

Research Article

Effects of Biochar Amendment on Tomato Bacterial Wilt Resistance and Soil Microbial Amount and Activity

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Received 11 April 2016; Accepted 3 August 2016

Academic Editor: Allen Barker

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Bacterial wilt is a serious soilborne disease of Solanaceae crops which is caused by *Ralstonia solanacearum*. The important role of biochar in enhancing disease resistance in plants has been verified; however, the underlying mechanism remains not fully understood. In this study, two different biochars, made from peanut shell (BC1) and wheat straw (BC2), were added to *Ralstonia solanacearum*-infected soil to explore the interrelation among biochar, tomato bacterial wilt, and soil microbial properties. The results showed that both BC1 and BC2 treatments significantly reduced the disease index of bacterial wilt by 28.6% and 65.7%, respectively. The populations of *R. solanacearum* in soil were also significantly decreased by biochar application. *Ralstonia solanacearum* infection significantly reduced the densities of soil bacteria and actinomycetes and increased the ratio of soil fungi/bacteria in the soil. By contrast, BC1 and BC2 addition to pathogen-infected soil significantly increased the densities of soil bacteria and actinomycetes but decreased the density of fungi and the ratios of soil fungi/bacteria and fungi/actinomycetes. Biochar treatments also increased soil neutral phosphatase and urease activity. Furthermore, higher metabolic capabilities of microorganisms by biochar application were found at 96 and 144 h in Biolog EcoPlates. These results suggest that both peanut and wheat biochar amendments were effective in inhibiting tomato bacterial wilt caused by *R. solanacearum*. The results suggest a relationship between the disease resistance of the plants and the changes in soil microbial population densities and activity.

1. Introduction

Bacterial wilt is a serious soilborne disease caused by *Ralstonia solanacearum* [1]. This disease is difficult to control because the pathogen can survive within a large temperature range (10°C to 41°C) and in diverse environments [2]. *R. solanacearum* can induce persistent latent infection in nursery plants even at low populations in soil or irrigation water [3]. Traditional control methods, including host resistance, crop rotation, and chemical methods, may be limited or elicit negative effects on food safety and environment [4]. Therefore, effective and eco-friendly approaches should be developed to reduce this disease.

Biochar, a product of the thermal degradation of organic materials in the absence of air (pyrolysis), is distinguished from charcoal in terms of usage. In particular, as a soil

amendment, biochar can exhibit long-term carbon sequestration potential and reduce greenhouse gas emission and in soil [5]. Biochar can also improve soil tilth [6] and increase crop productivity and competitive ability [7, 8]. Biochar application can also enhance crop response to disease [9], and this enhancement can be attributed to an increase in soil pH [10], nutrient retention [6, 11], cation exchange capacity in soil [11], transformations and turnover of P and S [12], and neutralization of phytotoxic compounds in soil [13].

It is reported that biochar can increase plant resistance to disease. For instance, biochar can reduce fungal foliar diseases caused by *Botrytis cinerea* and *Oidiopsis sicula* in tomato (*Solanum lycopersicum* L.) and pepper (*Capsicum annuum* L.) [14]. Harel et al. suggested that strawberry defense responses mediated by biochars are functionally similar to induced systemic resistance [15]. Moreover, biochar can reduce

TABLE 1: Basic properties of biochars used in the experiment.

Type	Feedstock	Temperature (°C)	pH	C ^a (%)	N ^a (%)	Available P (mg kg ⁻¹)	Available K (mg kg ⁻¹)	Ash ^b (%)	C/N ratio (%)
BC1	Peanut shell	500 × 2 h	9.89	17.41	0.60	611.3	3872	70.70	29.02
BC2	Wheat straw	500 × 2 h	10.02	47.38	0.98	325.0	3820	47.60	48.35

^aMolar ratios, analyzed with an elemental analysis apparatus, vario TOC cube, Elementar Analysensysteme GmbH, Germany.

^bMass % w/w analyzed by dry combustion in a muffle furnace at 600°C for 2 h.

soilborne diseases caused by bacteria and fungi [16]. Nerome et al. found that bacterial wilt (*R. solanacearum*) in tomato was reduced by adding biochar derived from municipal biowaste [17].Suppressions of plant diseases by biochar were attributed to several mechanisms [18–20], including the following. The chemical components of biochar may provide direct inhibition of pathogens and the porous structure of biochar may provide microbial habitats which is good for bacterial abundance. Biochar can also promote plant growth by providing nutrients and improving nutrient solubilization and uptake. To some extent, biochar sorption may change the mobility and activity of pathogens or modify signaling between pathogens and plants [19].

Densities, activity, and diversity of soil microorganisms can be important indicators to evaluate soil health status and soil quality [21, 22]. Pathogen infection may change soil microbial properties; thus, soil can be converted from a highly fertile bacterial type to poorly fertile fungal type [23, 24]. Biochar amendments to soils may alter soil function and fertility in various ways, including through induced changes in the microbial community. Kelly et al. found a significant decline in the fungi/bacteria ratio in the Colorado soil with switchgrass biochar [25]. Considering that biochar is implicated in soilborne disease reduction, we hypothesized that disease resistance mediated by biochar may be related to changes in microbial properties. This study aimed to investigate the interrelation among biochar, bacterial wilt resistance, and soil microbial components and activity.

2. Materials and Methods

2.1. Plant Materials and Soil Conditions. Tomato cv. Taiwan Red cherry (tomato genotype; produced by Kefeng Seed Co., Ltd., Changchun, Jilin, China), which is susceptible to *R. solanacearum*, was used in this experiment. Tomato seeds were stored in a fridge at 4°C and steeped in water at room temperature for 2 h before use. The seeds were surface-sterilized in water at 50°C for 15 min and then germinated on moist filter paper in Petri dishes. After 2 d, the seeds were sown in nursery soil (tomato farm soil disinfected at 150°C for 4 h) in a growth chamber with the following conditions: 30°C/25°C (day/night), 14 h light, and 200 μmol·m⁻²·s⁻¹ light intensity. After five weeks, tomato plants were transplanted to a polyethylene plastic pot (170 mm in diameter, 165 mm in height) filled with 2 kg of soil, each pot with 2 plants. The plants were maintained at 28°C in a controlled greenhouse for 1.5 months until the end of the experiment.

The soil collected from a continuous cropping cultivation tomato field (Zhucun Village, Zengcheng City, Guangdong,

China) was sandy loam where the amount of bacterial wilt is relatively high; the proportions of sand, silt, and clay particles at 0–20 cm soil layer were 73%, 22%, and 5%, respectively. The contents of soil organic matter, soil-available N, P, and K, and soil-total N, P, and K were 16.30 g kg⁻¹, 115.47 mg kg⁻¹, 151.10 mg kg⁻¹, 82.54 mg kg⁻¹, 0.948 g kg⁻¹, 1.347 g kg⁻¹, and 29.2 g kg⁻¹, respectively. Soil pH was 5.89 and soil C/N ratio was 9.82. The soil samples were sieved at <2 mm and stored at 4°C until analysis to characterize relevant physical and biochemical attributes of the soil. Biochar was amended to soil before transplantation of tomato plants.

2.2. Biochar. Two kinds of biochar (Sanli New Energy Resources Co., Ltd., Shangqiu, Henan, China) were used in this experiment: biochar made from peanut shell (BC1) and wheat straw (BC2). Biochar was prepared via pyrolysis in a vertical kiln with a temperature at 500°C and a residence time of about 2 h in an anaerobic condition (Sanli New Energy Resources Co., Ltd.). The basic properties of the two biochar treatments are shown in Table 1. Scanning electron microscope images are shown in Figure 1.

2.3. Experimental Design. Four treatments were assessed in this experiment: no biochar and no *R. solanacearum* inoculation (CK); *R. solanacearum* inoculation (Rs); peanut shell biochar (BC1) amendment and *R. solanacearum* inoculation (BC1 + Rs); and wheat straw biochar (BC2) amendment and *R. solanacearum* inoculation (BC2 + Rs). The experiment was arranged in a completely randomized design with four replications; each replication had one pot with 2 tomato plants. Our preliminary experiment showed that 2% w/w of the two biochar amendments had the best effects in inhibiting bacterial wilt of tomato. Thus, 2% w/w ratio was used in this experiment. 2% w/w ratio of peanut and wheat biochar was used in this experiment. 28 d after *R. solanacearum* inoculation, when the plants of *R. solanacearum* treatment all died, soil from all treatment groups was collected to determine the amount of *R. solanacearum* in soil, soil microbial population densities (bacteria, fungi, and actinomycetes), and soil microbial activities (soil sucrase, urease, and neutral phosphatase). Biolog EcoPlate experiment was also conducted when the soil was collected.

2.4. *R. solanacearum* Inoculation. *R. solanacearum* strain biovar 3 (provided by College of Horticulture, South China Agricultural University, Guangzhou 510642, China) was used to inoculate tomato plants. This strain is a highly aggressive species. The isolate was cultured on 2,3,5-triphenyltetrazolium chloride (TTC) medium and incubated

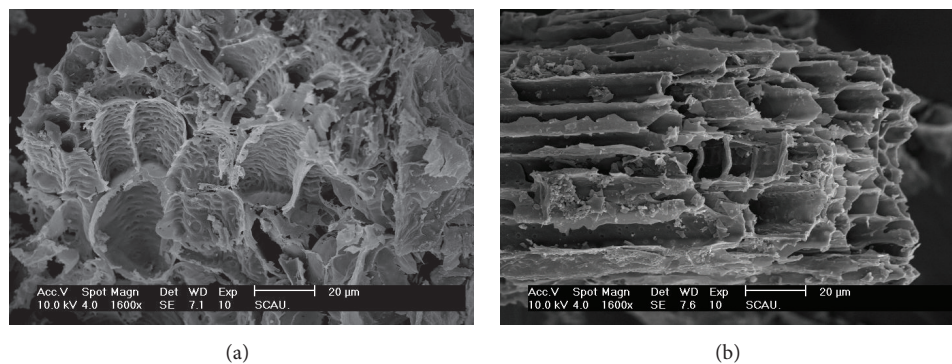


FIGURE 1: Scanning electron microscope images of two biochar used in this study. (a) Biochar made by peanut shell; (b) biochar made by wheat straw.

at 30°C for 48 h; cell density was adjusted to 10^8 CFU·mL⁻¹ before the isolate was inoculated. As the sixth tophyllia of the tomato plant appeared, the roots of each tomato plant were lightly stabbed and inoculated with *R. solanacearum* by pouring 10 mL of the bacterial suspension into each pot. CK plants were also stabbed, but the same volume of deionized water was added. After all of the plants of the Rs treatment died, the plants were harvested, and the soil was collected.

2.5. Pathogen Evaluation. Data collection was conducted at an interval of 2 d after pathogen infection by using a disease score [26] based on 10 plants per treatment. The investigation began when the tomato leaf exhibited symptoms of wilt. The following scoring method was used: 0: no symptom; 1: one leaf wilted; 3: two or three leaves wilted; 5: all except the top shooting leaves wilted; 7: all leaves wilted; and 9: stems collapsed or plants died.

Disease index was calculated using the following equation: disease index (%) = $[\sum(r \times Nr)/(R \times n)] \times 100\%$, where r is the rating value, Nr is the number of infected leaves with a rating of r , R is the value of the most serious disease severity, and n is the total number of tested plants [26].

2.6. Determination of the Amount of *R. solanacearum* in Soil. The amount of *R. solanacearum* in soil was determined according to the method described by Wang et al. [27] with minor modification. At 25 d after pathogen inoculation, 10 g of fresh soil was collected, added to flasks with 90 mL of sterile water, and diluted to 10^{-5} . The soil-suspending liquid was spread with TTC medium (bacterial general medium with $100 \mu\text{L}\cdot\text{mL}^{-1}$ 2,3,5-triphenyltetrazolium chloride) and then incubated for 2 d at 30°C in an incubator (GXZ Intelligent, Jiangnan Instrument Plant). Plate culture count was performed to record the amount of *R. solanacearum* in soil.

2.7. Determination of Soil Microbial Population Densities. The population densities of bacteria, fungi, and actinomycetes were determined using the dilution method described by Martin [28]. The media (Guangdong Huankai Microbial Sci. & Tech, Co., Ltd., China) for cultivating bacteria, fungi, and actinomycetes were nutrient agar, rose Bengal agar, and gauze's medium number 1, respectively. Fresh soil (10 g) was

added to a flask with 90 mL of sterile water and shaken in a shaker (TaiCang Experimental Factory & Suzhou Bing Lab Equipment Co., Ltd., Suzhou, China) for 30 min at 150 rpm. Afterward, 100 μL of supernatant fluid from each sample was extracted into a 2 mL sterile centrifuge tube with 900 μL of sterile water. Vortex Genius was used to mix the solution in the centrifuge tube. The solution was diluted to 10^{-5} , 10^{-3} , and 10^{-5} for bacterial, fungal, and actinomycetes analyses, respectively. The dilution was spread in the corresponding medium and then placed in an incubator (GXZ Intelligent, Jiangnan Instrument Plant) at 30°C. Bacteria, fungi, and actinomycetes were cultured for 2, 5, and 5 d, respectively. After these microorganisms were incubated, the number of colonies was recorded to determine the densities of different microbial populations.

2.8. Determination of Soil Enzyme Activity. Soil urease activity was determined using the method described by Yao and Huang [29]. Air-dried, finely sifted soil (5 g, passed through a 2 mm sieve) was placed in a 50 mL conical flask. Approximately 1 mL of toluene was added to the flask. After 15 min, 10 mL of 10% urea solution and 20 mL of citrate buffer (pH 6.7) were added. The flasks were immediately shaken and placed in an incubator (GXZ Intelligent; Jiangnan Instrument Plant) at $37 \pm 1^\circ\text{C}$ for 24 h. The sample was incubated and filtered using a filter paper. Soil urease activity was colorimetrically determined at 578 nm with a UV/Vis spectrophotometer (T90 UV/Vis spectrophotometer; PGeneral, Beijing, China) for 1 h.

Considering that soil pH was mostly neutral in this study, we determined soil neutral phosphatase enzyme activity; soil neutral phosphatase is the main phosphatase enzyme [30]. Neutral phosphatase was determined using a previously described chemical method [27], similar to urease measurement. Soil neutral phosphatase activity was colorimetrically determined at 660 nm with a UV/Vis spectrophotometer (T90 UV/Vis spectrophotometer; PGeneral, Beijing, China).

Soil sucrase activity was tested according to the method described by Ling and Zhang [31]. In brief, 2 g of air-dried soil (sieved through <1 mm), 15 mL of 8% glucose solution, 5 mL of 0.2 M phosphate buffer (pH 5.5), and 1 mL of toluene were added to a 50 mL conical flask. The solution was incubated

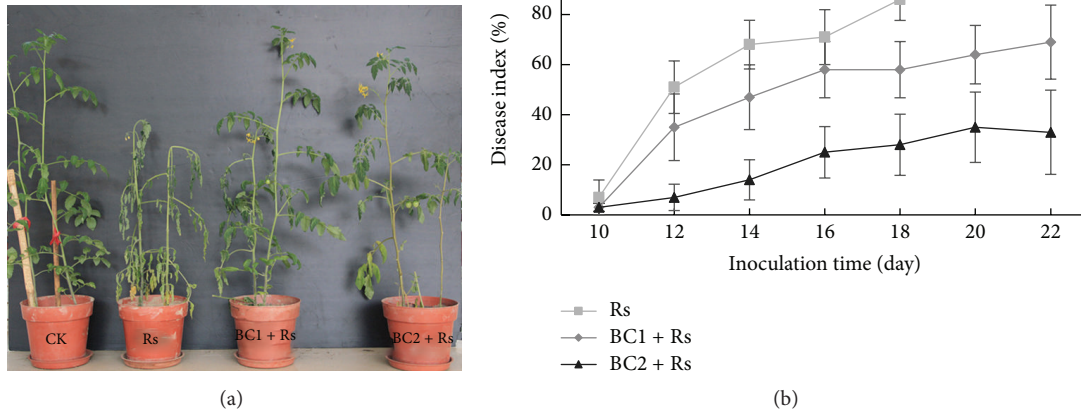


FIGURE 2: Suppressive effects of biochar amendment on *R. solanacearum* of tomato plant (a) and effects of biochar amendment and *R. solanacearum* inoculation on the disease index (%) of bacterial wilt in tomato plants (b). CK, no biochar and no *R. solanacearum* inoculation; Rs, *R. solanacearum* inoculation; BC1 + Rs, peanut biochar amendment and *R. solanacearum* inoculation; BC2 + Rs, wheat biochar (BC2) amendment and *R. solanacearum* inoculation.

for 24 h at $37 \pm 1^\circ\text{C}$ and filtered; afterward, 1 mL of aliquot was transferred to a test tube with 3 mL of 3,5-dinitrosalicylic acid (DNS) solution. The sample was heated in boiled water for 5 min, cooled in water for 3 min, and colorimetrically quantified at 508 nm by using a spectrophotometer (T90 UV/Vis spectrophotometer; PGeneral, Beijing, China).

2.9. Microbial Community Analysis with Biolog EcoPlates. The intensity and the diversity of bacterial metabolism were evaluated using Biolog EcoPlates [32]. Ten grams of fresh soil aliquots was added to 90 mL of sterile saline solution (0.85% w/v NaCl) and diluted to 10^{-3} with the same solution. Afterward, 150 μL of diluted solution was added to each well of Biolog EcoPlate (Biolog, CA) and incubated at 30°C for 168 h. Absorbance at 590 nm was recorded with Biolog MicroStation (Bio Tec Instruments, Inc., CA, USA) at an interval of 24 h; data were analyzed using average well color development (AWCD).

Biolog data obtained after 168 h of incubation in AWCD were subjected to Shannon diversity (H') analysis to determine microbial functional diversity. H' was calculated using the following equation [33]:

$$H' = -\sum_{i=1}^{31} (P_i \times \ln P_i), \quad (1)$$

$$P_i = \frac{(C_i - R)}{\sum_{i=1}^{31} (C_i - R)},$$

where C_i is the color production within each well and R is the absorbance value of the plate's control well.

2.10. Soil pH Analysis. As the soil was acidic soil from southern part of China, soil pH was determined with two different kinds of solutions (1M KCl for CK and Rs treatments; aqueous solution for BC1 and BC2 treatments). The ratio of soil and solution was 1:2.5. Briefly, 10 g aliquots of

air-dry soil from the four treatments were each added to 25 mL solution. The mix solution was shaken for 30 min at 150 rpm and then stood for 1 h. Subsequently, soil pH was measured, respectively, by PHS-3C PH Meter (Shanghai REX Instrument Factory, Shanghai, China).

2.11. Statistical Analysis. Data in the figures were expressed as mean \pm standard error of four replicates and analyzed by one-way ANOVA in SPSS17.0 (Statistical Analysis Systems Institute; SPSS Inc., Chicago, IL, USA). Statistical differences among treatments were determined by Duncan's test ($P < 0.05$). Graphs were constructed using SigmaPlot 12.5 (Systat Software, Inc., San Jose, CA, USA).

3. Results

3.1. Effects of Biochar Application on Disease Severity. The symptoms of bacterial wilt in *R. solanacearum*-inoculated treatment were observed at 4 d postinoculation (dpi). By contrast, these symptoms in biochar-amended treatment were observed only at 10 dpi, indicating that biochar amendment delayed pathogen development. Both biochar treatments significantly suppressed disease development and increased disease resistance of tomato plants (Figure 2). Compared with Rs treatment, BC1 + Rs treatment reduced the disease index of bacterial wilt by 60.00%, 30.61%, 32.26%, and 30.56% at 10, 14, 20, and 24 dpi, respectively. BC2 + Rs treatment reduced the disease index by 60.00%, 79.59%, 67.74%, and 66.67%, respectively. These results showed that BC2 could effectively reduce bacterial wilt to a greater extent than BC1.

3.2. Effects of Biochar Application on the Density of *R. solanacearum* in Soil. Compared with CK treatment, the density of *R. solanacearum* was markedly increased by 80.43% after pathogen inoculation. However, biochar treatments significantly decreased the density of *R. solanacearum* by

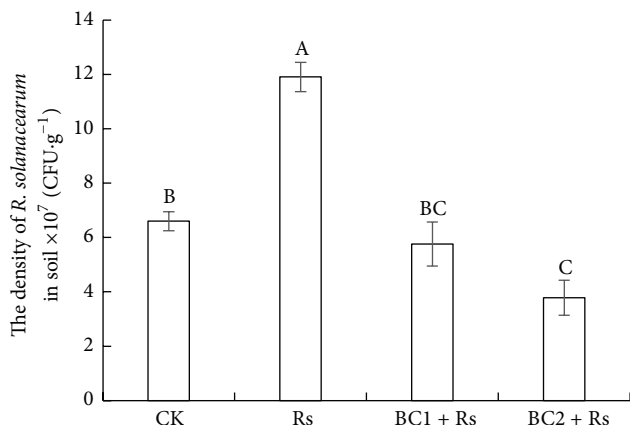


FIGURE 3: Effects of biochar amendment and *R. solanacearum* inoculation on *R. solanacearum* density in soil. CK, no biochar and no *R. solanacearum* inoculation; Rs, *R. solanacearum* inoculation; BC1 + Rs, peanut biochar amendment and *R. solanacearum* inoculation; BC2 + Rs, wheat biochar (BC2) amendment and *R. solanacearum* inoculation. Different letters on the columns denote a significant difference at $P < 0.05$ using the DMRT (Duncan's new multiple range tests) method.

51.63% (BC1) and 68.22% (BC2) compared with Rs treatment alone (Figure 3).

3.3. Effects of Biochar Application on Soil Microbial Population Densities. Biochar amendment and *R. solanacearum* inoculation significantly influenced the density of soil microbial population (Figure 4). Compared with CK treatment, *R. solanacearum* inoculation significantly decreased the amounts of soil bacteria and actinomycetes by 57.55% and 26.45%, respectively. By contrast, *R. solanacearum* inoculation increased the amount of fungi by 272.58%. However, biochar in BC1 + Rs and BC2 + Rs treatments significantly increased soil bacteria by 57.39% and 96.42% and soil actinomycetes by 42.47% and 63.33% and reduced soil fungi by 65.87% and 29.73%, respectively.

R. solanacearum inoculation significantly increased soil fungi/actinomycetes ratio and fungi/bacteria ratio by 418.44% and 735.14%, respectively. However, these ratios were decreased by 76.81% and 78.57% in BC1 + Rs and by 56.76% and 63.34% in BC2 + Rs treatments, respectively, compared with Rs treatment (Figure 5).

3.4. Effects of Biochar Application on Soil Enzyme Activity. Soil neutral phosphatase and urease activities were significantly affected by *R. solanacearum* inoculation (Figure 6). Compared with CK treatment, *R. solanacearum* infection reduced soil neutral phosphatase activity by 83.39% and soil urease activity by 4.52%. Biochar amendments increased soil enzyme activities. In particular, BC1 + Rs and BC2 + Rs treatments increased soil neutral phosphatase by 458.70% and 329.15%, respectively (Figure 6(a)); likewise, these treatments increased soil urease activity by 15.36% and 14.08%, respectively (Figure 6(b)). *R. solanacearum* inoculation did not significantly affect soil sucrase activity; however, biochar

amendments decreased soil sucrase activity (Figure 6(c)) by 29.94% (BC1 + Rs) and 27.97% (BC2 + Rs).

3.5. Effects of Biochar Application on Physiological Profiles at a Microbial Community Level. Biolog assay was originally developed to identify microbial isolates based on substrate utilization profiles. This assay is commonly performed to obtain substrate utilization profiles at a community level [32]. Color intensity was determined by calculating the AWCD of each plate. The AWCD values of different treatments at different stages are shown in Figure 7. Biochar treatments yielded higher AWCD than nonbiochar treatments, especially after 72 h; this result indicated that the tested metabolic capabilities of biochar treatments were higher than those of nonbiochar treatments. The physiological profiles of biochar treatments significantly differed at specific representative stages (96, 144, and 168 h after incubation).

We selected 144 h after cultivation as a specific time to identify differences in AWCD value of each treatment because 144 h is the logarithmic period of soil microbes. The results showed that Rs treatment decreased the AWCD value by 3.29% compared with CK treatment; by contrast, biochar treatments significantly increased AWCD by 15.21% (BC1 + Rs treatment) and 15.53% (BC2 + Rs treatment), compared with Rs treatment.

3.6. Effects of Biochar Application on Soil pH. Soil pH was significantly increased after biochar amendment regardless of pathogen inoculation (Figure 8). Pathogen inoculation decreased soil pH by 3.16% compared with CK treatments. Biochar amendment treatments increased soil pH by 27.53% (BC1) and 22.67% (BC2) compared with Rs treatment, respectively. The soil was increased to around neutral pH by biochar amendment (Figure 8).

4. Discussion

The beneficial role of biochar inducing plant diseases resistance has been investigated in several foliar and soilborne pathosystems [14, 15, 34]. In the present study, 2% w/w peanut and wheat biochar amendment delayed *R. solanacearum* development, significantly reduced the severity of disease incidence, and increased tomato plant resistance. The density of *R. solanacearum* in soil was also significantly decreased by biochar application. And also due to biochar amendment, soil pH got back to the neutral level which is good for bacteria growth.

The soil microbial population structure is critical to soil function and ecosystem services, which affect soil structure and stability, nutrient cycling, aeration, water use efficiency, disease resistance, and C storage capacity [35]. In previous reports, the increase in bacterial densities is associated with the enhanced resistance of amended soils against southern blight of processing tomatoes, *Phytophthora* root rot of alfalfa, and potato scab [36, 37]. Beneficial microbiota can compete with pathogens for space and nutrients or produce microbial agents, thereby improving plant health [38]. Our results showed that the density of soil bacteria and actinomycetes

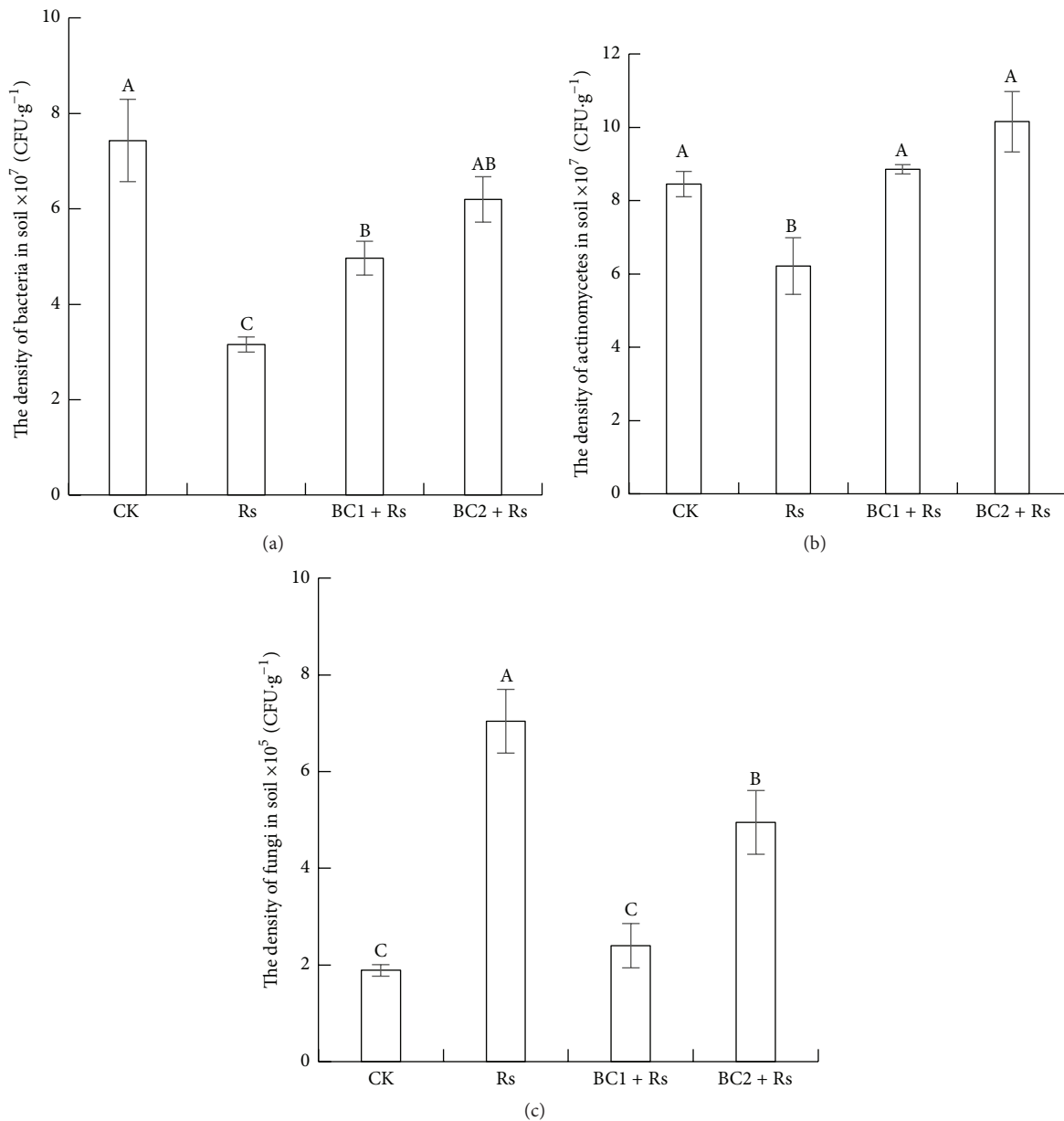


FIGURE 4: Effects of biochar amendment and *R. solanacearum* on the amount of soil bacteria (a), actinomycetes (b), and fungi (c). CK, no biochar and no *R. solanacearum* inoculation; Rs, *R. solanacearum* inoculation; BC1 + Rs, peanut biochar amendment and *R. solanacearum* inoculation; BC2 + Rs, wheat biochar (BC2) amendment and *R. solanacearum* inoculation. Different letters on the columns denote a significant difference at $P < 0.05$ using the DMRT (Duncan's new multiple range tests) method.

was significantly decreased after *R. solanacearum* was inoculated; by contrast, the amount of soil fungi increased. Thus, soil was converted from “bacterial type” to “fungal type,” and this result is similar to that of Larkin [23]. Interestingly, biochar addition to *R. solanacearum*-infected soil could increase the amount of soil bacteria and actinomycetes; conversely, biochar addition could decrease the amount of soil fungi, soil fungi/bacteria ratio, and fungi/actinomycetes ratio to reverse the change in soil microorganism composition that resulted from pathogen infection. And this finding is similar to that in a previous study in which silicon supply resulted

in the change of soil microbial components under pathogen inoculation [27].

Our results also showed that higher metabolic capabilities were found in the two biochar amendment treatments at 96 and 144 h (two sensitive stages during cultivation), indicating that biochar addition resulted in high substrate utilization capability of microorganism. What is more, a good relationship is observed between soil fertility and soil microorganisms. In plants, soil microbes interact to mediate and influence various exchanges that contribute to plant growth and productivity [21]. The extent of these effects

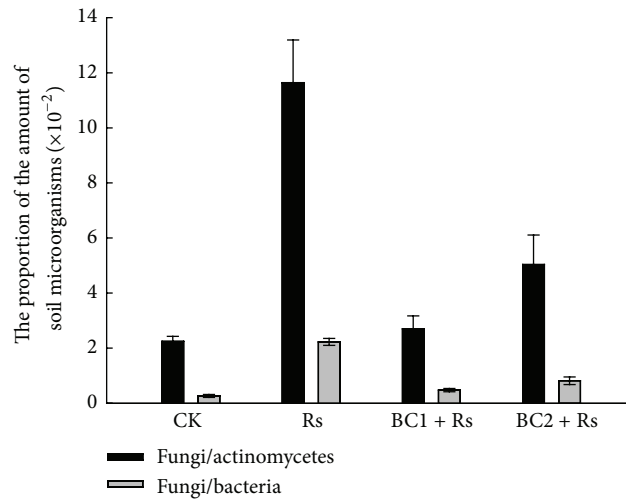


FIGURE 5: Effects of biochar amendment and *R. solanacearum* on the ratio of soil microorganism: fungi/actinomycetes and fungi/bacteria. CK, no biochar and no *R. solanacearum* inoculation; Rs, *R. solanacearum* inoculation; BC1 + Rs, peanut biochar amendment and *R. solanacearum* inoculation; BC2 + Rs, wheat biochar (BC2) amendment and *R. solanacearum* inoculation.

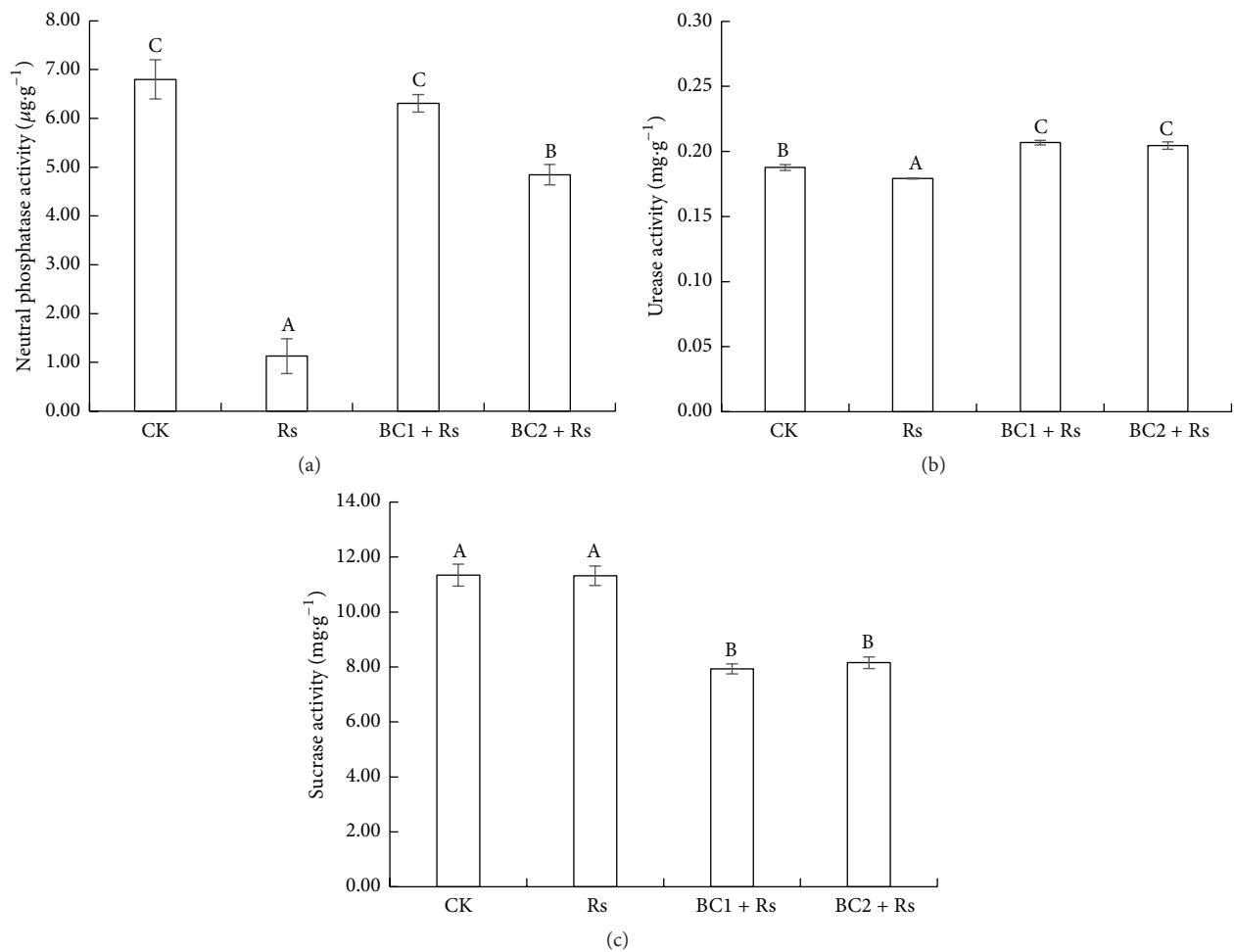


FIGURE 6: Effects of biochar amendment and *R. solanacearum* inoculation on the activity of soil neutral phosphatase (a), urease (b), and sucrose (c). CK, no biochar and no *R. solanacearum* inoculation; Rs, *R. solanacearum* inoculation; BC1 + Rs, peanut biochar amendment and *R. solanacearum* inoculation; BC2 + Rs, wheat biochar (BC2) amendment and *R. solanacearum* inoculation. Different letters on the columns denote a significant difference at $P < 0.05$ using the DMRT (Duncan's new multiple range tests) method.

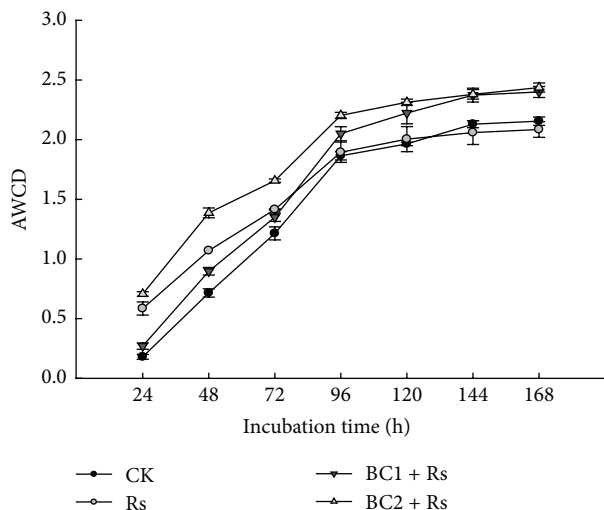


FIGURE 7: Effects of biochar amendment and *R. solanacearum* on soil microorganism community-level substrate utilization profiles. CK, no biochar and no *R. solanacearum* inoculation; Rs, *R. solanacearum* inoculation; BC1 + Rs, peanut biochar amendment and *R. solanacearum* inoculation; BC2 + Rs, wheat biochar (BC2) amendment and *R. solanacearum* inoculation.

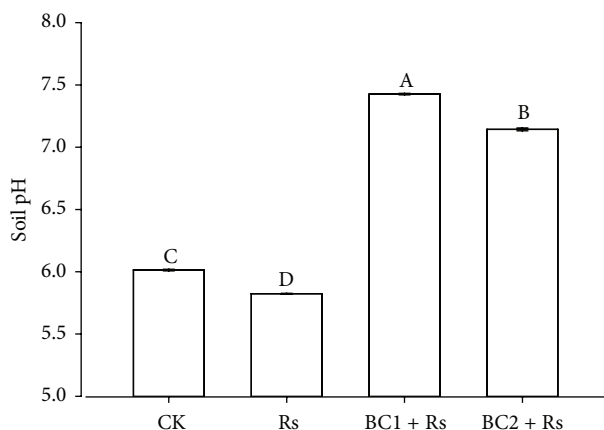


FIGURE 8: Effects of treatment on soil pH. CK, no biochar and no *R. solanacearum* inoculation; Rs, *R. solanacearum* inoculation; BC1 + Rs, peanut biochar amendment and *R. solanacearum* inoculation; BC2 + Rs, wheat biochar (BC2) amendment and *R. solanacearum* inoculation. Different letters on the columns denote a significant difference at $P < 0.05$ using the DMRT (Duncan's new multiple range tests) method.

likely depends on biochar production conditions and feedstock, which control macrostructures and microstructures of biochar particles [12]. Brussaard et al. suggested that organic amendments are possibly among the most important strategies of soil biodiversity management [39].

Soil enzymes are direct mediators of biological catabolism of soil organic and mineral components. As an integral part of nutrient cycling in soil, soil-specific enzyme activities, including dehydrogenase and phosphatase activities, can be used to estimate soil microbial activity and evaluate soil

health [40]. Soil microbial activity is implicated in quantifying soil function, such as C and N cycles and organic matter decomposition [41, 42]. Some enzymes (e.g., hydrolase and glucosidase) facilitate the breakdown of organic matter; other enzymes (e.g., amidase, urease, phosphatase, and sulfatase) are involved in nutrient mineralization. Urease, phosphatase, and arylsulphatase are important in the mineralization of nitrogen, phosphorous, and sulfur compounds [40]. Moreover, soil enzymes may exhibit a strong relationship with disease suppression. Some enzymes (e.g., chitinases and/or glucanases) may reinforce plant resistance to pathogens by breaking down polysaccharides, chitin, and β -glucans responsible for the rigidity of fungal cell walls, thereby destroying cell wall integrity [43]. Baek et al. found that chitinase enzyme activity is positively correlated with suppression ability of *Rhizoctonia solani*-incited cotton seedling disease [44]. Woo et al. reported that biocontrol activity against *B. cinerea* on bean leaves was reduced when chitinase activity is disrupted [45]. In this study, *R. solanacearum* infection significantly reduced soil neutral phosphatase and urease activities; by contrast, biochar amendment increased enzyme activities and maintained a relatively higher soil microbial activity. The activity of microorganisms related to soil neutral phosphatase and urease was enhanced.

In conclusion, our study indicates that both peanut and wheat biochar applications significantly reduced the severity of bacterial wilt caused by *R. solanacearum*. Biochar amendment could increase the population densities of soil bacteria and actinomycetes, modify soil fungi/bacteria and fungi/actinomycetes ratio, increase soil microbial activity, and suppress *R. solanacearum* distribution to establish a healthy soil environment. The pathogen resistance of tomato after biochar amendment is closely related to the changes in soil microbial activity and community structure. Further studies could be conducted by molecular microbiology and sequencing technologies to identify specific soil microbial group or species with antagonistic roles against *R. solanacearum*.

Abbreviations

TTC: 2,3,5-Triphenyltetrazolium chloride

Rs: *R. solanacearum*.

Competing Interests

The authors declare that they have no competing interests.

Acknowledgments

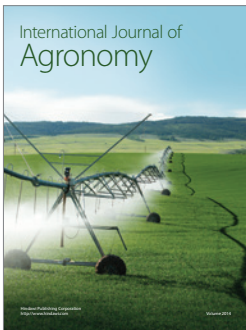
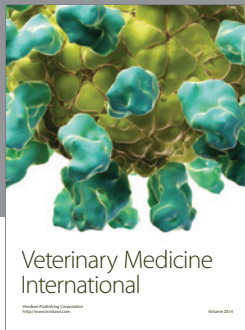
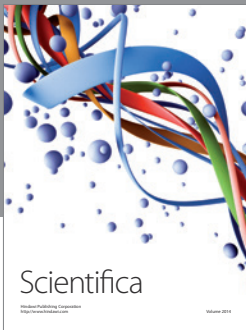
This study was financially supported by grants from the National Key Basic Research Funds of China (2011CB100400), the National Natural Science Foundation of China (31370456), Doctoral Foundation of the Ministry of Education of China (20124404110007), and the Natural Science Foundation of Guangdong Province (S2012010010331). They would like to thank Professor Shiming Luo at South China Agricultural University, China,

for reviewing this paper and giving useful suggestions and comments.

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