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Full Length Article

Control of Fusarium Stalk Rot of Corn by Addition of Biochars

Liang Liu[1†], Chunlai Liu[2†], Guoqing Shen[3], Fan Yang[2], Shuang Wang[2], Xifeng Jiang[2] and Xinmin Li[2*]
[1]*Heilongjiang Academy of Agricultural Sciences Postdoctoral Programme, Heilongjiang, 150086, China*
[2]*Institute of Plant Protection (IPP), Heilongjiang Academy of Agricultural Sciences, Scientific Observing and Experimental Station of Crop Pest in Harbin, Ministry of Agriculture, Heilongjiang, 150086, China*
[3]*Department of Environment and Resource, School of Agriculture and Biology, Shanghai Jiao Tong University, Shanghai, 200240, China*
[*]For correspondence: shengfangshi408@126.com; liuliang0423@126.com
[†]These author contributed equally to this work

Abstract

The efficacy of biochar prepared from rice husk and bamboo at 500°C against stalk rot of corn caused by *Fusarium graminearum* was tested at biochar doses of 0, 0.5, 1, 2 and 3% (w/w). In general, the application of biochar controlled the disease. Compared with the 0% control, biochar addition at the low concentration of 0.5% enhanced corn growth. In addition, biochar addition at various concentrations decreased the disease severity. The disease control potential varied based on the biochar type and dose. The most effective dose for controlling the disease was 2% for rice husk biochar and 1% for bamboo biochar. A terminal restriction fragment length polymorphism analysis revealed changes in the soil microbial community after biochar addition. In conclusion, our results provide a basis for applying rice husk and bamboo biochar as a soil amendment for the suppression of corn stalk rot caused by *F. graminearum*. © 2019 Friends Science Publishers

Keywords: Biochar; Corn stalk rot; Control effect; *Fusarium graminearum*; Plant growth promotion

Introduction

Corn stalk rot is a highly destructive fungal soil-borne diseases and it significantly reduces the yield and quality of corn. In some corn-production areas, the incidence of corn stalk rot reaches 30–50% (He *et al*., 2019). *Fusarium graminearum, F. incarnatum, F. brachygibbosum, F. verticillioides, F. temperatum, F. proliferatum* and *Pythium* species are the dominant causal pathogens of corn stalk rot around the world (Yu *et al*., 2017; Kim *et al*., 2018). Corn stalk rot caused by *F. graminearum* mainly leads to substantial annual yield losses in the cool growing regions of North China (Zhang *et al*., 2017; Li *et al*., 2018; He *et al*., 2019). However, attaining the complete control of corn stalk rot caused by pathogens is a great challenge (Hashem *et al.*, 2018). Conventional strategies, such as resistant cultivar cultivation, seed coatings and biological control, do not completely control this disease (Li *et al.*, 2016b). Identifying alternative control methods that present high efficiency, low costs and limited environmental impacts is of high priority for corn stalk rot control.

Reports have demonstrated that the utilization of organic amendments, including animal and green manure, organic waste, compost and peat, is effective for controlling diseases caused by soil-borne pathogens (Bonanomi *et al*., 2010; Heck *et al*., 2019; Tayyab *et al*., 2019). Despite the potential of this technique, several side effects limit the practical application of organic amendments. Researchers have shown that unprocessed organic compounds and mature compost can damage plant roots, which predispose them to pathogen attacks and promote plant disease. However, biochar appears to be a new and promising tool to control many plant diseases (Bonanomi *et al*., 2015). Biochar occurs naturally in soil and can improve soil quality, increase crop yield and influence the interactions among soil, biochar, crop and soil-borne pathogens (Kammann and Graber, 2015). These functionalities are due to several physical and chemical properties of biochar that allow them to provide nutrients and alter the pH, adsorption ability, water-holding capacity, cation exchange capacity and redox activity of the soil (Lehmann *et al*., 2011; Abid *et al*., 2014; Lu *et al*., 2015; Novak *et al*., 2019). Matsubara *et al.* (2002) first reported that biochar addition to soil affected the progress of root rot diseases caused by *Fusarium*. They found that the values of *Fusarium* root rot disease indices were reduced when biochars were added (Matsubara *et al*., 2002). To date, various types of biochar have been discovered to influence the occurrence and spread of soil-borne diseases (Graber *et al*., 2014). Biochar can potentially affect a variety of soil-borne diseases, including Fusarium root rot (*F. oxysporum* f. spp. *asparagi*), Fusarium crown rot (*F. proliferatum*), bacterial wilt (*Ralstonia solanacearum*), stem canker (*Phytophthora cinnamomi* and

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P. cactorum) and damping-off and root rot (*Rhizoctonia solani*) (Elmer and Pignatello, 2011; Jaiswal *et al*., 2014; Rawat *et al.,* 2019). However, the studies to date have examined only a few potential distinct pathosystems and biochar types.

In the soil, disease-causing pathogens and biochar form complex soil-biochar-pathogen systems. To date, at least five different hypotheses have been proposed to explain biochar suppression of disease, including the sorption of toxic compounds, the induction of systemic resistance in hosts, the improvement of soil quality, the enhancement of the activities of beneficial microbes and toxicity to microbes (Bonanomi *et al*., 2015; Jaiswal *et al*., 2019). However, only a few pathosystems have been studied. Moreover, research on disease organisms has been focused on only fungi and related organisms. The mechanisms of pathosystems involving bacteria, nematodes, viruses and other organisms remain to be researched (Yang *et al.,* 2017). Diverse sources and concentrations of biochar should be tested on various soil types in greenhouse and field experiments under a range of climatic conditions. The main objective of this paper was to explore the control effects of various biochars on corn stalk rot caused by *F. graminearum.* The specific aims were to determine how the different biochars influence (a) corn plant growth under *F. graminearum* inoculation, (b) corn stalk rot control and (c) soil microbial diversity.

Materials and Methods

Biochar and Soil Preparation

Two types of biochar were produced from air-dried rice husk and bamboo biomass by slow pyrolysis (oxygen limited, 500°C for 4 h) in a muffle furnace (Thermo Fisher Scientific, Inc., Pittsburgh, U.S.A.). The biochar parameters were measured at Shanghai Jiao Tong University, Shanghai, China. The biochar characteristics are shown in Table 1. Soils were collected from Heilongjiang Modern Agricultural Demonstration Area (126.86 N, 45.85 E) in Harbin, Heilongjiang, China. After naturally drying the soils and passing them through a 2 mm sieve, the physico-chemical nature of the soil was characterized using standard methods (Liu *et al*., 2015). The pH value of the soil was 6.11 ± 0.02, and the organic matter, nitrogen (N), phosphorus (P), potassium (K_2O) and zinc (Zn) contents in soil were 27.5 ± 0.52 mg/kg; 137 ± 0.95 mg/kg; 22.6 ± 0.64 mg/kg; 136 ± 1.53 mg/kg and 1.77 ± 0.05 mg/kg, respectively. The mechanical composition of soil included sand (0.01–1.0 mm) at 51.49 ± 0.44%, silt (0.001–0.01 mm) at 28.37 ± 0.06% and clay (<0.001 mm) at 20.14 ± 0.03%.

Corn Planting and Incubation Conditions

Corn seedlings (Tian Nong 9, Heilongjiang, China) were grown from seeds in an incubator at 25°C. After corn seed germination, 10 uniform seedlings (48 h old) were transplanted into individual pots (10 cm in height and 13 cm in diameter) containing 1 kg of (<2 mm) air-dried soil per pot and one of several concentrations of rice husk or bamboo biochar. The pots were placed in an incubation cabinet and incubated at 25°C and 60% moisture content (Liu *et al*., 2015).

Table 1: The physico-chemical characteristics of biochars

Biochar	pH	Ash (%)	C (%)	N (%)	SSA (m^2/g)
Rice husk	11.06 ± 0.04	51.27 ± 0.03	40.22 ± 0.02	1.23 ± 0.04	71.32 ± 0.16
Bamboo	10.21 ± 0.01	45.87 ± 0.07	46.32 ± 0.07	0.08 ± 0.04	58.01 ± 0.12

Pathogen Isolation, Culture and Inoculation

Pure *F. graminearum* (isolated from a corn plant in Northeast China in 2017, GenBank NO.: MH231314) was cultured on potato dextrose agar medium (25°C, 3 days). Three fungi-agar disks were cut out along the colony edges using an 8 mm hole punch and were inoculated in a 250 mL sterilized (121°C for 40 min) bottle containing 150 g of sorghum. The sorghum culture was incubated for 10 days at 121°C. Then, the pathogen-sorghum mixture was mixed with soil at a concentration of 3% (w/w).

Experimental Treatment

The rice husk and bamboo biochars were produced at a pyrolysis temperature of 500°C, and their effects at concentrations of 0.5%, 1%, 2% and 3% (w/w) were compared in two sets of experiments. Soil inoculated with *F. graminearum* was used as a control (0%). Each experimental treatment had three replicates, and all experiments were conducted three times.

Corn Growth-Parameter Measurement and Soil Sampling

Twenty days after planting, the growth parameters and disease grade of corn were evaluated. Stem diameter was measured at the stem base, and corn height was measured from the stem base to the top. Dry weight was calculated after placing the fresh corn in an 80°C air circulation oven for 10–15 days to constant dry weight. Rhizosphere soil from each pot was collected and maintained at 4°C for analysis at the end of the experiments.

Determination of Bacterial and Fungal Community Structure by T-RFLP

Total DNA was extracted from 0.5 g of soil using the soil DNA extraction kit (Omega BioTek, Inc., Norcross, USA) according to the manufacturer's instructions. DNA quality and quantity were evaluated by 1.2% agarose gel electrophoresis. For this analysis, DNA in the soil was amplified with primers for bacterial 16S rDNA, 5’ FAM-

labeled 8f (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492r (5'-TACCTTGTTACGACTT-3') and for fungal internal transcribed spacer (ITS) regions, 5'FAM-labeled (5'-CTTGGTCATTTAGAGGAAGTAA-3') and ITS4 (5'-TCCTCCGCTTATTGATATC-3'). PCR amplification was performed in 50 μL reaction volumes under the following cycling conditions: 95°C for 5 min; followed by 35 cycles of 94°C for 1 min, 42°C or 55°C (for bacterial or fungal DNA, respectively) for 1 min and 72°C for 1.5 min and a final elongation step at 72°C for 7 min (Liang *et al*., 2015; Gumiere *et al*., 2016). The PCR products were purified using a gel extraction kit (Axygen Biosciences, Hangzhou, China) according to the manufacturer's instructions. DNA was then resuspended in GE buffer. The quantity and quality of the purified DNA were analyzed spectrophotometrically with a NanoDrop 2000 system (ND-2000, Peqlab, Germany). Amplified DNA was digested with endonuclease MspI (HpaII) or HaeIII (Thermo Scientific, Waltham, U.S.A.). Each reaction mixture (30 μL) contained 10 μL of DNA, 3 μL of 10× Buffer Tango and 1 μL of MspI (HpaII) or HaeIII restriction enzyme. Nuclease-free water was added to obtain a total volume of 30 μL. For the restriction-enzyme reactions, the mixtures were incubated at 37°C for 3–5 h and then the mixtures were incubated at 80°C for 20 min for enzyme inactivation. The 20 μL digested products (T-RFs) were detected through the ABI PRISM® 3700 Genetic Analyzer System (Applied Biosystems, Foster City, USA), and GS500LIZ (Life Technologies, Foster City, USA) was used as a size standard.

Data Processing and Analysis

The sample data were analyzed by one-way analysis of variance (ANOVA) followed by Duncan's multiple range test (SPSS version 19.0, $P < 0.05$) (SPSS Inc., Chicago, U.S.A.). The degree of disease was recorded 20 days after the F. graminearum strain and biochar treatments according to the classification standards of corn stalk rot and the disease index was calculated with the following formula (Li *et al*., 2016a):

$$\text{disease index} = \sum (\text{number of corn seedlings in each disease stage} \times \text{level value}) / (\text{the highest level} \times \text{total number of corn seedlings} \times 100)$$

Disease reduction was calculated with the following formula:

$$\text{disease reduction} = \text{the disease index of 0\% biochar (soil inoculated with } \textit{F. graminearum}) - \text{disease index after treatment with } \textit{F. graminearum} \text{ strain and biochar/disease index of 0\% biochar}$$

The seeding index was calculated with the following formula:

$$\text{seeding index} = (\text{stem diameter}/\text{corn height}) \times \text{dry weight}$$

A diversity and clustering analysis (unweighted pair group with mathematical averages, UPGMA) based on the Jaccard similarity between different soil microbial community terminal restriction fragment length polymorphism (T-RFLP) profiles was performed using Paleontological Statistics (PAST) software version 3.22 (http://folk.uio.no/ohammer/past/) (Hammer *et al*., 2001).

Results

Corn Growth Parameters

Corn growth and biomass data are shown in Fig. 1. Both rice husk and bamboo biochar had pronounced impacts on plant height and dry weight parameters at all levels of biochar addition in the presence of the pathogen. For plant height (Fig. 1a), bamboo biochar yielded a superior performance over rice husk biochar. The highest plant height was obtained at a 0.5% biochar concentration. The growth parameters of corn were significantly improved at 0.5% biochar relative to the other biochar concentrations, including 0%. Compared with the 0% treatment, at 0.5%, the rice husk and bamboo biochar increased plant height by 29.33 and 17.07%, respectively ($P < 0.05$). At 1, 2 and 3%, there was no significant difference in plant height between the different biochar treatments. In addition, the biochars significantly improved the dry weight of corn at all concentrations (Fig. 1b). At 2% rice husk biochar and 1% bamboo biochar, dry weight was significantly increased by 10.21% and 6.70%, respectively, relative to the corresponding weights at 0%. The measured dry weight parameters were lower at 3% biochar addition than at the other biochar concentrations. All of these parameters generally produced U-shaped dose-response curves.

Control of Corn Stalk Rot

Data related to the disease index and seeding index are provided in Fig. 2. Compared to the 0% treatment, all of the other biochar treatments improved the disease index and seeding index of corn. The most significant ($P < 0.05$) decreases in the disease index were observed in the treatments with 2% rice husk biochar (32.50) and 1% bamboo biochar (17.00) (Fig. 2a). Compared with the biochar application at a rate of 0%, the application of 2% rice husk biochar and 1% bamboo biochar increased the seeding index by 0.0041 and 0.0049, respectively (Fig. 2b). In general, the application of biochar led to a decrease in the corn stalk rot incidence, with disease reductions of 68.42% (2% rice husk biochar) and 35.79% (1% bamboo biochar), and its effects were concentration dependent (Fig. 3).

Diversity of Soil Microbial Communities Based on T-RFLP Profile Analyses

T-RFLP analyses of 16S rRNA and ITS gene fragments were performed to compare the community compositions of

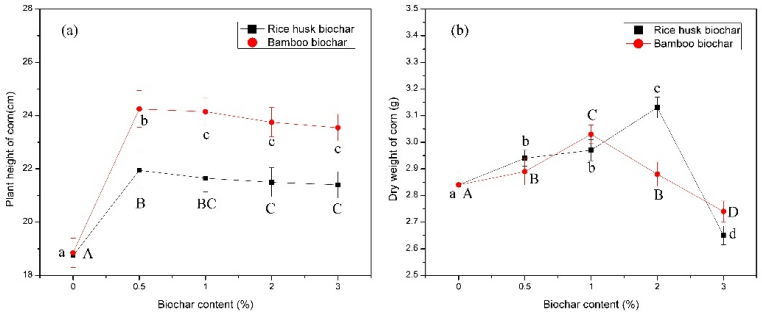

Fig. 1: Effect of biochar (0, 0.5, 1, 2 and 3% w/w) on (**a**) plant height and (**b**) dry weight with *Fusarium graminearum* inoculation. The results shown are the mean ± SEM (n = 3). Data points labeled with a common capital letter and small letter are not significantly different within rice husk and bamboo biochar at the $P < 0.05$ level according to Duncan's Multiple Range Test

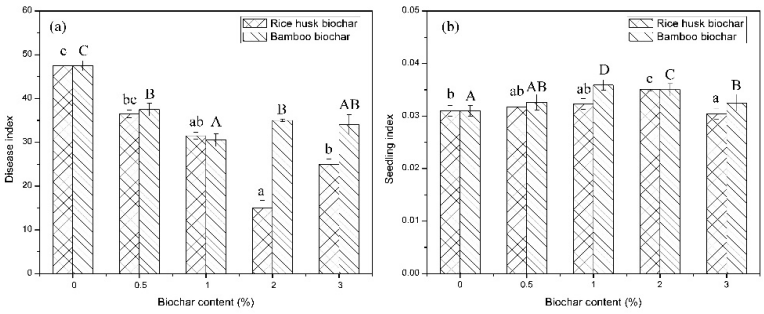

Fig. 2: Effect of biochar (0, 0.5, 1, 2 and 3% w/w) on (**a**) the disease index and (**b**) the seedling index in corn stalk rot caused by *Fusarium graminearum.* The results shown are the mean ± SEM (n=3). Columns labeled by a common capital letter and small letter show no significant difference within rice husk and bamboo biochar by Duncan's Multiple Range Test at $P < 0.05$

soil inoculated with *F. graminearum* and biochars. The T-RFLP profiles were clustered by UPGMA based on Jaccard similarity and visualized using a dendrogram. The indices were expressed as the mean and standard deviation of three replicates of each sample. Cluster analysis of T-RFs digested with MspI indicated marked effects of the biochars on the community structure of rhizospheric bacteria (Fig. 4a). The major bacterial communities were mainly divided into two major groups and three distinct clusters. Group I corresponded to the 0.5% rice husk biochar treatment, and group II corresponded to the other treatments. In addition, the bacteria in the 0.5% bamboo biochar and 2% rice husk biochar treatments formed one cluster. The differences amended with 0.5% rice husk biochar were greater than those in the 0%, 1%, 2% and 3% biochar treatments. Fig. 4a shows that pronounced changes occurred in the soil bacterial community under the addition of 0.5% rice husk biochar. However, no changes were observed in the other biochar treatments. Fig. 4b presents the relative abundances of bacterial community members (in base pairs) of soils (for those tax with relative abundance >2%). In the T-RFLP profiles, fragments with sizes of 92, 141, 148, 198, 288, 293, 486, and 500 bp were detected as major detection peaks, and these T-RFs represented dominant bacterial populations in soil. Fragments of 138, 396, 411, 432, 483,

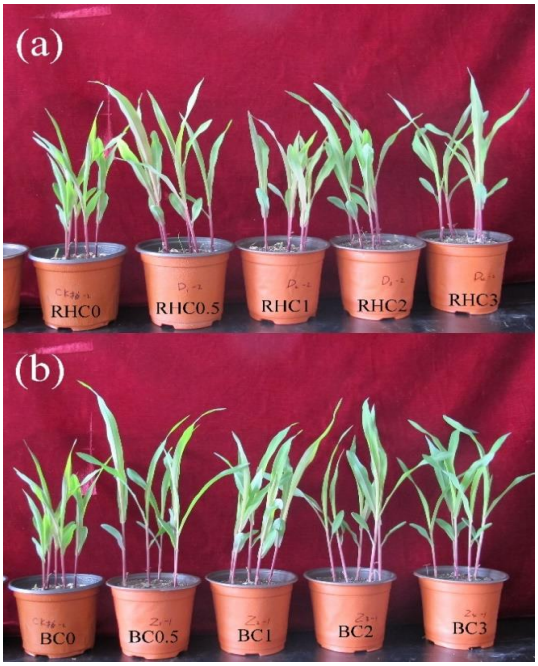

Fig. 3: Effect of biochar treatment (0, 0.5%, 1%, 2%, and 3% w/w) on corn stalk rot caused by *Fusarium graminearum.* (**a**) rice husk biochar; (**b**) bamboo biochar. RHC: rice husk biochar; BC: bamboo biochar

490, 509, and 541 bp were less abundant in the biochar-treated soils than in the 0% biochar soils (relative abundance<1%). The relative abundances of 472, 486 and 483 bp fragments were greater in the 0.5% rice husk biochar treatment than the other treatments and reached values of 16.04%, 16.16% and 5.02%, respectively (P <0.05). These results showed that biochar stimulated the growth of some beneficial bacteria in the soil.

The results of the cluster analysis revealed the effects of the different biochars on the fungal community. Two distinct statistically significant (P < 0.05) fungal clusters identified using HaeIII are shown in Fig. 4c. Group I corresponded to the 3% bamboo biochar and 1% rice husk biochar treatments, whereas group II corresponded to the other treatments. In group II, the fungal clusters in the 2% rice husk biochar treatment formed one cluster, whereas those of the 3% rice husk biochar treatment clustered with those of the 0.5% and 0% rice husk biochar treatments. The cluster analyses suggested that the soil fungal communities in the 0.5% and 0% rice husk biochar treatments had high similarity. Fig. 4d shows the relative abundances of fungal community members. The T-RFLP profiles revealed that 145, 292, 536 and 539 bp fragments were major peaks (relative abundance >3%). Fragments of 145, 292, 539 and 655 bp were major peaks in the 0% treatment. In the 2% rice husk biochar treatment, the 128, 145, 536, 539, 642 and 655 bp fragments were major peaks. The relative abundance of the 539 bp fragment was higher in the 2% rice husk biochar treatment than in the other treatments and reached 47.21% (P<0.05). This result suggests that the role of biochar on the soil fungal community involves the suppression of corn stalk rot.

Discussion

Achieving the effective control of corn stalk rot could greatly increase the yield and quality of corn worldwide. In this study, rice husk and bamboo biochar were applied in an attempt to identify a more effective method of managing corn stalk rot caused by *F. graminearum*. The growth parameters and disease grade of corn were measured and evaluated. Based on an analysis of the data, the disease index, disease reduction and seeding index of corn were calculated. We also performed a T-RFLP analysis to reveal changes in the soil microbial community after biochar addition. These results provide a basis for applying rice husk and bamboo biochar as soil amendments for the suppression of corn stalk rot caused by *F. graminearum*.

Studies have shown that various types of biochar can prevent and control diseases caused by soil-borne pathogens and the optimum suppressive ability is mainly influenced by its source feedstock (crop residues, wood, municipal waste, sewage sludge, manure, animal bones, etc.), pyrolysis temperature (<600°C) and application concentration (<3%) (Graber *et al*., 2014; Bonanomi *et al*., 2015; Rawat *et al*., 2019). In our study, we selected rice husk and bamboo as source feedstocks, produced the biochar under slow pyrolysis at 500°C for 4 h with oxygen limited availability, used doses of biochar <3% in the soil. Until now, systematic studies on the control of corn stalk rot caused by *F. graminearum* via rice husk and bamboo biochar application have not been performed, although the application of various types of biochar to soil has been shown to reduce damping-off and root rot (Eo *et al.,* 2018; Hassan *et al.,* 2018).

Our research shows that the optimum levels of biochar addition for controlling corn stalk rot caused by *F. graminearum* were 2% for rice husk biochar and 1% for bamboo biochar. At other application rates, biochar provided disease control but at lower control efficiencies. The disease severity under rice husk and bamboo biochar soil amendments demonstrated a U-shaped response curve. Such dose-response effects are termed 'hormesis' effects. In general, low doses of biochar suppress disease, whereas high doses have negative impacts on disease progression (Huang and Gu, 2019). This type of curve (U-shaped) has been reported for several types of biochar and plant diseases (Frenkel *et al.,* 2017). It is thought that biochar applied at high doses damages the crop root system and enhances the attacking ability of pathogens (Gale and Thomas, 2019). However, the exact mechanisms that result in a U-shaped dose-response curve for biochar are not yet clear.

The maximum plant height of corn occurred at a low application rate of rice husk biochar and bamboo biochar (0.5%) and not at any of the higher application rates (1%, 2% and 3%). Bamboo biochar was more conducive to stimulating growth than rice husk biochar. Correspondingly, the bamboo biochar treatments resulted in greater plant heights compared with the rice husk biochar treatments. Bamboo biochar has a high carbon content (46.32%), whereas rice husk biochar has a high pH, ash and N content and specific surface area (SSA) (Table 1). In theory, these parameters could play roles in promoting plant growth (Cross *et al*., 2016; Yu *et al*., 2019). Alternatively, some characteristics of biochar may affect the beneficial microflora in soil.

In our study, the different application rates of biochar were all effective in controlling corn stalk rot caused by *F. graminearum*. However, the extent of corn stalk rot suppression varied with the feedstock type and application rate of biochar. The differences in the control efficiency of corn stalk rot between rice husk and bamboo biochar could reflect the different physicochemical compositions of the biomass. Therefore, the types of biochar and their concentration should be considered and evaluated before the application of biochar to control corn stalk rot. The plant height of corn was not correlated with disease suppression in any of the biochar treatments. This result suggests that disease suppression at different levels of biochar may be related to *F. graminearum*-induced corn systemic resistance against stalk rot and may indicate that biochar

addition can have long-term effects (Rogovska *et al*., 2017; Peiris *et al*., 2019; Zhou *et al*., 2019). However, biochar stimulates plant growth at low concentrations (Sun *et al*., 2017; Gale and Thomas, 2019).

Conclusion

The corn growth-parameter measurements and T-RFLP profile analyses of the diversity of soil microbial communities suggested that the application of rice husk or bamboo biochar can suppress corn stalk rot caused by *F. graminearum*. The most effective dose for controlling the disease was 2% for rice husk biochar and 1% for bamboo biochar. The physical and chemical properties of biochar and the application amount affected the disease-suppression capability of biochar.

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