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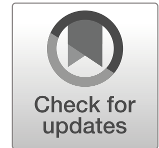

Suppression of Phytophthora blight of pepper by biochar amendment is associated with improved soil bacterial properties

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Abstract
Biochar amendment effectively controlled the Phytophthora blight of pepper and suppressed the pathogen abundance, with biochar applied just before transplanting (BC0) producing greater effects than that applied 20 days before transplanting (BC20). Biochar treatments stimulated the proliferation of total bacteria, *Bacillus* spp., *Pseudomonas* spp. , *Streptomyces* spp., and *Sphingomonas* spp. The proliferative effect of BC20 treatment gradually weakened compared to that of BC0 treatment with extended planting time. Moreover, biochar amendment strongly promoted the antagonist percentage and antagonistic ability of total bacteria, *Bacillus* spp., and *Pseudomonas* spp. and the promoting effect of BC0 treatment was stronger than that of BC20 treatment. Biochar-enriched *Bacillus* and *Streptomyces* strains, followed by *Pseudomonas* strains, were the best in terms of reducing the abundance of *P. capsici* and controlling Phytophthora blight of pepper. In addition, MiSeq sequencing data indicated that biochar treatments altered the soil bacterial community and enriched some beneficial bacteria, with BC0 treatment producing greater effects than BC20 treatment. Overall, the biochar-induced improvement of soil properties (particularly the abundance of biocontrol bacteria such as *Bacillus* spp., *Pseudomonas* spp. and *Streptomyces* spp. and bacterial antagonisms against *P. capsici*) may constitute one of the important mechansims of biochar-mediated control of Phytophora blight of pepper.

Keywords Biochar · Application time · Phytophthora blight of pepper · Biocontrol bacteria · Bacterial antagonism

Introduction

Plant soil-borne diseases in protected cultivation have increased drastically due to long-term intensive agriculture, leading to enormous economic losses (Oerke et al. 2012). Organic amendment, including crop residues, compost, and organic waste, is a promising strategy for the control of soil-borne diseases because of its strengths such as safety, environmental protection, cost-effectiveness, and resource utilization (Bonanomi et al. 2018; Scotti et al. 2015). Biochar is a novel organic soil amendment, with special physical and chemical properties, that potentially functions to increase soil fertility, improve soil health, and promote plant growth (El-Naggar et al. 2019; Lehmann et al. 2011; Novak et al. 2016). In recent years, there have been many reports showing the ability of biochar to suppress soil-borne diseases, such as bacterial wilt of tobacco, damping-off of cucumber, root rot of asparagus, and Fusarium wilt of tomato (Frenkel et al. 2017; Graber et al. 2014).

Previous studies showed that biochar significantly influenced bacterial community composition at the phylum, family, and genus levels, and significantly influenced bacterial richness and diversity (Abujabhah et al. 2018; Chen et al. 2018; Jenkins et al. 2017; Kolton et al. 2016; Yao et al. 2017). A close correlation between soil-borne disease control and improvement of the bacterial community composition

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under biochar amendment has been reported previously. According to Jaiswal et al. (2017 and 2018), biochar reduced the severity of tomato Fusarium wilt and cucumber Pythium damping-off due partly to the increase in microbial activity, bacterial diversity, and abundance of some beneficial microorganisms. The results obtained by Zhang et al. (2016a) matched those obtained by Jaiswal et al. (2017 and 2018); i.e., the biochar-mediated control of tobacco bacterial wilt correlated with the enhancement of bacterial richness and diversity and enrichment of some beneficial bacteria.

Various beneficial bacteria such as *Pseudomonas* spp., *Bacillus* spp., *Flavobacterium* spp., *Rhizobium* spp., *Streptomyces* spp., *Brevibacillus* spp., and *Mesorhizobium* spp. have been reported to be enriched by biochar (Elmer and Pignatello 2011; Graber et al. 2010; Jaiswal et al. 2018; Kolton et al. 2011; Zhang et al. 2016a). However, no in-depth study has been conducted to evaluate the relationship between the abundance of beneficial bacteria and disease suppression under biochar amendment. Organic amendments can enhance disease suppression by strongly stimulating bacterial antagonism against soil-borne pathogens (Huang et al. 2006; Perez et al. 2008). Therefore, it is crucial to know how soil bacterial properties including the bacterial community composition, abundance of beneficial bacteria, and bacterial antagonism respond to biochar application.

Steinbeiss et al. (2009) demonstrated that the soil microbial activity decreased with the time after biochar amendment. A similar finding was also reported by Thies (2013), who observed that biochar amendment had significantly influnced soil microbial abundance, activity, and diversity and that the influence declined over time. Thus, it is speculated that the control effect of biochar may be affected by a time-dependent influence of biochar application on soil microbial properties.

Phytophthora blight of pepper caused by *Phytophthora capsici* Leonian is one of the most serious soil-borne diseases for pepper production worldwide (Babadoost et al. 2015). We previously demonstrated that Phytophthora blight of pepper was effectively controlled by biochar application (Wang et al. 2017). In this study, we hypothesized that (1) disease control is associated with biochar-induced changes in the soil bacterial properties; (2) biochar application time is an important factor influencing soil bacterial properties and the control of Phytophthora blight of pepper; and (3) biochar amendment can enrich biocontrol bacteria contributing disease suppression.

Materials and methods

Biochar and soil

Biochar was produced from corn straw at a pyrolysis temperature of 500 °C as described by Lu et al. (2014). The biochar was sieved with a 0.45-mm sieve before mixing with soil. The biochar had a pH of 9.73 and an electric conductivity of 5715 μs cm^{-1}, and contained 489.9 g kg^{-1} total C, 22.8 g kg^{-1} total H, 145.7 g kg^{-1} total O, 17.5 g kg^{-1} total N, 324.2 g kg^{-1} ash, 285.5 g kg^{-1} organic matter, 2.22 g kg^{-1} available phosphorus, and 24.7 g kg^{-1} available potassium.

Sandy loam soil was used in pot experiments. The soil was collected from the arable layer (0–20 cm) in a pepper greenhouse. The greenhouse was located in Huaian, Jiangsu Province, eastern China (33° 30′ N, 119° 05′ E). The soil had a pH of 7.44 and contained 29.4 g kg^{-1} organic matter, 3.05 g kg^{-1} total N, 2.02 g kg^{-1} total phosphorus, 12.6 g kg^{-1} total potassium, 25.5 mg kg^{-1} ammonium N, and 116.7 mg kg^{-1} nitrate N.

Pot experiments

Three treatments were set up: (a) soil incubated for 20 days before transplanting (CK); (b) soil incubated for 20 days and amended with 1.33% (w/w) biochar just before transplanting (BC0); and (c) soil amended with 1.33% (w/w) biochar and incubated for 20 days before transplanting (BC20). Each treatment was placed in a plastic box in triplicate and incubated in a greenhouse at 15–30 °C for 20 days. During incubation, soil water content was maintained at approximately 20% by daily adjustment according to weight loss. After incubation, all treatments were inoculated with a *P. capsici* zoospore suspension to a concentration of 100 zoospores per gram of soil. Each treatment included three replicates with 20 pots per replicate (total 60 pots per treatment). Each pot (diameter 12 cm and height 15 cm) was filled with 600 g of soil and transplanted with one 5-week-old pepper seedling. All pots were incubated at greenhouse temperatures ranging from approximately 15–30 °C for 45 days and watered daily as needed.

Disease severity was recorded every 5 days from May 18 to June 31, 2016. The disease index was evaluated using a 0–4 scale (0 = healthy plant; 1 = leaves slightly wilted or small brownish lesions on stems; 2 = leaves wilted with brownish lesions on stems; 3 = leaves significantly wilted with large brownish lesions on stems; 4 = dead plant). Disease index = [∑(number of diseased plants in this index × disease index rating from 0 to 4)/(4 × number of plants investigated)] × 100%.

Soil sampling and DNA extraction

Before transplanting, soil samples were taken from each replicate of all treatments. At 15, 30, and 45 days after transplanting, five plants were randomly excavated from each replicate of all treatments. Soil tightly adhering to the root surface was brushed off and collected as the rhizosphere sample. After sieving through a 2-mm screen, each soil sample was stored at − 70 °C for total DNA extraction. In addition,

soil samples at 30 days after transplanting were stored at 4 °C for later screening of biochar-enriched biocontrol bacteria and analysis of antagonist percentage and antagonistic ability of bacteria. Soil DNA was extracted from 0.5 g of soil using a FastDNA SPIN Kit for Soil (MP Biomedicals, USA).

Quantitative real-time PCR

All qPCR assays were performed using an ABI 7500 Sequence Detection System (Applied Biosystems, USA). A SYBR Green-based qPCR for total bacteria, *P. capsici*, *Bacillus* spp., *Pseudomonas* spp., and *Sphingomonas* spp. was performed with SYBR Premix Ex Taq (TaKaRa Biotechnology Dalian Co., Ltd.) according to the manufacturer's instructions. The bacterial 16S rRNA gene copy number was determined with primer pair 338F/518R (Wang et al. 2014). *P. capsici*, *Bacillus*, *Pseudomonas*, and *Sphingomonas* populations were quantified using genus-specific primer pairs CAPFW/CAPRV1 (Wang et al. 2014), BacF/1378R (Drigo et al. 2009), Psf/Psr (Drigo et al. 2009), and Sphingo108f/Sphingo420r (Shi and Bending, 2007), respectively. TaqMan qPCR for *Streptomyces* spp. was performed using Premix Ex Taq (Probe qPCR) according to the manufacturer's instructions. The primers/probes used in the TaqMan qPCR of *Streptomyces* were StrepF/StrepR/Strep-Probe (Kettleson et al. 2013). The target gene copy numbers of bacteria and *P. capsici* were estimated from plasmid-based standard curves. For *Bacillus* spp., *Pseudomonas* spp., *Sphingomonas* spp., and *Streptomyces* spp., standard curves were developed from 10-fold dilution of genomic DNA by serial dilution in sterile ultrapure water. Each qPCR plate contained triplicate reactions for DNA samples, a series of standards, and a negative control. Sterile water was used as the negative control (Schöler et al. 2017; Vestergaard et al. 2017).

Illumina MiSeq sequencing and data analysis

Soil DNA samples at 30 days after transplanting were submitted to Majorbio Co., Ltd. (Shanghai, China) for Illumina MiSeq sequencing. The primers 515F (5′-GTGCCAGCMGCCGCGG-3′) and 907R (5′-CCGTCAATTCMTTTRAGTTT-3′) were used for PCR amplification of the bacterial 16S rRNA target gene (Xiong et al. 2012). PCR products were sequenced using the MiSeq sequencing technique following their standard protocols. All sequences were clustered into operational taxonomic units (OTUs) based on an identity level of 97% using UPARSE. Based on the saturation of rarefaction curves (Schöler et al. 2017; Vestergaard et al. 2017), the bacterial diversity for each site was evaluated by calculating richness (number of OTUs), Shannon index, coverage, and the richness estimator indices Chao1 and ACE (abundance-based coverage estimation) using mothur software (Schloss et al. 2009). OTUs were classified using the Silva (SSU123) 16S rRNA database (Amato et al. 2013). Principal coordinate analysis (PCoA) based on the weighted UniFrac distances was performed to determine the differences in the bacterial community composition.

Isolation of soil bacteria and analysis of the antagonistic bacteria

For each replicate of all treatments, 100 isolates of bacteria, 50 isolates of *Bacillus* spp., 50 isolates of *Pseudomonas* spp., and 50 isolates of *Streptomyces* spp. were isolated based on colony morphology by the dilution-plate method. The media used were as follows: beef extract peptone agar for bacterial isolates and *Bacillus* spp. (Graber et al. 2010), King's B agar for *Pseudomonas* spp. (Graber et al. 2010), and STR agar for *Streptomyces* spp. (Conn et al. 2007).

All isolates were tested for their antagonistic ability against *P. capsici* by coculturing on agar plates. An agar disk containing *P. capsici* mycelia was placed in the center of a PDA plate, and the bacterial isolate was placed 20 mm from the edge of the plate. Each isolate was inoculated four times on the same plate and incubated at 28 °C for 4 days. Isolates that could form a halo zone were scored as antagonistic to *P. capsici*. The antagonist percentage was equal to the number of antagonistic isolates divided by the total number of isolates (Minaeva et al. 2008).

Screening and evaluation of biochar-enriched biocontrol bacteria

Preliminary screening

Strains of *Bacillus*, *Pseudomonas*, and *Streptomyces* spp. enriched by BC0 treatment compared to CK treatment described in 3.6 were selected and placed on new PDA plates to obtain pure colonies. In the same manner, strains of *Sphingomonas* spp. enriched by BC0 treatment were also screened and subcultured on *Sphingomonas* selective agar (Sphing agar) (Yim et al. 2010). Morphological features such as colonies, mycelia, and spores were used to avoid replication of isolates. The remaining strains were cultured in beef extract-peptone broth and their genomic DNA was extracted from the bacterial suspensions using the Bacterial DNA Mini kit (Omega Bio-Tek, USA). These bacterial strains were identified by sequencing the 16S rRNA regions, and nucleotide sequence similarity searches were performed using the NCBI-GenBank database (Meyer-Dombard et al. 2015).

Rescreening

Soil was divided into two parts: one not amended with biochar and the one amended with 1.33% (w/w) biochar. Both

biochar-unamended and biochar-amended soils were inoculated with the bacterial suspensions as already described. The inoculum concentration of the bacterial suspension was adjusted to 10^7 CFU per gram of soil. Both treatments for each strain consisted of three replicates (one pot per replicate). A 5-week-old pepper seedling was transplanted into each pot (diameter 12 cm and height 15 cm) filled with 600 g of soil. All pots were incubated in a greenhouse between 15 and 30 °C and watered as required. Fifteen days later, the abundance of the target strains in the rhizosphere was determined by qPCR as already described. Bacterial strains that were more abundant in the biochar-amended rhizosphere than in the biochar-unamended rhizosphere were considered biochar-enriched bacteria.

Biocontrol assay against Phytophthora blight of pepper

A *P. capsici* zoospore suspension was mixed with soil at a concentration of 100 zoospores per gram of soil. The soil was divided into several treatments of biochar-enriched bacteria and one control. The bacterial inoculum concentration was adjusted to achieve 10^7 CFU per gram of soil. Soil inoculated with only the pathogen was used as a control. Each treatment consisted of three replicates (15 pots per replicate). Each 5-week-old pepper seedling was transplanted into a pot (diameter 12 cm and height 15 cm) filled with 600 g of soil. All pots were incubated in a greenhouse at 15–30 °C and watered daily as required. Disease severity was recorded at 15 and 30 days after transplanting. Disease index was evaluated as already described. Biocontrol efficacy = [(disease index of control − disease index of fungal treatment)/disease index of control] × 100% (Zhan et al. 2011).

Statistical analysis

All statistical analyses were performed using SPSS version 19.0 statistical software (IBM, USA). The significant difference between the treatments was determined by one-way analysis of variance (ANOVA). A probability of $p < 0.05$ was accepted as statistically significant.

Results

Effect of biochar on severity of Phytophthora blight of pepper and abundance of *P. capsici*

Disease symptoms of Phytophthora blight of pepper appeared in transplants in CK treatment at 5 days after transplanting. By contrast, disease symptoms appeared in BC0 and BC20 treatments at 15 and 10 days after transplanting, respectively (Fig. 1a). Compared with CK treatment, BC0 and BC20 treatments reduced disease index at 20 days after transplanting by 81.79% and 53.2%, respectively, and at 45 days after transplanting by 62.52% and 34.83%, respectively. These results indicated that biochar amendment delayed and reduced disease development and BC0 treatment had a significantly higher control efficacy than BC20 treatment ($p < 0.05$).

Up to 15 days after pathogen inoculation, *P. capsici* abundance declined in all treatments. Later, the abundance of *P. capsici* increased between days 15 and 30 and then decreased slightly between days 30 and 45 (Fig. 1b). Compared with CK treatment, biochar treatments decreased the abundance of *P. capsici* at 15 days after transplanting by 95.11% (BC0) and 57.50% (BC20) and at 30 days after transplanting by 77.65% (BC0) and 46.34% (BC20). It was notable that the suppression of *P. capsici* by biochar

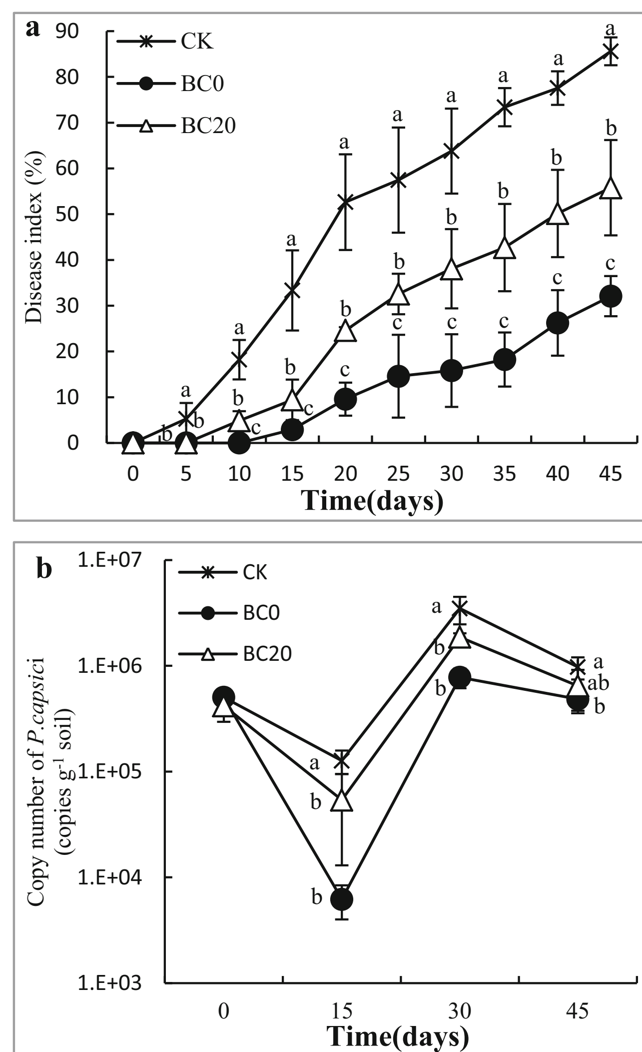
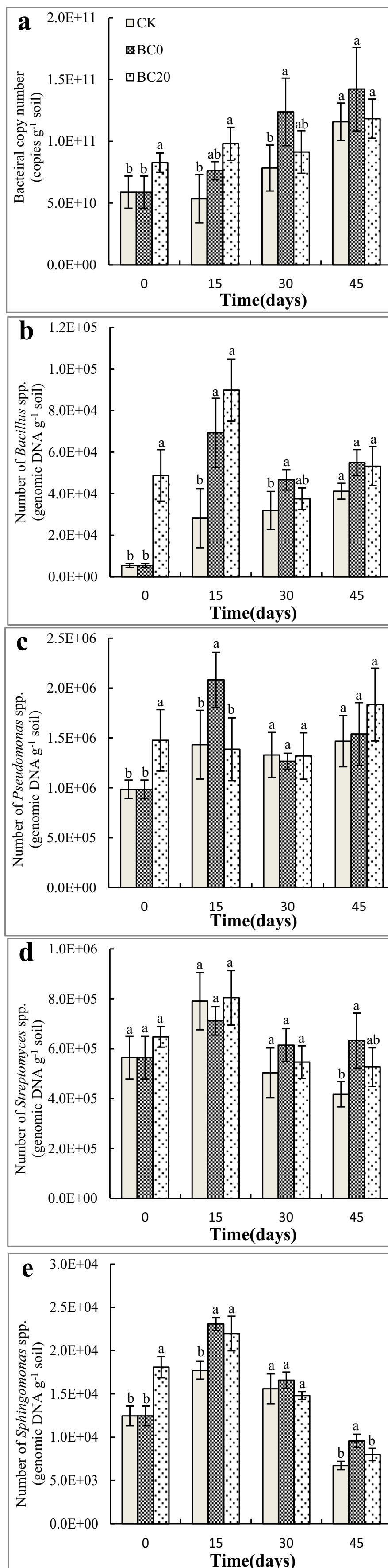


Fig. 1 Temporal effects of biochar application at different times on disease index (**a**) and abundance of *P. capsici* (**b**). *CK*, soil not amended with biochar; *BC0*, soil amended with biochar 0 days before transplanting; *BC20*, soil amended with biochar 20 days before transplanting. Bars represent the standard error of each mean. Different letters represent significant differences among the three treatments following Duncan's test ($p < 0.05$)



◀ **Fig. 2** Temporal effects of biochar application at different times on the abundance of total rhizosphere bacteria (**a**) and potential biocontrol bacteria. Potential biocontrol bacteria are *Bacillus* (**b**), *Pseudomonas* spp. (**c**), *Streptomyces* spp. (**d**), and *Sphingomonas* spp. (**e**). *CK*, soil not amended with biochar; *BC0*, soil amended with biochar 0 days before transplanting; *BC20*, soil amended with biochar 20 days before transplanting. Bars represent the standard error of each mean. Different letters represent significant differences among the three treatments following Duncan's test ($p < 0.05$)

diminished with time. In addition, BC0 treatment was more suppressive to *P. capsici* than BC20 treatment during the entire growing period.

Effect of biochar on bacterial abundance

qPCR results demonstrated that both biochar treatments increased the abundance of total bacteria, *Bacillus* spp., *Pseudomonas* spp., *Sphingomonas* spp., and *Streptomyces* spp. (Fig. 2). BC20, after incubation with biochar for 20 days, had 0.41-, 7.98-, 0.50-, 0.45-, and 0.15-fold increased abundance of total bacteria, *Bacillus* spp., *Pseudomonas* spp., *Sphingomonas* spp., and *Streptomyces* spp., respectively, compared with that of CK. The abundance of total bacteria, *Bacillus* spp., *Streptomyces* spp., and *Sphingomonas* spp. in BC20 subsequently increased slightly and then deceased but were always higher than those in CK and those of BC20 were lower than those of BC0. BC0 showed a strong consistent increase in the abundance of total bacteria, *Bacillus* spp., and *Sphingomonas* spp. during the entire growing period. At 30 days and 45 days after transplanting, BC0 showed a relatively strong proliferative effect on *Streptomyces* spp. Compared with CK, BC0 increased the abundance of total bacteria, *Bacillus* spp., *Sphingomonas* spp., and *Streptomyces* spp. by 0.23-, 0.33-, 0.42-, and 0.52-fold at 45 days after transplanting, respectively, while that of BC20 was increased by 0.03-, 0.29-, 0.26-, and 0.19-fold, respectively. Biochar also increased the abundance of *Pseudomonas* spp.; however, the proliferative effect was sustained for a short time only; BC20 showed a proliferative effect only before transplanting, while BC0 showed this effect at 15 days after transplanting.

Therefore, biochar stimulated the growth of all bacteria, *Bacillus* spp., *Pseudomonas* spp., *Sphingomonas* spp. and *Streptomyces* spp. and this effect gradually declined with time. In addition, the shorter biochar application time led to a stronger proliferative effect on potential biocontrol bacteria.

Pearson's correlation analysis was performed to assess any correlations between disease severity and abundance of *P. capsici*, total bacteria, and biocontrol bacteria at 15, 30, and 45 days after transplanting (Table 1). The abundance of *P. capsici* was positively correlated with disease severity, suggesting that disease severity was influenced by the abundance of *P. capsici*. Negative correlations were observed between the abundance of *Bacillus and Streptomyces* with disease severity and abundance of *P. capsici*. The abundance of total bacteria,

Table 1 Relationship of disease severity and pathogen abundance with rhizosphere bacterial abundance based on correlation analysis of multitime point data (15, 30, and 45 days after transplanting)

	Disease severity	*P. capsici*
P. capsici	0.532**	–
Total bacteria	− 0.339	− 0.276
Bacillus	− 0.538**	− 0.478*
Pseudomonas	− 0.147	− 0.303
Streptomyces	− 0.533**	− 0.486*
Sphingomonas	− 0.368	− 0.17

**Correlation is significant at the $p < 0.01$ level; *Correlation is significant at the $p < 0.05$ level

Pseudomonas spp., and *Sphingomonas* spp. were negatively correlated to some extent with both disease severity and the abundance of *P. capsici*.

Effect of biochar on antagonistic bacteria

Biochar treatments significantly increased both antagonist percentage and average antagonistic ability of total bacteria, *Bacillus* spp., and *Pseudomonas* spp. ($p < 0.05$); however, the effect on *Streptomyces* spp. was slight (Fig. 3). The antagonist percentage and average antagonistic ability in BC0 were higher than those in BC20, but these differences were not significant ($p > 0.05$).

Correlation analysis was also performed between disease severity, the abundance of *P. capsici*, and antagonist percentage and antagonistic ability of bacteria at 30 days after transplantation (Table 2). The antagonist percentage and antagonistic ability of total bacteria, *Bacillus* spp., and *Pseudomonas* spp. were significantly correlated with disease severity and the abundance of *P. capsici* ($p < 0.05$).

Screening and evaluation of biochar-enriched biocontrol bacteria

Four *Bacillus*, three *Pseudomonas*, five *Streptomyces*, and two *Sphingomonas* strains enriched by BC0 were screened by comparing the colony characteristics on selective media plates from CK and BC0 and further molecular identification (Table S1). Among the strains, three *Bacillus* (BA1, BA2, and BA3), three *Pseudomonas* (PS1, PS2, and PS3), three *Streptomyces* (ST1, ST4, and ST5), and two *Sphingomonas* (SP1 and SP2) strains were confirmed to be enriched by biochar by comparing their colonization in the pepper rhizosphere during biochar amendment and non-biochar amendment (Table S2).

Eleven antagonistic bacterial strains showed considerable variation in disease severity reduction (Table 3). *Bacillus* BA1 and BA2 had strong control effects at 15 and 30 days after transplanting, and their control efficacies ranged between 44 and 58%. *Streptomyces* strains also had strong control effects, with control efficacies of 59.18% and 40.82% at 15 days after transplanting for ST1 and ST5, respectively; however, their control efficacies declined to 35.90% and 10.26%, respectively, at 30 days after transplanting. The control effects of *Pseudomonas* strains were weaker than those of *Bacillus* and *Streptomyces* strains. The control efficacies of PS1 and PS3 were 34.29% and 26.53%, respectively, at 15 days after transplanting; however, their control efficacies declined to 14.29% and 29.49%, respectively, at 30 days after transplanting. In addition, *Sphingomonas* strains showed no control effect at 15 and 30 days after transplanting; in fact, they even aggravated the disease to some extent. In general,

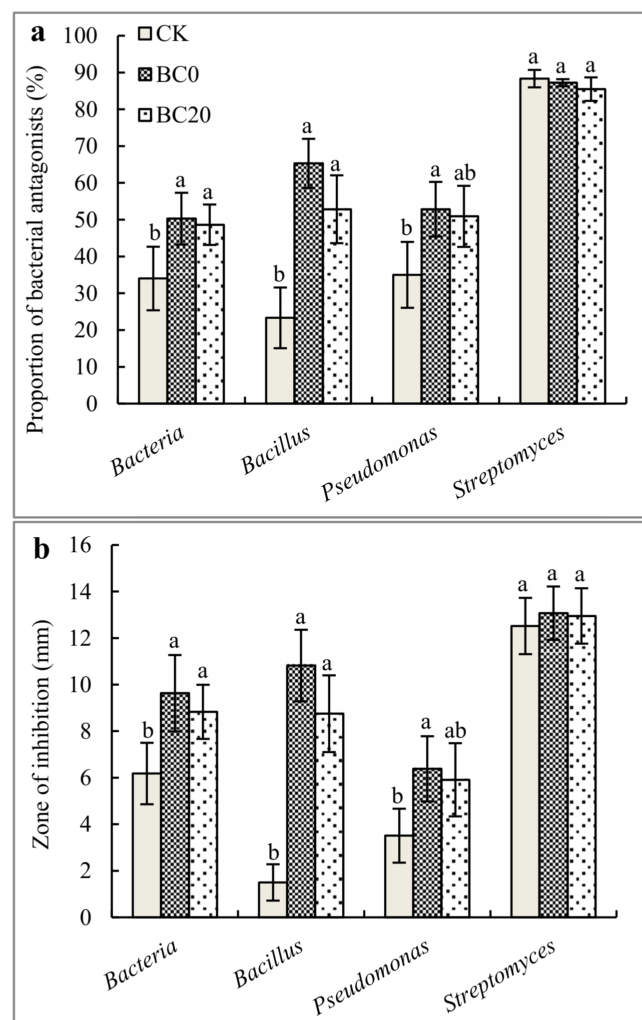


Fig. 3 Effects of biochar application at different times on antagonist percentage (**a**) and average antagonistic ability (zone of inhibition) of bacteria (**b**) at 30 days after transplanting. *CK*, soil not amended with biochar; *BC0*, soil amended with biochar 0 days before transplanting; *BC20*, soil amended with biochar 20 days before transplanting. Bars represent the standard error of each mean. Different letters represent significant differences among the three treatments following Duncan's test ($p < 0.05$)

Table 2 Relationship of disease severity and pathogen abundance with rhizosphere bacterial antagonists at 30 days after transplanting

		Disease severity	*P. capsici*
Antagonist percentage	Total bacteria	− 0.661*	− 0.817**
	Bacillus	− 0.884**	− 0.853**
	Pseudomonas	− 0.713*	− 0.723*
	Streptomyces	0.147	0.344
Antagonistic ability	Total bacteria	− 0.742*	− 0.850**
	Bacillus	− 0.813**	− 0.847**
	Pseudomonas	− 0.792*	− 0.690*
	Streptomyces	− 0.095	− 0.154

**Correlation is significant at the $p < 0.01$ level; *Correlation is significant at the $p < 0.05$ level

Bacillus and *Streptomyces* strains exerted a strong control effect, followed by *Pseudomonas* strains; however, *Sphingomonas* strains exerted no control effect against Phytophthora blight of pepper.

Similar results were obtained from the pepper rhizosphere infection by *P. capsici*. *Bacillus*, *Pseudomonas*, and *Streptomyces* strains significantly inhibited pathogen infection ($p < 0.05$), with *Bacillus* inhibiting more than *Pseudomonas* and *Streptomyces* at 15 days after transplanting. The differences in pathogen abundance among treatments at 30 days after transplanting, similar to those at 15 days, were reduced to some extent. Notably, *Pseudomonas* showed a slight reduction in pathogen abundance. Thus, *Bacillus* strains showed the best suppression of *P. capsici*, followed by *Streptomyces* strains, and finally *Pseudomonas* strains.

Effect of biochar on the bacterial community composition

At 30 days after transplanting, the differences between CK and biochar treatments were extremely small both in terms of bacterial richness indices (OTUs, Chao1, and ACE) and Shannon's diversity index (Table S3). However, principal coordinates analysis (PCoA) based on the OTU abundance revealed a separation between CK and biochar treatments with respect to bacterial community composition. In addition, BC0 treatment was slightly separated from BC20 treatment (Fig. S1).

Biochar treatments had great influence on several bacterial genera (average relative abundance > 0.2%) (Fig. 4 and S2). Figure 3 shows some biochar-enriched bacterial genera that have beneficial effects on plant performance or disease suppression. Compared to CK, biochar treatments enriched *Streptomyces* spp., *Mesorhizobium* spp., Gammaproteobacteria_unclassified, *Lysobacter* spp., *Nocardioides* spp., and *Steroidobacter* spp., with BC0 producing a greater effect than BC20. In addition, BC0 increased the relative abundance of *Bacillus* spp., *Pseudomonas* spp., and *Sphingomonas* spp. to some extent. Correlation analysis showed that the relative abundance of *Bacillus* spp., *Streptomyces* spp., *Mesorhizobium* spp., Gammaproteobacteria_unclassified, *Lysobacter* spp., *Nocardioides* spp., and *Steroidobacter* spp. was negatively correlated with disease severity and abundance of *P. capsici* (Table 4).

Table 3 Disease severity, control efficacy, and pathogen abundance relative to biocontrol strain

Treatment	15 days after transplanting			30 days after transplanting		
	Disease severity (%)	Control efficacy (%)	Pathogen concentration (10^4 copies g^{-1})	Disease severity (%)	Control efficacy (%)	Pathogen concentration (10^5 copies g^{-1})
PC	34.03 ± 7.02^{abc}	–	13.88 ± 1.98^{ab}	54.17 ± 12.15^{ab}	–	3.48 ± 0.50^{a}
PC + BA1	14.58 ± 3.47^{e}	57.14	4.61 ± 0.93^{ef}	29.02 ± 7.02^{d}	46.43	2.39 ± 0.13^{cd}
PC + BA2	17.50 ± 5.73^{de}	48.57	3.18 ± 1.09^{f}	30.31 ± 9.19^{d}	44.05	1.82 ± 0.42^{d}
PC + BA3	35.00 ± 7.02^{abc}	− 2.86	6.96 ± 2.21^{def}	50.94 ± 6.26^{abc}	5.95	2.78 ± 0.55^{abc}
PC + PS1	22.36 ± 6.57^{bcde}	34.29	7.90 ± 2.96^{cde}	46.43 ± 7.96^{abcd}	14.29	2.96 ± 0.36^{abc}
PC + PS2	35.97 ± 11.56^{ab}	− 5.71	11.38 ± 3.22^{bc}	57.78 ± 9.02^{a}	− 6.67	3.23 ± 0.21^{ab}
PC + PS3	25.00 ± 4.59^{bcde}	26.53	9.00 ± 1.51^{cd}	38.19 ± 8.56^{bcd}	29.49	2.63 ± 0.45^{bc}
PC + ST1	13.89 ± 3.61^{e}	59.18	7.28 ± 1.55^{cdef}	34.72 ± 8.91^{cd}	35.90	2.40 ± 0.18^{cd}
PC + ST4	31.25 ± 10.56^{abcd}	8.16	6.91 ± 1.26^{def}	55.56 ± 14.77^{ab}	− 2.56	2.87 ± 0.30^{abc}
PC + ST5	20.14 ± 11.56^{cde}	40.82	6.03 ± 1.91^{def}	48.61 ± 11.82^{abc}	10.26	2.84 ± 0.23^{abc}
PC + SP1	43.75 ± 9.19^{a}	− 28.57	14.69 ± 3.18^{ab}	58.33 ± 7.96^{a}	− 7.69	2.70 ± 0.41^{bc}
PC + SP2	37.50 ± 11.30^{ab}	− 10.20	17.52 ± 3.48^{a}	61.11 ± 9.19^{a}	− 12.82	2.88 ± 0.46^{abc}

Different letters after the values of the same column refer to Duncan's test $p < 0.05$

PC, soil inoculated with *P. capsici* zoospores. *Bacillus* strains: *BA1*; *BA2*; *BA3*. *Pseudomonas* strains: *PS1*; *PS2*; *PS3*. *Streptomyces* strains: *ST1*; *ST4*; *ST5*. *Sphingomonas* strains: *SP1*; *SP2*

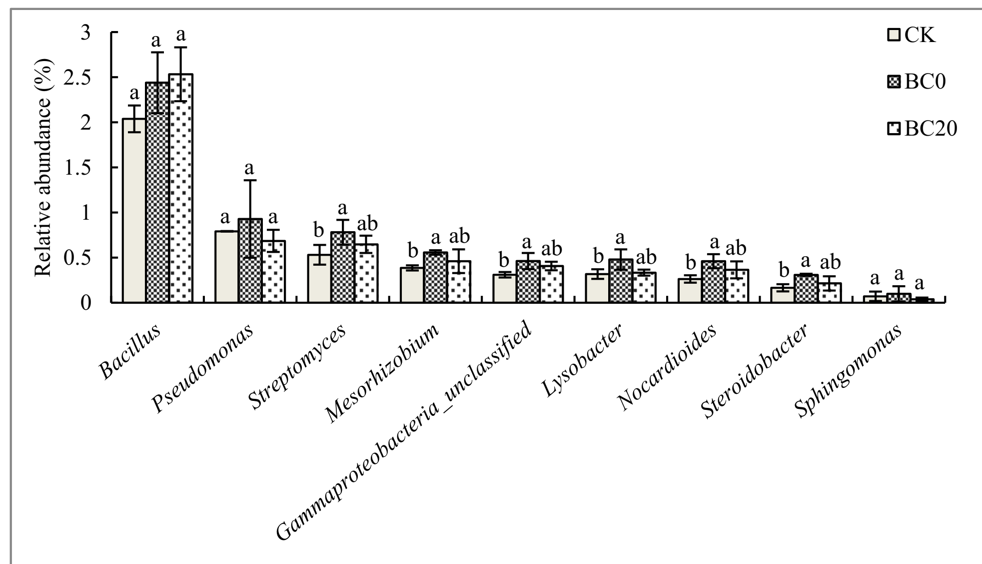


Fig. 4 The relative abundances of bacterial genera changed by biochar amendment at 30 days after transplanting. *CK*, soil not amended with biochar; *BC0*, soil amended with biochar 0 days before transplanting; *BC20*, soil amended with biochar 20 days before transplanting. Bars represent the standard error of each mean. Different letters represent significant differences among the three treatments following Duncan's test ($p < 0.05$)

Discussion

This study confirmed that biochar amendment effectively suppressed Phytophthora blight of pepper, supporting the ability of biochar to protect plants against soil-borne disease observed in previous studies (Frenkel et al. 2017; Graber et al. 2014). A positive correlation between the abundance of *P. capsici* and the severity of Phytophthora blight of pepper was observed during the entire growing period. Thus, the biochar-mediated control of Phytophthora blight is linked to the suppression of *P. capsici* colonization in the rhizosphere.

The impact of application time on the biochar-mediated control effect was investigated for the first time. Application of biochar 0 days in advance of pepper seedling planting (BC0) presented a stronger control effect than application of biochar 20 days in advance (BC20). Their difference in the control effect may be caused by differences in the suppression of *P. capsici* colonization. The influence of soil bacterial properties on soil health and pathogen colonization is particularly important (Chaparro et al. 2012; Gómez Expósito et al. 2017). Therefore, we assessed the responses of the soil bacterial properties to BC0 and BC20 and explored their relationship with suppression of *P. capsici* and disease severity.

Biochar amendment led to a proliferative effect on bacterial abundance confirming previous studies (Abujabhah et al. 2018; Prayogo et al. 2014; Teutscherova et al. 2017; Yao et al. 2017). The proliferative effect gradually weakened to some extent with extended planting time; however, over the entire growing period, bacterial abundance in biochar treatments remained higher than that in the control. Thus, biochar-mediated control of Phytophthora blight may be partly dependent on its improvement of bacterial abundance.

Our previous study showed that biochar amendment increased abundance of several culturable microorganisms, such as *Bacillus* spp., *Pseudomonas* spp., and *Streptomyces* spp., in the rhizosphere of pepper (Wang et al. 2017). These microorganisms have been frequently reported to control disease caused by *Phytophthora* spp. (Sadeghi et al. 2017; Sang and Kim, 2014; Zhang et al. 2016b) due to their ability to promote plant growth, produce antagonistic compounds against pathogens, and induce plant disease resistance. In addition, denatured gradient gel electrophoresis profiles revealed that biochar at high application rates increased the abundance of *Sphingomonas* spp. (Wang et al. 2017) and several studies reported a certain facilitation effect of *Sphingomonas* for plant growth and disease suppression (Hrynkiewicz et al. 2009; Wachowska et al. 2013). To clearly understand the relationship between these four plant-beneficial bacteria and disease suppression under biochar amendment, a dynamic analysis of the abundance of these bacteria and pathogens in the rhizosphere during the entire growing period was conducted by qPCR. The results showed that biochar amendment remarkably increased the abundance of *Bacillus* spp., *Pseudomonas* spp., *Sphingomonas* spp., and *Streptomyces* spp., confirming the findings by Elmer and Pignatello (2011), Graber et al. (2010), Jaiswal et al. (2017 and 2018), and Zhang et al. (2016a). *Streptomyces* spp. and *Sphingomonas* spp. may have been enriched via biochar amendment as they can degrade the aromatic constituents of biochar (Ferradji et al. 2014; Fida et al. 2013). Correlation analysis showed that the *P. capsici* abundance and disease severity were negatively correlated with the proliferative effect for *Bacillus* spp. and *Streptomyces* spp. and, to some extent, were also correlated with the proliferative effect for *Pseudomonas* spp. and *Sphingomonas* spp. Thus, the biochar-mediated improvement in abundance of biocontrol bacteria contributed greatly to the ability of biochar to suppress *P. capsici* and disease severity. Although the abundance of

Table 4 Relationship of disease severity and pathogen abundance with the relative abundance of bacterial genera at 30 days after transplanting

	Bacillus	*Pseudomonas*	*Streptomyces*	*Mesorhizobium*	Gammaproteobacteria_unclassified	*Lysobacter*	*Nocardioides*	*Steroidobacter*	*Sphingomonas*
Disease index	−0.56	−0.24	−0.837**	−0.763*	−0.893**	−0.572	−0.891**	−0.706*	−0.131
P. capsici	−0.667*	−0.116	−0.765*	−0.637	−0.736*	−0.608	−0.788*	−0.667*	0.044

**Correlation is significant at the $p < 0.01$ level; *Correlation is significant at the $p < 0.05$ level

biocontrol bacteria in BC20 was significantly higher than that of BC0 before transplanting, the control effect of BC20 was noticeably lower than that of BC0. This may be associated with the short-term proliferative effect of biochar on biocontrol bacteria. The abundance of biocontrol bacteria in BC0 in the mid and late growing periods was higher than that in BC20.

Biochar amendment not only strongly stimulated the proliferation of antagonists of *P. capsici* in total bacteria, *Bacillus* spp., and *Pseudomonas* spp., but also increased their antagonistic ability. Soils associated with higher antagonist percentage and antagonistic ability are likely to have higher disease suppression (Alabouvette and Steinberg, 2006). In many cases, organic materials added to soil protected plants from soil-borne pathogens by enhancing antagonistic potential (Huang et al. 2006; Perez et al. 2008). In the present study, the Pearson correlation analysis clearly showed a positive correlation between antagonist percentage and antagonistic ability of bacteria and soil disease suppression. Thus, it is likely that bacterial antagonism enhanced by biochar amendment plays an important role in the suppression of *P. capsici* and Phytophthora blight of pepper. In addition, it was notable that a shorter biochar application time led to a more significant effect on antagonistic microorganisms. The fact that the control effect of BC0 was higher than that of BC20 was likely correlated with the antagonist percentage and antagonistic ability being higher in BC0 than in BC20. When biochar was added to the soil, the antagonist percentage of total bacteria increased markedly. Considering that *Bacillus* spp. and *Pseudomonas* spp. only account for a small portion of total bacteria, biochar must have also increased the proportion of other potential biocontrol bacteria. To clarify this, further studies are necessary.

As mentioned above, significant enrichments of *Bacillus* spp., *Pseudomonas* spp., *Streptomyces* spp., and *Sphingomonas* spp. were observed in biochar treatments. In order to ascertain whether these potential biocontrol bacteria contribute to the suppression of Phytophthora blight of pepper, biochar-enriched strains belonging to these bacterial groups were isolated and investigated for suppression ability against *P. capsici* and Phytophthora blight of pepper. Three *Bacillus*, three *Pseudomonas*, three *Streptomyces*, and two *Sphingomonas* strains were identified as biochar-enriched potential biocontrol bacteria. *Bacillus* and *Streptomyces* strains exerted inhibitory effects against *P. capsici* and Phytophthora blight of pepper, while the effects of *Pseudomonas* strains were slight, and *Sphingomonas* strains completely lacked such an effect. In addition, qPCR results showed a long-term growth-promoting effect of biochar on *Bacillus* and *Streptomyces* but a short-term growth-promoting effect on *Pseudomonas*. Therefore, the enrichment of *Bacillus* and *Streptomyces* in biochar-amended soil may contribute largely to the disease suppression ability of biochar. *Pseudomonas* may also be an important biocontrol bacteria that was enriched by biochar amendment.

Analysis of Illumina sequenced 16S rRNA genes was conducted to further investigate the effect of biochar amendment on the soil and to explore the correlation between the biochar-mediated bacterial community composition and disease suppression. Biochar amendment either had no effect on bacterial richness and diversity or lowered the bacterial richness indices ACE and Chao1 to some extent. This result contrasts with other studies by Jaiswal et al. (2017) and Zhang et al. (2016a), which indicated that the suppression of soil-borne diseases under biochar amendment was correlated with improved bacterial richness and diversity. However, PCoA results showed that the bacterial communities in biochar-amended soils grouped away from those in biochar-unamended soils, which was in agreement with other studies showing that biochar amendment altered the bacterial community composition (Jaiswal et al. 2017; Kolton et al. 2016; Yao et al. 2017; Zhang et al. 2016a). Moreover, different application times of biochar can alter the bacterial community composition to different degrees.

The relative abundance of several important bacterial genera was markedly altered by biochar amendment. Consistent with qPCR results, high-throughput sequencing results demonstrated that biochar amendment significantly facilitated the growth of *Bacillus* spp., *Pseudomonas* spp., *Streptomyces* spp., and *Streptomyces* spp. In addition, biochar treatments showed higher relative abundance of *Mesorhizobium* spp., Gammaproteobacteria_unclassified, *Lysobacter* spp., *Nocardioides* spp., and *Steroidobacter* spp. Such biochar-induced enrichment in *Mesorhizobium* spp. (Graber et al. 2010; Zhang et al. 2016a) and *Steroidobacter* spp. (Zhang et al. 2016a) have also been reported elsewhere. *Mesorhizobium* is a common rhizobacterium that has been reported to promote plant growth (Brígido et al. 2017). *Lysobacter* has been reported as biocontrol agent (Islam et al. 2005). *Steroidobacter* spp. have a positive effect on plant development. *Nocardioides* spp. can degrade alkanes as well as polycyclic aromatic compounds in biochar (Galitskaya et al. 2016). Further correlation analysis showed that biochar-mediated disease suppression might be related to the enrichment of these genera. The proliferative effect of BC0 on these genera was higher than that of BC20, which might be a contributing factor for high disease suppression in BC0 treatment. Such bacterial genera may be considered in future research in controlling plant soil-borne disease by using biochar.

Conclusions

Based on this study, biochar-induced changes in bacterial properties, especially the increased abundance of biocontrol bacteria and antagonist percentage and antagonistic ability of bacteria, played a key role in the suppression of *P. capsici* and Phytophthora blight of pepper. However, biochar-mediated soil disease suppression does not depend on its effect on bacterial diversity. Biochar-enriched *Bacillus* spp., *Pseudomonas* spp., and *Streptomyces* spp. were the main biocontrol bacteria that have functional importance in suppressing the disease. The short-term biochar promotion of the abundance of biocontrol bacteria and bacterial antagonism against *P. capsici* may explain why the disease suppression by biochar weakened with the application time. Future research could focus on enhancing the control effect of Phytophthora blight of pepper by combined application of biochar and biochar-enriched biocontrol microorganisms.

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