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Chemical properties and microbial responses to biochar and compost amendments in the soil under continuous watermelon cropping

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ABSTRACT

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Biochar added to soil can improve crop growth and productivity. However, the mechanisms of crop growth improvement by biochar application are not well understood, particularly in the presence of soil-borne pathogens caused by continuous monocropping. Thus, a two-year field experiment was carried out to study the chemical and microbiological response of Lixisols (pH 5.8) to the amendment of biochar and its effect on watermelon productivity and *Fusarium* wilt disease incidence. Biochar was added alone or together with compost before watermelon transplanting. Mixed application of biochar with compost significantly increased watermelon yield as compared to adding compost or biochar alone. However, biochar had no effects on *Fusarium* wilt disease incidence in both years. Combined application of biochar with compost significantly increased contents of soil NH_4^+-N, available phosphorus (P) and available potassium (K). Soil Biolog data indicated that the Shannon-Weaver diversity index and evenness index were increased significantly in the combined application of biochar with the compost treatment. There was a significant positive correlation between watermelon yield and soil NH_4^+-N, available P, available K, microbial diversity or microbial evenness in the continuous watermelon monocropping system.

Keywords: soil fertility; organic amendment; microbial community; fungal disease; crop productivity

Biochar is a co-product from thermal degradation of organic material in low or zero oxygen environment (pyrolysis). Biochar can have very different properties as well as stability depending on the feedstock and generation procedures used (Graber et al. 2014). Hence, wide variations in crop productivity treated with biochar as the soil amendment have been reported in the literature. Most attention to date has focused on biochar effects on grain crops, and plant growth responses to biochar amendment varied (Gathorne-Hardy et al. 2009, Van Zwieten et al. 2010, Abrishamkesh et al. 2015). There is paucity of information of biochar effects on vegetable crops (Elmer and Pignatello 2011, Ghosh et al. 2015), indicating a need to generate robust understanding of how biochar can be effectively used in vegetable crop production. Biochar effects on crop yield have been mainly attributed to soil chemical and biological responses, including greater amounts of plant-available water (Jeffery et al. 2011), increased cation exchange capacity (CEC) and enhanced retention of basic nutrients (Lehmann et al. 2003), and greater pH and base saturation (Lehmann et al. 2003, Major et al. 2010). Moreover, biochar has also been shown to affect soil enzyme activity

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and soil microbial community composition and abundance (Lehmann et al. 2011). Recently, some studies have found that biochar amendment to soil usually increases the soil enzyme activities involved in nitrogen (N) and phosphorus (P) cycles but reduces the soil enzyme activities related to carbon (C) cycle (Bailey et al. 2011). Conversely, other studies have reported inconsistent results (Lammirato et al. 2011, Paz-Ferreiro et al. 2014), which suggests that biochar has variable effects on different soils, enzymes, and assay types. C and N cycles in soil are driven by soil microorganisms. Biochar addition to soils has been recently shown to affect the abundance of soil microorganisms and microbial community structure (Wang et al. 2015). These changes may influence nutrient cycle, which affects plant growth directly.

Fusarium wilt of watermelon, caused by *Fusarium oxysporum* f. sp. *niveum* (FON), commonly occurs in locations where the crop has been grown for a number of seasons. There is some evidence that biochar can alter the severity of diseases caused by soil-borne plant pathogens in various trees and crops (Elmer and Pignatello 2011, Zwart and Kim 2012, Jaiswal 2013, Jaiswal et al. 2014, Graber et al. 2014); however, there is no literature on the influence of biochar on any soil-borne pathogens affecting watermelon. Therefore, the aims of this study were to investigate biochar applied individually or in combination with compost to a soil in continuous watermelon cropping in order to (1) determine watermelon productivity and *Fusarium* wilt disease occurrence in the field; (2) characterize the impact on soil chemistry as well as biological characteristics through plate counting and community level physiological profiles (Biolog); and (3) elucidate the relationship between watermelon yield and soil microbiological and chemical characteristics.

MATERIAL AND METHODS

A field study was established in fall 2013 near Huai'an, Jiangsu, China (32°4'N, 118°2'E). The soil was a Lixisol. The soil texture is light loamy soil and the properties are described in Table 1. Compost and biochar chemical characteristics are presented in Table 1. The raw materials for the compost were pig manure and rice straw. Biochar, was derived from rice straw and created by fast pyrolysis at 700°C.

The experiment was set up in a randomized complete block design with three replicates and four treatments: (1) chemical fertilizer alone (F); (2) chemical fertilizer plus biochar (FB); (3) chemical fertilizer plus compost (FC); (4) chemical fertilizer plus compost and biochar (FCB). Plots were 3.3 m wide and 6.2 m long and included four planted rows and 60 plants were grown in each plot. The compost and biochar were hand-applied to the soil surface at rates of 15 t/ha and 6 t/ha, respectively. Plots received 93.7 kg N/ha, 32.7 kg P/ha and 91 kg K/ha after collecting initial soil samples but before biochar application. Topdressing to all treatments was carried out one month after transplanting. The amounts of N, P and K applied to each plot were shown in Table 2. The field was roller harrowed after watermelon harvest in 2014, and kept fallow until March 2015. The experiment was repeated from March 10, 2015 to June 10, 2015. The same amounts of fertilizer, biochar and compost were applied to appropriate plots.

Watermelon yield and *Fusarium* wilt disease incidence were recorded at harvest for each season. Disease incidence was expressed as the percentage of diseased plants over the total number of plants in each plot. Soils were sampled in late October 2014 and again in late June 2015. Soil pH and electrical conductivity (EC) were determined with a soil:water ratio of 1:2.5 (w:v ratio) using a pH electrode (LE 438, Mettler Toledo, Spain) and a conductivity indicator (DDS-307, INESA Instrument), respectively. Organic carbon was determined by oxidation with potassium dichromate. N was determined by Kjeldahl digestion.

Table 1. Selected chemical properties of biochar and compost applied to the experimental plots in 2014 and 2015 and soil properties in August 2014

Property	Unit		Biochar	Compost
Moisture	(%)	–	9.8	35
pH		5.79	10.3	7.4
Electrical conductivity	(µS/cm)	642	4389	3210
Organic carbon	(%)	1.09	37.8	17.6
N_{tot}	(g/kg)	–	1.66	10.1
N_{hydrol}	(mg/kg)	97.3	–	–
P_{avail}	(mg/kg)	132	212	168
K_{avail}	(mg/kg)	196	2824	436

– not determined

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Table 2. The amounts of nitrogen (N), phosphorus (P), potassium (K) added to different plots each year (kg/ha)

Treatment	Mineral fertilizer			Compost			Biochar		
	N	P	K	N	P	K	N	P	K
F	118	40.6	116.1	–	–	–	–	–	–
FB	109.1	40.6	116.1	–	–	–	8.9	3.2	15.2
FC	20	40.6	116.1	98	17	95.6	–	–	–
FCB	11.1	40.6	116.1	98	17	95.6	8.9	3.2	15.2

F – chemical fertilizer; FB – chemical fertilizer + biochar; FC – chemical fertilizer + compost; FCB – chemical fertilizer + compost and biochar; – not determined

Soil alkali-hydrolyzable nitrogen was distilled with 2 mol/L NaOH for 24 h, the liberated NH_3 was quantified by conductometric titration. Available P (P_{avail}) in soil was extracted by sodium bicarbonate and determined using the molybdenum blue method. Available K (K_{avail}) in soil was extracted by ammonium acetate and determined by flame photometry. Dissolved organic carbon (DOC) and dissolved organic nitrogen (DON) in soil were extracted with distilled water (1:10 w/v ratio) and were determined using a TOC/TN analyzer (multi N/C 3000, Analytik Jena AG, Germany). The NO_3^--N, NH_4^+-N concentrations were extracted by 2 mol/L KCl and measured by an AutoAnalyzer (AA3, Bran and Luebbe, Germany) (Bao 2000).

Four soil enzymes were selected for this study: β-glucosidase, fluorescein diacetate (FDA) hydrolysis, protease, and alkali phosphatase. FDA hydrolysis activity was determined according to Adam and Duncan (2001). Activity of β-glucosidase, alkali phosphatase and protease activities were determined according to Guan (1986).

Biolog Eco microliter plates (Matrix Technologies Corporation) were used to determine the nutritional versatility of microbial metabolic potential from the various treatments. The Biolog analysis was conducted according to Liu et al. (2015). The adjusted absorbance was analysed by principal components analysis (PCA) based on a correlation matrix using the Canoco software (Microcomputer Power, Ithaca, USA).

One-way analysis of variance was used to assess differences in soil chemical properties between treatments. Pearson's correlation coefficients were calculated using the SPSS to determine the relationship between watermelon yield and soil chemical properties. The normalized carbon source utilization data were subjected to principal components analysis (PCA) to reduce complex multidimensional data and to allow for a more straightforward interpretation of results. Functional diversity from the Biolog data was evaluated by calculating the Shannon's substrate diversity index (H) and Shannon's evenness index (E). All the measurements reported refer to the 96 h time point according to Liu et al. (2015).

RESULTS AND DISCUSSION

Generally, the nutrient availability and the watermelon yield were largely affected by the application of treatments, significantly in the short-term. However, these significant effects were more pronounced in mixed than single treatment applications. Treatment effects on soil nutrient contents in June 2015 are shown in Table 3. Mineral fertilizer application alone decreased the pH of soil significantly. FC and FCB treatments increased organic C 1.23- and 1.20-fold, respectively, compared to the control. The FCB treatment contained the greatest quantity of alkali-hydrolyzable N (95.8 mg/kg). Relative to the control, FCB increased NH_4^+-N and NO_3^--N 1.3-fold, 1.8-fold, respectively, while compost or biochar alone produced no significant increase in NH_4^+-N. FCB treatment increased soil available K and available P 1.7-fold, 1.3-fold, respectively as compared to the control (Table 3).

Watermelon yields in 2014 and 2015 were significantly affected by the amendments applied. In general, watermelon yield was greater in 2015 than that in 2014. Mixed application of biochar with compost significantly increased watermelon yield as compared to adding compost or biochar alone (Figure 1). Specifically, NH_4^+-N, P_{avail}, K_{avail} contents in soil were positively and significantly correlated with watermelon yield [$r = 0.626$ ($P < 0.05$), $r = 0.726$ ($P < 0.05$), $r = 0.890$ ($P < 0.01$), respectively] (Figure 2). These results are consistent with many previous reports (Lehmann et al. 2003,

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Table 3. Mean soil (0–20 cm depth) pH, electrical conductivity, organic carbon, alkali-hydrolyzable nitrogen (N_{hydrol}), available K (K_{avail}), available P (P_{avail}), NH_4^+-N, NO_3^--N, dissolved organic carbon (DOC) and dissolved organic nitrogen (DON) after the second harvest (*n* = 3)

Treatment	pH	EC (μS/cm)	Organic C (%)	N_{hydrol}	NH_4^+-N	NO_3^--N	K_{avail}	P_{avail}	DOC	DON
				(mg/kg)						
F	6.02^b	841^b	0.83^c	84.9^b	39.6^b	84.4^c	127^c	89.9^c	60.2^b	49.9^c
FB	6.67^a	1094^a	0.99^b	91.5^{ab}	59.9^b	215.9^a	176^b	105.7^b	67.72^b	189.3^a
FC	6.55^a	1165^a	1.19^a	92.9^{ab}	47.7^b	160.6^b	185^b	102.7^b	69.43^{ab}	126.7^b
FCB	6.66^a	1099^a	1.22^a	95.8^a	67.6^a	152.6^b	212^a	120.5^a	74.84^a	118.4^b

F – chemical fertilizer; FB – chemical fertilizer + biochar; FC – chemical fertilizer + compost; FCB – chemical fertilizer + compost and biochar. Values with the same letters are not significantly different at the 5% level

Van Zwieten et al. 2010), where the application of biochar was shown to increase crop production and fertility in soils. The increased yield of watermelon in plots that received mixed biochar and compost treatments, might be due to the potentially reduced nutrient leaching and increased the nutrient holding capacity of the soil (Partey et al. 2014).

Continuous cropping of watermelon led to sever occurrence of *Fusarium* wilt. The average disease incidence was 79.7% in 2014 and 69.8% in 2015. However, *Fusarium* wilt disease incidence in both years exhibited no significant difference among treatments (Figure 3). The results indicated that the rice residue-derived biochar had no significant effects on the severity of *Fusarium* wilt disease of watermelon caused by FON. However, Akhter et al. (2016) evaluated the response of *Fusarium oxysporum* f. sp. *lycopersici* chlamydospores to tomato plants grown in biochar amended soil, and found that soil amendment with garden waste biochar exhibit a great potential in suppressing *Fusarium* chlamydospore infectivity in tomato plants (Akhter et al. 2016). Until now, biochar soil amendment has been reported to affect the progress of diseases caused by soil-borne pathogens in six distinct pathosystems (Graber et al. 2014). The discrepancy of plant disease intensity between different studies could be due to the differences in biochar precursor, dose of addition, or the soil-pathogen-plant system (Graber et al. 2014).

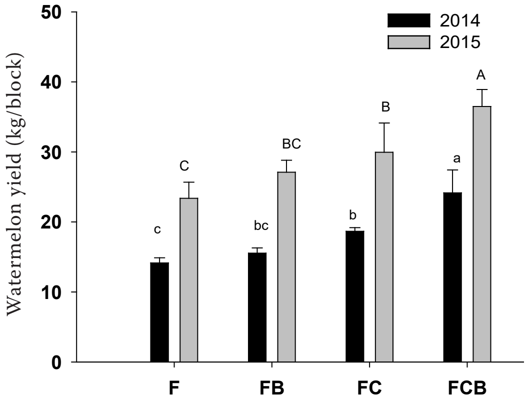

Figure 1. Watermelon yield in 2014 and 2015 of different treatments (*n* = 3). Bars with the same letter for each season are not significantly different at $P < 0.05$. F – chemical fertilizer; FB – chemical fertilizer + biochar; FC – chemical fertilizer + compost; FCB – chemical fertilizer + compost and biochar

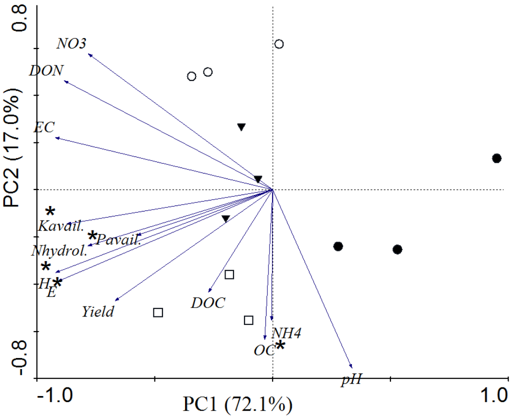

Figure 2. Principal component analysis (PCA) diagram showing correlation beween the soil variables and watermelon yield after second harvest (● – F; ○ – FB; ▼ – FC; □ – FCB). Arrows with * have a significant correlation with the watermelon yield. OC – organic carbon; N_{hydrol} – alkali-hydrolyzable N; DOC – dissolved organic carbon; DON – dissolved organic nitrogen; P_{avail} – available phosphorus; K_{avail} – available potassium; EC – electric conductivity; H – Shannon-Weaver index of diversity; E – Shannon-Weaver index of evenness

Soil extracellular enzymes are the proximate agents of organic matter decomposition and nutrient cycle (Nannipieri et al. 2002). All of the

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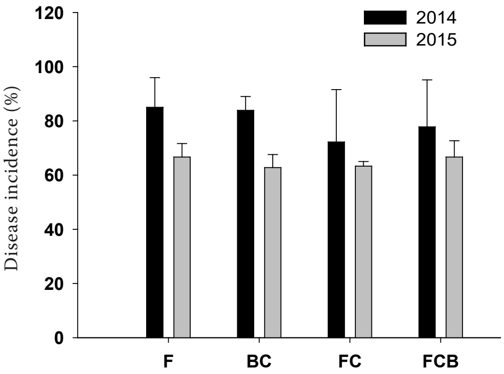

Figure 3. Watermelon *Fusarium* wilt disease incidences in 2014 and 2015 of different treatments ($n = 3$). F – chemical fertilizer; FB – chemical fertilizer + biochar; FC – chemical fertilizer + compost; FCB – chemical fertilizer + compost and biochar

selected soil enzyme activities were affected by any of the soil amendment (Table 4). Potential activities of β-glucosidase and FDA hydrolysis were the highest in plots receiving compost plus biochar. Mixed application of biochar with compost significantly increased the activities of β-glucosidase and FDA hydrolysis 1.5-fold, 1.3-fold, respectively, over F. The activity of soil alkali phosphatase in FCB treatment was more than doubled over CF. Adding biochar alone increased the activities of β-glucosidase, protease and alkali phosphatase, although the differences were not significant. These results indicate that biochar addition could increase the activities of soil enzymes involved in C, N and P cycles, and combined addition of compost and biochar has more significant effects as compared to F. Similar results were reported by Wang et al. (2015), who stated that addition of maize biochar C at 0.5% could increase the activities of soil enzymes involved in C and N cycles. Conversely, alkali phosphatase activity decreased with increasing maize biochar addition rate. In low fertility tropical soils, the enzymes involved in C, N and P cycles were significantly increased by biochar addition, however, for the more fertile soil, biochar addition resulted in lower phosphomonoesterase activity (Paz-Ferreiro et al. 2014). The inconsistent influence of biochar on enzymatic activities might be due to its high dependence on soil type. The increased activity of β-glucosidase and FDA hydrolysis could be either due to stimulation of a specialized subset of the microbial community by the biochar or growth of biomass in response to initially labile C (Kolb et al. 2009, Bailey et al. 2011, Qayyum et al. 2014). The increase in alkali phosphatase activity by biochar amendment could have been due to a chemical enhancement of enzyme function caused by the interaction with biochar (Jindo et al. 2012). In our study, the addition of biochar reduced protease activity in soil, which may be due to a decreased availability of inorganic N (Chintala et al. 2014). Protease activity was different from the previous study reported by Oleszczuk et al. (2014). Possible reasons for the inconsistent results might be due to the different treatments of soils and the types of biochar (Lehmann et al. 2003).

The physico-chemical properties of biochar, as well as the biochar-induced changes in soil physico-chemical properties can alter the activities of soil microorganisms (Lehmann et al. 2011). Total bacterial (7.85-fold) and actinomycetic (~ 3-fold) counts were greater in FCB treatments as compared to F and FB (Table 5). The AWCD values in Biolog assays in the F and FB were lower than those in FC and FCB. The FCB treatment had the highest AWCD compared to all other treatments during the 168 h of the Biolog culture (Figure 4a). The promotion of microbial populations and Biolog AWCD values after biochar and compost application might reflect the improved nutritional conditions (Watzinger et al. 2014, Hale et al. 2015).

Biochars are frequently reported to promote the microbial community structure of soils, which is expected to result in a shift in the bacterial and

Table 4. Mean soil (0–20 cm depth) enzyme activities (µg product/g/h) after the second harvest ($n = 3$)

Treatment	β-glucosidase	Fluorescein diacetate hydrolysis	Protease	Alkali phosphatase
F	13.47^{c}	22.79^{b}	66.42^{ab}	47.05^{c}
FB	14.13^{bc}	22.15^{b}	77.19^{a}	56.41^{bc}
FC	17.56^{ab}	21.95^{b}	67.39^{ab}	64.21^{b}
FCB	21.25^{a}	28.77^{a}	61.14^{b}	123.06^{a}

F – chemical fertilizer; FB – chemical fertilizer + biochar; FC – chemical fertilizer + compost; FCB – chemical fertilizer + compost and biochar

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Table 5. Mean populations of soil culturable microorganisms and microbial diversity and evenness after the second harvest (n = 3)

Treatment	Bacteria × 10^6 CFU/g	Actinomycetes × 10^5 CFU/g	Fungi × 10^3 CFU/g	*Fusarium oxysporum* × 10^3 CFU/g	Shannon diversity index	Shannon evenness index (E)
F	1.23^{b}	1.65^{b}	5.36^{a}	3.39^{a}	0.62^{c}	0.17^{c}
FB	4.85^{ab}	1.33^{b}	3.67^{a}	2.13^{a}	1.08^{b}	0.36^{b}
FC	5.88^{ab}	4.60^{a}	4.84^{a}	3.81^{a}	1.21^{ab}	0.40^{ab}
FCB	9.66^{a}	4.81^{a}	7.38^{a}	2.01^{a}	1.56^{a}	0.57^{a}

F – chemical fertilizer; FB – chemical fertilizer + biochar; FC – chemical fertilizer + compost; FCB – chemical fertilizer + compost and biochar

fungal community structure. In the current study, Biolog data after 96 h incubation were subjected to principal component analysis (PCA) for further determination of functional diversity indices of the microbial communities under different treatments, as shown in Figure 3. Communities separated along PC1 according to whether they had received compost or not. Communities from F and FB soils grouped along the positive regions of PC1, and clearly separated from FC and FCB plots (Figure 4b). Shannon-Weaver diversity and evenness indices, which were positively and significantly correlated with watermelon yield (r = 0.696 (P < 0.05), r = 0.716 (P < 0.05), respectively) (Figure 2), were significantly increased in FCB treatments (Table 4). It is likely that the specific chemical properties of biochar allowed the development of highly specialized bacteria that were not dominant in the soil (F) or in the compost (FC) (Atkinson et al. 2010), as shown by the higher bacterial abundance in the presence of biochar in soil (Doan et al. 2014). The results of this study demonstrated a positive synergistic effect of applying rice residue-derived, fast-pyrolysis biochar with compost to a Lixisol. As inferred from the study, biochar interaction with compost was more evident than the interaction with chemical fertilizers in relation to watermelon yield and soil nutrient supply. The positive effect of biochar on crop growth could result from higher microbial abundance, activity and diversity, which may cause a significant change in nutrient cycle activities and N, P, K availability. However, little effect on *Fusarium* wilt disease incidence was observed after biochar addition alone or in combination with compost, hence, if growers wish to acquire high and stable production in a continuous cropping soil, an integrated agricultural management including pretreatment of soil to reduce the background value of FON in soil, together with soil organic amendment should be introduced.

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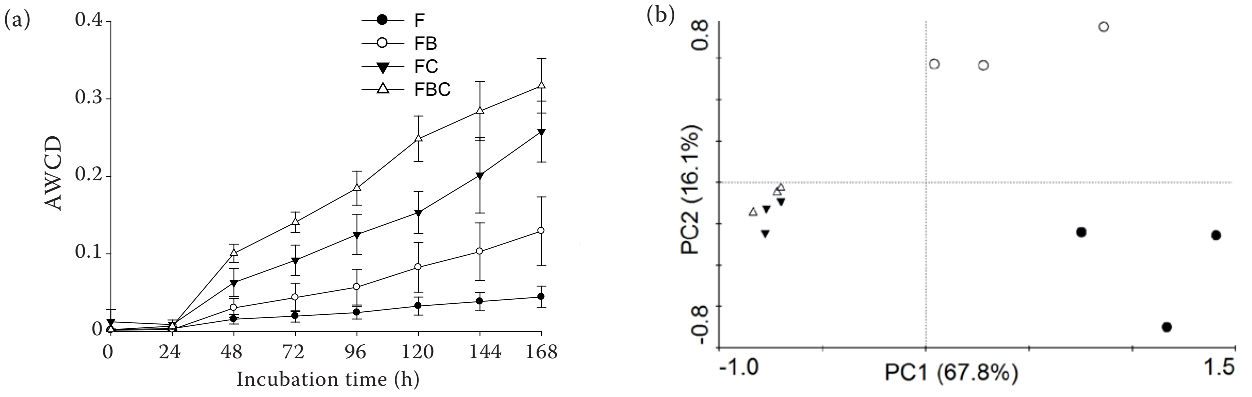

Figure 4. Average well color development (AWCD) for different treatments (a) and plot of principal components analysis of substrate utilization profiles for soil microbial communities exposed to various treatments (b)

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