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Bacillus subtilis SL-13 biochar formulation promotes pepper plant growth and soil improvement

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Abstract: The use of microbial fertilizers can help to avoid the harmful effects of traditional agricultural fertilizers and pesticides; however, there are many constraints on the practical application of such fertilizers. In this study, microbial biochar formulations (MBFs) were obtained by loading biochar, created from agricultural waste, with *Bacillus subtilis* SL-13. The effects of the MBF on pepper plant growth and soil fertility were studied in pot experiments. The MBF improved the soil texture and environment and favored plant growth. Compared with *B. subtilis* SL-13-only and biochar-only treatments, the MBF treatments exhibited a significant increase in pepper plant growth and physiological indices and a significant improvement in the physical–chemical properties and activities of several enzymes in the soil. Therefore, the present study demonstrated that MBFs not only retain the beneficial effect of biochar in improving the soil properties but also improve the performance of *B. subtilis* SL-13 in promoting plant growth.

Key words: biochar, *Bacillus*, carrier, environmentally friendly, soil.

Résumé : L'utilisation de fertilisants microbiens peut permettre d'éviter les effets nocifs des fertilisants et des pesticides agricoles traditionnels; toutefois, il existe plusieurs contraintes à l'application pratique de tels fertilisants. Dans cette étude, des formulations de biocharbon microbien (FBM) ont été obtenues en chargeant du biocharbon créé à partir de déchets agricoles avec *Bacillus subtilis* SL-13. Les effets des FBM sur la croissance de plants de poivrier et sur la fertilité du sol ont été étudiés lors d'expériences en pots. Les FBM amélioraient la texture et l'environnement du sol et favorisaient la croissance des plants. Comparativement aux traitements avec *B. subtilis* SL-13 seul ou le biocharbon seul, la croissance des plants de poivrier et les indices physiologiques augmentaient significativement, et les propriétés physicochimiques et l'activité de plusieurs enzymes du sol étaient significativement améliorées par les traitements aux FBM. Ainsi, l'étude actuelle a démontré que non seulement les FBM conservent l'effet bénéfique du biocharbon en améliorant les propriétés du sol, mais elles améliorent aussi la performance de *B. subtilis* SL-13 en favorisant la croissance végétale. [Traduit par la Rédaction]

Mots-clés : biocharbon, *Bacillus*, transporteur, respectueux de l'environnement, sol.

Introduction

The long-term application of chemical fertilizers and the low utilization of fertilizer by plants ultimately causes soil environmental degradation, including soil compaction, fertility reduction, and a decrease in biological activities. Deterioration of the soil environment decreases plant growth capacity and agricultural product yields. Fertilizer and pesticide residues in the soil also leach into the groundwater system, indirectly polluting drinking water and lakes (Mahboob et al. 2015). These negative effects of long-term chemical fertilizer application have led to the development and gradual promotion of alternative fertilizers, such as microbial fertilizers. Various types of microbial fertilizers have been developed. One such microbial fertilizer is plant-growth-promoting rhizobacteria (PGPR), which has been shown to be very effective (Wu et al. 2012).

The development of PGPR microbial fertilizers has been restricted by the difficulties in transporting them and by the strict storage conditions that are required (Okon and Labandera-Gonzalez 1994). Attempts to solve these problems have been made by loading PGPR onto

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biocompatible solids. For example, bacterial survival during transportation and storage was improved by adding the bacteria to microcapsules, peat soil, and vermiculite (Wu et al. 2014; Hale et al. 2015; Maheshwari et al. 2015).

Biochar is a common soil amendment used in agriculture because it effectively decreases the bulk density (BD), increases the water-retention (or water-holding) capacity (WHC), improves the cation exchange capacity (CEC) of the soil, and adsorbs heavy metals from the soil (Major et al. 2010). Biochar application can effectively improve the soil environment for crop cultivation. In addition, the larger pore structure of biochar reportedly provides a suitable environment for microorganisms, i.e., improves their survival and reduces predation risk (Lehmann et al. 2011; Hale et al. 2014; Tao et al. 2018). Hale et al. (2014) found that inoculating PGPR into different types of biochar effectively promoted the growth and development of cucumber plants, improved soil WHC, and decreased the soil BD. Wang et al. (2017) used the biochar made from corn stalks and pig manure as a *Bacillus subtilis* B38 carrier for remediating soil heavy metal contamination. Soil treatments using *B. subtilis*-inoculated biochar increased the yields of crops grown in contaminated soil and decreased the heavy metal contents of the crops (Wang et al. 2017).

The *B. subtilis* SL-13 used in the study by Liu et al. (2011) was obtained from cotton field soil in Xinjiang Province, China, and was shown to promote chitinase activity and increase sprouting and seedling growth in tomato plants. In addition, *B. subtilis* SL-13 was also shown to be a suitable biological control agent and promotor of plant growth in relatively unstable soils; with broad application prospects (Liu et al. 2011). In previous studies, biochar has been shown to promote bacterial growth during cultivation and improve the survival rates of *B. subtilis* SL-13 (Tao et al. 2018).

In the present study, we investigated if biochar could serve as a suitable carrier for PGPR and if the performance and growth of plants could be improved by the application of *B. subtilis* SL-13-inoculated biochar. Soil samples were treated with three kinds of applications: (*i*) only *B. subtilis* SL-13, (*ii*) uninoculated biochar, and (*iii*) biochar loaded with *B. subtilis* SL-13, to determine the effect of microbial biochar formulation (MBF) on the performance of pepper plants and on soil properties.

Materials and methods

Bacterial strain and medium

Bacillus subtilis SL-13 (GenBank accession No. EF508705) strain used in the present study was obtained from tomato fields in Xinjiang Province. *Bacillus subtilis* SL-13 was cultivated in nutrient broth (50 mL), which contained 10 g/L tryptone (Aoxing Bio-Tech Ltd., Beijing, China), 5 g/L beef extract (Aoxing Bio-Tech Ltd., Beijing, China), and 5 g/L NaCl (Yongshen Ltd., Tianjin, China), at 30 °C and with shaking at 170 r/min for 36 h. The pH of the medium was maintained between 7.0 and 7.2. The cell suspension was used as *B. subtilis* SL-13 seed broth.

Table 1. Physicochemical properties of the original soil.

Property	Value
pH	8.35
EC (ms/cm)	0.16
OM (g/kg)	12.8
Avl. N (mg/kg)	58.64
Avl. P (mg/kg)	4.15

Note: EC, electrical conductivity; OM, organic matter; Avl. N, available nitrogen; Avl. P, available phosphorus.

A 2% mixture of the seed broth in 50 mL of nutrient broth was cultivated at 30 °C in a flask shaken at 170 r/min for 24 h. The mixture was centrifuged at 6000 r/min (4024*g*) at 4 °C for 30 min. The cells were then washed twice with sterilized 0.85% NaCl solution and suspended in 10 mL of sterile 0.85% NaCl solution. The viable cell count of the cell suspension was determined using the dilution plating method, i.e., the suspension was plated onto nutrient agar (NA) plates and the colony-forming units (CFU) were counted. The viable cell counts of the bacterial suspensions ranged from 1×10^8 to 1×10^9 CFU/mL. *Bacillus subtilis* SL-13 suspensions were added to the biochar.

Preparation of biochar from cotton straw

Pulverized cotton stalks were placed in cuboid porcelain bowls (60 mm × 30 mm × 15 mm) and gradually pyrolyzed in a N_2 gas atmosphere for 2 h in a tube furnace. One batch of cotton stalks was pyrolyzed at 400 °C and another at 600 °C, and the products were labeled as BC400 and BC600, respectively.

Preparation of MBFs

Biochar (2 g, 160 to 180 mesh) and cell suspension (10 mL) in 0.85% NaCl solution were mixed in a 50 mL conical flask, incubated at 30 °C, and shaken at 130 r/min for 24 h. The optimal conditions for the adsorption of *B. subtilis* SL-13 on biochar were determined in our previous study (Tao et al. 2018).

Pot experiments

Pot experiments were performed to assess the differences in the effects of *B. subtilis* SL-13 (only), biochar (only), and the MBF applications on pepper plant growth. Polyvinyl chloride pots were filled with 250 g of field soil. The physicochemical properties of the original soil are shown in Table 1. The potted soils were treated with different formulations (see Table 2). The *B. subtilis* SL-13 suspension was mixed with the soil at a ratio of 1:10 (*v*/*m*), and the biochar or MBF additions were thoroughly mixed with the soil at a ratio of 2% (*m*/*m*). The densities of *B. subtilis* SL-13 in the suspension mixture and in the inoculated BC400 and BC600 applications were 9.12 $\log_{10}$ CFU/mL,

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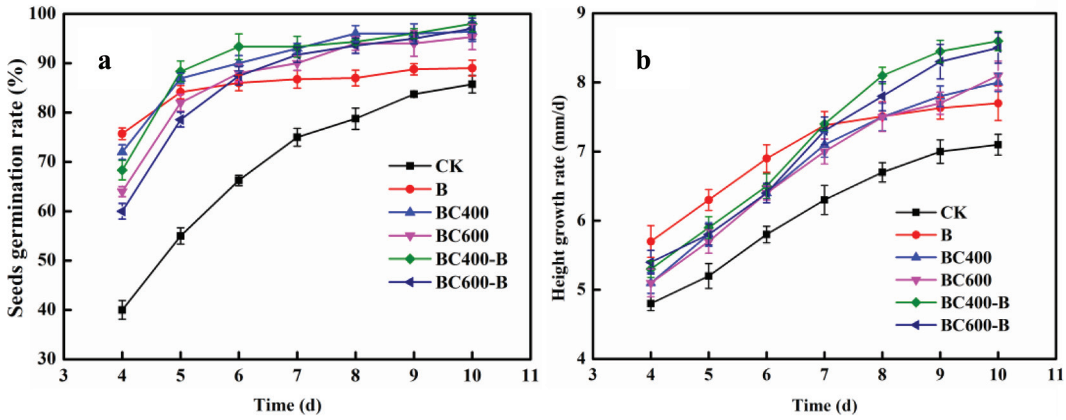

Fig. 1. Germination rates of pepper seeds (*a*) and height growth rates (*b*) of pepper plants in the different treatment groups between 4 and 10 days after sowing. CK, control check; B, *Bacillus subtilis* SL-13; BC400, biochar prepared at 400 °C; BC600, biochar prepared at 600 °C; BC400-B, bacterial BC400 formulation; BC600-B, bacterial BC600 formulation. [Colour online.]

Table 2. The treatments prepared for the pot experiment.

Treatment	Description
CK	Unamended soil
B	Soil + bacterial suspension of *Bacillus subtilis* SL-13
BC400	Soil + BC400
BC600	Soil + BC400
BC400-B	Soil + microbial BC400 formulation
BC600-B	Soil + microbial BC600 formulation

Note: BC400, biochar from cotton stalks pyrolyzed at 400 °C; BC600, biochar from cotton stalks pyrolyzed at 600 °C.

8.91 $\log_{10}$ CFU/g biochar, and 8.36 $\log_{10}$ CFU/g biochar, respectively.

Pepper seeds were surface sterilized with 2% NaClO for 10 min and then with ethanol (95%) for 5 min. Ten pepper seeds were added to each pot and five replicates of each treatment were performed. The pots were watered regularly to maintain the soil WHC at 70%. Seed germination was recorded daily between 4 and 10 days after sowing, and the seed germination rate (GR) was calculated as follows:

$$\mathrm{GR} = N/T \times 100\%$$

where *N* is the number of germinated seeds and *T* is the total number of seeds planted. The daily height growth rate (HGR) was also measured after 20, 30, and 40 days of germination, and the available N and P contents of the soil were determined after harvesting. The dry masses, fresh masses, root lengths, and stem lengths of the pepper seedlings were measured 40 days after sowing. Pepper leaf indices (leaf area, number of leaves, chlorophyll content, and soluble sugar content), soil BD, WHC, soil pH, organic matter content, CEC, and invertase, catalase, and urease activities were measured (Liu et al. 2017).

Statistical analysis

Statistical significances were determined using an analysis of variance (ANOVA) followed by the Tukey's test for multiple comparisons, with *P* values of <0.05 considered statistically significant. All statistical analyses were performed using the SPSS version 17.0 (Chicago, Illinois, USA) software package.

Results and discussion

Effect of the MBFs on the pepper seed GR and HGR

The pepper seed GRs in the different treatments over seven consecutive days are shown in Fig. 1. GRs were higher in the bacteria, biochar, and MBF treatments than in the unamended soil (control, CK). Plants treated with *B. subtilis* SL-13 only showed the highest GRs. This finding was consistent with that of a previous study, which showed that PGPRs (*Pseudomonas putida*, *Pseudomonas fluorescens*, and *B. subtilis*, etc.) promoted the GRs of barley, bean, canola, etc. (Zablotowicz et al. 1991). Plants in the CK group still had the lowest GRs after 4 days. The GR of plants in the *B. subtilis* SL-13-only treatment group gradually increased after 4 days, possibly because the number of bacteria in the soil gradually decreased. The GR of plants in the biochar-only treatments increased rapidly after 4 days. Compared with the GRs of plants in the CK group, those of plants in all biochar-addition treatments were higher and were significantly higher (14.2%) in the BC400 MBF treatment group. The HGR of pepper plants was higher in all treatment groups 4–10 days after sowing (Fig. 1*b*); plants in the *B. subtilis* SL-13 treatment group had faster HGRs than those of other treatment groups 4–7 days after sowing. Plants in the MBF treatment groups reached the fastest HGRs, which exceeded those of plants in the *B. subtilis* SL-13 treatment group after 7 days. This finding may have been attributed to the synergistic effects of changes in soil BD, WHC, and CEC in response to the biochar treatment and potential plant-growth-promoting activities of the inoculum. The bacteria in the biochar may have been more adaptable to the soil environment than the no-carrier bacteria, and the *B. subtilis* SL-13 in the biochar may have competed better with other microorganisms in the soil, i.e., they were

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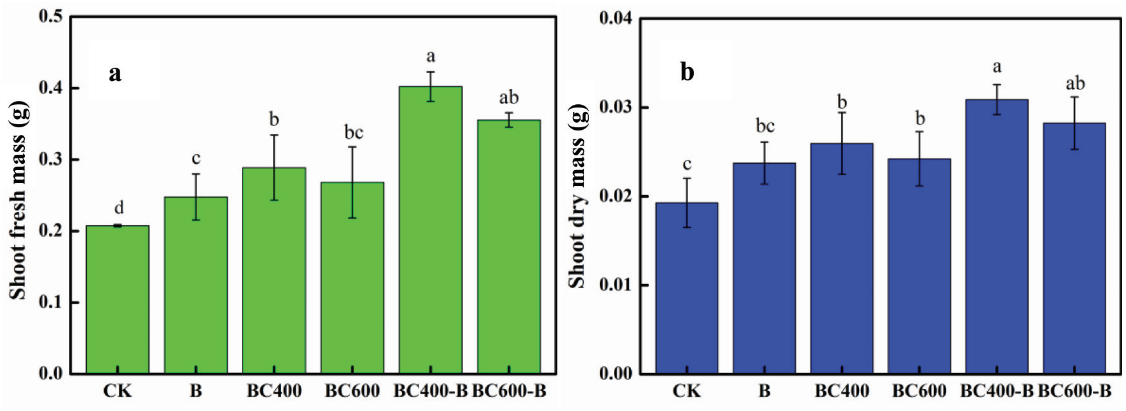
Fig. 2. Effects of the different treatments on the fresh (*a*) and dry (*b*) masses of pepper plant shoots 40 days after sowing. Different lowercase letters indicate significant differences ($P \leq 0.05$) between treatments.



able to survive longer in the soil due to the presence of biochar. Tripti et al. (2017) demonstrated similar effects on tomato seed germination after treatment with biochar loaded with *Bacillus* sp. A30 or *Burkholderia* sp. L2.

Effects of the MBFs on the pepper growth parameters

Development of the aerial parts of the pepper plants were measured after 40 days. The fresh and dry masses of the pepper stems reflected the effects of the different MBFs on pepper plant growth (Fig. 2). Pepper plants treated with biochar and MBFs had higher biomasses ($P < 0.05$) than those in the CK group, i.e., biochar and MBF treatment increased the biomass of the aerial plant parts. Silber et al. (2010) demonstrated that nutrient released from biochar increased the soil CEC, which lead to the improvement of soil indicators. Other studies showed that biochar addition to soil increased the stem and root biomass of pines and birches (Wardle et al. 1998; Vaccari et al. 2011), and increased wheat biomass and yield by 30%. In the present study, the fresh pepper plant masses were significantly higher in the MBF-treated (both BC400-B and BC600-B) plants ($P < 0.05$) than in those in the other treatments. The dry masses of pepper plants were 30.07% and 19.0% higher in the BC400-B and BC600-B MBF treatments, respectively, than in the *B. subtilis* SL-13 treatment (treatment B). These findings confirm those of previous studies that have shown that biochar application promotes plant growth by increasing soil WHC and reducing soil BD and by increasing the population densities of plant-growth-promoting bacteria by serving as an inoculum carrier (Hale et al. 2014). The increased plant biomass may also be attributed to small amounts of nutrients released to the soil from the biochar. Silber et al. (2010) demonstrated a positive correlation between the changes in soil properties after biochar addition and plant growth.

Interestingly, the fresh and dry masses of the pepper stems were significantly higher in the *B. subtilis* SL-13-treated (treatment B) plants than in the control (treatment CK) plants. This may have been because *B. subtilis* produces various phytohormones (indoleacetic acid, abscisic acid, organic acid, gibberellins, and cytokinins, etc.) that induce pepper stem growth (Tu et al. 2016).

Effects of different treatments on pepper leaf growth

The leaf is the main organ for photosynthesis and respiration, thus improved leaf growth is extremely important for higher crop yields. The effects of the different treatments on the pepper seedling leaves are shown in Fig. 3. Leaf area was significantly higher in the plants in the biochar and MBF treatment groups than the CK group. Leaf area of the pepper seedlings was also slightly higher in the *B. subtilis* SL-13 treatment group than in the CK group. Leaf area in plants in the BC400 and BC600 treatment groups were 33.36% and 22.31% higher than the CK group, respectively. The maximum leaf area percentages in the BC400-B and BC600-B MBF treatment groups and were 112.21% and 85.36% significantly higher, respectively, than those in the CK group. Eventually, the leaf area was higher in the BC400-B and BC600-B treatment groups (by 85.38% and 55.33%, respectively) than in the *B. subtilis* SL-13 treatment group. In a previous study, biochar treatment was found to increase the leaf area and leaf length of pepper and tomato plants. Previously, the influence of biochar on plant growth was associated with changes in soil WHC and BD, and the potential residues on biochar that may have plant stimulatory effects (Graber et al. 2010). In the present study, leaf area and the number of leaves increased to a larger degree in pepper plants grown in soil that was treated with MBFs than in any of the other treatments.

Effects of different treatments on pepper plant height and root length

The heights of the pepper seedlings were measured 20, 30, and 40 days after sowing, and directly reflected the growth of the plants in the different treatments (Fig. 4*a*). Shoot lengths were slightly (but not significantly) higher in plants in the *B. subtilis* SL-13, biochar, and MBF treatment groups than in the CK group. Shoot lengths were most clearly higher in the biochar and MBF treatment groups than in the CK group after 20 days germination,

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Fig. 3. Effects of the different treatments on the leaf area (*a*) and number of pepper leaves (*b*). Different lowercase letters indicate significant differences ($P \leq 0.05$) between treatments.

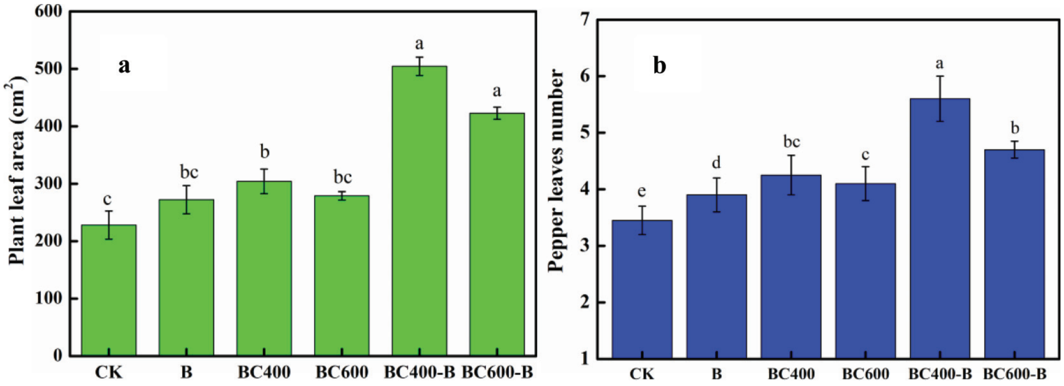
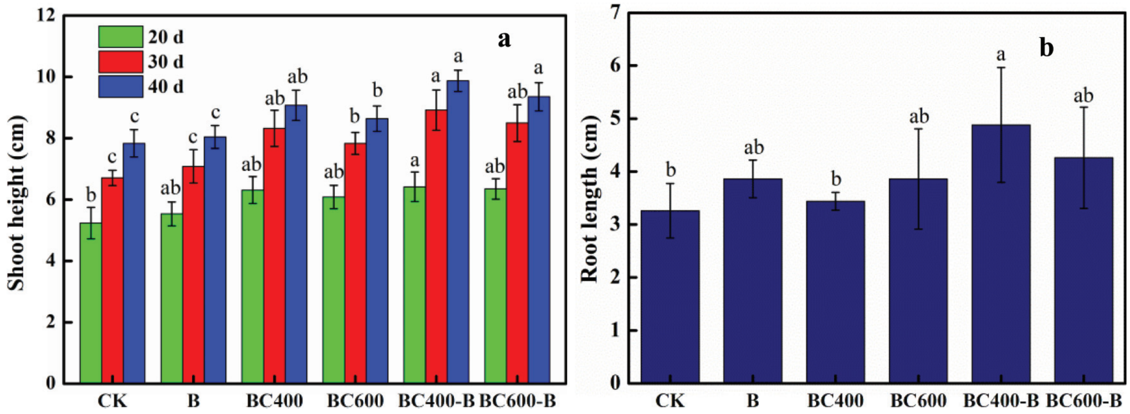


Fig. 4. Effects of the different treatments on the shoot height (*a*) and root length (*b*) of the pepper plants. Different lowercase letters indicate significant differences ($P \leq 0.05$) between treatments on the same sampling day. [Colour online.]



and the highest pepper shoot lengths were found in plants treated with MBF. Shoots were 22.74% and 8.81% longer in the BC400-B treatment group than in the *B. subtilis* SL-13 and BC400 treatment groups, respectively. Similarly, the shoots in the BC600-B treatment group were 16.27% and 8.26% longer than in the *B. subtilis* SL-13 and BC600 treatment groups, respectively. These results indicated the benefit of MBF applications as a result of the synergistic effects of biochar and *B. subtilis* SL-13. The dual amendments also had greater effects during the late stages of plant growth, which may be explained by previously reported findings (Hale et al. 2015), i.e., that the higher porosity and WHC of biochar were important promotors of microbial survival and plant growth.

Biochar has different physical and chemical properties due to it being composed of different raw materials. The pH value of cotton straw biochar increases with the increase in pyrolysis temperature, thus the pH value of BC600 was higher than BC400. In this study, the higher soil improvement effect of BC400 than BC600 may be attributable to the fact that the initial soil was alkaline. Moreover, in our previous study, we found that the C/N of BC600 was higher than that of BC400 (Tao et al. 2018). A high C/N ratio leads to N limitation in the decomposition of organic compounds, which results in low initial decomposition rates of hemicellulose and cellulose, and thus a lower microbial biomass in MBF in BC600 (Jindo et al. 2012).

The effects of the different treatments on the length of pepper plant roots are shown in Fig. 4*b*. Root growth was slightly higher in the BC400 and BC600 treatment groups than in the CK group. Plants in the BC400-B MBF treatment group had the longest roots, which were 26% longer than those in the CK group. Because biochar decreases soil BD, plants are more easily able to form roots in biochar-treated soils.

The effects of the different treatments on pepper plant growth are shown in Fig. 5. Pepper plant growth was higher in the MBF treatment groups than in the other treatment groups. Pepper seedlings exhibited strong morphologies in the *B. subtilis* SL-13, biochar, and the MBF treatment groups, and were more robust after MBF treatments than after any of the other treatments. The leaves and stems in the MBF treatment groups were thriving. These findings indicated that biochar and *B. subtilis* SL-13 had clear synergistic effects on pepper plant growth.

Effects of different treatments on the chlorophyll contents of pepper leaves

Chlorophyll is the main pigment in plants that is involved in photosynthesis. Chlorophyll content is a strong

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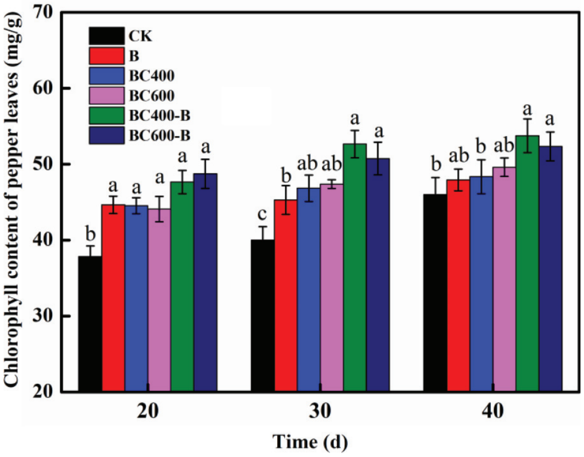
Fig. 5. Photographs of plants grown in untreated soil (CK) (*a*), soil with *Bacillus subtilis* SL-13 (*b*), soil with biochar (*c*), soil with microbial biochar formulations (*d*). [Colour online.]

indicator of the photosynthetic capacity and health status of plants (Van der Mescht et al. 1999). The chlorophyll content in the leaves of pepper plants from the different treatments are shown in Fig. 6. Chlorophyll contents were higher in the *B. subtilis* SL-13, biochar, and MBF treatment groups than in the CK group 20 days after sowing. After 30 days, the chlorophyll contents in the biochar and MBF treatment groups were higher than in the *B. subtilis* SL-13 treatment group. Eventually, the chlorophyll contents were higher in the MBF treatment groups than in any of the other treatment groups, with the contents being 16.8% and 13.7% higher in the BC400-B and BC600-B MBF treatment groups than in the CK group, respectively. These findings can be explained by the fact that photosynthesis is mainly regulated by the stimulation of endogenous signals and the environment, e.g., *B. subtilis* SL-13 may regulate signal transduction pathways that are related to photosynthesis, and biochar alters the soil environment, which results in plant growth stimulation (Zhang et al. 2008).

Fig. 6. Effects of the different treatments on the chlorophyll contents of the pepper leaves. Different lowercase letters indicate significant differences ($P \leq 0.05$) between treatments on the same sampling day. [Colour online.]



Effects of the different treatments on the soluble sugar contents of the pepper plants

Soluble sugar provides energy and metabolic intermediates for plant growth and development but is also an important plant hormone regulator. The soluble sugar contents in the pepper plants of each treatment, 40 days after sowing, are shown in Fig. 7. The soluble sugar contents were higher in the BC400 and BC600 treatment groups than the CK group. The soluble sugar contents of the BC400-B and BC600-B MBF treatment plants were 40.49% and 39.02% higher than the CK plants, respectively. This could be explained by results previously reported that soluble sugar content can affect plant resistance and biochar and increase plant tolerance (Feng et al. 2002; Elad et al. 2011); this may be due to an increase in the soluble sugar content of plants growth in improvement soil of biochar. In a previous study by Waqas et al. (2017), a combination of biochar and plant endophytes were applied to the soil of soybean plants, which resulted in an increase in the nutrient uptake, and thus an increase in the accumulation of nutrients, such as sugar and amino acids, in the soybean plants. In our study, pepper plant growth was higher in response to treatment with the MBFs (i.e., a mixture of biochar and *B. subtilis* SL-13) than treatment with either biochar or *B. subtilis* SL-13 only.

Effects of the treatments on the physical and chemical properties of the soil

The physical and chemical properties of the soil used in the different treatments are shown in Table 3. Biochar

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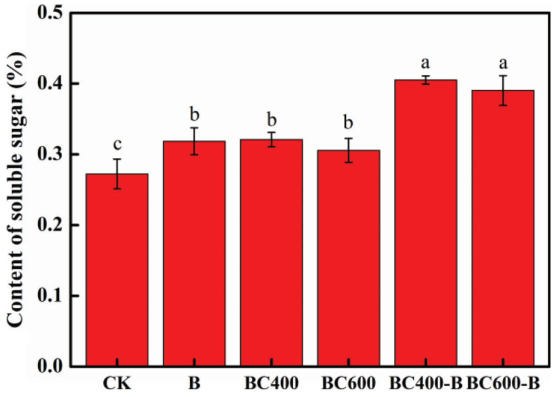
Table 3. Effects of different treatments on the physical and chemical properties of soil.

Treatment	BD (g/cm^3)	WHC (%)	pH	OM (g/kg)	CEC (cmol/kg)
CK	1.46±0.018a	26.84±3.71b	8.35±0.12a	12.80±1.34b	16.57±0.53b
B	1.42±0.032a	24.75±4.21b	7.93±0.14b	14.31±0.58b	15.68±1.16b
BC400	1.36±0.026b	29.53±2.49a	8.26±0.38a	17.36±1.62a	18.14±0.74a
BC600	1.34±0.021b	30.94±2.87a	8.32±0.24a	17.29±0.94a	18.63±0.96a
BC400-B	1.31±0.051b	31.61±1.04a	8.01±0.22b	18.46±1.18a	17.81±1.21a
BC600-B	1.30±0.043b	30.29±2.15a	8.18±0.15b	17.49±1.54a	18.26±1.07a

Note: BD, bulk density; WHC, water-holding capacity; OM, organic matter; CEC, cation exchange capacity. Different lowercase letters indicate significant differences ($P \leq 0.05$) between treatments.

Fig. 7. Effects of the different treatments on the soluble sugar content of the pepper plants. Different lowercase letters indicate significant differences ($P \leq 0.05$) between treatments.



addition clearly reduced the soil BD compared with that of the CK soil, which is consistent with numerous reports in the literature. Soil BDs in the BC400, BC600, BC400-B MBF, and BC600-B MBF treatment groups were 6.85%, 8.22%, 10.27%, and 10.96% lower than in the CK soil, respectively. The WHC increased after the addition of biochar, and the highest WHC (17.77% higher than the CK soil) was found in the BC400-B MBF treatment. In addition to increasing the soil porosity, promoting microorganism activity, reducing soil aggregation, and decreasing the soil BD, biochar also has a high specific surface area and water adsorption capacity (Brussaard et al. 2007) and can thus also increase soil WHC when applied. The findings of the present study confirmed this notion.

The addition of the *B. subtilis* SL-13 suspension decreased the soil pH (Table 3). This may have been attributed to humic acid produced by microbial activity and metabolism. Because biochar contains organic matter, biochar treatments increase the soil organic matter content, an indicator of soil fertility (Prayogo et al. 2014). The decomposition of organic matter in soil produces organic acids and carbon dioxide and, thus, may explain the observed decrease in soil pH in the biochar and MBF treatments.

Modifying the CEC of soil is an important motivation for soil amendment. The CEC of the soil in the BC600 treatment was 12.49% higher than that of the CK soil (Table 3), while the addition of the *B. subtilis* SL-13 suspension did not increase the soil CEC. Because biochar has a high CEC, biochar addition directly increases the soil CEC. The findings of the present study confirmed that biochar is an excellent soil modifier and that its application improves soil texture. Abujabhah et al. (2016) also found that the addition of biochar improved the soil physical and chemical properties, regulated soil fertility, and increased crop yields.

Effects of the treatments on the available N and P contents of the soil

Both N and P are essential elements that are involved in plant metabolism. Available N and P can be absorbed directly through plant roots. Available N includes ammonium nitrogen, nitrate nitrogen, amino acids, amides, and easily decomposed protein nitrogen. Available P includes water-soluble phosphorus, soluble weak acid phosphorus, and colloid-adsorbed phosphorus (Bosatta and Ågren 1996). In the present study, the total amount of available N and P in the soil decreased as the plants grew. The available N and P contents of the soil in the different treatments are shown in Fig. 8. A slight difference in the available N content was observed in the different treatment groups 20 days after sowing. However, the available N content of soils in the biochar treatments was higher than in the *B. subtilis* SL-13 treatment 40 days after sowing. These findings may be attributed to the fact that biochar reduces the leaching of NH_4^+ and nitrate (Lehmann et al. 2003). The available P content was slightly higher in the soils treated with biochar and MBF than in the CK soil and *B. subtilis* SL-13 treatment soil, which may be attributed to the fact that biochar can release soluble P into the soil environment (Wang et al. 2014). Biochar also has a high nutrient-holding capacity, i.e., nutrients are not easily lost from biochar-treated soil (Elad et al. 2012).

Effects of the treatments on enzyme activities in the soil

The activities of most enzymes in the soil environment are related to the nutrient content, microorganism content, and respiration intensity of the soil. In general, enzyme activity increases as soil fertility increases. Soil enzyme activities can be characterized by the intensity of the biological activity in the soil and can be used as an index for soil fertility (Rietz and Haynes 2003). The invertase, catalase, and urease activities in the soil in the different treatments are shown in Fig. 9. The invertase and

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Fig. 8. Effects of the different treatments on the available N (*a*) and available P (*b*) contents of the soil. Different lowercase letters indicate significant differences ($P \leq 0.05$) between treatments on the same sampling day. [Colour online.]

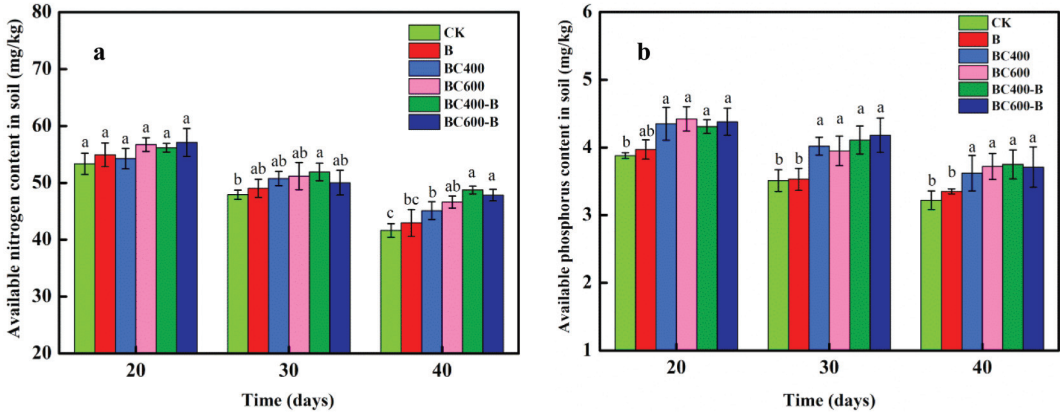
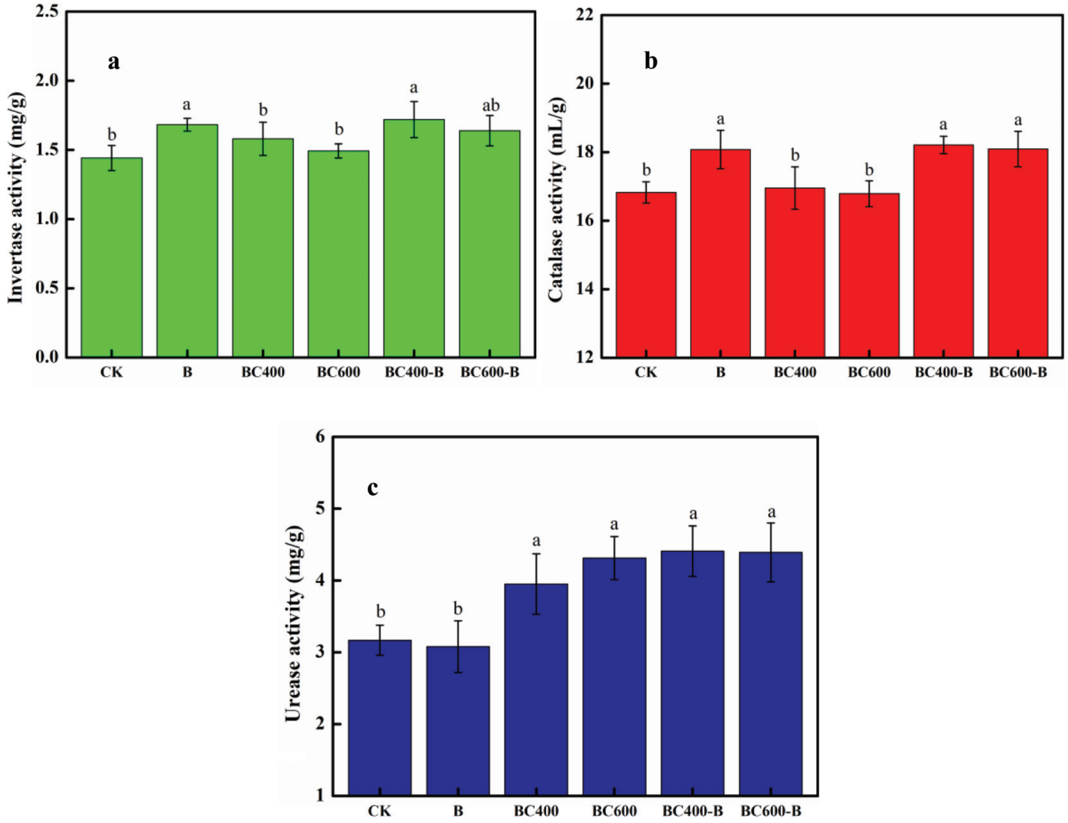


Fig. 9. Invertase (*a*), catalase (*b*), and urease (*c*) activities in the soils of the different treatments. Different lowercase letters indicate significant differences ($P \leq 0.05$) between treatments on the same sampling day.



catalase activities were higher in the *B. subtilis* SL-13 and MBF treatment groups than in the CK group. This may be because the increased amount of bacteria in the soil improved the bacterial metabolic activity and directly improved the invertase and catalase activities in the soil (Sahin et al. 2011). In the present study, the addition of biochar (only) did not markedly increase the invertase and catalase activities; however, the urease activity was improved by the addition of biochar and the MBFs. Increased urease activity has been shown to improve plant utilization of fertilizers (Liang et al. 2003). The addition of bacteria and biochar was found to improve the activities of some enzymes in soil, which resulted in improved pepper plant growth. We concluded that biochar and PGPR addition improved the enzyme activities to a greater extent than the addition of either biochar or PGPR alone because of synergistic effects.

Conclusions

Biochar loaded with *B. subtilis* SL-13 effectively increased the GR of pepper seeds, fresh and dry masses of pepper plants, chlorophyll contents, and sugar accumu-

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lation in pepper leaves. The MBFs also decreased the soil BD, and increased the WHC, organic matter content, CEC, and available N and P contents compared with the CK soil or soil with only *B. subtilis* SL-13 added. The addition of bacteria and biochar improved the activities of some enzymes in the soil. Biochar-fertilized soil was found to provide a more suitable environment for plant cultivation than soil without biochar treatment. In conclusion, the MBFs improved the soil and promoted plant growth and, therefore, can be considered as environmentally safe formulations for agricultural application. In addition, the use of MBFs can decrease the usage of chemical fertilizers and pesticides. The results of this study highlight the effectiveness of MBFs and support the use of such formulations in future agricultural endeavors.

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