



Negative interactive effects between biochar and phosphorus fertilization on phosphorus availability and plant yield in saline sodic soil



Gang Xu^a, You Zhang^{a,d}, Junna Sun^{a,c}, Hongbo Shao^{a,b,*}

^a Yantai Institute of Coastal Zone Research, Chinese Academy of Sciences, Yantai 264003, China

^b Institute of Agro-Biotechnology, Jiangsu Academy of Agriculture Sciences, Nanjing 210014, China

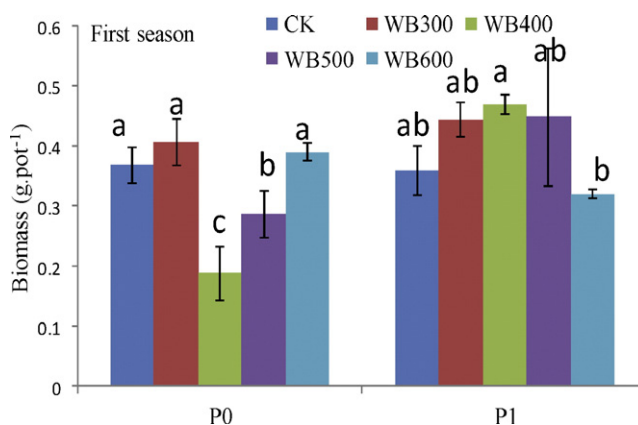
^c School of Life Science, Ludong University, Yantai 264025, China

^d University of Chinese Academy of Sciences, Beijing 100049, China

HIGHLIGHTS

- Lower pyrolysis temperature (<400°C) biochar retained P availability and increased plant growth.
- Negative (antagonistic) interaction occurred between biochar and P fertilization on the biomass production and plant P concentration
- Very limited utility value of biochar application occurred in saline sodic soil.

GRAPHICAL ABSTRACT



The growth of *Suaeda salsa* in biochar amended saline sodic soils with (P₁) or without (P₀) P fertilization.

ARTICLE INFO

Article history:

Received 1 May 2016

Received in revised form 11 June 2016

Accepted 12 June 2016

Available online 18 June 2016

Keywords:

Biochar
Phosphorus
Saline sodic soil
Interaction effect
Bioassay test

ABSTRACT

Little is known about the interactive effects between biochar application and phosphorus (P) fertilization on plant growth and P uptake. For this purpose, five wheat straw biochars (produced at 25 °C, 300 °C, 400 °C, 500 °C and 600 °C for 4 h) with equal P (36 mg kg⁻¹) amount, with and without additional P fertilization (100 mg kg⁻¹) were applied in a pot experiment to investigate the growth of *Suaeda salsa* and their uptake of P from biochar and P fertilization amended saline sodic soil. Soil P fractions, dry matter yield, and plant P concentrations were determined after harvesting 90 days. Our results confirmed that relatively lower pyrolysis temperature (<400 °C) biochar retained P availability and increased plant growth. The plant P concentration was significantly correlated with NaHCO₃-P_i (P < 0.05), and NaOH-P_i (P < 0.1) during early incubation time (4 days) for biochar amended soil. As revealed by statistical analysis, a significant (P < 0.05) negative (antagonistic) interaction occurred between biochar and P fertilization on the biomass production and plant P concentration. For plant biomass, the effects size of biochar (B), P, and their interaction followed the order of B × P (0.819) > B (0.569) ≈ P (0.568) based on the partial Eta squared values whereas the order changed as P (0.782) > B (0.562) > B × P (0.515) for plant P concentration. When biochar and P fertilization applied together, phosphate precipitation/sorption reaction occurred in saline sodic soil which explained the decreased plant P

* Corresponding author at: Yantai Institute of Coastal Zone Research, Chinese Academy of Sciences, Yantai 264003, China Institute of Agro-biotechnology, Jiangsu Academy of Agricultural Sciences, Nanjing 210014, China.

E-mail address: shaohongbochu@126.com (H. Shao).

availability and plant yield in saline sodic soil. The negative interaction effects between biochar and P fertilization indicated limited utility value of biochar application in saline sodic soil.

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1. Introduction

Biochar amendments increased soil phosphorus (P) availability and therefore enhanced crop productivity especially for low fertility soils (Blackwell et al., 2010; Biederman and Harpole, 2013; Marks et al., 2014; Subedi et al., 2016). When incorporation into soil, biochar can increase P availability through directly P release from biochar and indirectly improve P use efficiency by changes in soil pH, CEC, structure, decrease P leaching and affect soil P related microbial activity (Xu et al., 2014; Jiang et al., 2015; Zhai et al., 2015; Christel et al., 2016). Recent review suggested that P values of biochar as a soil amendment were highly depended on soil type, biochar type, and biochar rate (Scott et al., 2014). The positive effects of biochar on P values have been mostly proved in highly weathered acidic soil whereas the response was complex in alkaline soils (Lentz and Ippolito, 2012; Parvage et al., 2013). With increasing biochar rate, increase plant yield or soil available P were observed in acidic soil (Blackwell et al., 2010). In addition, animal-derived biochar supplied more P for plant growth than plant-derived biochar (Liang et al., 2014). In field experiment, different feedstocks biochar were generally tested for possible amendment in highly weather soils (Macdonald et al., 2014). However, pyrolysis temperature was more important factors determining the properties of biochar (Cantrell et al., 2012; Christel et al., 2013). The previous studies suggested that pyrolysis temperature greatly affect P transformation from crop residue to biochar (Xu et al., submitted). However, little is known about plant response to different pyrolysis temperature biochar based on a bioassay tests. This kind of information is important for the production and evaluation of biochar as a potential P source.

In addition, the directly P supply from biochar was thought to be short-lived and the indirectly P value with biochar application should to be verified in the long-term experiment (Wang et al., 2015). Most importantly, the interaction effects between biochar and P fertilizer need to be clarified in agricultural field (Tammeorg et al., 2014; Jain et al., 2016). Chan et al. (2010) found a positive (synergistic) interaction between biochar and nitrogen fertilizer where they found biochar application significantly increased plant yield and N (nitrogen) use efficiency in the presence of N fertilization (Chan et al., 2008). However, the interactive effects between biochar and P fertilizer on the plant growth and P use efficiency are still limited (Schulz and Glaser, 2012).

In acidic soils, the liming effects of biochar were regarded as the main mechanism for enhancing plant productivity (Farrell et al.,

2014). However, in a saline sodic soil, we hypothesized that biochar application could reduce P availability and decrease crop productivity due to P sorption or precipitation. The present study aimed to compare the effects of biochar made at different pyrolysis temperatures on P availability and crop productivity, to evaluate the interaction effect between biochar and P fertilization on P availability and crop productivity.

2. Materials and methods

2.1. Experimental setup

The soil sample was collected from Dongying, Shandong Province, China. The soil properties and sampling process were detailed described in previous studies (Wu et al., 2014). The fresh wheat straw were carefully rinsed with purified water and then air-dried and milled through 2-mm sieve (WB25). The feedstock was pyrolyzed under oxygen-limited atmosphere in muffle furnace at 300 °C, 400 °C, 500 °C, and 600 °C for 4 h respectively (Xu et al., 2013).

Twelve treatment combinations (six pyrolysis temperature produced biochar with equal amount P of 36 mg P kg⁻¹ of soil, with and without P fertilization (KH₂PO₄) of 100 mg P kg⁻¹ of soil). The single biochar application referred as WB25, WB300, WB400, WB500, and WB600, respectively while biochar together with P fertilization defined as PWB25, PWB300, PWB400, PWB500, and PWB600, respectively. The treatments were arranged in a completely randomized block design with three replicates. For each treatment, three pre-germinated *Suaeda salsa* seeds were planted into a pot containing fresh soil (500 g oven-dry based). The *Suaeda salsa* was grown for 90 days in a glasshouse. 100 ml of Hoagland nutrient solution without P was added after plant transportation and the water was regularly supplied to ensure plant growth. The plant growth experiment was carried out for two growth season. After harvest, the plant was dried at 70 °C for 48–72 h in an oven. Another set of pot without plant growth was also carried out for soil P fractions at day 4 and day 90.

2.2. Chemical analysis

Soil P fractions was sequential extracted with a modified Hedley fractionation (Tiessen and Moir, 1993). Generally, 0.5 g samples were extracted step by step using 30 ml each of deionized water (18.2 MΩ), 0.5 M NaHCO₃, 0.1 M NaOH, 1 M HCl shaking for 16 h each addition.

Table 1

Effects of different biochar addition into saline soil on the soil phosphorus fractions. Data in the table indicate means of five replicates (±SD). Different letters on same column indicate significant difference at P < 0.05.

	H ₂ O-P	NaHCO ₃ -P _i	NaHCO ₃ -P _o	NaOH-P _i	NaOH-P _o	HCl-P _i
<i>4 days</i>						
CK	1.1 ± 0.1a	22.0 ± 0.7ab	38.3 ± 7.1bcd	6.7 ± 0.8ab	13.4 ± 7.0a	483.6 ± 18.9abc
WB25	0.7 ± 0.2a	19.3 ± 2.2a	52.2 ± 5.5e	7.7 ± 0.8ab	12.4 ± 0.9a	497.6 ± 10.3bc
WB300	1.6 ± 1.2a	34.4 ± 18c	29.0 ± 8.7b	8.9 ± 1.4b	18.3 ± 1.2a	493.4 ± 5.6bc
WB400	3.9 ± 0.1ab	29.0 ± 1.2abc	28.0 ± 2.4b	6.7 ± 0.8ab	20.7 ± 13.4ab	506.7 ± 28.6bc
WB500	4.7 ± 1.4ab	28.3 ± 1.6abc	31.1 ± 5.8bc	6.1 ± 0.4ab	15.5 ± 1.2a	510.9 ± 12.4c
WB600	7.2 ± 0.2b	28.8 ± 1.6abc	29.9 ± 6.0bc	5.8 ± 0.4ab	13.2 ± 0.6a	509.5 ± 1.4c
<i>90 days</i>						
CK	2.0 ± 1.0a	21.3 ± 2.6ab	39.8 ± 6.5bcde	7.3 ± 5.7ab	48.8 ± 19.0d	448.2 ± 53.8a
WB25	5.4 ± 0.5ab	19.8 ± 0.7a	34.8 ± 3.3bc	7.3 ± 1.3ab	39.5 ± 4.2 cd	470.3 ± 22.2ab
WB300	5.5 ± 0.2ab	26.6 ± 2.7abc	40.3 ± 13.3bcde	8.5 ± 1.8b	32.9 ± 4.4bc	488.6 ± 12.0bc
WB400	14.0 ± 8.2c	30.9 ± 2.5bc	15.7 ± 0.04a	5.8 ± 0.6ab	36.3 ± 3.8c	489.3 ± 26.9bc
WB500	16.9 ± 0.3c	32.5 ± 3.4c	43.4 ± 5.76cde	5.0 ± 0.6a	33.3 ± 3.1bc	480.5 ± 16.9abc
WB600	12.8 ± 5.8c	33.3 ± 3.1c	48.9 ± 12.1de	4.9 ± 0.6a	33.4 ± 6.5bc	470.7 ± 16.1ab

Table 2
Effects of biochar and phosphorus addition into saline soil on the soil phosphorus fractions. Data in the table indicate means of five replicates (\pm SD). Different letters on same column indicate significant difference at $P < 0.05$.

	H ₂ O-P	NaHCO ₃ -P _i	NaHCO ₃ -P _o	NaOH-P _i	NaOH-P _o	HCl-P _i
4 days						
P	131 \pm 9 cd	143 \pm 7bcd	26 \pm 0a	40 \pm 4ef	60 \pm 1abc	612 \pm 39ab
PWB300	139 \pm 10 cd	157 \pm 8de	34 \pm 25a	42 \pm 6f	47 \pm 11ab	617 \pm 15ab
PWB400	123 \pm 20c	134 \pm 4ab	39 \pm 19a	38 \pm 2def	67 \pm 9bcd	621 \pm 18ab
PWB500	136 \pm 10 cd	151 \pm 12 cd	49 \pm 7a	33 \pm 3bcd	53 \pm 14abc	617 \pm 12ab
PWB600	125 \pm 13 cd	142 \pm 7bc	44 \pm 8a	25 \pm 2a	69 \pm 13 cd	601 \pm 8a
90 days						
P	71 \pm 2a	147 \pm 5bcd	48 \pm 19a	56 \pm 3 h	126 \pm 13e	604 \pm 12a
PWB300	91 \pm 3b	129 \pm 6a	60 \pm 18ab	43 \pm 2f	83 \pm 13d	613 \pm 22ab
PWB400	82 \pm 3ab	152 \pm 3 cd	43 \pm 15a	36 \pm 3cde	46 \pm 11a	640 \pm 12b
PWB500	76 \pm 4ab	147 \pm 8bcd	45 \pm 26a	31 \pm 1bc	46 \pm 10a	614 \pm 24ab
PWB600	74 \pm 2a	166 \pm 13e	87 \pm 33b	30 \pm 1b	43 \pm 5a	624 \pm 23ab

Organic P in NaHCO₃ and NaOH was calculated as the difference between total and inorganic P. The plant biomass was weighted after oven drying. Total P content in the plant was determined after digestion with concentrated H₂SO₄/H₂O₂ (Tiessen and Moir, 1993).

2.3. Statistical analysis

All results are reported as mean \pm standard deviation. One-way ANOVA followed by LSD test was used to test the differences in plant production and P concentration among treatments. In order to determine the main effect as well as to identify if there is a significant interaction among biochar and phosphorus, a multivariate ANOVA test was adopted, and estimates of effect size (Eta squared value) were calculated when the effects were significant at $P < 0.05$.

3. Results

3.1. Influence of biochar and phosphorus fertilization addition on P pools in saline sodic soil

As is shown in Table 1, the P forms were significantly affected by the different biochar and the incubation time (day 4 and day 90). In general, the P forms with higher availability were much affected by the biochar addition and the incubation time.

Biochar addition significantly increased H₂O-P content in saline soil which followed the order of WB600 > WB500 > WB400 > WB300 > WB25 irrespective of the incubation time. The H₂O-P concentration increased by 1.8–7.7 folds from day 4 to day 90 in the amended soils. The concentration of NaHCO₃-P_i increased proportionally with increased pyrolysis temperature of biochar especially at day 90. Biochar

application showed little effects on NaHCO₃-P_o while the feedstock (WS25) seemed to greatly enhance this labile organic phosphorus. The content of NaOH-P_i and NaOH-P_o were not significantly affected by biochar application, but NaOH-P_o significantly increased from day 4 to day 90. Biochar addition seemed to have a little effect on HCl-P_i at all biochars and incubation time.

Compared with single biochar amendment, the effects of biochar on P pools were significantly decreased in the presence of P fertilization (Table 1, Table 2). The H₂O-P_i was less affected by biochar application, but was greatly reduced over incubation time. Except for WB600, biochar addition seemed to have a little effect on NaHCO₃-P_i and NaHCO₃-P_o in amended soils while the NaHCO₃-P_o concentration was increased from day 4 to day 90. The content of NaOH-P_i and NaOH-P_o were significantly decreased with biochar application especially at higher pyrolysis temperature. Consistence with single biochar amendment, the content of HCl-P_i was less affected by biochar application when P fertilizer applied.

3.2. The growth of *Suaeda salsa* in biochar amended soils with and without P fertilization

In the first growth season, the plant biomass was not enhanced with single biochar application (Fig. 1). On the contrast, WB400 and WB500 significantly depressed the growth of *Suaeda salsa* compared with control ($P < 0.05$). When combined with P fertilization, biochar amendment showed the tendency of enhancing the plant biomass productivity compared with un-amended treatment. The plant biomass of *Suaeda salsa* was greatly reduced from the first to the second growth season. For the second growth season, biochar addition with or without P fertilization also did not increase the plant biomass although the WB300 greatly enhanced the plant growth.

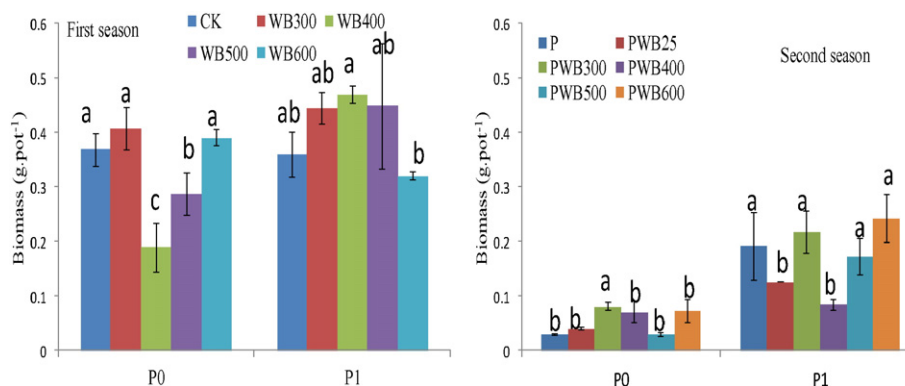


Fig. 1. The growth of *Suaeda salsa* in biochar amended saline sodic soils with (P₁) or without (P₀) P fertilization. Data in the figures indicate means of five replicates (\pm SD). Different letters on same column indicate significant difference at $P < 0.05$.

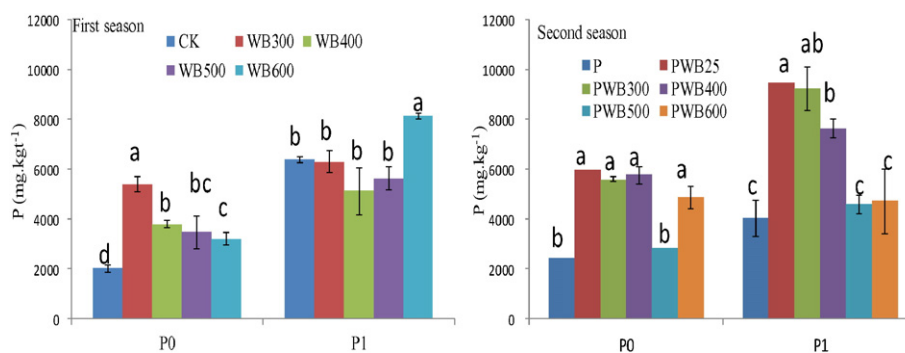


Fig. 2. Plant phosphorus concentration in biochar amended saline sodic soils with (P_1) or without (P_0) P fertilization.

3.3. The phosphorus concentration of *Suaeda salsa* in biochar amended saline sodic soils with or without P fertilization

Compared with plant biomass, biochar addition greatly enhanced plant P concentration of *Suaeda salsa* over the two growth seasons although the relatively lower pyrolysis temperature (≤ 400 °C) biochar seem to be favor for plant P uptake. With P fertilization, biochar addition significantly increased plant P concentration for the second growth season. Whereas, little effects was observed between treatments without P fertilization except for the higher plant P content in WB600 for the first growth season (Fig. 2).

4. Discussions

4.1. The response of plant phosphorus content on biochar and phosphorus fertilization addition in saline sodic soil

It was reported that the P pools in biochar were greatly affected by pyrolysis temperature. During pyrolysis treatment, the water soluble or organic liable P were transformed into more stable P pool which suggested that the relatively lower pyrolysis temperature retains more P availability. Our bioassay analysis confirmed that the relatively lower pyrolysis temperature biochar (< 400 °C) present more available P for plant uptake in the present study. The stable P mineral species, such as whitlockite, crandallite, and brushite, were found to explain the decrease P availability at high pyrolysis biochar (Cao and Harris, 2010). In a field experiment, Farrell et al. (2014) reported that biochar produced at 550 °C was less effective for plant P uptake compared with P fertilization because of the lower soluble P content in higher pyrolysis biochar. In result, a relatively lower pyrolysis temperature was recommended for biochar production.

The ANOVA analysis suggested that the effects of P fertilization, biochar and their interaction on the plant P concentration followed the order of $P (0.782) > B (0.562) > B \times P (0.515)$ based on the partial Eta squared values. The data suggested that P fertilization was effective than biochar to increase plant P concentration because the P supplication from P fertilization (100 mg kg^{-1}) was three fold compare to biochar addition (36 mg kg^{-1}). In addition, the results indicated that a significantly negative (antagonistic) interaction effects between biochar

and P fertilization was occurred on the plant P concentration because the size of measured effects were significantly lower than the size of sum of single biochar or P fertilization. The results indicated that plant P availability was greatly decreased because a negative interaction occurred upon addition of biochar and P fertilization into saline soils. Soluble P was reported very low (5 mg kg^{-1}) in our saline sodic soil (Wu et al., 2014). The release of P from biochar, P fertilization and their combination would determine P availability for plant growth. It is very interesting to note that the P supply from mixture ($B \times P$) was greatly lower than expected value even lower than single biochar or P fertilization. The P availability was highly pH dependent in our soil and biochar enhance soil pH was generally reported in previous studies (Yuan and Xu, 2011). Higher solution pH values increase the precipitation of phosphate to less soluble forms (Marks et al., 2014). In alkaline soils, the large amount of free Ca^{2+} , Mg^{2+} , Al^{3+} and Fe^{3+} oxides contained in biochar could be P sorption sites (Xu et al., 2013). Our results indicated that phosphate precipitation/sorption reaction occurred in saline sodic soil when biochar and P fertilization applied together. The interaction effects between biochar and P fertilization will depress P availability in saline sodic soils.

4.2. The response of *Suaeda salsa* growth to biochar and phosphorus fertilization addition in saline soil

We have identified very low available P content (5 mg kg^{-1}) in this saline sodic soil (Wu et al., 2014). The intrinsic low P availability would inhibit plant growth (Farrell et al., 2014). However, salt stress has been identified as major factor constraining crop productivity in saline sodic soils (Qadir et al., 2000). Soil salinity greatly reduced plant performance by imposing osmotic stress, hormonal imbalance, nutrient deficiencies and specific ion toxicity (Qadir et al., 2000). In the present study, ANOVA analysis suggested that the effects of P fertilization, biochar, and their interaction on the biomass production of *Suaeda salsa* followed the order of $B \times P (0.819) > B (0.569) \approx P (0.568)$ based on the partial Eta squared values (Table 3). It is very interesting to note that almost same biomass production was reported between biochar and P fertilization application although the P supplication from P fertilization (100 mg kg^{-1}) was three fold compare to biochar addition (36 mg kg^{-1}). Here, the results indicated that only adequate application of P fertilization to the saline soil will not guarantee for higher plant

Table 3

A two-way ANOVA for effects of P fertilization, biochar and their interaction on the biomass of *Suaeda salsa*, and plant P content for the first growth season.

Source	df	Biomass production (g pot^{-1})				Plant P content (mg kg^{-1})			
		Mean square	F	P	η^2	Mean square	F	P	η^2
Biochar (B)	4	0.008	6.562	0.002	0.568	3,671,830.295	6.418	0.002	0.562
Phosphorus (P)	1	0.032	26.422	0.000	0.569	41,150,580.483	71.922	0.000	0.782
$B \times P$	4	0.028	22.596	0.000	0.819	3,038,631.273	5.311	0.004	0.515
Model	9	0.019	15.895	0.000	0.877	7,554,714.084	13.204	0.000	0.856
Error	20	0.001				572,151.935			

Table 4
The correlation coefficient of biomass and TP content of *Suaeda salsa* cultivated in alkaline soil added biochars with different forms of phosphorus in soil at different periods.

treatment		H ₂ O-P	NaHCO ₃ -P _i	NaHCO ₃ -P _o	NaOH-P _i	NaOH-P _o	HCl-P _i
Biochar (4 days)	Biomass	-0.13	0.10	0.32	0.34	-0.62	-0.46
	P content	-0.20	0.95**	-0.69	0.81*	0.65	0.07
Biochar + phosphorus (4 days)	Biomass	0.58	0.67	-0.27	0.49	-0.66	0.21
	P content	-0.32	-0.35	-0.83*	0.48	0.24	0.01
Biochar (90 days)	Biomass	-0.59	-0.35	0.82*	0.45	0.08	-0.40
	P content	0.01	0.14	-0.13	0.48	-0.66	0.80*
Biochar + phosphorus (90 days)	Biomass	0.38	-0.75	0.17	0.16	0.17	-0.16
	P content	0.22	-0.14	0.05	0.64	0.54	0.12

* Significance of $P < 0.1$ (two tails).

** Significance of $P < 0.05$ (two tails).

production. There were increasing evidences suggested that biochar application could help combat salinity stress to plant and improve productivity in saline croplands (Drake et al., 2015; Lashari et al., 2015; Hammer et al., 2014). Akhtar et al. (2015) have reported that incorporation of biochar into salt-affected soil could alleviate salinity stress mainly because of its high salt adsorption potential, decreasing osmotic stress by enhancing soil moisture content, and releasing mineral nutrients. Biochar addition improved soil physical properties such as reduce bulk density, increase surface area and porosity and possibly increase aggregate stability which implied improvement of saline sodic soil aeration, water holding capacity, and nutrient use efficiency (Atkinson et al., 2010). In results, biochar application showed higher plant performance than single P fertilization in the saline sodic soil.

As revealed by statistical analysis, the size of interaction effect ($B \times P$) was higher than single biochar or P application for biomass production. The result confirmed that biochar application showed more improvement in plant production than P fertilization in saline sodic soil. Furthermore, our data suggest that the plant production in mixed biochar and phosphorus amendments was lower than the predicted sum of plant production of individual amendments estimated by effect size (Eta squared value). The data indicated a negative (antagonistic) interaction occurred between biochar and P fertilization on the biomass production. Similar result was reported in a calcareous soil where effect of banded biochar on wheat yields was insignificant in the presence of P fertilization (Blackwell et al., 2010). In alkaline soils, biochar application depressed P availability in the present study. Therefore, the interaction between biochar and P fertilization would suppress P availability for plant growth (Table 3). In a meta-analysis, Biederman and Harpole (2013) concluded that there was limited evidence of a super-additive or synergistic effect when both biochar and fertilizer are applied for plant production. The results suggested that a limited improvement of plant production with biochar application in saline sodic soils in the short term experiment.

4.3. The correlation analysis between the plant yield, plant phosphorus content and phosphorus fractions in soil at different incubation time

As is shown in Table 4, the correlation analysis showed that the dry matter yield of *Suaeda salsa* was less affected by the P fractions in soil over incubation time. The fact confirmed that solely addition of adequate P fertilization into the saline soil will not guarantee for high productivity of plant in Yellow River Delta. The result also suggested that successful amelioration of saline soil need to complete improvement of physical, chemical and microbial properties of soil.

The plant P concentration were positively correlated with NaHCO₃-P_i ($P < 0.05$), and NaOH-P_i ($P < 0.1$) during early incubation time (4 days) for single biochar amended soil. After fertilizing with extra P or incubating for 90 days, this correlation became very weak. The results indicated that the initial content of NaHCO₃-P_i or NaOH-P_i could be good indicator of plant available P in biochar amended soils.

5. Conclusions

The present bioassay test suggested that relatively lower pyrolysis temperature (<400 °C) biochar retained P availability and increased plant growth in saline sodic soil. However, the lower pyrolysis biochar showed lower stability and pronged to degrade in long term experiment. As a result, we need to carefully balance the P availability and carbon stability before making the decision for biochar production.

As revealed by statistical analysis, a significant ($P < 0.05$) negative (antagonistic) interaction occurred between biochar and P fertilization on the biomass production and plant P concentration. Our first hypothesis was confirmed that biochar application decreased P availability in saline sodic soil because of the sorption/precipitation of phosphate with biochar amendments. However, biochar can also alleviate salinity stress which seemed to increase plant production. In generally, the negative interaction effects between biochar and P fertilization indicated limited utility value of biochar application in saline sodic soil.

Acknowledgements

This research was supported by the National Natural Science Foundation of China (41573120; 41501309; 41171216; 41001137), Jiangsu Autonomous Innovation of Agricultural Science & Technology [CX(15)1005], Shuangchuang Talent Plan of Jiangsu Province, 135 Development Plan of Yantai Institute Zone of Research and the Science & Technology Development Plan of Shandong Province (2013YD10013).

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