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RESEARCH

Effects of biochar on seedling root growth of soybeans

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ABSTRACT

Biochar application is an effective method for agriculture production. In order to study the biochar effect on soybean (*Glycine max* [L.] Merr.) seedling root growth, this study was conducted in a sand culture experiment using two soybean cultivars with different P efficiencies. Soybean was pot-grown at four biochar rates (0%, 0.15%, 0.75% and 1.5%, w/v). The results showed no difference in Cultivar × Biochar interaction on root morphologic characteristics. At 7 d after germination (DAG), biochar slightly enhanced root growth; at 10 DAG, biochar significantly increased total root length (TRL) and total root surface area (TRSA) highest by 48.4% and 27.4% ($P < 0.05$) at 1.5% biochar rate, respectively, compared to the control. The positive effects on root morphology by biochar were especially concentrated on fine roots (< 0.5 mm). In addition, root vitality and leaf soluble sugar content were significantly increased by both 0.75% and 1.5% biochar; shoot biomass increased maximally by 65.6% at 1.5% biochar rate compared to the control; inversely, root/shoot ratio significantly reduced by 32.3% and 23.5% at 1.5% biochar rate, respectively, at 7 and 10 DAG. We suggest that biochar had positive effects on soybean seedling growth through improving root morphology and root vitality, regardless of different P efficient cultivars.

Key words: Dry matter production, *Glycine max*, RAD, root vitality, TRL, TRSA.

INTRODUCTION

The application of biochar to soil is an effective method for improving soil quality that has been widely employed in recent years. As biochar has large porosity and high specific surface area (Braida et al., 2003), it has strong adsorption ability. In addition, biochar contains Ca^{2+}, K^{+}, Mg^{2+} and other base ions, which can improve soil base saturation and adjust soil pH values (Chen et al., 2013). The physical and chemical properties of biochar are conducive to improving root nodule N fixation, nutrient retention in farmland soil, nutrient utilization and microbial habitat while also reducing the excessive loss of some nutrients from the soil. Although biochar is widely applied in agriculture, its effects differ depending on soil type and plant variety (Asai et al., 2009; Uzoma et al., 2011; Noguera et al., 2012). Biochar has various effects on crop growth in different soil types, and soil type also affects the application level and function of biochar (Macdonald et al., 2014). Crane-Droesch et al. (2013) performed a detailed statistical analysis of the effects of biochar on crop yields in different soil types, finding that soil cation exchange capacity and organic C content are closely related to changes in crop yields, which depend on soil type. In addition, different plant species react differently to biochar: Kloss et al. (2014) found that the biomass of mustard, barley and alfalfa varies to different degrees in the presence of biochar.

Previous studies have compared the effects of biochar on different plant species but not between different varieties of the same plant species. Soybean (*Glycine max* [L.] Merr.) is an important crop for food and oil production. Different soybean varieties have different levels of P absorption and utilization efficiency, which leads to huge differences in growth in response to the soil environment (Ao et al., 2014). Agricultural crops absorb almost all water and nutrients through the root system, root morphology traits and root vitality are a reflection of the plant's capacity for water and nutrient absorption, and it is also used as an indicator of root development (Dai et al., 2017). Thus, roots play an important role in crop growth and yield formation (Min et al., 2014). When biochar is applied to the soil, it comes in close contact with the plant root and has a direct effect on root growth, thereby affecting root morphology (Olmo et al., 2016), which in turn has a profound impact on the growth of the plant shoot. Therefore, it is important to investigate the effects of biochar on plant root growth. Root morphology changes with different soil conditions, such as soil fertility, soil bulk density and soil water content (Souza et al., 2016; Li et al., 2017). Biochar has special properties, and its existence in soil could influence plant growth. To explore the primal reaction in root and compare the difference between crop varieties, in this study, we used two soybean cultivars with different P efficiencies grown in quartz sand (to avoid the interference of soil type) to investigate root morphological growth and production in soybean at seedling stage, in order to explore the plant's responses to the application of biochar at early growth stage.

MATERIALS AND METHODS

Plant material and experimental conditions

The present study was carried out in the greenhouse facility in Shenyang, Liaoning province, China, in 2017. A sand culture experiment was conducted with two different soybean varieties with different P efficiencies: P-efficient soybean 'Liaodou 13' and P-inefficient soybean 'Tiefeng 3'. The culture pots comprised PVC pipes (150 mm diameter, 250 mm height) equipped with pored bottom and filled with quartz sand (2-3 mm particle size, with no mineral nutrients used). The biochar, which was produced by Liaoning Jin He Fu Agriculture Development Company, was derived from pyrolysis of rice husks at 450 °C for 1 to 2 h (Chen et al., 2012). Biochar had a pH of 8.4 (1:5 biochar/water ratio), containing 6.7 mg g^{-1} total N, 37.2 mg kg^{-1} alkaline N, 0.9 mg g^{-1} total P, 61.3 mg kg^{-1} available P, and 1.3 mg g^{-1} available K.

The experiment involved four treatments: CK, B1, B2 and B3, representing an amount of biochar per pot volume of 0%, 0.15%, 0.75% and 1.5% (w/v, g cm^{-3}), respectively. A randomized block design was utilized, and each treatment was performed with three replicates. Biochar at the desired rate was mixed into the quartz sand. Before the experiment, quartz sand was washed thoroughly with distilled water, and quartz sand or mixed medium was placed into each culture container to a height of 23 cm. Soybean was sown using four seeds per pot, which was thinned to two plants per pot after emergence. After the seed was sown, distilled water was gently poured onto the medium twice per day at 08:00 h and 16:00 h, before germination (even days after sowing, as the cotyledons came out from the quartz sand). After the cotyledons just unfolded, the plants were watered daily with nutrient solution at 08:00 h and distilled water (pH 4.5, to remove excess salt) at 16:00 h. The pH of the nutrient solution was adjusted to 6. Both nutrient solution and distilled water were applied at a rate of 500 mL per pot respectively. The nutrient solution formula was based on the formula described by Villagarcia et al. (2001) and was modified according to the results of our preliminary experiments: 3.6 mM $CaSO_4·2H_2O$, 2 mM KNO_3, 18 μM KCl, 250 μM $MgSO_4·7H_2O$, 18 μM $FeSO_4·7H_2O$, 9.3 μM H_3BO_3, 0.9 μM $MnSO_4·H_2O$, 0.9 μM $ZnSO_4·7H_2O$, 0.18 μM $CuSO_4·5H_2O$ and 0.18 μM $(NH_4)_6$ $MO_7O_{24}·4H_2O$.

Sampling and measurement

Samples were harvested at two different growth stages of seedling: 7 d after germination (DAG), with one trifoliate, fully expanded compound leaf; and 10 DAG, with two trifoliate, fully expanded compound leaves. Two plants were harvested and pooled in each pot to represent a replicate. The roots were gently removed from the medium and washed with water to remove the quartz sand and biochar. The root and shoot were then separated from the cotyledonary node. An image analysis system (WinRHIZO Pro LA2400; Regent Instruments Canada Inc., Quebec, Canada) was used to scan and analyze root morphological traits, whole root systems were placed on a scanner (Epson Expression 10000XL; Seiko Epson Corporation, Suwa, Nagano, Japan), in a transparent plastic tray filled with water. The root morphological variables included total root length (TRL), total root surface area (TRSA), root average diameter (RAD) and total root volume (TRV). To investigate the effects of biochar on the distribution of soybean root diameter class, soybean roots were grouped into five classes according to root diameter: 0.0-0.5, 0.5-1.0, and > 3.0 mm. We analyzed the values and distributions of RL, RSA, and RV in each class.

After scanning, the root and shoot were dried to a constant weight at 80 °C for biomass and carbohydrate content measurements.

Chemical analysis

Root vitality was performed according to the reducing ability of dehydrogenase, it was measured by using the triphenyltetrazolium chloride (TTC) method as described by Clemensson-Lindell (1994) with some modifications. Root tip samples (0.2 g, 1.0 cm length from the top) were placed into test tubes, followed by the addition of 10 mL 0.4% (w/v) TTC in 0.06 M Na_2HPO_4-KH_2PO_4. The samples were vacuum-infiltrated for 10 min before being incubated at 37 °C for 2 h in the dark, followed by the addition of 2 mL 1 M H_2SO_4 to terminate the reaction. The samples were then extracted in 10 mL 100% (v/v) methanol at 30 °C for 7 h. The absorbances were recorded at 484 nm using an ultraviolet spectrophotometer (UV-7200; Shimadzu, Kyoto, Japan).

Dry samples were placed into a tube with 4 mL 80% ethanol and heated for 30 min in a boiling water bath. After cooling, the solution was centrifuged for 10 min at 3000 rpm and the supernatant was collected to quantify the soluble sugars with anthranone-H_2SO_4 in an ultraviolet spectrophotometer (U-3900, Hitachi High-Tech Science Corporation, Tokyo, Japan) at a wavelength of 620 nm, using glucose as a standard (Zhu et al., 2014). Sucrose content was estimated by the resorcinol method (Cardini et al., 1955): after destroying the fructose by heating 10 min at 100 °C in 0.01 M NaOH, 0.1% (m/v) resorcinol and 10 N HCl were added to the tube, which was then heated for 10 min at 100 °C. The cooled solution was used to determine sucrose content via a colorimetric assay.

Statistical analysis

The data were analyzed using SPSS 22.0 software (IBM, Armonk, Nueva York, USA) with a two-way ANOVA, with four biochar treatments and two soybean cultivars as the independent variables. Treatment means were compared by Tukey's test at $P \leq 0.05$.

RESULTS

Soybean root morphology

At 7 DAG, TRL and TRSA were affected individually by cultivar and biochar significantly (Table 1). 'Liaodou 13' had higher RAD, but lower TRL and TRSA than did 'Tiefeng 3'. Biochar additions significantly increased soybean TRL and TRSA at B2 treatment, respectively, by 25.6% and 21.9%, compared with the control. There was a significant Cultivar × Biochar interaction in TRV, as the TRV of 'Tiefeng 3' but not 'Liaodou 13' was significantly increased under B2 treatment.

At 10 DAG, there were no differences in cultivars and Cultivar × Biochar interaction, root morphology was significantly affected by biochar additions. In general, higher biochar rate induced higher TRL, TRSA and TRV. 'Liaodong 13' TRL significantly increased by 16.1%, 23.7% and 52.1% relative to control TRL, respectively with B1, B2 and B3; and that of 'Tiefeng 3' accordingly increased by 24.8%, 28.7% and 44.6%. The TRSA significantly increased under B3 treatment by 31.5% and 23.2% relative to control TRSA, respectively, for 'Liaodou 13' and 'Tiefeng 3'. In contrast, the RAD values of both soybean varieties were obviously reduced under all biochar treatments compared to the control. The lowest RAD was observed under B2, with reductions of 17.5% and 23.8%, respectively, for 'Liaodou 13' and 'Tiefeng 3'.

Distributions of root length (RL), root surface area (RSA) and root volume (RV) in various diameter classes

There were nonsignificant Cultivar × Biochar interactions for root morphology in various diameter classes. Biochar addition increased RL, RSA, and RV in 0.0-0.5 and 0.5-1.0 mm diameter, and the enhancement was pronounced at 10 DAG (Table 2, Figure 1). At 10 DAG, in the 0.0-0.5 mm diameter class, higher biochar rate always induced higher RL, RSA and RV, with a highest RL increment of 80.0% under B3 treatment relative to the control; while in 0.5-1.0 mm diameter class, the according values slightly reduced in B2 treatment, but remained peak at B3 treatment. Overall, the main effects of biochar on soybean root morphology concentrated on 0.0-1.0 mm root diameter class, which indicated that biochar addition increased the growth of fine root, and also improved the ratio of fine/coarse root.

In other words, the allocation proportions of RL, RSA, and RV were different in various diameter classes. In general, over 50% RL was in 0.0-0.5 mm diameter, and 0.5-1.0 mm diameter roots occupied about 50% of RSA, and over 40% of RV totally. Biochar addition increased root allocation proportion in 0.0-0.5 mm diameter class, but reduced that in > 1.0 mm diameter class (Table 3). Totally, the allocation proportion in 0.0-0.5 mm diameter class significantly increased with biochar addition by 5.1%, 16.9%, and 29.2%, respectively, of RL, RSA, and RV, at 7 DAG; and accordingly increased by 24.5%, 45.1%, and 60.3% at 10 DAG.

Table 1. Effects of biochar on root morphology in soybean varieties with different P efficiencies at 7 d after germination (DAG) and 10 DAG.

	Cultivar	Treatment	TRL	RAD	TRSA	TRV
			cm	mm	cm^2	cm^3
7 DAG	Liaodou 13	CK	295.7 ± 3.85b	0.67 ± 0.04a	62.66 ± 4.32a	1.06 ± 0.14a
		B1	307.19 ± 27.30b	0.66 ± 0.06a	63.86 ± 3.85a	1.06 ± 0.12a
		B2	372.33 ± 22.70a	0.59 ± 0.03a	69.51 ± 6.74a	1.03 ± 0.15a
		B3	340.21 ± 14.88ab	0.6 ± 0.01a	62.36 ± 3.64a	0.92 ± 0.07a
		Average	328.86B	0.63A	64.60B	1.02A
	Tiefeng 3	CK	334.94 ± 27.88b	0.58 ± 0.02a	60.99 ± 3.41b	0.88 ± 0.04b
		B1	374.97 ± 6.34ab	0.58 ± 0.03a	68.35 ± 2.50b	0.99 ± 0.09b
		B2	419.41 ± 19.28a	0.62 ± 0.01a	81.04 ± 4.41a	1.25 ± 0.09a
		B3	372.73 ± 53.44ab	0.58 ± 0.03a	67.26 ± 6.48b	0.97 ± 0.05b
		Average	375.51A	0.58B	69.41A	1.02A
	ANOVA (P value)	Cultivars (C)	0.001	0.005	0.022	0.896
		Biochar rate (B)	0.001	0.174	0.001	0.017
		C×B	0.684	0.021	0.150	0.024
10 DAG	Liaodou 13	CK	405.94 ± 15.36c	0.63 ± 0.06a	80.1 ± 5.58b	1.26 ± 0.20ab
		B1	470.88 ± 37.90b	0.54 ± 0.01b	79.5 ± 5.53b	1.07 ± 0.07b
		B2	502.11 ± 5.93b	0.52 ± 0.00b	81.28 ± 0.98b	1.05 ± 0.01b
		B3	617.46 ± 16.40a	0.54 ± 0.03b	105.31 ± 2.70a	1.43 ± 0.1a
		Average	499.10A	0.56A	86.55A	1.20A
	Tiefeng 3	CK	384.51 ± 39.71c	0.63 ± 0.02a	76.32 ± 10.25b	1.21 ± 0.20a
		B1	479.97 ± 17.00b	0.54 ± 0.02b	82.17 ± 5.49b	1.12 ± 0.11a
		B2	502.47 ± 26.91ab	0.51 ± 0.02b	80.08 ± 1.60b	1.02 ± 0.02a
		B3	555.83 ± 19.46a	0.54 ± 0.02b	94.04 ± 5.37a	1.27 ± 0.12a
		Average	478.79A	0.55A	81.85A	1.13A
	ANOVA (P value)	Cultivars (C)	0.058	0.55	0.086	0.198
		Biochar rate (B)	< 0.001	< 0.001	< 0.001	0.002
		C×B	0.116	0.685	0.317	0.56

Values are means of three replicates. Means within a column followed by different lower-case letters are significantly different at the 0.05 probability level between biochar rates; means with different uppercase letters are significantly different at 0.05 probability level between two soybean cultivars.
CK, B1, B2 and B3, represents biochar rate of 0%, 0.15%, 0.75% and 1.5% (w/v, g cm^{-3}), respectively. TRL: Root total length; RAD: root average diameter; TRSA: root total surface area; TRV: root total volume.

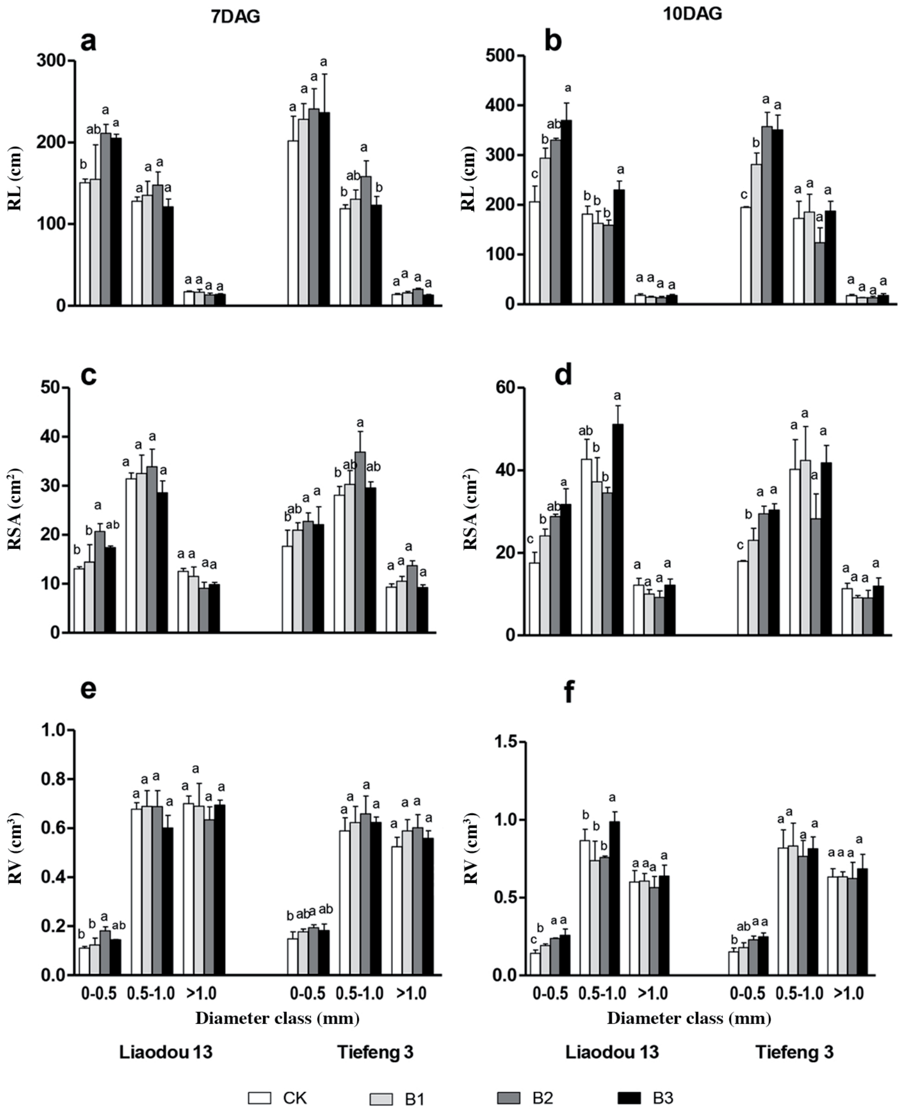
Table 2. Effects of cultivars (C), biochars (B) and their interaction (C×B) on the distribution of root length (RL), root surface area (RSA) and root volume (RV) in different root diameter classes at 7 d after germination (DAG) and 10 DAG.

	Root diameter class (mm)	Cultivars (C)		Biochars (B)		C×B	
		7 DAG	10 DAG	7 DAG	10 DAG	7 DAG	10 DAG
RL, cm	0.0-0.5	0.001	0.686	0.019	< 0.001	0.500	0.381
	0.5-1.0	0.953	0.146	0.002	0.003	0.599	0.144
	> 1.0	0.298	0.881	0.409	0.063	0.067	0.863
RSA, cm^2	0.0-0.5	< 0.001	0.705	0.002	< 0.001	0.460	0.807
	0.5-1.0	0.726	0.181	0.006	0.002	0.236	0.176
	> 1.0	0.373	0.595	0.445	0.053	0.056	0.929
RV, cm^3	0.0-0.5	< 0.001	0.542	0.001	< 0.001	0.335	0.798
	0.5-1.0	0.511	0.224	0.019	0.002	0.080	0.214
	> 1.0	0.574	0.313	0.647	0.09	0.093	0.972

Root vitality

Root vitality in both soybean varieties increased with increasing biochar application rate, and significant increments were found in B2 and B3 treatments (Figure 2). At 7 DAG, the root vitality of 'Liaodou 13' increased by 226.4% and 384.7% relative to the control, respectively, in B2 and B3 treatment; and that of 'Tiefeng 3' accordingly increased by 109.7% and 225.7%. At 10 DAG, the root vitality of 'Liaodou 13' increased by 135% in B2 treatment relative to the control; and that of 'Tiefeng 3' increased by 50% and 92.7%, respectively, in B2 and B3 treatment.

Figure 1. Effects of biochar on the distribution of root length (RL), root surface area (RSA) and root volume (RV) of different P efficient soybeans in different root diameter classes at 7 d after germination (DAG) and 10 DAG.



Diameter classes include: 0.0-0.5 mm, 0.5-1.0 mm and > 1.0 mm. Different letters above vertical bars indicate significant difference ($P < 0.05$) between biochar rates.
a: RL at 7 DAG, b: RL at 10 DAG, c: RSA at 7 DAG, d: RSA at 10 DAG, e: RV at 7 DAG, f: RV at 10 DAG.
CK, B1, B2 and B3, represents biochar rate of 0%, 0.15%, 0.75% and 1.5% (w/v, g cm^{-3}), respectively.

Sucrose and soluble sugar content in leaves and roots

Sucrose is the main transported form of plant photosynthesis. Sucrose produced in the leaf would be transported to non-photosynthetic tissues to provide plant energy. Slight difference in sucrose content was found between soybean cultivars (Table 4). There was a significant Cultivar × Biochar interaction for leaf sucrose content at 10 DAG, as biochar addition significantly increased it for ‘Tiefeng 3’, but not for ‘Liaodou 13’. The increment was 57.2% and 97.9% relative to the control, respectively, under B2 and B3 treatment.

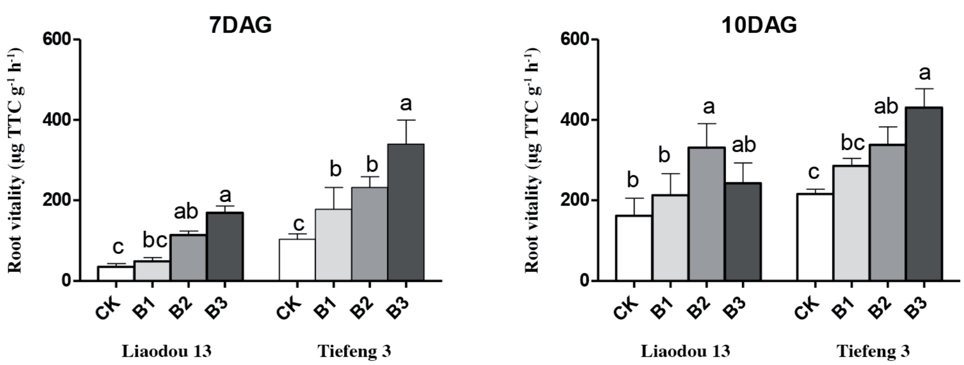
‘Tiefeng 3’ had much higher soluble sugar content in leaf and root than did ‘Liaodou 13’ at 7 DAG (Table 5). While at 10 DAG, root soluble sugar content of ‘Tiefeng 3’ sharply decreased, and was significantly lower than that of ‘Liaodou 13’. There were significant differences in biochar treatments and Cultivar × Biochar interactions for leaf soluble sugar

Table 3. Effects of biochar on root allocation proportion in various diameter classes.

Stage	Cultivar	Treatment	RL 0.0-0.5	RL 0.5-1.0	RL > 1.0	RSA 0.0-0.5	RSA 0.5-1.0	RSA > 1.0	RV 0.0-0.5	RV 0.5-1.0	RV > 1.0
			%			%			%		
7 DAG	Liaodou 13	CK	50.95	43.21	5.84	22.92	55.11	21.97	6.95	42.65	50.40
		B1	50.45	44.07	5.48	24.71	55.62	19.67	8.24	45.85	45.91
		B2	56.71	39.71	3.58	32.49	53.23	14.28	12.93	48.99	38.08
		B3	60.27	35.58	4.15	31.18	51.17	17.65	10.78	44.87	44.35
	Tiefeng 3	CK	60.29	35.51	4.20	32.08	50.99	16.93	11.78	46.63	41.59
		B1	60.91	34.80	4.29	33.92	49.04	17.04	12.73	44.86	42.41
		B2	57.47	37.70	4.83	31.01	50.28	18.71	11.03	43.25	45.72
		B3	63.39	33.06	3.55	36.31	48.53	15.16	13.38	45.69	40.93
10 DAG	Liaodou 13	CK	50.78	44.70	4.52	24.25	58.88	16.87	8.29	50.73	40.98
		B1	62.42	34.53	3.05	33.81	52.13	14.06	12.50	48.00	39.50
		B2	65.78	31.62	2.60	39.67	47.58	12.75	16.29	45.04	38.67
		B3	59.91	37.22	2.87	33.37	53.77	12.86	12.99	49.76	37.25
	Tiefeng 3	CK	50.63	44.88	4.49	25.85	57.87	16.28	9.44	51.08	39.48
		B1	58.55	38.69	2.76	30.90	56.85	12.25	11.58	53.87	34.55
		B2	69.10	28.06	2.84	44.13	42.27	13.60	17.43	42.87	39.70
		B3	63.06	33.70	3.24	36.10	49.71	14.19	14.18	46.59	39.23

Values are means of three replicates. Means within a column followed by different lower-case letters are significantly different at the 0.05 probability level between biochar rates; means with different uppercase letters are significantly different at 0.05 probability level between two soybean cultivars.
CK, B1, B2 and B3, represents biochar rate of 0%, 0.15%, 0.75% and 1.5% (w/v, g cm^{-3}), respectively. RL: Root length; RSA: root surface area; RV: root volume, refers to the root volume value within different root diameter classes; proportion refers to the percentage of root volume to total root volume within a certain diameter class.

Figure 2. Effects of biochar on root vitality (mean ± SD, n = 3) in soybean varieties with different P efficiencies at 7 d after germination (DAG) and 10 DAG.



Different letters above vertical bars indicate significant difference (P < 0.05) between biochar rates.
CK, B1, B2 and B3, represents biochar rate of 0%, 0.15%, 0.75% and 1.5% (w/v, g cm^{-3}), respectively.
TTC: Triphenyltetrazolium chloride.

content at 7 and 10 DAG. B3 treatment increased ‘Liaodou 13’ leaf soluble sugar content by 23% and 38.6%, respectively, at 7 and 10 DAG; while B2 treatment accordingly increased ‘Tiefeng 3’ leaf soluble sugar content by 22.9% and 30.9%. In addition, at 7 DAG, biochar addition significantly increased root soluble sugar content by 45.3% and 24.0%, respectively for ‘Liaodou13’ and ‘Tiefeng 3’.

Plant DM production

Biochar displayed significant effects on root dry weight at 10 DAG, but there was a significant Cultivar × Biochar interaction for it, as root dry weight of ‘Liaodou 13’ significantly increased by 29% under B3 treatment, while that of ‘Tiefeng 3’ significantly increased by 35% under B1 treatment, relative to respect controls. In contrast, biochar addition significantly increased shoot dry weight began at 7 DAG. As the shoot dry weight of ‘Liaodou 13’ significantly increased

Table 4. Effects of biochar on sucrose content in soybean varieties with different P efficiencies.

Cultivar	Treatment	Leaf 7 DAG	Leaf 10 DAG	Root 7 DAG	Root 10 DAG
		mg g^{-1}		mg g^{-1}	
Liaodou13	CK	13.26 ± 2.22a	14.82 ± 0.72a	12.58 ± 2.07b	10.48 ± 2.02a
	B1	14.76 ± 2.67a	14.85 ± 0.89a	15.85 ± 1.23ab	13.1 ± 1.8a
	B2	14.51 ± 2.81a	15.28 ± 0.67a	18.01 ± 2.89a	13.01 ± 1.7a
	B3	14.03 ± 1.33a	16.22 ± 0.45a	20.92 ± 3.03a	13.15 ± 1.94a
	Average	14.03A	15.65A	16.97A	12.28A
Tiefeng 3	CK	17.27 ± 2.53a	13.03 ± 0.91c	16.67 ± 0.37a	9.74 ± 1.25a
	B1	16.25 ± 1.91a	13.98 ± 1.17bc	17.88 ± 0.89a	10.55 ± 1.46a
	B2	15.09 ± 2.12a	20.48 ± 1.52b	16.95 ± 2.54a	12.79 ± 1.87a
	B3	15.93 ± 2.02a	25.78 ± 0.92a	18.04 ± 3.16a	11.41 ± 2.11a
	Average	16.16A	15.78A	17.62A	11.07A
ANOVA (P value)					
	Cultivars (C)	0.1483	0.7674	0.6232	0.3598
	Biochar rate (B)	0.9683	< 0.001	0.1434	0.4589
	C×B	0.9061	< 0.001	0.2872	0.9572

Values are means of three replicates. Means within a column followed by different lower-case letters are significantly different at the 0.05 probability level between biochar rates; means with different uppercase letters are significantly different at 0.05 probability level between two soybean cultivars.
CK, B1, B2 and B3, represents biochar rate of 0%, 0.15%, 0.75% and 1.5% (w/v, g cm^{-3}), respectively; DAG: d after germination.

Table 5. Effects of biochar on soluble sugar content in soybean varieties with different P efficiencies.

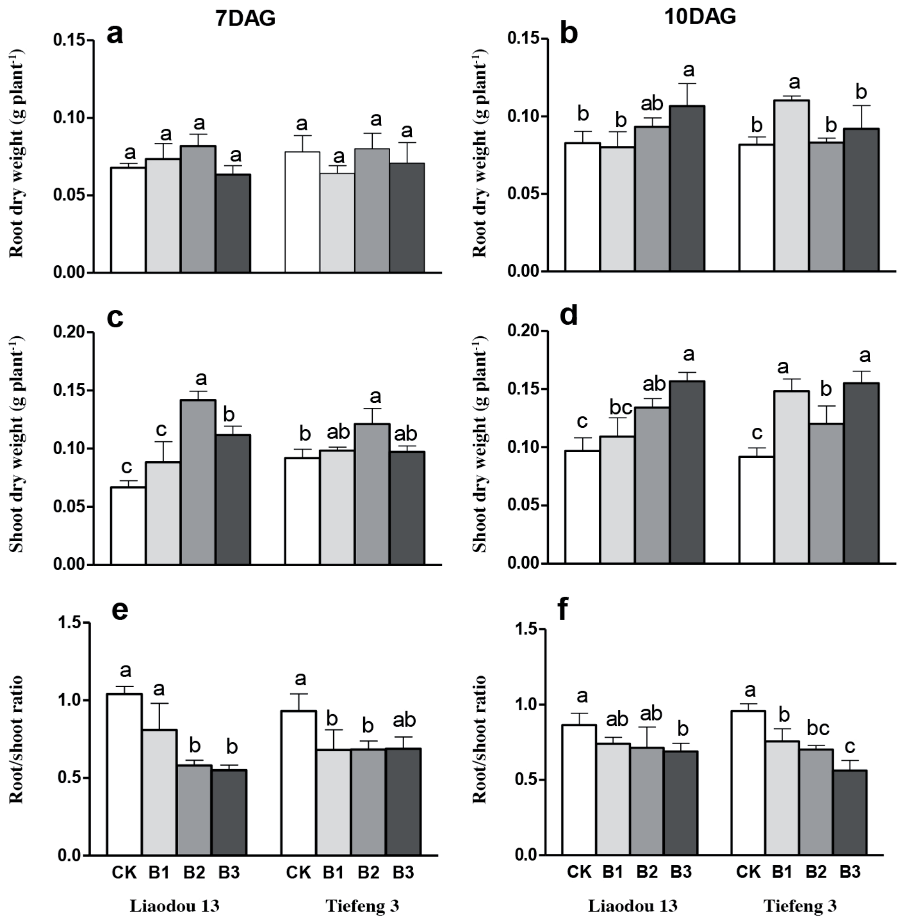
Cultivar	Treatment	Leaf 7 DAG	Leaf 10 DAG	Root 7 DAG	Root 10 DAG
		mg g^{-1}		mg g^{-1}	
Liaodou13	CK	38.71 ± 1.46b	45.33 ± 2.49b	21.09 ± 2.21b	35.43 ± 5.16a
	B1	40.91 ± 1.6b	54.01 ± 8.22ab	26.17 ± 4.06ab	38.91 ± 2.17a
	B2	44.62 ± 1.28ab	52.84 ± 1.07b	33.25 ± 3.13a	42.63 ± 2.28a
	B3	47.6 ± 2.95a	62.81 ± 1.49a	32.51 ± 1.17a	38.6 ± 3.1a
	Average	42.95B	53.74A	28.35B	38.89A
Tiefeng 3	CK	42.16 ± 2.35b	45.15 ± 0.14b	30.5 ± 6.98b	26.99 ± 0.39a
	B1	48.74 ± 0.39a	52.53 ± 1ab	31.77 ± 2.21b	24.26 ± 3.74a
	B2	51.81 ± 2.31a	59.09 ± 1.21a	43.52 ± 1a	28.03 ± 1.89a
	B3	46.32 ± 1.53ab	53.1 ± 0.57ab	38.19 ± 3.88ab	30.82 ± 2.35a
	Average	47.25A	52.46A	35.94A	27.52A
NOVA	Cultivars (C)	< 0.001	0.4316	< 0.001	< 0.001
(P value)	Biochar rate (B)	< 0.001	< 0.001	< 0.001	0.1124
	C×B	0.0258	0.0211	0.7163	0.1768

Values are means of three replicates. Means within a column followed by different lower-case letters are significantly different at the 0.05 probability level between biochar rates; means with different uppercase letters are significantly different at 0.05 probability level between two soybean cultivars.
CK, B1, B2 and B3, represents biochar rate of 0%, 0.15%, 0.75% and 1.5% (w/v, g cm^{-3}), respectively; DAG: d after germination.

under B2 and B3 treatments, and that of 'Tiefeng 3' increased under B2 treatment at 7 DAG (Figure 3c). At 10 DAG, most biochar rates significantly increased shoot dry weight of both soybeans, with maximum increments of 65.6% under B3 treatment relative to the control (Figure 3d).

Biochar addition significantly reduced the root/shoot ratios of both soybeans. The lowest values were observed at B3 treatment. The root/shoot ratio decreased by 37.9% and 26.6% relative to respect controls, respectively, for 'Liaodou 13' and 'Tiefeng 3', at 7 DAG; and respectively by 17.3% and 29.6% at 10 DAG (Figures 3e, 3f).

Figure 3. Effects of biochar on root dry weight (a, b), shoot dry weight (c, d) and root/shoot ratio (e, f) (mean ± SD, n = 3) of soybeans varieties with different P efficiencies at 7 d after germination (DAG) and 10 DAG.



Different letters above vertical bars indicate significant difference ($P < 0.05$) between biochar rates.
CK, B1, B2 and B3, represents biochar rate of 0%, 0.15%, 0.75% and 1.5% (w/v, g cm^{-3}), respectively.

DISCUSSION

Plant roots play an important role in water and nutrient absorption. Morphology and physiological activity directly influences the absorption area and capacity of the root (Ding et al., 2014). Recent studies (Brennan et al., 2014; Olmo et al., 2016) indicated that the addition of biochar at high rates increased specific root length but reduced both root diameter and root tissue mass density. In this study, we used two soybean varieties with different P efficiencies. The results showed little difference in seedling root response to biochar of soybeans with different P efficiencies. The effects of biochar on root morphology in this study were consistent with these findings. Biochar additions increased soybean root length, root surface area, and root volume, but reduced root diameter. These positive effects of biochar application on soybean root morphology, led to a luxuriant root system, which enlarged the space occupied by roots and increased their absorption area in the soil, especially for less mobile elements such as P.

Consistent with the result that biochar addition induced fine root proliferation of Olmo et al. (2016), we further found that biochar application mainly accelerated the growth of fine roots in 0.0-0.5 mm diameter class, subsequently promoted total root growth. Recent studies have shown that fine pore channels with narrow diameters do not restrict root elongation but they instead exert radial pressure on the root, resulting in the compression of the root tissues and reduced root diameter

(Bengough, 2003; Kolb et al., 2012). Biochar, produced from pyrolysis processes, possess large porosity and specific surface area (Lehmann and Joseph, 2009). The porous structure and nutrient content of biochar contribute to its effects on fine root growth (Olmo et al., 2016). Significant increment induced by biochar of RL, RSA and RV in 0.0-0.5 mm diameter, was responsible for a large ratio of fine/coarse root. Root diameter plays a significant role in soil penetration (Materechera et al., 1992), and fine roots can take up nutrients from a larger soil volume per unit root surface than coarse roots (Jungk and Claassen, 1997). Furthermore, the turnover rate of root tissues in crop plants (Guo et al., 2004; 2008) and the degree of mycorrhizal colonization in the grassland (Reinhardt and Miller, 1990) are dependent on root size distribution.

As for plant growth, slight increase induced by biochar was found in root weight, while shoot weight significant increased with biochar application at 7 and 10 DAG. Accordingly, root/shoot ratio was significantly decreased with biochar applications. The incongruous growth of root and shoot could be explained by the reality that biochar had extrusive effects on fine root growth, as well as root vitality. Root vitality directly affects nutrient absorption of the root, vastly supported the growth of shoots. In addition, biochar addition enhanced soluble sugar content in leaf and root. Soluble sugar is the primary product of photosynthesis in higher plants, played a key role as building blocks of macromolecules controlling plant growth and development (Gibson, 2000). The enrichment of soluble sugar in soybean leaf and root induced by biochar had been regarded to serve as a trigger role in control of osmotic pressure at the cellular level, contributed to nutrient absorption and plant growth (Cha-um et al., 2009).

In this study, we used quartz sand as culture medium, mixed with biochar at different rates, researched the influence of biochar on soybean root growth directly, and maximally avoided the interferences by other factors. While in actual agricultural production, there are lots of potential interactive factors, further research is needed for more accurate results.

CONCLUSIONS

The results of this study indicate that biochar treatment can promote soybean root growth at seedling stage, especially in roots 0.0-0.5 mm in diameter, which also affects plant growth. The application of biochar can help maintaining adequate root biomass while simultaneously accelerating plant growth and keeping the proper root/shoot ratio. Biochar treatment also enhances root vitality, maintaining the proper root absorption capacity for nutrient uptake. The increase in seedling plant biomass under biochar application lays the foundation for subsequent plant growth.

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