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Biochar – manure compost in conjunction with pyroligneous solution alleviated salt stress and improved leaf bioactivity of maize in a saline soil from central China: a 2-year field experiment

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Abstract

BACKGROUND: Salinity is a major stress threatening crop production in dry lands. A 2-year field experiment was conducted to assess the potential of a biochar product to alleviate salt-stress to a maize crop in a saline soil. The soil was amended with a compost at 12 t ha^{-1} of wheat straw biochar and poultry manure compost (BPC), and a diluted pyroligneous solution (PS) at 0.15 t ha^{-1} (BPC-PS). Changes in soil salinity and plant performance, leaf bioactivity were examined in the first (BPC-PS1) and second (BPC-PS2) year following a single amendment.

RESULTS: While soil salinity significantly decreased, there were large increases in leaf area index, plant performance, and maize grain yield, with a considerable decrease in leaf electrolyte leakage when grown in amendments. Maize leaf sap nitrogen, phosphorus and potassium increased while sodium and chloride decreased, leaf bioactivity related to osmotic stress was significantly improved following the treatments. These effects were generally greater in the second than in the first year.

CONCLUSION: A combined amendment of crop straw biochar with manure compost plus pyroligneous solution could help combat salinity stress to maize and improve productivity in saline croplands in arid/semi-arid regions threatened increasingly by global climate change.

Keywords: salt stress; biochar manure compost; pyroligneous solution; plant growth performance; leaf bioacativity; soil amendment; saline soil

INTRODUCTION

Among all abiotic stresses, salt stress is widely considered as the most significant environmental constraint for crop production in arid and semi-arid regions of the world.[1,2] Salt stress triggers inhibition in plant growth and development by imposing osmotic stress, increasing abscisic acid concentration, decreasing xylem pH and conductivity caused by specific ion toxicity on plant.[2,3] Salt stress could also affect the activity of major cytosolic enzymes with disturbance of intercellular potassium homeostasis and cause oxidative stress in plant cells.[1,3] Accumulation of reactive oxygen species in plant tissue under salt stress could cause death in root cells[3] and massive depletion of cytosolic K^+ in plant roots. In addition, serious salt stress may even cause injuries in plant condition such as tissue burning, leaf abscission, root death and yield loss over different timescales,[4,5] impairment of mineral nutrition, inactivation of photosynthesis,[6] and reduced water potential due to high Na^+ concentrations.[1]

Biochar is an organic carbon-rich porous material produced via pyrolysis of plant biomass generally for use as a soil amendment.[7] In earlier studies,[8–10] systemic plant resistance of several prominent foliar pathogens and disease resistance were promoted,[8] while improvement of soil structure improved and salt stress alleviated[9,10] with biochar soil amendment in salt affected soils. Pyroligneous solution (PS), often known as wood vinegar, is a liquid by-product of pyrolysis of crop biomass,[11] which contains hundreds of dissolved organic chemicals.[12] PS has been widely used as an organic amendment for foliar fertilisation and polluted soil remediation,[13] for reducing pest problems and disease infection,[8] and for regulating plant growth through enhancement of growth hormones.

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Business-scale technology for biochar production through pyrolysis of crop wastes has been well developed in China for the last few years.[11] In a previous study[10] dealing with soil amendment using biochar manure compost and pyroligneous solution, a remarkable decline in soil salinity and an increased wheat yield were observed. However, the effects on growth and production of maize, which is increasingly and extensively cropped in drylands of China and is being challenged by climate change, have not been characterised.[14] This study investigates the effects of soil amendment of biochar manure compost and pyroligneous solution (BPC-PS) on alleviating major abiotic stress and improving physiological functions, bioactivity, crop growth and yield. In this study, summer maize as a typical dryland crop is used in the experiment in saline soil.

Table 1. Basic properties of the topsoil (0–20 cm), biochar and pyroligneous solution and biochar poultry manure compost before used for the experiment

	Sample			
Property	Top soil	Biochar	PS	BPC
pH (in H_2O)	8.25	10.35	9.37	7.50
TOC (g kg^{-1})	5.13	467	3.87	419
Total N (g kg^{-1})	0.70	5.90	0.55	25.0
Salt (g kg^{-1})	12.68	41.97	ND	ND
CEC (cmol kg^{-1})	21.26	21.70	ND	ND
Bulk density (g cm^{-3})	1.33	0.65	ND	1.00

PS, pyroligneous solution; BPC, biochar and poultry manure compost; TOC, total organic carbon; CEC, cation exchange capacity; ND, not detected.

MATERIALS AND METHODS

Experiment site, climate and soil conditions

As in the previous study,[10] the field experimental site was located in Kangzhuang Village (34° 32′ N, 115° 30′ E) of Shangqiu municipality of Henan province, China. This area is semi-arid and a semi-humid temperate monsoon climate prevails. On average for the period of 2008–2012, a mean annual temperature was 13.9 °C and a mean total evaporation was 1735 mm. A mean annual total sunshine time was 2510 h with 230 frost-free days per year. Mean annual precipitation was 770 mm and 785 mm, respectively, for 2011 and 2012. The tested soil is an Aqui-Entisol according to Chinese Soil Taxonomy,[15] which was derived from the paleo-sediment of the Yellow River. The soil properties are shown in Table 1. The conventional cropping system had been a yearly rotation of summer maize and winter wheat.

Biochar and pyroligneous solution used

Biochar used for the compost was produced through pyrolysis of wheat straw at 480 °C in a vertical kiln, developed by Sanli New Energy Company in Shangqiu, China.[11] The basic properties of biochar and the chemical composition of PS were reported previously.[12,16] Prior to use for composting, biochar was ground to pass a 2 mm sieve, homogenised thoroughly and the PS was diluted five-fold using distilled water.

Biochar poultry manure compost

For developing biochar manure compost, poultry manure was collected from a local poultry farm and kept for 1 week under ambient conditions in order to lose excess moisture. Then the manure was mixed with the biochar material in a ratio of 1:3 (PM:BC, v/v) for composting for 6 weeks. The biochar manure compost (BPC) produced was thoroughly mixed prior to soil amendment. The compost was a dark loose neutral (pH in water 7.5) organic material containing 419 g kg^{-1} of organic carbon, 25 g kg^{-1} of total N, and 0.82 g kg^{-1} of alkaline-releasable N, 12.2 mg kg^{-1} of Olsen-P and 0.83 mg kg^{-1} of NH_4NO_3 exchangeable K as well.

Experiment design

An abandoned salinised cropland was treated with combined amendment of BPC-PS. After the wheat harvest in early June 2010, diluted PS at 0.15 t ha^{-1} was directly sprayed onto the soil surface. BPC was broadcast on the soil surface at 12 t ha^{-1} 1 week after PS amendment. The broadcasted BPC was thoroughly mixed with the topsoil by machine ploughing to a depth of approximately 20 cm and then machine raked for levelling.

For comparison, we used a plot of unreclaimed salinised soil as background control (CK), which was similarly fertilised and ploughed but without BPC-PS. The maize cultivar and chemical fertilisers used under CK were the same as under the BPC-PS treatment. The experiment was repeated in 2011 so that the treatment effects persisted. For this, a new plot (BPC-PS1, hereafter) was treated with the same procedure as in 2010. However, the plots treated in 2010 received no more BPC but only PS in same dosage before maize sowing in 2011 (BPC-PS2). The same plot of control was used for comparison in 2011. Through this design, the cross-year effect of BPC treatment can be addressed in terms of variation with years. The experiment was repeated in triplicates with a complete randomised block design of the individual plots in an area of 0.15 ha. All the crop production management practices, including sowing intensity, fertilisation, and weed and pest control were consistent across the treatment plots. No irrigation was carried out during maize production.

Maize cultivation and fertilisation

Seeds of a maize cultivar (Zheng Dan 958) were directly sown after the soil preparation in mid-June each year. Basal fertilisers were applied while sowing. Following the local conventional fertilisation, basal fertilisers of N as urea, P as calcium super-phosphate and K as potassium sulfate were applied at 112.5 kg N ha^{-1}, 112.5 kg P_2O_5 ha^{-1} and 112.5 kg K_2O ha^{-1}, respectively. At the early development stage after 40 days of germination, additional N as urea at 120 kg N ha^{-1} was ditch applied between the maize rows to a depth of 10–15 cm and covered by the soil using hand-operated tools.

Soil sampling and analysis

Topsoil sampling was done after the maize harvest. A composite sample of six sub-samples was collected at a depth of 0–20 cm in an S-shaped pattern across each experimental plot using an Eijkelkamp core sampler. The collected soil samples were stored in sealed plastic bags and shipped to the laboratory. Visible plant detritus and gravels, if any, were removed from the soil before air drying at room temperature. A dried sample was ground to pass a sieve with 2 mm openings, and a portion of the fine soil (<2 mm) was further ground to pass a sieve with 0.15 mm openings and saved for chemical analysis.

Soil properties were analysed following the protocols described in a laboratory analytical manual.[17] Briefly, pH of soils was measured in 1:2.5 w/v ratio soil suspensions and electrical conductivity (EC) was determined from saturated paste extract. Total dissolved salt content was determined by extracting the soil with water, which was oven dried and weighed. Exchangeable cations were measured with ammonium acetate leaching and the concentration of Na^+, Ca^{2+} and Mg^{2+} determined by flame photometry (FP-6410; Xinyi Instruments Co. Ltd., Shanghai, China) and atomic adsorption spectrophotometry (TAS-986; Persee Analytical Instruments Ltd., Beijing, China). The concentration of Cl^- was measured with water extraction and determined using the silver nitrate titration method. Finally, the sodium adsorption ratio (SAR), exchangeable sodium ratio (ESR) and exchangeable sodium percentage (ESP) were estimated with the ion concentrations as described, using the following equations:

$$\mathrm{SAR} = \frac{\mathrm{Na}^+ \sqrt{\mathrm{Ca}^{2+} + \mathrm{Mg}^{2+}}}{2} \tag{1}$$

$$\mathrm{ESR} = -0.013 + 0.015\,\mathrm{SAR} \tag{2}$$

$$\mathrm{ESP} = \frac{100 \times \mathrm{ESR}}{1 + \mathrm{ESR}} \tag{3}$$

Plant observation, sampling and analysis

Field observation and sampling of plants

At the early growth stage 40 days after sowing, plant density was measured by counting plant geometry in 10 randomly selected rows, calculated and expressed as x thousand plants ha^{-1}. Plant growth performances were examined under the field conditions, using 15 randomly selected plants in each plot. For analysis of leaf chlorophyll content, electrolyte leakages, ion content of sap and bioactivity, the second top fully expanded leaves of the selected plants were collected, removed of any mid-rubs and rinsed once with tap water and subsequently twice with distilled water, chopped into small pieces of 2 cm length prior to analysis.

When ripened, all maize cobs were harvested in each experimental plot and dried for 10 days. After threshing using an electric thresher, the maize grains obtained were directly weighed for evaluating the maize yield (kg ha^{-1}) under the treatments.

Determination of plant relative water content and electrolyte leakage

Relative water content of growing maize plants was determined using a recommended protocol.[18] In detail, a portion of collected leaves of the sampled 15 plants were crashed, mixed and packed in a plastic bag to weigh the fresh weight (W_f). A random portion was air dried for five days at the laboratory, and then oven dried at 72 °C for 60 h to determine the dry weight (W_d). Finally, the relative water content (PWC) of maize plant was calculated as the moisture loss during drying divided by the fresh weight.

Electrolytes leakage (EL) was measured following the method described by Lutts *et al.*[19] A portion of the collected fresh leaves was washed with distilled water three times and then dried/pressed with clean towels, chopped into small pieces and removed of midribs. Leaf sample of approximately 0.3 g was placed in a 30 mL test tube with 20 mL of distilled water, incubated for 2 h at 25 °C for measuring the initial conductivity (C_i) with a digital conductivity meter. Then the test tube was tightly sealed and autoclaved at 120 °C for 30 min to completely destroy the tissue and release the total electrolytes in water. Conductivity was then measured with the same procedure and expressed as C_{max}. Finally, leaf electrolyte leakage was estimated as the portion (%) of C_i to $C_{max.}$

Measurement of leaf chlorophyll content

A portion (2.0 g) of the collected fresh leaves was homogenised in a 800 mL L^{-1} acetone solution in a glass tube. The tube was tightly sealed, covered with a black polyethylene sheet and chilled at 4 °C for 36 h for removing all the green pigment. The solution was subsequently diluted 10 times and absorbance of the acetone extracts was measured with a colorimeter at 663 nm for Chl_a, at 645 nm for Chl_b, and at 652 nm for Chl_t, respectively. The content of chlorophyll species (mg L^{-1}) was calculated with the following equations:[20]

$$\mathrm{Chl_a} = \left(12.72 \times A_{663}\right) - \left(2.69 \times A_{645}\right) \tag{4}$$

$$\mathrm{Chl_b} = \left(22.88 \times A_{645}\right) - \left(4.68 \times A_{663}\right) \tag{5}$$

$$\mathrm{CHl_t} = \left(20.21 \times A_{652}\right) - \left(2.69 \times A_{663}\right) \tag{6}$$

where A_{663}, A_{645} and A_{652} are the concentrations of Chl_a, Chl_b and Chl_t, respectively. Ultimately, the total chlorophyll content was calculated as the sum of the above-measured individual chlorophylls.

Determination of leaf bioactivity indicators

Free proline of maize leaves was extracted following the method of Bates *et al.*[21] In detail, a portion (0.3 g) of the oven-dried maize leaves was ground in a mortar with 5 mL of a 300 mL L^{-1} sulfosalicylic acid and then centrifuged at 12 000 × *g* for 15 min. Subsequently, 2 mL of the clear supernatant were mixed with 2 mL of acetic acid and 2 mL of acid ninhydrin reagent in a 15 mL glass tube. The mixture was boiled at 98.5 °C in a water bath for 1 h and then cooled in an ice bath, followed by extraction with toluene using a separation funnel. Finally, the absorbance of the toluene layer was measured with a colorimeter at 520 nm. The free proline content of leaves was calculated using a calibration curve of proline standards and expressed as moles g^{-1} dried leaf tissue.

Content of malonaldehyde (MDA), an indicator of lipid peroxidation, of maize leaf was determined with a modified protocol using the thiobarbituric acid (TBA) reaction.[22] A portion (0.3 g) of fresh maize leaves was ground in a mortar in 2 mL of 100 mL L^{-1} trichloroacetic acid (TCA) and 10 mL L^{-1} sodium dodecyl sulfate salt. The homogenised material was transferred to a 15 mL centrifuge tube with an additional 8 mL of TCA before centrifuging at (15 000 × *g*) for 10 min. Subsequently, 2 mL of the supernatant was transferred to a glass tube containing 3 mL of 100 mL L^{-1} TCA and 6 mL L^{-1} thiobarbituric acid and heated in a water bath at 98.5 °C for 15 min and then quickly cooled in an ice bath. Absorbance of the mixture was measured for the leaf MDA content with a colorimeter at 450 nm, 532 nm and 600 nm.

Soluble sugar, amino acids and ascorbic acid, and ion content in leaf sap

Leaf soluble-sugar content was determined with colorimetry.[23] A portion of homogenised fresh leaves from a plot was extracted with water at room temperature. The extract was reacted with phenol and sulfuric acid, and measured with a colorimeter at 485 nm. Leaf amino acids content was determined following a conventional method[24] while the leaf content of ascorbic acid was determined using a TCA extraction method described by Singh *et al.*[25]

For analysis of ion content in leaf sap, a portion (about 4.0 g) of the crushed fresh leaves was chopped into small pieces, placed in

Table 2. Chemical properties of topsoil (0–20 cm) under (BPC-PS) treatment of the salt stressed soil over experimental control under maize cultivation

	Treatment		
Property	CK	BPC-PS1	BPC-PS2
pH (in H_2O)	7.80 ± 0.12^a	7.63 ± 0.08^a	7.43 ± 0.03^b
EC ($dS\ m^{-1}$)	8.26 ± 0.27^a	5.00 ± 0.21^b	4.83 ± 0.14^c
Total salt ($g\ kg^{-1}$)	8.21 ± 0.11^a	5.13 ± 0.06^b	4.84 ± 0.04^c
SAR	16.71 ± 1.65^a	11.09 ± 0.47^b	9.00 ± 1.42^c
ESP	21.00 ± 2.50^a	14.40 ± 0.71^b	11.34 ± 2.16^c
Na^+ ($g\ kg^{-1}$)	5.36 ± 0.13^a	3.42 ± 0.20^b	3.26 ± 0.06^b
Ca^{2+} ($g\ kg^{-1}$)	1.26 ± 0.17^c	1.54 ± 0.01^b	1.87 ± 0.04^a
Cl^- ($g\ kg^{-1}$)	0.30 ± 0.05^a	0.15 ± 0.03^b	0.12 ± 0.02^b

Different letters in a same row indicate significant differences ($P < 0.05$) between the treatments in a single year.
SAR, sodium adsorption ratio; ESP, exchangeable sodium percentage; EC, electrical conductivity; CK, background control; BPC, biochar and poultry manure compost; PS, pyroligneous solution.

an Eppendorf centrifuge tube and frozen at −20 °C for 4 weeks. The frozen leaf samples were further homogenised using glass rods and then centrifuged at 12 000 × *g* for 20 min to obtain the supernatant as the maize leaf sap. For total nitrogen and phosphorus analyses, 5 mL of the supernatant leaf sap was digested with concentrated H_2SO_4:H_2O_2 at 280 °C on an electric hotplate. For determination of ion contents of Na^+, K^+ and Cl^-,[5,26] the leaf sap was diluted 1:50 with deionised water and measured directly using methods mentioned above.

For all the analyses above, a blank sample was processed with the same procedure in a single batch of measurement.

Data processing and statistical analysis

All analytical data were expressed as mean ± 1 SD. Data processing was performed with Microsoft Excel 2003. Statistical analysis was done with SPSS, version 16.0, 2001 (SPSS Institute, Chicago, IL, USA). Significance for differences between the treatment means was examined by one-way analysis of variance (ANOVA), with a probability defined at 0.05.

RESULTS

Soil salinity and plant salt stress

Results of changes in electric conductivity (EC), pH, total salt content and the ion concentration of Na^+, Ca^{2+} and Cl^- in soil extracts of the soil samples with the treatments are listed in Table 2. There was a significant reduction in EC by 28% and 41%, in SAR by 34% and 46%, and in ESP by 31% and 48% over control though relatively unchanged soil pH respectively under treatments of BPC-PS1 and BPC-PS2. Meanwhile, total salt, exchangeable Na^+ and soluble Cl^- were all significantly reduced by 30% and 50%, and by 40% and 60%, respectively, under BPC-PS1 and BPC-PS2 compared to CK. In contrast, there was a significant increase in exchangeable Ca^{2+} by 18% and 33%, respectively, under BPC-PS1 and BPC-PS2. Furthermore, there was a significant reduction in Na^+ by 33% and 48%, and in Cl^- by 41% and 48% in maize leaf sap, in electrolyte leakage by 9% and 11% under BPC-PS1 and BPC-PS2 over control (Fig. 1). Moreover, leaf water content increased by 13% under both amendment treated plots compared to untreated.

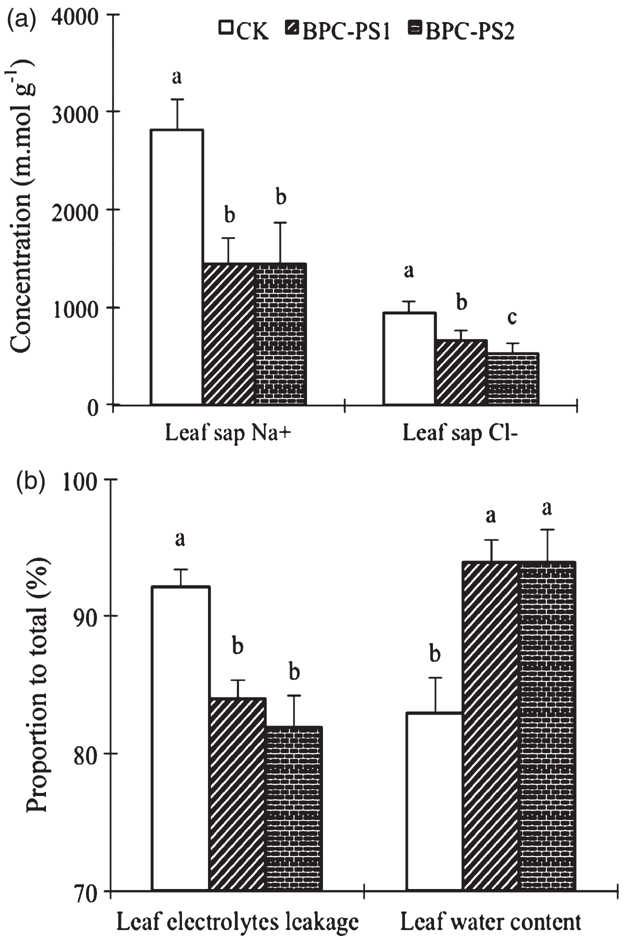

Figure 1. (A) Concentration of leaf sap sodium and chloride; (B) leaf electrolyte leakage [proportion to total released electrolytes (%)] and water content [proportion to total leaf mass (%)] of growing maize in response to BPC-PS treatment. Different letters in the same block indicate significant differences ($P < 0.05$) between the treatments in a single year. CK, control; BPC, wheat straw biochar and poultry manure compost; PS, diluted pyroligneous solution.

Plant growth, performance and productivity

Data of maize growth observed 40 days after sowing and grain yield at harvest are shown in Table 3 and Fig. 2. Remarkably, significant increases were observed in plant height by 23% and 39%, in root length by 47% and 53%, in plant density by 76% and 81% and in leaf areas index by 65% and 110%, respectively, with BPC-PS1 and BPC-PS2 over the control. There was a concurrent large increase in maize grain yield by 140% and 195%. Maize leaf chlorophyll content was greatly increased in BPC-PS treated plots, with chlorophyll a by 28.5% and total chlorophyll by 22.7%, although the chlorophyll b content was unchanged compared to the control.

Leaf bioactivity

Leaf sap nutrient contents are listed in Table 4. A greater increase in nutrients of N, P and K in leaf sap was found by 64–54%, 32–45% and 24–27% under the BPC-PS treatments over the control. K/Na ratio in leaf sap was seen higher by 61–63% under the treatments over control. However, total soluble sugar and amino acids showed a decrease by 48–49% and by 45–57% under the amendments over control.

Table 3. Plant heights, root length, plant density, leaf area index and chlorophyll content of maize leaf under biochar manure compost with pyroligneous solution (BPC-PS) treatment of the salt stressed soil over experimental control

	Treatment		
Property	CK	BPC-PS1	BPC-PS2
Plant height (cm)	84.00 ± 3.33^c	103.00 ± 3.22^b	117.00 ± 3.52^a
Plant density (10^3 ha^{-1})	123.33 ± 49.33^b	216.67 ± 41.63^a	223.33 ± 37.86^a
Root length (cm)	16.69 ± 1.61^b	24.55 ± 0.98^a	25.47 ± 1.07^a
Leaf area index (m^2)	2.89 ± 0.69^c	4.77 ± 0.57^b	6.08 ± 0.66^a
Chl_a (mg L^{-1})	3.16 ± 0.17^b	3.31 ± 0.26^b	4.06 ± 0.27^a
Chl_b (mg L^{-1})	0.93 ± 0.06	1.01 ± 0.07	1.69 ± 0.86
Chl_t (mg L^{-1})	5.04 ± 0.28^b	5.29 ± 0.41^b	6.52 ± 0.37^a

Different letters in a same row indicate significant differences ($P < 0.05$) between the treatments mean ($n = 15$), three replicated plot of each treatment with 15 random maize plant were observed.
CK, background control; Chl, chlorophyll; BPC, biochar and poultry manure compost; PS, pyroligneous solution.

Table 4. Maize leaf bioactivity and major nutrient content in leaf sap under BPC-PS treatment

	Treatment		
Nutrient	CK	BPC-PS1	BPC-PS2
Free proline (moles g^{-1})	142.44 ± 5.61^a	46.53 ± 4.44^b	24.60 ± 2.76^b
Ascorbic acid (mg g^{-1})	33.56 ± 3.10^a	16.97 ± 3.43^b	17.28 ± 1.72^b
Soluble sugar (mg g^{-1})	46.98 ± 4.34^a	23.75 ± 4.80^b	24.20 ± 2.42^b
Amino acids (mg g^{-1})	9.43 ± 0.87^a	5.21 ± 1.06^b	4.02 ± 0.34^b
MDA (moles g^{-1})	33.92 ± 2.29^a	29.24 ± 0.93^b	27.39 ± 2.17^c
Leaf sap N (mmol g^{-1})	1500 ± 186^c	4188 ± 131^a	3286 ± 194^b
Leaf sap P (mmol g^{-1})	97.96 ± 12.43^c	144.52 ± 30.87^b	176.88 ± 28.61^a
Leaf sap K^+ (mmol g^{-1})	2251 ± 139^b	2959 ± 168^a	3110 ± 177^a
Leaf sap K/Na	0.81 ± 0.13^b	2.10 ± 0.40^a	2.20 ± 0.34^a

Different superscript letters in a same row indicate significant differences ($P < 0.05$) between the treatments mean ($n = 3$).
MDA, malonaldehyde; CK, background control; BPC, biochar and poultry manure compost; PS, pyroligneous solution.

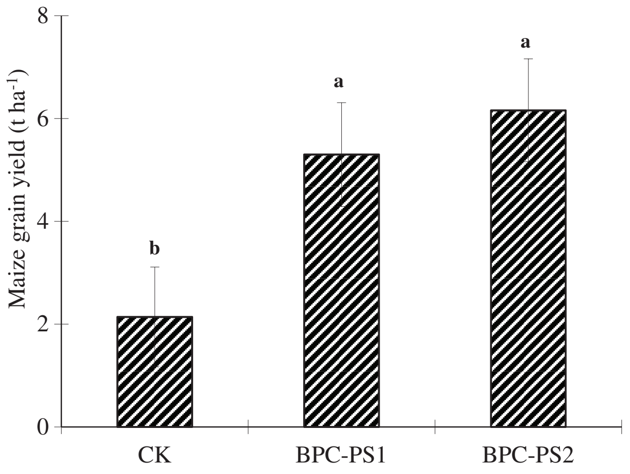

Figure 2. Maize grain yield in response to BPC-PS treatment. Different letters over the bar indicate significant differences ($P < 0.05$) between the treatments in a single year. CK, control; BPC, wheat straw biochar and poultry manure compost; PS, diluted pyroligneous solution.

Meanwhile, free proline content of maize leaf was significantly lower under the treatments than under control, being by 83% in BPC-PS2 plot. Coincidently, the amount of lipid peroxidation MDA in maize leaf was significantly ($P < 0.05$) lower by 13.8% and 19%, respectively, under BPC-PS1 and BPC-PS2 treatment over the control. Similarly, the leaf concentration of total ascorbic acid was found significantly lower by 48–49% under BPC-PS treatments over the control.

DISCUSSION

Effect of the BPC-PS treatment on plant growth and grain yield

Salt stress is a major abiotic stress on upland crops worldwide because of its direct impact on seed germination, root penetration, growth and finally yield of many crop species.[27,28] The results here showed that the amendment of BPC-PS caused a significant decrease in salt stress to, and thus growth improvement of, the maize crop. In detail, EC, Na^+ and Cl^-, as well-recognised indicators of soil salinity,[10,29] were all reduced by over 30% in treated maize plots with biochar and associated organic material. This was seen to be coincident with a reduction in Na^+ and Cl^- contents in maize leaf sap by a similar magnitude. Interestingly, soil exchangeable Ca^{2+} was increased by over 25% and 50%, and soil pH was decreased by 0.2 unit and 0.4 unit, respectively, under BPC-PS1 and BPC-PS2 over control, which was in agreement with a reduction in ESP by similar amounts under the two treatments (Table 2). Both crop straw biochar and poultry manure may contain large amounts of calcium, which could be released through biological degradation and thus increase the pool of exchangeable Ca^{2+} after amended to soil.[30] On the other hand, pyroligneous solution containing plenty of dissolved organic molecules and may have considerable effects on leaching the soluble salts and thus help improve soil structure and moisture content when used in combination with biochar manure compost. Nevertheless, a single amendment of BPC with repeated application of PS was performed in the 2-year experiment in this study. This could bring a synergic contribution to decreased soil salinity and enhanced soil nutrient supply in the soil. Thus, the treatment could significantly improve nutrient uptake and greatly recover crop productivity in the salt-stressed cropland, which has already been reported for wheat production in a previous study.[10]

However, the present study provides further information of reduced salt stress to plant growth with the treatment of BPC-PS on the saline soil. In a short-term greenhouse experiment amendment of biochar was shown to mitigate negative effects of salt addition on plants, via improvement of biological growth and photosynthetic activity, which was dramatically degraded in cropland with salinity.[31] Our findings here showed greatly reduced salt stress to plants, and maize plant growth/performance profoundly simultaneously recovered in the tested saline soil under BPC-PS treatment. Following BPC-PS treatment, a significant reduction of over

10% was observed in leaf electrolyte leakage, and in leaf sap Na^+ and Cl^- in the range 33–48% and of 41–48% (Fig. 1). However, large increases of 65% and 110% in leaf area index and 76% and 81% in plant density, together with a moderate increase in plant root length of 47% and 53%, respectively, under BPC-PS1 and BPC-PS2 were observed (Table 3). An increase in plant height was shown in range of 23–39% and almost no significant change in chlorophyll b content under both BPC-PS treatments.

The enhanced plant density due to successful germination of maize seeds along with an increase in leaf area index provided maize growth after greatly reduced salt stress with the treatments over the untreated controls. Biochar amendment to salt soil increased plant biomass production by ~50% and recovered growth performance similar to non-saline soil, though chlorophyll contents and photosynthetic carbon assimilation were not significantly affected.[31] Nevertheless, reduction in photosynthetic activity and chlorophyll content in plant leaves have often been reported.[32] Of course, nutrient improvement could potentially contribute to the greatly improved plant growth and performance with enhanced silicon availability.[33] Wheat biochar contains a considerable pool of silicon in high pH reaction, which has been already shown beneficial effects for rice production in field experiments.[33] Moreover, plant nutrient uptake was greatly (by comparable altitude to that of plant performance improvement in) promoted under the BPC-PS treatments (data in Table 4). In particular, considerably and significantly increased K^+ and K^+/Na^+ ratio in leaf sap also indicated the alleviation of salt stress to maize plants under the treatments. Increased leaf content of phosphorus and potassium could play a vital role in mitigating salt stress damage to plant physiology,[34] as high sodium and poor phosphorus created specific ion toxicity in plant cells. The great increase in plant growth, performance and thus maize yield could be explained, in part at least, by the great improvement of seed germination, plant density, nutrient uptake and enhanced leaf area following reduction in salt stress to plants under the treatments. Thus, the fact of no change in photosynthesis rate did not comprise these beneficial effects on increasing the maize productivity following the reduction in salt stress to plants.

Effect of BPC-PS amendment on leaf bioactivity and health

Free proline, as an inert compatible osmolyte, could play a key role in regulating the osmotic potential in plant cell and protecting sub-cellular structures and macromolecules from osmotic stress.[35] Also, it is often considered as a potent reactive oxygen species scavenger associated with the prevention of apoptosis, like plasma cell death.[36] Thus, the content of free proline could be one important bio-indicator of osmotic stress to plant health. To defend abiotic stress from salinity, free proline content is generally increased in plant cells via protein biosynthesis or metabolism, including hydrolysis and oxidative degradation of proteins. While a high content of free proline (up to 142 moles g^{-1}) was found in leaves from untreated plots, the content was four and six times lower under BPC-PS1 and BPC-PS2, respectively, indicating a great alleviation of osmotic stress to plant cells of maize. This is further supported by the changes in other bioactive parameters in maize leaf with the treatments.

The soluble sugar, amino acids and ascorbic acid compounds all contribute to osmotic adjustment of plants grown under abiotic stresses. Salt stress generally induces increases in soluble sugar, ascorbic acid and other biocompounds with disordered biochemical activity in plant life cycle.[37] Furthermore, as one of the main products of bioactivity lipid peroxidation, MDA has an important role in abiotic stress since it reduces salt-induced oxidation damage by increasing the activity of antioxidant enzymes and by lowering lipid peroxidation.[38] A higher level of MDA was reported for aquatic plants in waters with higher level of salinity.[39] In the present study leaf contents of soluble sugar, amino acids and ascorbic acids were all significantly decreased under BPC-PS treatments over the control (Table 4). Accordingly, accumulation of MDA in the plant cell was seen to be significantly reduced under the BPC-PS treatments without considerable difference between plants treated for 1 and 2 years. The significant changes in the leaf contents of these osmotic stress regulating agents followed the trend in reduction in leaf sap salinity but was by much lower magnitude as compared to those in plant growth and performance, and the changes in free proline contents under the treatments. While all these further give evidence for alleviating salt stress to plants, the contents of these compounds did not exert great response to the BPC treatment nor changes with experiment length. The finding here suggests leaf bioactivity and plant health were greatly improved with much less accumulation of free radicals and their damage to plant cell under BPC-PS treatment, and content of free proline in leaf could be used as a key indicator for alleviating salt stress to plant cells with the BPC-PS amendment to saline soil, which seems sensitive both to the treatment effect and the experiment length effect. Here we could propose that the amendment of biochar–manure compost (BPC-PS) is not only effective for reducing salt stress in the cropland but also equally important for maize biochemical processes.

CONCLUSION

The present study clearly shows the significant beneficial effects of biochar–manure compost in conjunction with pyroligneous solution on improving maize growth and performance, and alleviating salt stress to plant following reduction in soil salinity in the saline soil. However, the BPC treatment effects are greater on maize growth, performance and grain yield than on individual plant bioactivity and health. While leaf chlorophyll content or photosynthetic carbon assimilation activity could not prove to be a useful indicator for the improvement of plant growth or productivity here, the leaf content of free proline could used for indicating an alleviation of salt stress and damage to plant cell under the treatment. Moreover, enduring and greater effects are shown in plots 2 years after a single BPC-PS treatment than in those for the first year, suggesting a sustainable role of the biochar-based material. Overall, combined use of biochar with manure compost aligned with leaching by pyroligneous solution from pyrolysis of crop straw could be taken as a key measure to combat the salinisation in croplands and sustain crop productivity in croplands increasingly threatened by global climate change in arid and semi-arid regions of the world.

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