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Effect of biochar on the bioavailability of difenoconazole and microbial community composition in a pesticide-contaminated soil

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ABSTRACT

Biochar is a soil amendment for carbon sequestration and contaminant remediation. However, there is relatively little information on the impact of biochar on soil microorganisms that play key roles in pollutant degradation. We investigated the interrelations between soil chemical properties, microbial community, and difenoconazole bioavailability after biochar amendments. Biochar was prepared by pyrolysis of flue-cured tobacco stalks. It was applied at four levels, 0%, 1.0%, 2.5%, and 5.0% (w/w), respectively, to soils in the presence and absence of tobacco plants (*K326*). Biochar increased soil pH, organic matter, total carbon, total nitrogen, C/N ratio and plant biomass. After 90 d incubation, 99% of the difenoconazole was degraded in planted and unplanted soils (control), whereas only 88% and 83% of difenoconazole was degraded in the 5.0% biochar-amended soil with and without plant, respectively. Difenoconazole had greater persistence in soil amended with biochar but plant uptake of difenoconazole was significantly decreased with increasing biochar application level. Compared to the control, the total plant residue of difenoconazole decreased by 24%, 41% and 45% in the 1.0%, 2.5% and 5.0% biochar treatments, respectively. Reduced plant uptake was related to increased sorption and microbial degradation of difenoconazole in biochar treated soils. High-throughput sequencing revealed that biochar altered soil bacterial community composition. Biochar amendment in planted soils increased the average relative abundance of *Sphingomonadaceae* and *Pseudomonadaceae* by 18% and 63%, respectively. When plants were absent, *Sphingomonadaceae* and *Pseudomonadaceae* increased by 46% and 110%, respectively. Therefore, biochar amendment enhances difenoconazole-degrading bacteria by modifying soil chemical properties, and eventually reduces difenoconazole bioavailability in contaminated soils.

1. Introduction

Agricultural soil contamination results from pesticide persistence and accumulation. This can alter microbial processes, increase plant uptake, and harm soil organisms. It also poses a threat to human and ecosystem health (Chen et al., 2015). Suitable methods are required to mitigate contamination (Powlson et al., 2011). We evaluated the suitability of biochar as a remediation tool for agricultural and other contaminated soils.

Biochar (BC) is produced by pyrolysis of biomass under anaerobic conditions. BC is a carbon-rich material that contains both non-carbonized organic matter (NCOM) and carbonized organic matter (COM) (Chen et al., 2008; Chiou et al., 2015). As a soil amendment, BC enhances plant growth and soil fertility by modifying the levels of physical and chemical properties in soils (Atkinson et al., 2010; Jones et al., 2012). The strong partitioning of NCOM and adsorption of COM by BC is particularly effective at sorbing and sequestering of organic pollutants in soils (Chen et al., 2008; Chiou et al., 2015). BC has up to 10–1000 times greater capacity for sorbing organic compounds than soil organic matter (Cornelissen et al., 2005; Wen et al., 2009). Therefore, BC can efficiently immobilize pollutants in contaminated soils and reduce the risk of pesticides entering the human food chain via agricultural products. There is evidence that use of BC as an amendment decreases the available concentrations of soil pesticides and reduces pesticides in plants (Yang et al., 2006; Yu et al., 2009). The irreversible sorption activity of BC can influence the microbial biodegradation of pesticides (Jones et al., 2011).

Biodegradation is the main process by which active ingredients in pesticides are broken down in soils. This is mainly dependent on the soil microbial community and activity. BC can directly influence soil microbial activity by providing a readily available carbon source and other nutrients (N, P and K) (Lehmann et al., 2011). BC application can also indirectly modify the indigenous microbial community that is normally responsible for contaminant decomposition and biotransformation (Jiang et al., 2016; Liu et al., 2015). The BC–microbe interaction can play an

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important role in pesticide degradation in agricultural soils. However, it is unclear if specific microorganism groups in soils amended with BC at different rates show a consistent response, particularly at sites polluted with high levels of the pesticide (difenoconazole). The effects of BC on bacterial populations will broaden our knowledge of difenoconazole biodegradation.

Difenoconazole (cis,trans-3-chloro-4-[4-methyl-2-(1H-1, 2, 4-triazol-1-ylmethyl) − 1,3-dioxolan-2-yl] phenyl 4-chlorophenyl ether) is a broad-spectrum triazole fungicide with a highly effective against the diseases caused by pathogenic fungi infecting tobacco, cereals, fruits, vegetables and other crops (Horsfield et al., 2010; Li et al., 2015; Munkvold et al., 2001). It inhibits fungal cytochrome P450 sterol 14α-demethylase (CYP51) activity and blocks ergosterol biosynthesis, which leads to blockage of synthesis of the fungal cell wall (Hamada et al., 2011; White et al., 1998). Thus, the ultimate result of these processes is death of the cell (White et al., 1998). In China, difenoconazole is one of the most important and widely-used pesticides for disease control (such as tobacco brown leaf spot *(Alternaria alternata* (Fries) Keissler) and powdery mildew *(Erysiphe cichoracearum* DC.)) and improving the quality of tobacco leaves and value of industrial use (Li et al., 2015; Tong et al., 2012). The characteristics of difenoconazole are high chemical and photochemical stability, low water solubility (15 mg L^{-1} at 20 °C), high hydrophobicity (log Kow = 4.4), and easy transportation (Chau et al., 2015; Wang et al., 2011). These properties make it stay in the environments (including soil, plant and water) and bioaccumulate in the food web. Therefore, its intensive applications may have led to undesirable side-effects on environment ecosystem (Kesavachandran et al., 2009). Monitoring the levels of difenoconazole in tobacco and other crops has received much attention due to a potential threat to human health.

In this paper, we examined changes of soil chemical properties, plant biomass, difenoconazole residues in plant and soil, and soil microbial communities following BC application at 0%, 1.0%, 2.5% and 5.0% (w/w) to soils contaminated with difenoconazole. Our objectives were to (i) determine the effectiveness of BC in reducing the uptake of difenoconazole into plant, and (ii) investigate the effects of BC addition on the change of microbial community composition in a difenoconazole- contaminated soil.

2. Materials and methods

2.1. Preparation and characteristics of BC

The BC was manufactured from tobacco stalks and sourced from Longgang Tobacco Experiment Station (26°52′24.8″ N, 107°06′ 40.8″E), Guizhou Academy of Tobacco Science, China. Air-dried tobacco stalks were charred under limited-oxygen conditions in a combined biomass particle carbonization furnace. BC derived from tobacco stem pyrolysis had a total surface area of 17.41 $m^2 g^{-1}$, a total pore volume of 0.0226 $cm^3 g^{-1}$, a microporous (< 2 nm) surface area of 5.85 $m^2 g^{-1}$, and a microporous volume of 0.0047 $cm^3 g^{-1}$. In addition, the resulting BC contained 760.09 g kg^{-1} C, 23.10 g kg^{-1} N, 52.20 g kg^{-1} K and 5.78 g kg^{-1} P, and had a pH (H_2O) of 9.77, and a C/N ratio of 32.95. BC was ground, and sifted through a 2-mm sieve before using as a soil amendment in this study.

2.2. Soil characteristics

The soil was collected from the plough layer (0–20 cm depth) of tobacco fields at the Longgang Tobacco Experiment Station, Kaiyang county, Guizhou province, China. This region has a humid subtropical monsoon climate with a mean annual temperature of 13.5–14.6 °C, a mean annual precipitation of 1130–1206 mm and four distinct seasons. The soil type is classified as a Limestone soil (Chinese Soil Taxonomy), equivalent to a Calcaric cambisol in the FAO/UNESCO Taxonomy. The average organic matter, total carbon, nitrogen, potassium and phosphorus content in the farmland soils were 43.67, 35.42, 1.92, 8.90 and 1.06 g kg^{-1}, respectively. Soil samples were air-dried, homogenized and sieved using a standard 2 mm sieve before experimental use.

2.3. Tobacco preparation and pot trial

The tobacco strain used was K326, a hybrid from the Northup King Seed Company. K326 tobacco is resistant to diseases and heat, and it is widely cultivated in China. It has a growing period of 120 d under field conditions. In the pot experiment, each plastic container (39 cm diameter × 28 cm height) was used to contain 15 kg of BC-free soil or BC-amended soil. Then the pot soil was spiked with 30 mL of 20 mg mL^{-1} difenoconazole solution (in acetone) to give a concentration of test compound of 40 mg kg^{-1}. The application concentration of difenoconazol, according to its half-life period, must be given at doses high enough to ensure satisfactory detection in plants and soils after the whole growth cycle of tobacco. For planting treatments, tobacco plants were grown in soils amended with 0%, 1.0%, 2.5% and 5.0% BC (w/w) (0% BP, 1.0% BP, 2.5% BP and 5.0% BP, respectively). Without tobacco plants, there were also four treatments of soils amended with 0%, 1.0%, 2.5% and 5.0% BC (0% BA, 1.0% BA, 2.5% BA and 5.0% BA, respectively). Finally, the soil amended with and without BC in each treatment was thoroughly mixed to obtain a uniform distribution of difenoconazole and then incubated at a water content of 50% water holding capacity (WHC).

After 7 d incubation, uniform size tobacco seedlings (0.2 cm diameter, 10 cm height) were transplanted directly from a seedbed into the pots. The tobacco plants were grown in a greenhouse with natural sunlight. The growth conditions were 26–34 °C, and a constant relative humidity of 70%. After a 90-d growth period, the aboveground parts (leaves and stems) were removed. The roots were also removed from the soil, washed with tap water for 10 min, and then air-dried for 24 h at room temperature. The fresh weight of each component was measured for each pot, and component subsamples were taken to determine moisture content and difenoconazole concentration. After plant removal, a soil sample from each pot was taken and divided into two parts. One subsample was quick-frozen in liquid nitrogen and then stored at −80 °C until used for DNA extraction. The other subsample was air-dried and sieved using a standard 2 mm sieve for analysis of chemical properties.

2.4. Analysis of BC characteristics

The total surface area, micropore surface area, micropore volume, and total pore volume of the BC were measured using an automatic adsorption analyzer (Autosorb iQ^2, Quantachrome Co., USA). To obtain the pore structure characteristics, BC samples were degassed for 16 h under vacuum condition at 150 °C, and then the adsorption and desorption isotherms were calculated for the N_2 sorption at 77 K. The surface area and pore size distribution of the BC were calculated from the N_2 adsorption isotherms by using the Brunauer–Emmett–Teller (BET) and Density Functional Theory (DFT) methods, respectively.

2.5. Soil chemical analysis

The pH was measured by adding soil to deionized water at a ratio of 1:2.5 (w/v) using a pH meter. Soil organic matter (SOM) was measured by the potassium dichromate volumetric method (Li et al., 2013). Soil total phosphorus (TP) content was analyzed by H_2SO_4:$HClO_4$ (20:1) acid digestion (Wang et al., 2007). Total potassium (TK) content was determined by the NaOH melt-flame photometry method (Li et al., 2013). Total carbon (TC), total nitrogen (TN) contents and the C:N ratio (C/N) in soils were measured by a Vario Macro Elemental Analyzer (Elementar, Analysensysteme, GmbH, Germany).

2.6. Residue extraction and clean-up

5.0 g sample powder (plant tissues and soil) was weighed into an Erlenmeyer flask (150 mL) and mixed with 50 mL of HPLC-grade acetonitrile. Then the mixture was shaken using a thermostatic oscillator (IS-RDV3) at 150 rpm for 1 h 4 g of anhydrous magnesium sulfate ($MgSO_4$) and 3 g of sodium chloride (NaCl) were slowly added, with continuous shaking (10 min). An aliquot (10 mL) after filtration was transferred into a round-bottom flask, and evaporated to near dryness using a rotary evaporator. The residue was dissolved in 5 mL of hexane and then passed through a florisil cartridge which had been preconditioned with 5 mL of a hexane–acetone mixture 9:1 (v/v) and then 5 mL hexane. Finally, the eluate was evaporated to dryness under a stream of nitrogen, followed by reconstituted to 2 mL with hexane and then filtered through a filter membrane (0.22 μm) for gas chromatograph analysis.

2.7. Residue analysis

The residue analysis was carried out on a gas chromatograph (GC-2010, Shimadzu) equipped with an electron capture detector (ECD), and a DB-1 capillary column (30 m × 0.25 mm × 0.25 μm). The temperatures of injector and detector were maintained at 230 and 300 °C, respectively. Carrier gas was ultra-high purity nitrogen, with a flow rate of 1 mL min^{-1}. The temperature of column was initially maintained at 70 °C for 1 min. Subsequently, it was increased 5 °C min^{-1} to 260 °C and then 10 °C min^{-1} to 280 °C with a 20-min hold. Samples were injected by an auto sampler and kept at split injection mode with split ratio of 10.

2.8. DNA extraction, illumina sequencing and data processing

Total DNA was extracted from 0.25 g subsample of soil with the PowerSoil® DNA Isolation Kit (MOBIO Laboratories, Carlsbad, CA, USA) following the manufacturer's protocol. DNA was analyzed using a NanoDrop Spectrophotometer to assess the quality. Then the DNA samples were diluted and immediately stored at −20 °C until further use.

The V4 hyper variable region of the 16S rRNA gene was amplified using the universal primers 515F (5′-GTGCCAGCMGCCGCGGTAA-3′) and 909R (5′-CCCCGYCAATTCMTTTRAGT-3′), with a 12-nt unique barcode. The PCR mixture (final volume of 25 μL) contained 1 × PCR buffer, 1.5 mM $MgCl_2$, 0.4 μM of each deoxynucleoside triphosphate (dNTPs), 1.0 μM of each primer, 0.5 Unit of *Ex* Taq polymerase (TaKaRa, Japan) and 10 ng of genomic DNA. The PCR conditions consisted of an initial denaturation step of 95 °C for 3 min, 30 amplification cycles of denaturation at 94 °C for 40 s, annealing at 56 °C for 1 min, and extension at 72 °C for 1 min, followed by a final extension at 72 °C for 10 min. Amplicon quality was visualized using electrophoresis and then purified using a SanPrep DNA Gel Extraction Kit (Sangon Biotech, Shanghai, China). All PCR products were quantified with a Nanodrop 2000 Spectrophotometer, and pooled together at equal molar amounts. The sequencing libraries were prepared using a TruSeq DNA sample preparation kit according to the manufacturer's instructions. In accordance with the Illumina library preparation protocols, the purified library was diluted, denatured, rediluted, and then mixed with PhiX (about 30% of final DNA amount). Afterward, the samples were prepared for sequencing on Illumina Miseq system.

All 16S rRNA raw sequence data were analyzed using Quantitative Insights Into Microbial Ecology (QIIME) v.1.7.0 pipeline (http://qiime.org/tutorials/tutorial.html). Briefly, all sequence reads were trimmed and assigned to samples based on their barcodes. The sequence libraries were split and denoised to avoid an overestimation of the diversity in some of the samples caused by sequencing errors, including sequences with average base quality score < 30, sequence lengths shorter than 250 bp, and sequences containing incorrect primer sequences or ambiguous base calls. All chimeric sequences were filtered out by the UCHIME algorithm. Then sequences were clustered into operational taxonomic units (OTUs) at the ≥97% identity threshold and singleton OTUs were removed. The OTUs were then used as a basis for downstream analysis. More details regarding the data processing steps used are provided by Yao et al. (2014). The original sequence data have been deposited in the European Nucleotide Archive (ENA) under accession number PRJEB22655 (http://www.ebi.ac.uk/ena/data/view/PRJEB22655).

2.9. Statistical analysis

The differences in soil chemical properties, plant biomass, plant uptake of pesticides, and pesticide residues in soils among different treatments were compared by a one-way analysis of variance (ANOVA) with the least significant difference (LSD) test. Differences between the relative abundances of pollutant-degrading microorganisms in soils amended with and without BC were analyzed by a *t*-test. The relationship between difenoconazole residues and carbon content in soil was studied using linear regression analysis. A value of $p < 0.05$ was accepted as significant. All statistical analysis was performed by the SPSS software package (Version 13.0, SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Effect of BC on soil chemical properties and plant biomass

The addition of BC significantly increased soil organic matter (OM) from 42.47 ± 1.13 g kg^{-1} in the unamended soil to 93.92 ± 2.84 g kg^{-1} in the 5.0% BC-amended soil without tobacco, while the corresponding values were 43.74 ± 0.87–94.24 ± 5.99 g kg^{-1} with tobacco (Table 1). The pH, TC, TN, and C/N ratio were also increased with increasing amounts of BC. Although the values of TK and TP concentration are different between treatments with the same BC application rates, there were no statistically

Table 1
Soil chemical properties under different treatments.

Treatments	pH	TC (g kg^{-1})	TN (g kg^{-1})	TP(g kg^{-1})	TK(g kg^{-1})	C/N	OM(g kg^{-1})
0% BA	7.55 ± 0.06^{b}	35.26 ± 1.22^{e}	1.73 ± 0.04^{e}	0.94 ± 0.03^{bc}	5.99 ± 0.10^{c}	20.41 ± 0.23^{cd}	42.47 ± 1.13^{d}
1.0% BA	7.62 ± 0.04^{ab}	39.91 ± 0.57^{de}	1.97 ± 0.03^{de}	0.93 ± 0.05^{c}	5.71 ± 0.67^{c}	20.26 ± 0.56^{d}	47.47 ± 5.47^{d}
2.5%BA	7.70 ± 0.04^{ab}	51.73 ± 6.03^{c}	2.41 ± 0.28^{c}	0.97 ± 0.02^{abc}	6.36 ± 0.18^{bc}	21.49 ± 0.04^{bc}	62.91 ± 0.25^{b}
5.0% BA	7.74 ± 0.01^{ab}	78.66 ± 2.79^{a}	3.49 ± 0.07^{a}	1.04 ± 0.01^{a}	7.46 ± 0.29^{a}	22.53 ± 0.36^{ab}	93.92 ± 2.84^{a}
0% BP	7.70 ± 0.12^{ab}	37.10 ± 1.22^{e}	1.91 ± 0.12^{de}	0.95 ± 0.05^{bc}	6.47 ± 0.15^{bc}	19.42 ± 0.89^{d}	43.74 ± 0.87^{d}
1.0% BP	7.75 ± 0.05^{a}	42.51 ± 0.50^{d}	2.11 ± 0.05^{d}	0.94 ± 0.00^{bc}	6.54 ± 0.72^{bc}	20.15 ± 0.73^{d}	45.15 ± 1.05^{d}
2.5% BP	7.85 ± 0.02^{a}	50.52 ± 2.68^{c}	2.26 ± 0.12^{cd}	0.93 ± 0.04^{c}	6.87 ± 0.21^{ab}	22.35 ± 0.10^{ab}	55.36 ± 0.79^{c}
5.0% BP	7.86 ± 0.18^{a}	66.76 ± 2.43^{b}	2.93 ± 0.10^{b}	1.01 ± 0.01^{ab}	7.11 ± 0.67^{ab}	22.74 ± 0.46^{a}	94.24 ± 5.99^{a}

0% BA, 1.0% BA, 2.5% BA and 5.0% BA represent 0%, 1.0%, 2.5% and 5.0% BC treated soil without plants, respectively. 0% BP, 1.0% BP, 2.5% BP and 5.0% BP represent 0%, 1.0%, 2.5% and 5.0% BC treated soil with plants, respectively. TC: total carbon content; TN: total nitrogen content; TP: total phosphorus content; TK: total potassium content; OM: organic matter in soils. Different letters in the same column between treatments indicate a significant difference at the 0.05 level.

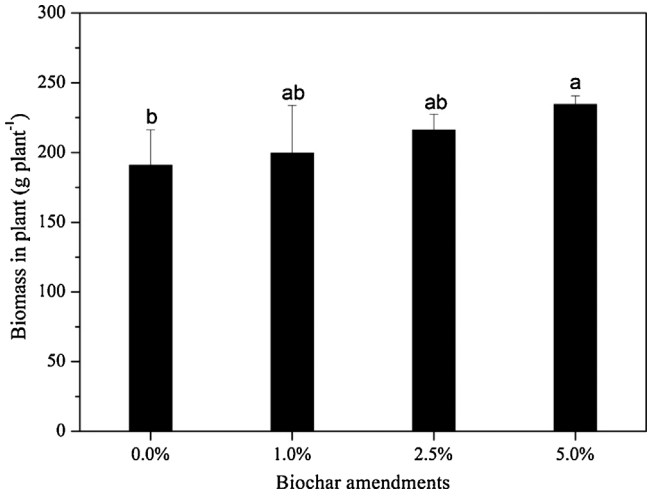

Fig. 1. Biomass of tobacco plants cultivated in a difenoconazole-contaminated soil amended with different BC application rates. Error bars show standard deviation. Bars with different letters are significantly different at the 0.05 level.

significant differences between BA and BP treatments. Planting tobacco also increased soil pH, and the effect was much greater than in soil treated with BC. However, planting tobacco had no significant effect on soil C/N ratios at the same application rate of BC.

Compared with the control soil, tobacco cultivated in the BC-amended soil produced greater biomass (Fig. 1). The increase rates of plant biomass (including root, stem and leaf) were 4.53%, 13.21% and 22.89% for the 1.0%, 2.5% and 5.0% BC-amended soils, respectively, in comparison with only 190.89 g plant^{-1} in the unamended soil. There was a significant increase of plant biomass in the 5.0% BC treatment compared to the control ($p < 0.05$). However, the values in the other BC treatments were not significantly different.

3.2. Effect of BC on difenoconazole residues in soils

At the end of the experiment, the difenoconazole residues in soils had significantly increased with increasing BC levels (Fig. 2). The level of difenoconazole in soil with tobacco plants increased from 0.38 mg kg^{-1} in the control to 4.62 mg kg^{-1} in the 5.0% BC-amended treatment. In soil without tobacco plants, the corresponding levels of difenoconazole increased from 0.40 mg kg^{-1} to 6.65 mg kg^{-1}. There

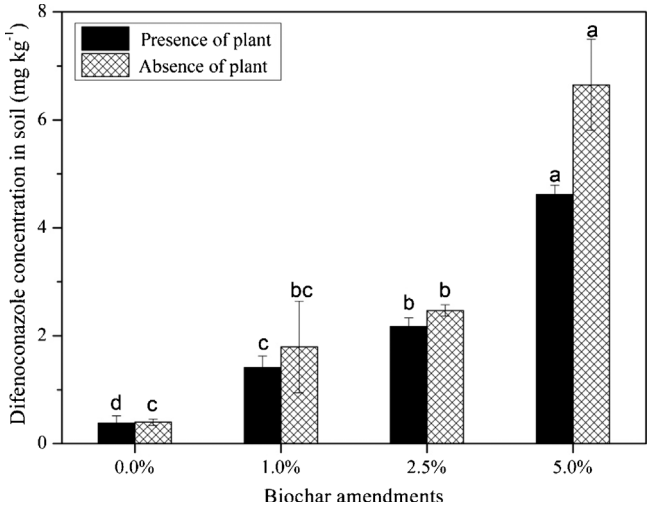

Fig. 2. Difenoconazole residues in a BC-amended soil with and without plants. Error bars show standard deviation. Bars with different letters are significantly different at the 0.05 level.

were significant differences of difenoconazole residues among the four treatments in the planted soils. The values in the unplanted soils were significantly different between 2.5%, 5.0% BC treatments and the control, but not between the 1.0% BC treatment and the control.

3.3. Effect of BC on the soil bacterial community

The ten most dominant microbial phyla were *Proteobacteria, Bacteroidetes, Acidobacteria, Chloroflexi, Actinobacteria, Crenarchaeota, Cyanobacteria, Gemmatimonadetes*, *Planctomycetes,* and *Verrucomicrobia,* which accounted for more than 94.19% and 94.02% of the sequences assigned to soil bacteria in the absence and presence of plants, respectively (Fig. 3). *Proteobacteria* was the largest bacterial phylum in all treatments. BC addition significantly increased *Proteobacteria* abundance from 18.51% in 0% BA to 29.38% in 5.0% BA, while the corresponding values were 24.31% in 0% BP to 27.83% in 5.0% BP. The abundance of *Actinobacteria*, *Gemmatimonadetes,* and *Planctomycetes* were also increased following BC applications. *Bacteroidetes* was the phylum most sensitive to BC, with relative abundance decreasing from 19.18% in 0% BA to 13.83% in 5.0% BA, and from 24.81% in 0% BP to 19.90% in 5.0% BP. *Acidobacteria* was also significantly decreased with the increasing of BC application, and a decrease of its relative abundance was 16.79% and 14.06% in BA and BP, respectively. However, the addition of BC had no significant impact on the abundances of *Chloroflexi*, *Crenarchaeota* and *Verrucomicrobia* with and without plants (Fig. 3).

Families, having pollutant degradation functions, were selected for analysis (Table 2). Regardless of tobacco planting, the degrading bacteria of *Sphingomonadaceae*, *Pseudomonadaceae*, *Bacillaceae*, *Micrococcaceae* and *Erythrobacteraceae* from BC-amended soils had greater abundance than those in unamended soils. With plant presence, an increase of 18% *Sphingomonadaceae* and 63% *Pseudomonadaceae* was observed along with an increase of 67% *Erythrobacteraceae* in BC-amended soils, although these results were not significant ($p > 0.05$). Similarly, without plants, there was an increase of 46% in *Sphingomonadaceae*, 110% in *Pseudomonadaceae* and 258% in *Erythrobacteraceae* compared to the controls. ANOVA showed that BC had a significant effect ($p < 0.05$) on the relative abundances of *Sphingomonadaceae* and *Erythrobacteraceae,* but not on the abundance of *Pseudomonadaceae*.

3.4. Effect of BC on difenoconazole residues in plants

After 90 d of growth, difenoconazole residue levels were measured in above-ground tissues (leaves and stems) and under-ground tissues (roots). Difenoconazole residues in leaves, stems, and roots were all lower in plants from soils that received BC than in control soils (Fig. 4). For instance, the root difenoconazole concentrations decreased from 1.79 ± 0.67 mg kg^{-1} in the control soil to 0.85 ± 0.11 mg kg^{-1} in the soil amended with 5.0% BC. The concentration of difenoconazole in stems decreased from 0.06 ± 0.03 to 0.02 ± 0.03 mg kg^{-1} in the control soil and 5.0% BC-amended soil, respectively. Similarly, the level of difenoconazole in leaves was from 0.02 ± 0.01 to 0.01 ± 0.00 mg kg^{-1} in the control soil and 5.0% BC-amended soil, respectively. Residues in leaves and stems were 91–122 and 32–45 times lower than those in roots, respectively. There were significant differences in difenoconazole residues between the control and 1.0%, 2.5%, 5.0% BC amendments, between the control, 1.0% BC amendment and 2.5%, 5.0% BC amendments, and between the control and 2.5%, 5.0% BC amendments in roots, stems, and leaves, respectively.

The BC was effective in reducing the uptake of difenoconazole into plant tissues from a contaminated soil, although the BC-amendment treatments also exhibited higher plant growth and biomass productivity (Fig. 1). To evaluate the influence of BC on difenoconazole residues in plants, the amount of uptake in a given tissue is derived from the residue concentration and plant biomass of the component (Table 3). Total uptake amounts of difenoconazole by the whole plant, or in the

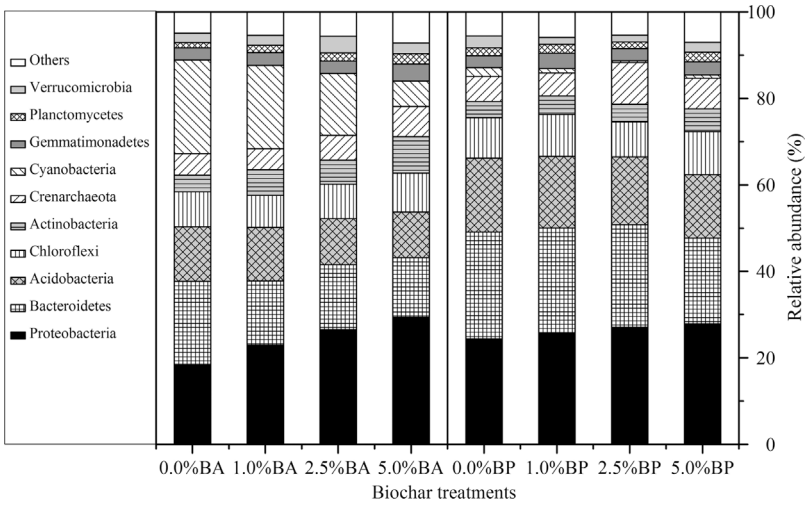

Fig. 3. The relative abundance of dominant phyla related to BC addition at different concentrations (0%, 1.0%, 2.5% and 5.0%). BA represents BC treated soil without plants. BP represents BC treated soil with plants.

plant tissues (root, stem, and leaf), after 90 d of growth, all decreased with increased BC application rates. After considering the variations of biomass, 5.0% BC-amended soil was still the most effective treatment, which decreased the total plant uptake of difenoconazole by 45% that of uptake in unamended soil. Even for the soil amended with 1.0% BC, the average reduction of plant difenoconazole uptake was up to 24%.

Accumulation of difenoconazole in roots could be described by the root concentration factor (RCF), which is defined as the ratio of the concentration in roots to that in soils on a dry weight basis. In this study, the RCF of difenoconazole gradually declined as the increasing of soil BC content. Compared with the control soil (4.71), the corresponding RCF values for difenoconazole were 0.91, 0.44 and 0.18 in the 1.0%, 2.5% and 5.0% BC-amended soils, respectively.

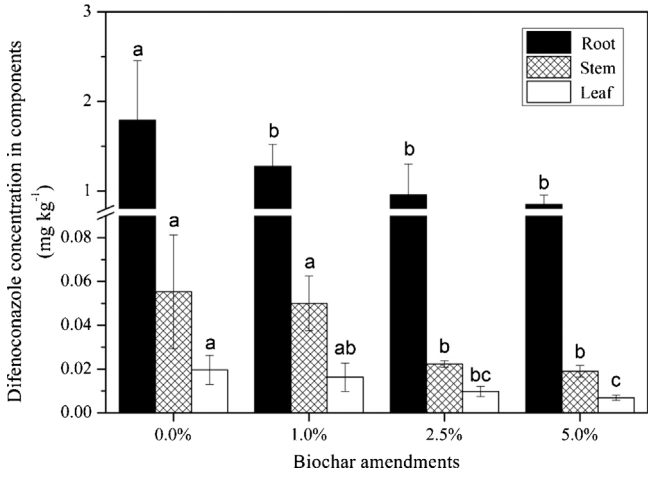

Fig. 4. Concentration of difenoconazole residues in the tissues (root, stem and leaf) of tobacco under different BC application rates. Error bars show standard deviation. Bars with different letters are significantly different at the 0.05 level.

4. Discussion

4.1. Plant biomass responses to BC addition

The BC-induced changes soil physical and chemical properties enhance plant growth, and this has been seen in previous studies (Cheng et al., 2008; Khorram et al., 2015; Rogovska et al., 2014). In the present study, a significant positive effect of BC on plant biomass was documented within 5.0% BC amendments. Similarly, a gradual increase in biomass with increasing BC application rates has also been discovered in other studies (Khorram et al., 2015; Rogovska et al., 2014). These findings may be attributed, at least in part, to the slow-release of nutrients from BC in amended soils (Cheng et al., 2008; Hagemann et al., 2017; Rogovska et al., 2014). For example, the levels of total N, K and P in tobacco BC were 23.10, 52.20 and 5.78 g kg^{-1}, respectively. The corresponding values in farmland soil were only 1.92, 8.80 and 1.04 g kg^{-1}, respectively (data not shown). Therefore, BC produced from incomplete combustion of stalks generally contained relatively high levels of nutrients (such as N, K and P, etc.), which could directly increase their accumulations in soils (Table 1) and promote plant growth. However, under field conditions, the level of BC addition is not always correlated with plant biomass increase. Some studies have showed no significant difference between BC-amended and unamended plots (Gaskin et al., 2010; Jones et al., 2012), while others have found that plant biomass decreased with BC application (Calderon et al., 2015; Gaskin et al., 2010). Therefore, the effect of BC amendment on plant biomass shows great variation perhaps due to the many combinations of feedstock types, pyrolysis conditions, field application rates, soil types, and crop types (Gaskin et al., 2010; Rajkovich et al., 2012).

Table 2
Changes in relative abundances (%) of soil functional bacteria among treatments.

Family	Plants present		Plants absent	
	Unamended soil	BC-amended soil	Unamended soil	BC-amended soil
Sphingomonadaceae	7.67 ± 0.65^{ab}	9.07 ± 1.25^{a}	4.65 ± 0.84^{c}	6.78 ± 0.55^{b}
Pseudomonadaceae	0.21 ± 0.05^{ab}	0.31 ± 0.07^{a}	0.11 ± 0.01^{b}	0.23 ± 0.09^{ab}
Bacillaceae	0.16 ± 0.04^{a}	0.17 ± 0.02^{a}	0.11 ± 0.08^{a}	0.15 ± 0.07^{a}
Micrococcaceae	0.38 ± 0.12^{a}	0.43 ± 0.14^{a}	0.34 ± 0.15^{a}	0.50 ± 0.14^{a}
Erythrobacteraceae	0.12 ± 0.02^{b}	0.20 ± 0.05^{b}	0.24 ± 0.08^{b}	0.86 ± 0.38^{a}

Different letters in a single row represent difference between treatments at the 0.05 level. The value obtained in the BC-amended soil was the average relative abundances of the three treatments (1.0%, 2.5% and 5.0% BC amendments).

Table 3
Estimated total amount of difenoconazole uptake (μg) in different parts of a tobacco under different BC treatments.

Treatments	M_T	M_R	M_S	M_L
0% BC	62.27 ± 12.13^{a}	57.17 ± 9.64^{a}	3.06 ± 2.23^{a}	2.04 ± 0.98^{a}
1.0% BC	47.59 ± 12.37^{ab}	42.96 ± 11.83^{ab}	2.85 ± 0.94^{a}	1.78 ± 0.56^{ab}
2.5% BC	36.80 ± 16.24^{b}	34.23 ± 16.64^{b}	1.44 ± 0.12^{a}	1.13 ± 0.31^{ab}
5.0% BC	34.39 ± 4.30^{b}	32.23 ± 4.37^{b}	1.25 ± 0.18^{a}	0.91 ± 0.17^{b}

M_T indicates the total amount of plant uptake of difenoconazole (μg). M_R, M_S and M_L indicate the amount of difenoconazole uptake (μg) in the root, stem and leaf of tobacco, respectively. Mean values with different letters in a single column are significantly different between different treatments at the 0.05 level.

4.2. Soil bacterial community composition responses to BC addition

Changes in microbial community structure, following soil BC addition, have often been studied (Ding et al., 2013; Hu et al., 2014; Khodadad et al., 2011). Interestingly, the effects of BC on soil microbial communities have shown considerable variability. Some studies found that BC amendment increased the relative abundance of members of the *Proteobacteria*, *Baceroidetes*, *Pseudomonas*, *Actinobacteria*, *Nitrospiraceae*, *Gemmatimonadetes, Chloroflexi*, *Firmicutes and Trichoderma* (Anderson et al., 2011; Ding et al., 2013; Graber et al., 2010; Hu et al., 2014; Khodadad et al., 2011; Kolton et al., 2011). Other studies demonstrated that BC decreased the abundance of *Proteobacteria, Acidobacteria*, *Firmicutes and Bacteroidetes* (Ding et al., 2013; Hu et al., 2014; Kolton et al., 2011; Wu et al., 2016). In the present study, *Proteobacteria, Actinobacteria*, *Gemmatimonadetes,* and *Planctomycetes* increased after BC additions. *Bacteroidetes and Acidobacteria* were decreased following the application of BC. However, *Chloroflexi*, *Crenarchaeota* and *Verrucomicrobia* showed no response to BC amendments (Fig. 3). There appears to be no obvious or unidirectional pattern of microbial community change following BC application. A number of factors may be responsible for these variable results. These include BC conditions (feedstock material, nutrient content, pyrolysis condition and application rate), initial soil conditions (soil organic matter, pH, temperature, moisture and aeration), and environmental conditions (land use, management intensity, and vegetation type) (Jenkins et al., 2016; Sun et al., 2016). All of these factors, individually or in combination, could modify the impact of BC addition on the structure diversity of soil microbial community.

In our study, BC amendment increased the soil bacterial population that has been used as bioremediation agents (Table 2). Several reports have shown the important role of *Erythrobacteraceae* for polycyclic aromatic hydrocarbon (PAH) degradation in soils and sediments (Sarma et al., 2004; Zhuang et al., 2015). Also, *Bacillaceae*, *Sphingomonadaceae* and *Pseudomonadaceae* are considered to be the most important bacteria for the biodegradation of a wide range of complex organic compounds, such as difenoconazole, PAH, and phenol in soils and sediments (Guo et al., 2005; Hasan and Jabeen, 2015; Leys et al., 2004). We found a clear increase in the relative abundance of these taxa in the soil as a result of BC application, along with *Micrococcaceae* which are considered the predominant microbial group that biodegrade methylpyridine and benzene in soils (Leigh et al., 2007; Lima-Morales et al., 2016). Therefore, our data indicate that BC-mediated degrading bacteria shifts have the potential to promote degradation of contaminants (such as difenoconazole), reduce their bioavailability and significantly decrease plant uptake of contaminants from soils.

4.3. Effect of BC on difenoconazole bioavailability

The BC act as appropriate sorbents for irreversible sorption of many types of pesticides (Martin et al., 2012; Yu et al., 2009). The concentrations of difenoconazole were 4, 6 and 16 times for the 1.0%, 2.5% and 5.0% BC-amended treatments, respectively, higher than those in the unamended soil. These data are consistent with earlier other results (Sopena et al., 2012; Yu et al., 2009). The sorption capacity pesticides on BC highly depends upon its characteristics and properties, including porous structure, charged surface, surface area and carbon content (Khorram et al., 2016; Sopena et al., 2012). In the present study, there was a significant positive correlation (R^2 = 0.973, p = 0.000) between the contents of difenoconazole and total carbon in soils. This finding may be attributted to a large accumulation of carbon in soils with BC amendment. The increased soil carbon level ranged from 35.26 g kg^{-1} in the unamended soil to 78.66 g kg^{-1} in the 5.0% BC-amended soil without tobacco plants, while the corresponding values ranged from 37.10 g kg^{-1} to 66.76 g kg^{-1} with tobacco plants (Table 1). Therefore, the soil with a high carbon content will easily increase sequestration of difenoconazole and reduce its bioavailability.

4.4. Effect of planting on difenoconazole residues in soil

Difenoconazole residues in soils were affected not only by BC amendments, but also by growing plants. The final residue concentrations of difenoconazole in the planted soils under different BC amendments were lower than those in the corresponding unplanted soils (Fig. 2). Planting can change the composition of the microbial community and facilitate the degradation of pesticides in rhizosphere soil by the release of enzymes and exudates from roots (Fang et al., 2001; Sun et al., 2004). *Sphingomonadaceae* and *Pseudomonadaceae* significantly increased in the presence of tobacco plants (Table 2) and may have played a major role in the degradation of difenoconazole (Cai et al., 2015; Sarkar et al., 2009). Previous studies demonstrated that selected species (*Sphingomonas* and *Sphingobium* spp.) in the family *Sphingomonadaceae* can degrade more than 90% of 100 mg L^{-1} difenoconazole concentrations within 24 h (Ahn et al., 2016). Selected species of *Pseudomonas nitroreducens* in the family *Pseudomonadaceae* can also degrade more than 90% of 180 mg L^{-1} of difenoconazole concentrations within 5 d (Cai et al., 2015; Sarkar et al., 2009). Therefore, it can be concluded that the increased degradation of difenoconazole observed in the presence of plants was due to increased relative abundance of these bacteria in the soils with or without BC addition (Table 3). Compared to unplanted soil, the reduction of difenoconazole residues in soils with growing plants occurs through the uptake of difenoconazole into plant parts and by the increasing populations microorganisms that degrade difenoconazole.

5. Conclusions

The increase of difenoconazole in BC-amended soil, along with the changes in bacterial community composition, demonstrates that BC application to soil can significantly decrease the bioavailability of difenoconazole to plant and soil microbial communities, and minimize difenoconazole residues in agricultural produce. Compared to unplanted soils, greater difenoconazole losses from planted soils occurred because of enhanced plant uptake and bacterial biodegradation. Therefore, this study provides evidence that BC–plant–microbe interactions can effectively remove soil contaminants such as difenoconazole. However, future studies are needed to determine if these findings can be developed in situ remediation methods of pesticide contaminated field.

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References

Ahn, J.H., Ro, Y.M., Park, I., Kim, W.G., Han, B., You, J., 2016. Isolation and characterization of the bacteria degrading the triazole fungicide difenoconazole. 43rd Annual Meeting and International Symposium, KMB. Korean Soc. Microbiol. Biotech., Korea, pp. 105.

Anderson, C.R., Condron, L.M., Clough, T.J., Fiers, M., Stewart, A., Hill, R.A., Sherlock, R.R., 2011. Biochar induced soil microbial community change: implications for biogeochemical cycling of carbon, nitrogen and phosphorus. Pedobiologia 54, 309–320.

Atkinson, C.J., Fitzgerald, J.D., Hipps, N.A., 2010. Potential mechanisms for achieving agricultural benefits from biochar application to temperate soils: a review. Plant Soil 337, 1–18.

Cai, H.M., Cao, Z.F., Zhao, J.Z., Wei, C.J., Yuan, S., Liang, D., 2015. Isolation, identification and degradative characters of difenoconazole-degrading bacteria BMJHZ-01. Chin. J. Anim. Nutr. 17, 590–595.

Calderon, F.J., Benjamin, J., Vigil, M.F., 2015. A comparison of corn (Zea mays L.) residue and its biochar on soil C and plant growth. PLoS One 10, e0121006.

Chau, N.D.G., Sebesvari, Z., Amelung, W., Renaud, F.G., 2015. Pesticide pollution of multiple drinking water sources in the Mekong Delta, Vietnam: evidence from two provinces. Environ. Sci. Pollut. Res. 22, 9042–9058.

Chen, B.L., Zhou, D.D., Zhu, L.Z., 2008. Transitional adsorption and partition of nonpolar and polar aromatic contaminants by biochars of pine needles with different pyrolytic temperatures. Environ. Sci. Technol. 42, 5137–5143.

Chen, M., Xu, P., Zeng, G.M., Yang, C.P., Huang, D.L., Zhang, J.C., 2015. Bioremediation of soils contaminated with polycyclic aromatic hydrocarbons, petroleum, pesticides, chlorophenols and heavy metals by composting applications, microbes and future research needs. Biotechnol. Adv. 33, 745–755.

Cheng, C.H., Lehmann, J., Engelhard, M.H., 2008. Natural oxidation of black carbon in soils: changes in molecular form and surface charge along a climosequence. Geochim. Cosmochim. Acta 72, 1598–1610.

Chiou, C.T., Cheng, J.Z., Hung, W.N., Chen, B.L., Lin, T.F., 2015. Resolution of adsorption and partition components of organic compounds on black carbons. Environ. Sci. Technol. 49, 9116–9123.

Cornelissen, G., Gustafsson, O., Bucheli, T.D., Jonker, M.T.O., Koelmans, A.A., Van Noort, P.C.M., 2005. Extensive sorption of organic compounds to black carbon, coal, and kerogen in sediments and soils mechanisms and consequences for distribution, bioaccumulation, and biodegradation. Environ. Sci. Technol. 39, 6881–6895.

Ding, G.C., Pronk, G.J., Babin, D., Heuer, H., Heister, K., Kogel-Knabner, I., Smalla, K., 2013. Mineral composition and charcoal determine the bacterial community structure in artificial soils. FEMS Microbiol. Ecol. 86, 15–25.

Fang, C.W., Radosevich, M., Fuhrmann, J.J., 2001. Characterization of rhizosphere microbial community structure in five similar grass species using FAME and BIOLOG analyses. Soil Biol. Biochem. 33, 679–682.

Gaskin, J.W., Speir, R.A., Harris, K., Das, K.C., Lee, R.D., Morris, L.A., Fisher, D.S., 2010. Effect of peanut hull and pine chip biochar on soil nutrients corn nutrient status, and yield. Agron. J. 102, 623–633.

Graber, E.R., Harel, Y.M., Kolton, M., Cytryn, E., Silber, A., David, D.R., Tsechansky, L., Borenshtein, M., Elad, Y., 2010. Biochar impact on development and productivity of pepper and tomato grown in fertigated soilless media. Plant Soil 337, 481–496.

Guo, C.L., Zhou, H.W., Wong, Y.S., Tam, N.F.Y., 2005. Isolation of PAH-degrading bacteria from mangrove sediments and their biodegradation potential. Mar. Pollut. Bull. 51, 1054–1061.

Hagemann, N., Kammann, C.I., Schmidt, H.P., Kappler, A., Behrens, S., 2017. Nitrate capture and slow release in biochar amended compost and soil. PLoS One 12, e0171214.

Hamada, M.S., Yin, Y.N., Ma, Z.H., 2011. Sensitivity to iprodione, difenoconazole and fludioxonil of Rhizoctonia cerealis isolates collected from wheat in China. Crop Prot. 30, 1028–1033.

Hasan, S.A., Jabeen, S., 2015. Degradation kinetics and pathway of phenol by *Pseudomonas* and *Bacillus* species. Biotechnol. Biotec. Equip. 29, 45–53.

Horsfield, A., Wicks, T., Davies, K., Wilson, D., Paton, S., 2010. Effect of fungicide use strategies on the control of early blight (*Alternaria solani*) and potato yield. Australas. Plant Pathol. 39, 368–375.

Hu, L., Cao, L.X., Zhang, R.D., 2014. Bacterial and fungal taxon changes in soil microbial community composition induced by short-term biochar amendment in red oxidized loam soil. World J. Microbiol. Biotechnol. 30, 1085–1092.

Jenkins, J.R., Viger, M., Arnold, E.C., Harris, Z.M., Ventura, M., Miglietta, F., Girardin, C., Edwards, R.J., Rumpel, C., Fornasier, F., Zavalloni, C., Tonon, G., Alberti, G., Taylor, G., 2016. Biochar alters the soil microbiome and soil function: results of next-generation amplicon sequencing across Europe. GCB Bioenergy 9, 591–612.

Jiang, X.Y., Denef, K., Stewart, C.E., Cotrufo, M.F., 2016. Controls and dynamics of biochar decomposition and soil microbial abundance, composition, and carbon use efficiency during long-term biochar-amended soil incubations. Biol. Fert. Soils 52, 1–14.

Jones, D.L., Edwards-Jones, G., Murphy, D.V., 2011. Biochar mediated alterations in herbicide breakdown and leaching in soil. Soil Biol. Biochem. 43, 804–813.

Jones, D.L., Rousk, J., Edwards-Jones, G., DeLuca, T.H., Murphy, D.V., 2012. Biochar-mediated changes in soil quality and plant growth in a three year field trial. Soil Biol. Biochem. 45, 113–124.

Kesavachandran, C.N., Fareed, M., Pathak, M.K., Bihari, V., Mathur, N., Srivastava, A.K., 2009. Adverse health effects of pesticides in agrarian populations of developing countries. Rev. Environ. Contam. Toxicol. 200, 33–52.

Khodadad, C.L.M., Zimmerman, A.R., Green, S.J., Uthandi, S., Foster, J.S., 2011. Taxa-specific changes in soil microbial community composition induced by pyrogenic carbon amendments. Soil Biol. Biochem. 43, 385–392.

Khorram, M.S., Wang, Y., Jin, X.X., Fang, H., Yu, Y.L., 2015. Reduced mobility of fomesafen through enhanced adsorption in biochar-amended soil. Environ. Toxicol. Chem. 34, 1258–1266.

Khorram, M.S., Zhang, Q., Lin, D.L., Zheng, Y., Fang, H., Yu, Y.L., 2016. Biochar: a review of its impact on pesticide behavior in soil environments and its potential applications. J. Environ. Sci. 44, 269–279.

Kolton, M., Harel, Y.M., Pasternak, Z., Graber, E.R., Elad, Y., Cytryn, E., 2011. Impact of biochar application to soil on the root-associated bacterial community structure of fully developed greenhouse pepper plants. Appl. Environ. Microbiol. 77, 4924–4930.

Lehmann, J., Rillig, M.C., Thies, J., Masiello, C.A., Hockaday, W.C., Crowley, D., 2011. Biochar effects on soil biota – a review. Soil Biol. Biochem. 43, 1812–1836.

Leigh, M.B., Pellizari, V.H., Uhlik, O., Sutka, R., Rodrigues, J., Ostrom, N.E., Zhou, J.H., Tiedje, J.M., 2007. Biphenyl-utilizing bacteria and their functional genes in a pine root zone contaminated with polychlorinated biphenyls (PCBs). ISME J. 1, 134–148.

Leys, N.M.E.J., Ryngaert, A., Bastiaens, L., Verstraete, W., Top, E.M., Springael, D., 2004. Occurrence and phylogenetic diversity of Sphingomonas strains in soils contaminated with polycyclic aromatic hydrocarbons. Appl. Environ. Microbiol. 70, 1944–1955.

Li, H.C., Wang, X., Ma, Y., Wen, Z.X., Li, J.Y., Zhang, H., Wu, Y.K., Lei, C.F., Wang, S.F., Wang, J.S., Geng, Z., Yang, C.Y., Li, W.D., Lu, W.G., 2013. Ecophysiological factors on phytic acid concentration in soybean seed. Crop Sci. 53, 2195–2201.

Li, Y.Q., Yang, L.Q., Sun, H.Q., Wang, X.G., Song, C., Zhou, Y.Q., Wang, N.S., Yu, C.F., Bi, Y.P., Wei, J.Y., Su, Z., 2015. Residues of four triazole fungicides in tobacco leaves under field condition and during curing. Int. J. Environ. Anal. Chem. 95, 808–815.

Lima-Morales, D., Jauregui, R., Camarinha-Silva, A., Geffers, R., Pieper, D.H., Vilchez-Vargas, R., 2016. Linking microbial community and catabolic gene structures during the adaptation of three contaminated soils under continuous long-term pollutant stress. Appl. Environ. Microbiol. 82, 2227–2237.

Liu, L., Chen, P., Sun, M.X., Shen, G.Q., Shang, G.F., 2015. Effect of biochar amendment on PAH dissipation and indigenous degradation bacteria in contaminated soil. J. Soils Sediments 15, 313–322.

Martin, S.M., Kookana, R.S., Van Zwieten, L., Krull, E., 2012. Marked changes in herbicide sorption-desorption upon ageing of biochars in soil. J. Hazard. Mater. 231, 70–78.

Munkvold, G.P., Martinson, C.A., Shriver, J.M., Dixon, P.M., 2001. Probabilities for profitable fungicide use against gray leaf spot in hybrid maize. Phytopathology 91, 477–484.

Powlson, D.S., Gregory, P.J., Whalley, W.R., Quinton, J.N., Hopkins, D.W., Whitmore, A.P., Hirsch, P.R., Goulding, K.W.T., 2011. Soil management in relation to sustainable agriculture and ecosystem services. Food Policy 36, S72–S87.

Rajkovich, S., Enders, A., Hanley, K., Hyland, C., Zimmerman, A.R., Lehmann, J., 2012. Corn growth and nitrogen nutrition after additions of biochars with varying properties to a temperate soil. Biol. Fert. Soils 48, 271–284.

Rogovska, N., Laird, D.A., Rathke, S.J., Karlen, D.L., 2014. Biochar impact on Midwestern Mollisols and maize nutrient availability. Geoderma 230, 340–347.

Sarkar, S., Seenivasan, S., Premkumar, R., 2009. Biodegradation of propiconazole by *Pseudomonas* putida isolated from tea rhizosphere. Plant Soil Environ. 55, 196–201.

Sarma, P.M., Bhattacharya, D., Krishnan, S., Lal, B.W., 2004. Degradation of polycyclic aromatic hydrocarbons by a newly discovered enteric bacterium, Leclercia adecarboxylata. Appl. Environ. Microbiol. 70 (5), 3163–3166.

Sopena, F., Semple, K., Sohi, S., Bending, G., 2012. Assessing the chemical and biological accessibility of the herbicide isoproturon in soil amended with biochar. Chemosphere 88, 77–83.

Sun, H.W., Xu, J., Yang, S.H., Liu, G.L., Dai, S.G., 2004. Plant uptake of aldicarb from contaminated soil and its enhanced degradation in the rhizosphere. Chemosphere 54, 569–574.

Sun, D.Q., Hale, L., Crowley, D., 2016. Nutrient supplementation of pinewood biochar for use as a bacterial inoculum carrier. Biol. Fert. Soils 52, 515–522.

Tong, Z.J., Jiao, T.L., Wang, F.Q., Li, M.Y., Leng, X.D., Gao, Y.L., Li, Y.P., Xiao, B.G., Wu, W.R., 2012. Mapping of quantitative trait loci conferring resistance to brown spot in flue-cured tobacco (Nicotiana tabacum L.). Plant Breed. 131, 335–339.

Wang, X., Lester, D.W., Guppy, C.N., Lockwood, P.V., Tang, C., 2007. Changes in phosphorus fractions at various soil depths following long-term P fertiliser application on a black vertosol from south-eastern Queensland. Aust. J. Soil Res. 45, 524–532.

Wang, C., Wu, Q.H., Wu, C.X., Wang, Z., 2011. Application of dispersion-solidification liquid–liquid microextraction for the determination of triazole fungicides in environmental water samples by high-performance liquid chromatography. J. Hazard. Mater. 185, 71–76.

Wen, B., Li, R.J., Zhang, S.Z., Shan, X.Q., Fang, J., Xiao, K., Khan, S.U., 2009. Immobilization of pentachlorophenol in soil using carbonaceous material

amendments. Environ. Pollut. 157, 968–974.
White, T.C., Marr, K.A., Bowden, R.A., 1998. Clinical, cellular, and molecular factors that contribute to antifungal drug resistance. Clin. Microbiol. Rev. 11, 382–402.
Wu, H.P., Zeng, G.M., Liang, J., Chen, J., Xu, J.J., Dai, J., Li, X.D., Chen, M., Xu, P., Zhou, Y.Y., Li, F., Hu, L., Wan, J., 2016. Responses of bacterial community and functional marker genes of nitrogen cycling to biochar, compost and combined amendments in soil. Appl. Microbiol. Biotechnol. 23, 1–9.
Yang, Y.N., Sheng, G.Y., Huang, M.S., 2006. Bioavailability of diuron in soil containing wheat-straw-derived char. Sci. Total Environ. 354, 170–178.
Yao, M.J., Rui, J.P., Li, J.B., Dai, Y.M., Bai, Y.F., Hedenec, P., Wang, J.M., Zhang, S.H., Pei, K.Q., Liu, C., Wang, Y.F., He, Z.L., Frouz, J., Li, X.Z., 2014. Rate-specific responses of prokaryotic diversity and structure to nitrogen deposition in the *Leymus* chinensis steppe. Soil Biol. Biochem. 79, 81–90.
Yu, X.Y., Ying, G.G., Kookana, R.S., 2009. Reduced plant uptake of pesticides with biochar additions to soil. Chemosphere 76, 665–671.
Zhuang, L.P., Liu, Y., Wang, L., Wang, W.P., Shao, Z.Z., 2015. Erythrobacter atlanticus sp nov.: a bacterium from ocean sediment able to degrade polycyclic aromatic hydrocarbons. Int. J. Syst. Evol. Microbiol. 65, 3714–3719.