistic bacteria with a broad spectrum of antifungal properties from soil and plants; however, few reports exist on the improvement of the soil microbial community and soil remediation by microbial agents prepared using microorganisms and crop residues. We selected *Bacillus amyloliquefaciens* JF-1 (GenBank accession number: MW578378), which was preserved in our lab that isolated through disease soil, with efficiently inhibition on *F. graminearum* and investigated its inhibitory effect on the growth of pathogenic fungus of maize stalk rot. Additionally, the effects on bacterial and fungal communities in soil were explored. The study findings can have profound implications for agricultural production, food safety, and soil ecology.

Materials and methods

Inhibitory effects of JF-1 on pathogenic fungal hyphae

After the activation of *B. amyloliquefaciens* JF-1 strain, it was inoculated in Luria Bertani (LB) medium (10.0 g peptone, 5.0 g yeast extract, 10.0 g NaCl, 20.0 g agar, 1000 mL distilled water, pH = 7.0). The sterile filtrate of JF-1 was prepared as follows: (1) JF-1 was inoculated in 100 mL LB liquid medium; (2) It was then incubated in a constant-temperature oscillator (Thermostatic HZQ-X300C, Shanghai Yiheng Science Instrument Co. Ltd., China) with constant shaking at 150 rpm and 30 ℃ for 24 h. JF-1 bacterial liquid was centrifuged in a cryogenic high-speed centrifuge LC-LX-HLR300D (Shanghai Lichen Technology Bangxi Instrument Technology Co. Ltd., China) at 7,104 g for 10 min. After centrifugation, the supernatant was filtered through a sterile filter membrane (0.22 μm), and the sterile filtrate was mixed with potato dextrose agar (PDA) medium at volume percentages of 5%, 10%, 20%, and 40%. The pathogenic fungus causing maize stalk rot (*F. graminearum*, GenBank accession number BGC0001600) was inoculated at the center

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of solid PDA medium, and the PDA plate without sterile filtrate was used as the blank control. The fungus was cultured in an incubator at 30 ℃ for 7 days. The fungal inhibition rate was calculated according to the following formula:

$$\text{Fungal inhibition rate} = [(A1 - A2) / A1] \times 100\% \quad \text{(Eq.1)}$$

where A_1 is the colony diameter without any sterile filtrate addition (blank control) and A_2 is the colony diameter treated with sterile filtrate.

JF-1 inhibition mechanisms on F. graminearum in maize

Determination of fungal cell wall degrading enzymes and plant auxin ability of JF-1

The ability of JF-1 to secrete protease, cellulase, chitinase, and indole-3-acetic acid (IAA) was quantitatively determined using an ELISA kit, as previously described (Jiang et al., 2020).

Preparation of JF-1 crude extract fungal inhibition

The implementation method referred to previous study (Du et al., 2017), $(NH_4)_2SO_4$ was added to the sterile filtrate of JF-1 LB culture until 60% saturation, rested at 4 ℃ for 2 h. The sterile filtrate of JF-1 LB culture was centrifuged at 7,104 g for 10 min, and then removed the supernatant, placed in a dialysis bag with the molecular weight cutoff of 8,000–14,000 DA; the dialysis bag was placed in 0.1 mol/L PBS buffer (pH 7. 0) overnight for dialysis. Finally, the solution in the dialysis bag was centrifuged for 10 min at 7,104 g and the supernatant was remained.

Determining the effect of JF-1 crude extract on ATP content of pathogenic fungal cells

Referring to the methods of research by Bajpai et al. (2015), 10 mL JF-1 crude extract was mixed with 100 mL fungal suspension (prepared by 0.1 mol/L PBS buffer) and inoculated in the oscillator at 28 ℃ and 150 rpm, 2 mL of the mixture at 0, 2, 4, 6, 8 and 10 h were took out and centrifuged at 7,104 g for 10 min and the supernatant was removed. The ATP content was measured with ATP assay kit (JianCheng, China), and 10 mL aseptic water was added as blank control. Each treatment was repeated thrice.

Determining the effect of JF-1 crude extract on nucleic acid leakage of pathogenic fungal cells

The nucleic acid contents were referring to a method of Souza et al. (2010), the change of cell membrane integrity was predicted by measuring the change of absorbance value of supernatant at 260 nm (Teethaisong et al., 2014). 10 mL JF-1 crude extract was mixed with 100 mL fungal suspension (prepared by 0.1 mol/L PBS buffer, the fungus was cultured by shaking in liquid PDA medium at 25 ℃ for 3~4 d and centrifuged at 7,104 g for 10 min and the supernatant was removed) and inoculated in the oscillator at 28 ℃ and 150 × g, respectively, 2 mL of the mixture at 0, 2, 4, 6, 8 and 10 h were took out and centrifuged at 7,104 g for 10 min, the absorbance value of the supernatant was determined by a UV-Vis spectrophotometer (Shimadzu UV-2600 spectrophotometer, Japan) at 260 nm, and 10 mL aseptic water was added as blank control. Each treatment was repeated thrice.

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Pot experiment of JF-1 on soil remediation of maize disease

Soil samples were taken from a patch of black soil (0-20 cm) with a high incidence of maize stalk rot and continuous cultivation for years, located in Jingyue District, Changchun City, Jilin Province, China, and belonging to the continental subhumid monsoon climate type of North temperate zone. Following this, 5 g each of sterilized distilled water, sterilized straw, and sterilized straw biochar were added into an equal volume of JF-1 bacterial suspension with a concentration of 1×10^8 CFU/mL at a ratio of 1: 40 (m/V), and the culture was centrifuged at 30 ℃ and 150 rpm for 12 h. Following this, the culture was centrifuged at 7,104 g for 10 min. The supernatant was removed to obtain a single JF-1 microbial agent, maize straw combined with JF-1 microbial agent, and maize straw biochar combined with JF-1 microbial agent, and they were stored in a refrigerator at 4 ℃ for subsequent use. The pot experiment was divided into three treatment groups, namely (1) T1 treatment: 10 g of single JF-1 microbial agent; (2) T2 treatment: maize straw combined with 10 g JF-1 microbial agent; and (3) T3 treatment: maize straw biochar combined with 10 g JF-1 microbial agent, and a CK blank control with no addition of substances. (Biochar preparation: An appropriate amount of maize stalk powder was placed into a crucible, sealed with aluminum foil paper, and placed into a muffle furnace. The pyrolysis temperatures were controlled at 400 ℃, using a heating rate of 10 ℃/min and a residence time of 1 h. The biochar samples were obtained through pyrolysis at the corresponding temperature for 1 h). Ten grams each of different microbial agents were evenly mixed with 500 g soil and they were added into small PVC pots. Each treatment was repeated thrice. The soil samples were inoculated with a highly pathogenic *F. graminearum* spores suspension with a concentration of 1×10^8 CFU·mL^{-1} at a ratio of 1:50 (spore suspension: soil, V/m). The soil moisture content was adjusted to 70% of the field moisture content, and the temperature was maintained at 30 ℃ during the day and 20 ℃ at night. After 90 days of culture, 100 g soil of each pot were collected and stored for future experiments and three maize seeds were planted in each pot, when the seedlings at the four-leaf stage, the plant height, fresh weight and root length (the length of the longest root was measured by avernier caliper) were measured and the disease index and control efficiency were calculated. The disease index and control efficiency were assayed as described by Wu et al. (2015); malondialdehyde (MDA) content in maize roots was measured by the thiobarbituric acid-reactive substances assay.

Determination of the physicochemical properties and enzyme activities of soil

The physicochemical properties of soil, including pH value, organic matter content, alkali-hydrolysable nitrogen, available phosphorus, available potassium, and cation exchange capacity, were measured according to previous studies (Qi et al., 2021; Wang et al., 2020). Soil enzymes include invertase, urease, catalase, and phosphatase, and the measurement method was based on previous studies (Chang et al., 2019).

Extraction and PCR amplification of microbial DNA from soil samples

Extraction of microbial DNA from soil samples

DNA was extracted from soil samples using an E.Z.N.A Mag-Bind Soil DNA Kit (Omega Corporation of America) according to manufacturer's instructions. Genomic

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DNA was accurately quantified using the Qubit 2.0 DNA test kit to determine the amount of DNA added in the PCR reaction mixture.

PCR amplification

Taking the total microbial DNA of soil samples as the template, PCR amplification was performed using the bacterial V3-V4 region-specific primer (338F 5'-ACTCCTACGGGAGCAG-3'; 806R 5'-GGACTACHVGGGTWTCTAAT-3') and fungal ITS specific primers (ITS1F 5'-CTTGGTCATTAGAGGAAGTAA-3'; ITS2 5'-TGCGTTCTTCATCGATGC-3'). PCR amplification was divided into the following steps: (1) 50 μL PCR amplification reaction contained 15 μL 2 × Taq master Mix, 1 μL of 10 μM Bar-primer F, 1 μL of 10 μM Bar-primer R, and 10-20 ng genomic DNA template filled with water; (2) Illumina bridge PCR compatible primers were introduced in the 50 μL amplification system, and the reaction contained 15 μL 2 × Taq master Mix, 1 μL of 10 μM primer F, 1 μL of 10 μM primer R, and 20 ng PCR product generated from the first step filled with water. The PCR products were determined using 1% agarose gel electrophoresis, and the DNA was purified and recovered. Beijing Auwigene Gene Technology Co., Ltd was commissioned to complete high-throughput sequencing.

Statistical analysis

The raw data were first screened to remove sequences < 200 bp in length and chimeras to obtain good quality sequences as clean-tags. Program vsearch 2.7.1 was used to perform OTU (Operational Taxonomic Units) classification on the processed sequences, and OTUs at 97% similarity level were clustered. Bioinformatic analysis was performed based on the results of OTUs clustering analysis. Venn diagrams were plotted by R to count the number of common and unique OTUs to multiple samples (Fouts et, al. 2012). Origin 2019 (OriginLab, USA) was used for statistical analysis. The average value for each treatment was calculated, and differences between the groups were calculated via one-way analysis of variance using the least significant differences test at 5% ($P < 0.05$) probability level. Chao1, Shannon, and Simpson indices were calculated by the following equations (Sun et al., 2022).

$$\boldsymbol{Chao}1 = \boldsymbol{S_{obs}} + \frac{\mathrm{n}\,(\mathrm{n}1\text{-}1)}{2\,(\boldsymbol{n}2+1)} \quad \text{(Eq.2)}$$

where Chao1 means the evaluated OTU number; Sobs means the observed OTU number; n1 means the OTU number with singletons; n2 means the OTU number with doubletons.

$$\text{Shannon} = -\sum_{i=1}^{S_{obs}} \frac{n_i}{\mathrm{N}} \ln \frac{n_i}{\mathrm{N}} \quad \text{(Eq.3)}$$

$$\text{Simpson} = 1 - \frac{\sum_{i=1}^{S_{obs}} \mathrm{n}_i\,(\mathrm{n}_i\text{-}1)}{\mathrm{N}\,(\mathrm{N}\text{-}1)} \quad \text{(Eq.4)}$$

where Sobs means the measured OTU number; ni means the OTU number with sequence of i; N means the number of all sequences.

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Results

Inhibitory effect of JF-1 on pathogenic fungus hyphae

According to the results in *Table 1*, the inhibition ability of JF-1 on the mycelia of pathogenic fungi increased with the increase in the concentration of the JF-1 sterile filtrate. When the concentration of the sterile filtrate reached 40%, the inhibition rate of mycelia was the highest (83.95%). As revealed in *Figure 1*, It was found by scanning electron microscopy that the mycelia of pathogenic fungi were enlarged and deformed, while the surface of the blank control group was smooth, full, and without any mycelial deformation. The results showed that the JF-1 sterile filtrate could inhibit *F. graminearum* by destroying its mycelia.

Table 1. *Inhibitory effect of JF-1 on hyphae of pathogenic fungi*

JF-1 concentration (%)	*F. graminearum*	
	CD (mm)	FIR (%)
Blank control	81 ± 3 a	-
5	52 ± 5 b	35.80 d
10	43 ± 6 c	46.91 c
20	25 ± 4 d	69.14 b
40	13 ± 5 e	83.95 a

All the presented values are means of three replicates. Means were subjected to analysis of variance and were separated by LSD test. Letters represent the significant difference among the mean values and ± are standard error values of the means. CD is colony diameter, FIR is fungal inhibition rate

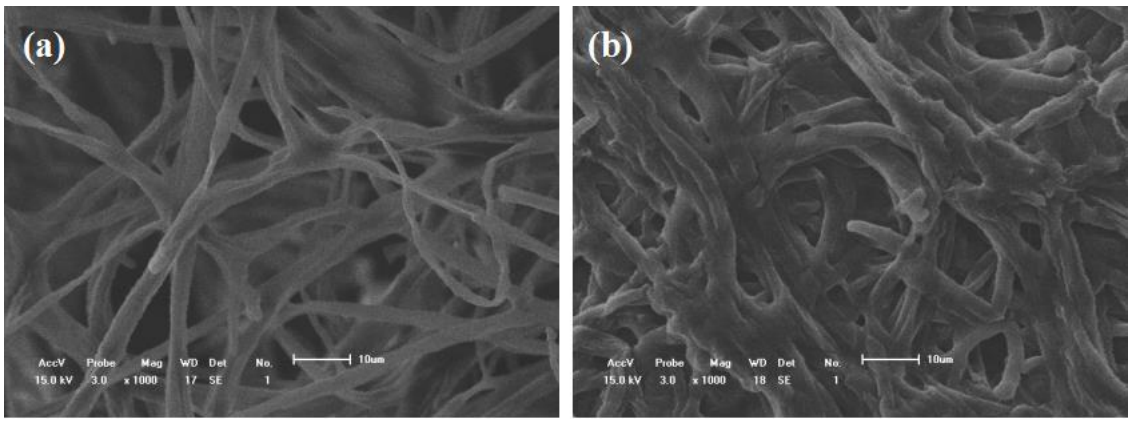

Figure 1. *Inhibitory effect of JF-1 on pathogenic fungus hyphae. Morphology of pathogenic fungus hyphae observed by scanning electron microscope (×1000), (a) is blank control and (b) is pathogenic fungus hyphae treated with JF-1*

JF-1 inhibition mechanisms on F. graminearum in maize

JF-1 produces fungal cell wall degrading enzymes and plant auxin

JF-1 bacterial liquid possessed a high content of main fungal cell wall hydrolases (*Table 2*). Among them, 18.79 pg mL^{-1} protease, 76.35 pg mL^{-1} cellulase enzyme, and 65.57 pg mL^{-1} chitinase facilitated plant growth and IAA production, and the level of

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the JF-1 strain reached 4.29 pmol L^{-1}, which were significantly higher than that of the blank control ($P < 0.05$).

Table 2. *Determination of fungal cell wall degrading enzymes and plant auxin ability of JF-1*

Metabolites	JF-1	Blank control
Pro (pg mL-1)	18.79 ± 0.07 a	3.74 ± 0.04 b
Cel (pg mL-1)	76.35 ± 0.38 a	6.73 ± 0.49 b
Chi (pg mL-1)	65.57 ± 0.87 a	5.53 ± 0.72 b
IAA (pmol L-1)	4.29 ± 0.03 a	0.48 ± 0.07 b

All the presented values are means of three replicates. Means were subjected to analysis of variance and were separated by LSD test. Letters represent the significant difference among the mean values and ± are standard error values of the means. Pro is Protease activity, Cel is Cellulase activity, Chi is Chitinase activity, IAA is indole-3-acetic acid.3.2.2 JF-1 decreased the ATP content of pathogenic fungal cells

JF-1 decreased the ATP content of pathogenic fungal cells

As illustrated in *Figure 2*, the ATP content of fungal cells treated with JF-1 crude extract decreased sharply (0.18 μmol/g) when compared with the blank control at 2 h. The decrease in ATP content reached a maximum value of 0.22 μmol/g after 8 h. The ATP content in fungal cells of the blank control was maintained at a stable level, whereas the ATP content of fungus treated with JF-1 crude extract was significantly lower than the blank control for the period of 0-10 h ($P < 0.05$). This may be due to the influence of JF-1 crude extract on the ability of *F. graminearum* to generate ATP, or due to the destruction of the cell membrane structure of *F. graminearum*, resulting in ATP leakage.

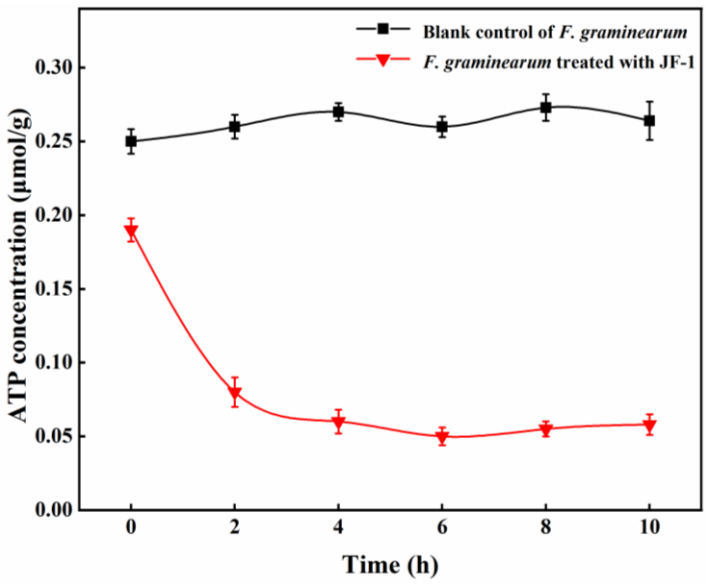

Figure 2. *JF-1 decreased the ATP content of pathogenic fungal cells. All the presented values are means of three replicates. Mean values were calculated and statistical analysis was performed using Origin 2019. All the mean values were subjected to analysis of variance, and means were separated at 5% probability. Error bars represent the standard error values of the means*

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JF-1 increased nucleic acid leakage of pathogenic fungal cells

Extracellular nucleic acid results from the leakage of nucleic acid from damaged cells and its content can be used as an indicator of the degree of cell damage and the formation of cell membrane holes. As shown in *Figure 3* in the period of 0–10 h, the absorbance of the supernatant of blank control at OD_{260nm} was always maintained at approximately 0.2. The absorption value of the supernatant treated with JF-1 crude extract reached 1.17 at 2 h. In the range of 0–10 h, the absorbance of the supernatant treated with JF-1 crude extract was always higher than that of blank control, indicating that the membrane permeability of *F. graminearum* cells had been changed by the JF-1 crude extract, resulting in nucleic acid leakage. However, at 6 h and 10 h, the fluctuation in the trend of absorption value may be due to the reuse of extracellular nucleic acid by *F. graminearum* cells as nutrients.

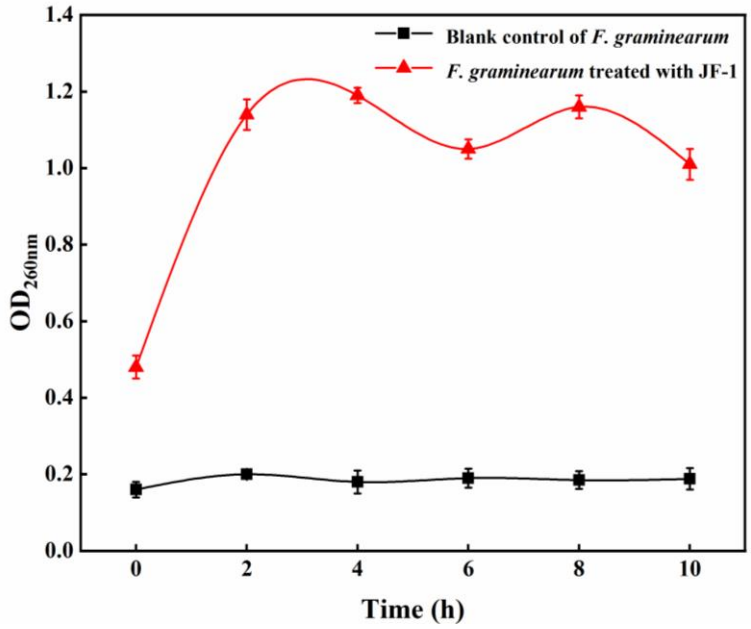

Figure 3. *Determining the effect of JF-1 on nucleic acid leakage of pathogenic fungal cells. All the presented values are means of three replicates. Mean values were calculated and statistical analysis was performed using Origin 2019. All the mean values were subjected to analysis of variance, and means were separated at 5% probability. Error bars represent the standard error values of the means*

Determination of the efficacy of JF-1 in reducing stalk rot in maize

Maize seedling experiment was carried out on the soil treated with T1, T2 and T3, all of which had a visible promotive effect on the growth of maize at the seedling stage, as shown in *Figure 4*. Notably, compared with blank control, T3 treatment increased the height, root length, and fresh weight of maize most significantly, increasing them by 19.71%, 9.04%, and 26.92%, respectively ($P < 0.05$). Simultaneously, compared with blank control, the control efficiency of T1, T2, and T3 treatments on stalk rot of maize seedlings reached 61.64%, 49.72%, and 65.36%, respectively, and the content of MDA in the root of maize seedlings decreased by 50.17%, 32.75%, and 67.60%, respectively.

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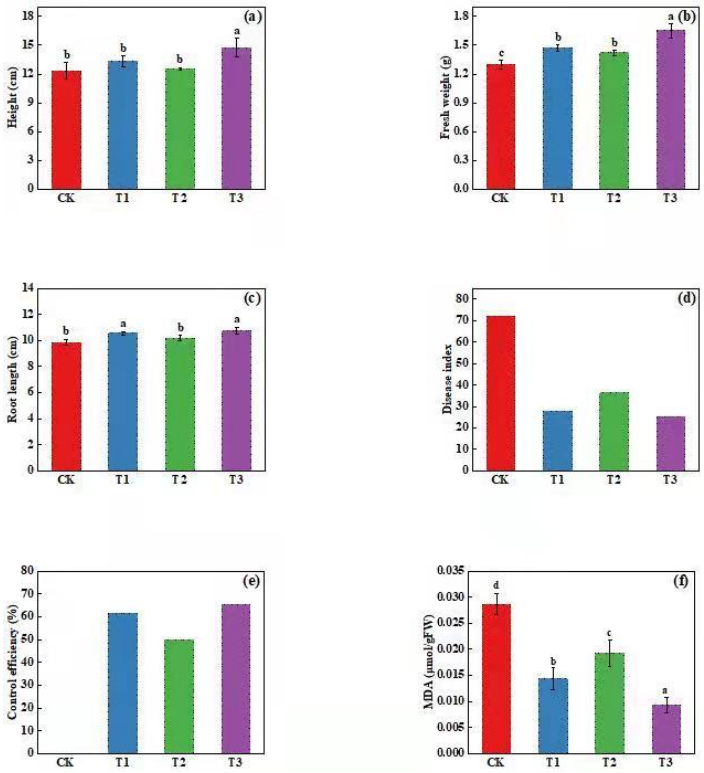

Figure 4. *The efficacy of JF-1 in reducing stalk rot in maize. All the presented values are means of three replicates. Means were subjected to analysis of variance and were separated by LSD test. Letters represent the significant difference among the mean values and ± are standard error values of the means. (a) is plant height; (b) is fresh weight; (c) is root length; (d) is disease index; (e) is control efficiency and (f) is MDA content. CK is blank control, T1 is single JF-1 microbial agent; T2 is maize straw combined with JF-1 microbial agent; T3 is maize straw biochar combined with JF-1 microbial agent*

Determination of the physicochemical properties and enzyme activities of soil

Maize straw combined with JF-1 and maize straw biochar combined with JF-1 significantly changed the physical and chemical properties and enzyme activities of soil ($P < 0.05$; *Table 3*). The soil pH values and cation exchange capacities of the T2 and T3 groups significantly increased ($P < 0.05$) compared to those of CK blank control group. The pH value increased by 3.37% and 5.86% and cation exchange capacity increased by 3.09% and 5.35% in T2 and T3 groups, respectively. After treatments with the three different JF-1 microbial agents, both the activities of soil nutrients and enzymes changed, and the effect of the T3 treatment were more obvious, compared with the CK blank control group, the organic matter, available N, available P, and available K content increased by 39.81%, 28.07%, 14.10%, and 4.91%, respectively. Thus, urease, invertase, phosphatase, and catalase increased by 94.51%, 43.40%, 52.78%, and 85.93%, respectively.

Results of soil microbial sequencing and differences in soil microbial community diversity

The DNA sequencing results of bacteria and fungi in the soil samples are shown in *Tables 4* and *5*, respectively. The sequencing coverage rate of all samples reached over

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98%, indicating that the sequencing depth of samples was sufficient to meet the requirements of subsequent numerical analysis. Alpha diversity is usually measured using the Chao1, Shannon, and Simpson indices. Among these, the Chao1 index reflects the richness of species, while the Shannon and Simpson indices reflect the diversity of species.

Table 3. *Effects of different treatments on soil physicochemical properties and enzyme activities*

Treatment	CK	T1	T2	T3
pH	5.63 ± 0.05 cd	5.68 ± 0.02 c	5.97 ± 0.03 b	6.15 ± 0.08 a
CEC (cmol kg^{-1})	20.73 ± 0.34 cd	20.85 ± 0.40 c	21.37 ± 0.93 b	21.84 ± 0.81 a
OM (g kg^{-1})	20.65 ± 0.73 d	21.88 ± 0.84 c	25.39 ± 0.61 b	28.87 ± 0.55 a
AN (mg kg^{-1})	96.84 ± 6.71 d	104.32 ± 5.16 c	115.37 ± 7.98 b	124.02 ± 6.34 a
AP (mg kg^{-1})	23.47 ± 0.26 cd	23.51 ± 0.19 c	26.73 ± 0.31 ab	26.78 ± 0.33 a
AK (mg kg^{-1})	214.83 ± 0.45 cd	215.47 ± 0.86 c	225.93 ± 0.59 a	225.38 ± 0.45 ab
URE (mg/g·d^{-1})	3.46 ± 0.25 d	3.87 ± 0.31 c	4.92 ± 0.18 b	6.73 ± 0.27 a
INV (mL/g·d^{-1})	8.25 ± 0.39 d	8.74 ± 0.26 c	10.69 ± 0.41 b	11.83 ± 0.33 a
PHO (μg/g·d^{-1})	0.36 ± 0.03 cd	0.39 ± 0.05 c	0.54 ± 0.01 ab	0.55 ± 0.02 a
CAT (mL/g·d^{-1})	3.34 ± 0.06 d	5.46 ± 0.11 c	5.78 ± 0.07 b	6.21 ± 0.09 a

All the presented values are means of three replicates. Means were subjected to analysis of variance and were separated by LSD test. Letters represent the significant difference among the mean values and ± are standard error values of the means. CK is blank control, T1 is single JF-1 microbial agent; T2 is maize straw combined with JF-1 microbial agent; T3 is maize straw biochar combined with JF-1 microbial agent. CEC is cation exchange capacity, OM is organic matter, AN is available nitrogen, AP is available phosphorus, AK is available potassium, URE is urease activity; INV is invertase activity; PHO is phosphatase activity; CAT is catalase activity

Table 4. *Effects of different treatments on the α diversity of bacterial community*

Sample	Number	Chao1	Coverage	OTUs	PD whole tree	Shannon	Simpson
CK	10682 ± 1983 d	5837.32 ± 142.89d	0.99	2868 ± 168 d	187 ± 18d	8.23 ± 0.56 cd	0.98
T1	12391 ± 1734 bc	6382.16 ± 127.67a	0.98	3128 ± 192 b	212 ± 21b	8.56 ± 0.41 c	0.98
T2	13095 ± 1802 a	6192.78 ± 138.24b	0.99	3207 ± 181 a	223 ± 15a	9.26 ± 0.53 a	0.99
T3	12893 ± 1879 b	6012.2 ± 165.43c	0.98	2949 ± 207 c	201 ± 19c	8.97 ± 0.62 b	0.99

All the presented values are means of three replicates. Means were subjected to analysis of variance and were separated by LSD test. Letters represent the significant difference among the mean values and ± are standard error values of the means. CK is blank control, T1 is single JF-1 microbial agent; T2 is maize straw combined with JF-1 microbial agent; T3 is maize straw biochar combined with JF-1 microbial agent

Table 5. *Effects of different treatments on the α diversity of fungal community*

Sample	Number	Chao1	Coverage	OTUs	PD whole tree	Shannon	Simpson
CK	10689 ± 1637 ab	1298.34 ± 128.13 b	0.99	1623 ± 119 a	182 ± 13 a	7.21 ± 0.51 a	0.94
T1	98201 ± 1702 c	1223.98 ± 102.32 c	0.99	1512 ± 103 c	163 ± 16 c	6.87 ± 0.67 b	0.98
T2	10735 ± 1629 a	1382.92 ± 138.27 a	0.98	1587 ± 138 b	175 ± 17 b	7.03 ± 0.46 ab	0.95
T3	95839 ± 1826 cd	1104.23 ± 129.74 d	0.99	1489 ± 121 cd	141 ± 11 d	5.83 ± 0.59 c	0.96

All the presented values are means of three replicates. Means were subjected to analysis of variance and were separated by LSD test. Letters represent the significant difference among the mean values and ± are standard error values of the means. CK is blank control, T1 is single JF-1 microbial agent; T2 is maize straw combined with JF-1 microbial agent; T3 is maize straw biochar combined with JF-1 microbial agent

Four soil samples were analyzed, the total operational taxonomic units (OTUs) of bacteria of the T1, T2, and T3 treatments increased by 9.07%, 11.82%, and 2.82%,

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respectively compared to those of CK blank control group. The Chao1 and Shannon indices of T1 treatment increased by 9.34% and 4.00%, respectively, those of T2 treatment increased by 6.09% and 12.52%, respectively, and those of T3 increased by 2.96% and 9.00%, respectively. The application of a single JF-1 microbial agent, maize straw combined with JF-1 microbial agent, and maize straw biochar combined with JF-1 microbial agent significantly increased the richness and diversity of the soil bacterial community ($P < 0.05$), and maize straw combined with JF-1 microbial agent showed the most significant increase ($P < 0.05$). As shown in *Figure 5a*, in the four treatments, the number of the same bacterial OTUs was 1,202. After T1, T2, T3, and CK blank control treatments, the OTU counts of unique bacteria in the soil samples were 577, 766, 368, and 457, respectively. Maize straw combined with a JF-1 microbial agent most significantly ($P < 0.05$) improved the abundance of unique bacteria in soil, by 67.61% as compared to that in the CK blank control group.

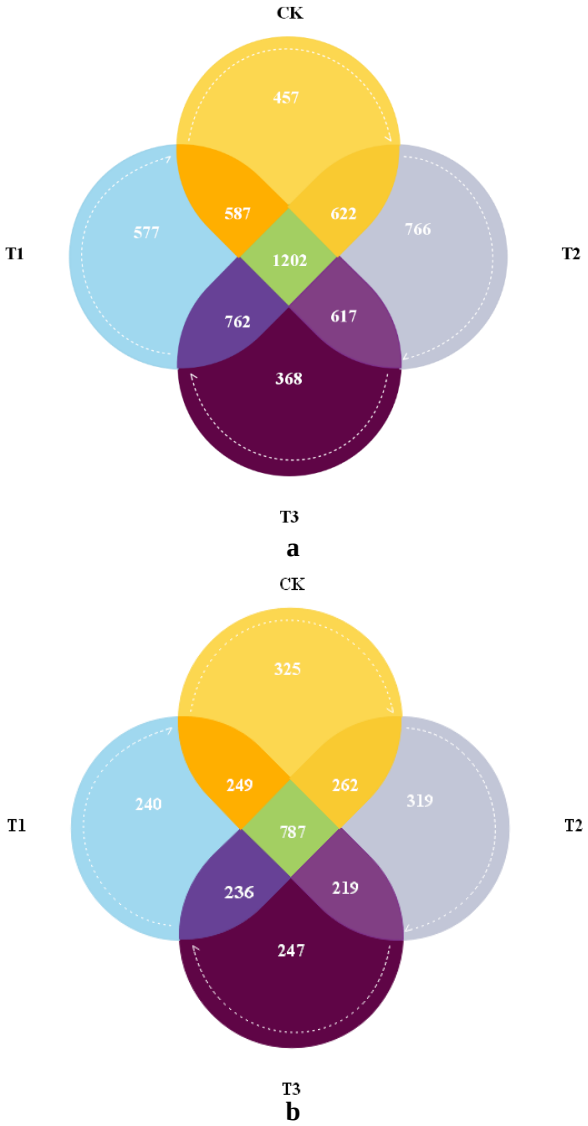

Figure 5. *OTUs number of bacterial and fungal communities in different treatments. The Venn diagrams showing the numbers of bacterial (a) and fungal (b) OTUs in different soil samples. CK is blank control, T1 is single JF-1 microbial agent; T2 is maize straw combined with JF-1 microbial agent; T3 is maize straw biochar combined with JF-1 microbial agent*

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The total OTUs of fungi in groups treated with T1, T2, and T3 decreased by 6.84%, 2.22%, and 8.26%, respectively, as compared to those in the CK blank control group. The Chao1 and Shannon indices of the T1 treatment decreased by 5.73% and 4.72%, respectively, as compared to those of CK blank control group. The Chao1 index of the T2 treatment increased by 6.51% and the Shannon index decreased by 2.50%. The Chao1 index of T3 treatment decreased by 14.95% and the Shannon index decreased by 19.14% as compared to those of CK blank control group. In conclusion, a single JF-1 microbial agent and maize straw biochar combined with JF-1 microbial agent significantly reduced the community richness and diversity of soil fungi ($P < 0.05$), and the effect of maize straw biochar combined with JF-1 microbial agent was more significant. However, maize straw combined with JF-1 microbial agent increased the richness of soil fungal community but decreased the diversity of soil fungal community. As shown in *Figure 5b*, among the four treatments, the number of the same fungi OTUs was 787 after T1, T2, T3, and CK blank control group treatments, and the OTU counts of unique fungi in the soil samples were 240, 319, 247, and 325, respectively, indicating that a single JF-1 microbial agent and maize straw biochar combined with a JF-1 microbial agent significantly reduced the abundances of unique soil fungi ($P < 0.05$) by 26.15% and 24.00%, respectively, as compared to the abundance in the CK blank control group.

Analysis of the differences in bacterial and fungal diversities at the phylum level between different treatments

The differences in bacterial community composition were analyzed at the phylum level (*Fig. 6a*). In the four soil samples, 41 phyla, 121 classes, 267 orders, 402 families, and 685 genera were detected. After T1, T2 and T3 treatments, the relative abundances of *Proteobacteria*, *Actinobacteria*, and *Gemmatimonadetes* were significantly higher ($P < 0.05$) than those in the CK blank control group, the relative abundance of *Acidobacteria* and *Chloroflexi* were significantly lower than that of CK blank control group ($P < 0.05$). After the single JF-1 microbial agent treatment, maize stalk combined with JF-1 microbial agent treatment, and maize stalk biochar combined with JF-1 microbial agent treatment, the relative abundances of *Proteobacteria*, which accounted for 23.42% in the CK blank control group, were increased by 10.04%, 6.37%, and 12.02%, respectively. The relative abundance of the second-most dominant bacteria, *Acidobacteria*, which accounted for 14.14% in the CK blank control group, decreased by 4.24%, 7.27%, and 10.56% in the T1, T2, and T3 groups, respectively, and it became the third dominant phylum in all three treatments groups. In contrast, the relative abundance of *Actinobacteria*, the third dominant bacteria accounting for 13.30% in CK blank control group, increased by 3.76%, 5.77%, and 9.16% in T1, T2, and T3 treatments, respectively, becoming the second dominant phylum in all three groups.

Differences in fungal community composition were analyzed at the phylum level (*Fig. 6b*). 13 phyla, 27 classes, 63 orders, 122 families, and 237 genera were detected in the four groups of soil samples. After T1, T2 and T3 treatments, the relative abundances of *Mortierellomycota* and *Basidiomycota* were significantly increased ($P < 0.05$) as compared to those of CK blank control group, the relative abundance of *Ascomycota* was significantly lower than that in the CK blank control group ($P < 0.05$). The relative abundance of *Ascomycota*, which accounted for 52.63% in CK blank control group, decreased by 5.99%, 12.45%, and 18.35% after a single JF-1 microbial agent treatment, maize stalk combined with JF-1 microbial agent treatment, and maize stalk biochar

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combined with JF-1 microbial agent treatment, respectively. The abundance of the second dominant phylum *Basidiomycota*, which accounted for 16.25% in the CK blank control group, increased by 5.04%, 6.02%, and 9.04% in T1, T2, and T3 groups, respectively, making it the third most dominant phylum in the three groups. The abundance of another dominant phylum, *Mortierellomycota*, which accounted for 14.37% in the CK blank control group, increased by 6.99%, 9.29%, and 12.64% after T1, T2, and T3 treatments, respectively, making it the second-most dominant phylum in all three treatment groups.

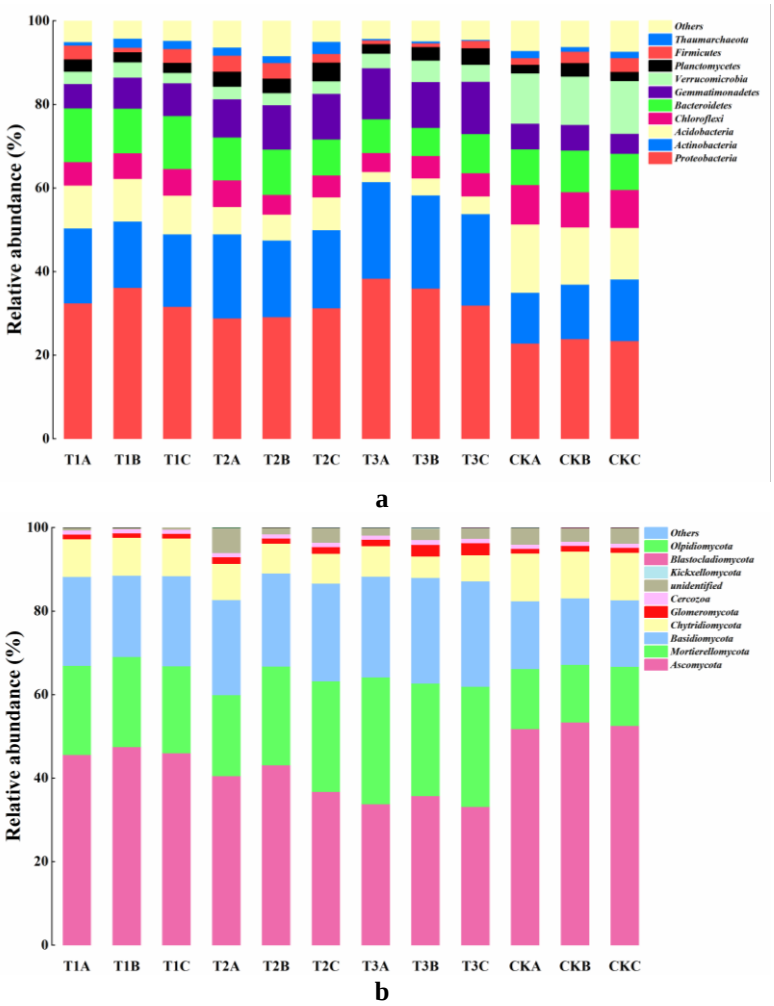

Figure 6. *Analysis of relative abundance of bacterial and fungal communities at phylum level. Bacterial diversity (a) and fungal diversity (b) in different soil samples at phylum level. CK is blank control, T1 is single JF-1 microbial agent; T2 is maize straw combined with JF-1 microbial agent; T3 is maize straw biochar combined with JF-1 microbial agent*

Discussion

Researchers have analyzed the inhibitory effect of antagonistic bacteria against plant diseases from different perspectives (Zhao et al., 2014; Leclère et al., 2005). In this study, the inhibition rates of the sterile fermentation filtrate of *B. amyloliquefaciens* JF-1 was 83.95% for *F. graminearum* mycelial growth; chitinase, cellulase, and protease, with high activities, were obtained from the JF-1 fermentation broth. Treatment of

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pathogenic fungi with JF-1 crude extract resulted in a decreased intracellular ATP content and nucleic acid leakage. The decrease in intracellular ATP content is a result of damage to the cell membrane, which affects its normal physiological activities (Olanya et al., 2015). Nucleic acid macromolecular substances penetrate the entire cell membrane and cytoplasm; the release of nucleic acid indicates that the integrity of the cell membrane has been destroyed (Kohanski et al., 2010). Additionally, JF-1 has the ability to secrete IAA. IAA can promote the growth of plant roots and facilitate the absorption of soil nutrients by plants (Mano and Morisaki, 2008). Therefore, it was inferred that JF-1 could promote the growth of plant roots.

The early infection of maize seedling roots with *F. graminearum* posed a serious hidden danger for a maize stalk rot outbreak. JF-1 immobilized with maize straw biochar had a 65.36% control effect on *F. graminearum* infection at the root of maize seedlings. The content of malondialdehyde (MDA) in the roots of maize seedlings decreased by 67.60%. The increase in MDA content in maize plants indicates that the damage to the maize cell membrane is aggravated (AbdElgawad et al., 2021). Therefore, JF-1 effectively protected maize seedlings from *F. graminearum.*

The ability of a microbial community to regulate the soil ecosystem depends on the stability of the soil structure and soil physical and chemical properties (Bissett et al., 2013). In this study, JF-1 microbial agents improved the physicochemical properties of maize disease soil. Among them, maize straw biochar combined with JF-1 microbial agent showed the most significant increase ($P < 0.05$), and the pH value, organic matter, and cation exchange capacity increased by 5.86%, 39.81%, and 5.35%, respectively. Many studies have shown that the genus *Bacillus* can activate the insoluble substances in soil for plant use and increase the content of available phosphorus and potassium in soil (Ogut et al., 2016; Ku et al., 2018; Lucio et al., 2018); The metal cation and ash contents in the biochar and straw can improve the cation exchange capacity of soil (Tan et al., 2017). Some metal ions in the soil participate in the life activities of microorganisms and play a key role in the expression of certain proteins and genes (Zhang et al., 2019). The activities of urease, invertase, phosphatase, and catalase increased using JF-1, which may be due to the colonization effect of JF-1 in soil. Compared to JF-1 application alone, straw and biochar combined with JF-1 increased the soil enzymes more significantly ($P < 0.05$), possibly because straw and biochar directly returned to the field, thus, affecting soil microorganisms and enzyme activities (Chen et al., 2014; Ji et al., 2014). To our knowledge, the environment was repaired by JF-1 microbial agents for the beneficial microorganisms in soil and growth of crops.

The soil microbial community is often called the “second genome of plants” (Berendsen et al., 2012). This study showed that after remediation with three treatments of JF-1 microbial agents, the microbial biomass of bacteria in maize disease black soil significantly increased ($P < 0.05$) and the richness and diversity of bacterial community improved. After different JF-1 microbial agent treatments, *Proteobacteria* and *Actinobacteria* in the soil samples were increased while *Acidobacteria* was decreased to a certain extent. This trend was most obvious in the soil samples treated with maize straw biochar combined with JF-1. *Actinobacteria* protects plant roots from infection by pathogenic fungi, and some species of *Actinobacteria* promote plant growth (Khamna et al., 2009). Studies have found that *Proteobacteria* and *Actinobacteria* are associated with the suppression of plant diseases (Mendes et al., 2011), and the increased relative abundance of *Proteobacteria* and *Actinobacteria* decreases the risk of soil diseases (Li

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and Xu, 2020). *Acidobacteria*, an acidophilic bacteria, mostly lives in soil with poor nutrition, and its abundance is often negatively correlated with soil pH (Jones et al., 2009). The decrease of *Acidobacteria* may be caused due to an increase in soil pH value after the application of microbial agents. Additionally, similar to *Acidobacteria*, *Chloroflexi* is also suitable for growing in oligotrophic environments (Fierer et al., 2007). After restoration with microbial agents, the increased nutrients in the soil may be an important reason for the decline in the abundance of *Acidobacteria* and *Chloroflexi*.

According to the analysis of fungal sequencing results at the phylum level, *Ascomycota*, *Mortierellomycota*, and *Basidomycota* were the three dominant phyla of maize disease black soil. After treatment with three groups of different JF-1 microbial agents, *Ascomycota* in soil samples showed a significant decrease ($P < 0.05$). This change was most significant in the soil treated with maize stalk biochar combined with JF-1 ($P < 0.05$); *Mortierellomycota* and *Basidomycota* showed different degrees of increase in each treatment, which may be because *F. graminearum* belongs to *Ascomycota*. The inhibition of *F. graminearum* using JF-1 led to a decrease in the relative abundance of *Ascomycota* in the soil. *Basidomycota*, an important decomposer in soil, plays an important role in the nutrient cycle (Yelle et al., 2008), and nutrients in microbial agents may promote its growth and reproduction. It has been reported that *Ascomycota* is closely related to the occurrence of soil diseases (Tedersoo et al., 2014). Yuan et al. (2020) showed that the relative abundance of *Ascomycota* is higher in soils with a high incidence of plant diseases, while the relative abundance of *Mortierellomycota* is higher in healthy soils, which was consistent with the results of this study. Interestingly, *Glomeromycota* (relative abundance > 1%) is a fungus that can form a complex with the roots of most plants to improve plant nutrient absorption and disease resistance (Liu et al., 2015; Caroline et al., 2017). The relative abundance of *Glomeromycota* was obviously increased in the soil treated with maize straw combined with JF-1 and maize straw biochar combined with JF-1; however, this change was not observed in the disease soil treated with a single JF-1 microbial agent. Therefore, the combination of straw biochar with JF-1 microbial agent had a good repairing effect on the chemical and microecological environment of maize disease soil, and it improves soil ecology and inhibits maize disease.

Conclusion

The bacterial biological control strain JF-1 can inhibit the mycelial growth of *F. graminearum* by secreting chitinase, protease, and other fungal cell wall-degrading enzymes. Maize straw biochar combined with JF-1 microbial agents can repair the physicochemical properties, soil enzyme activities, and microbial community structure of maize disease soil. The biological control bacterium JF-1 can be used as an efficient and cheap microbial agent; thus, JF-1 has a good development potential and application prospect for the prevention and control of maize stalk rot. In addition, the composite application of multiple functional microorganisms and the optimization of carriers are the directions of future research.

Conflict of interests. All authors declare that there is no conflict of interests in this study.

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