EFFECTS OF STRAW RETURN AND BIOCHAR APPLICATION ON SOIL NUTRIENTS AND OSMOTIC REGULATION IN COTTON UNDER DIFFERENT SOIL SALINITY LEVELS

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(Received 1st Sep 2022; accepted 7th Dec 2022)

Abstract. To clarify the effects of straw return and biochar application on the amelioration of soils with different salinity levels and cotton growth, in this soil column simulation test, straw and biochar were applied to soils with different salinity levels (1.5 (S1), 5 (S2) and 10 (S3) g/kg), with application rates equal to the same amount of carbon (two carbon levels: 6 t/hm^2 of straw (C1) = 2.25 t/hm^2 of biochar (B1); 12 t/hm^2 of straw (C2) = 4.5 t/hm^2 of biochar (B2)). Then, changes in soil physicochemical properties and cotton physiology were determined. The results showed that straw return and biochar application reduced soil salinity at S1, S2, and S3 levels. Besides, both of them increased soil water, total nitrogen, organic matter, available phosphorus, and available potassium contents, but there was no difference between S3 soil and S1 soil and between S3 soil and S2 soil ($p > 0.05$). Straw return and biochar application also increased chlorophyll and soluble sugar contents in cotton leaves ($p < 0.05$), and decreased the relative conductivity and malondialdehyde content in S1 and S2 soils ($p < 0.05$). However, in S3 soil, there was no difference between C1 treatments and CK and between B1 treatments and CK ($p > 0.05$). Thus, straw return and biochar application can improve the physicochemical properties of saline soil and regulate osmolytes in cotton, but the amelioration effect of low application rates on high-salinity soils is insignificant ($p > 0.05$).

Keywords: *biochar, cotton physiology, chemical property, salinized soil, straw*

Introduction

Soil salinization is one of the important causes of soil degradation. According to the Food and Agriculture Organization of the United Nations (FAO), the global saline soil area reaches 9.32×10^8 hm^2, accounting for about 2.25% of the world's arable land (Daliakopoulos et al., 2016). Especially, in 2021, the theme of World Soil Day was "Halt soil salinization, boost soil productivity". This indicates that soil salinization has become a global concern (Hassani et al., 2021). Saline soils have high contents of soluble salts such as Na^+, Ca^{2+}, Cl^-, and SO_4^{2-}, and poor physical, chemical, and biological properties, resulting in low soil productivity and degradation of ecological functions. The improvement of saline soils is of great significance for increasing arable land area and food security (Litalien et al., 2020).

Soil salinization always causes salt stress to crop (Isayenkov, 2012), leading to ion poisoning (Zhang et al., 2018; Zörb et al., 2019) and yield reduction (Butcher et al., 2016), which seriously restricts the sustainable development of agriculture (Yang et al., 2020). Xinjiang has the largest area of saline soil in China (about 2.18×10^7 hm^2). Besides, Xinjiang is also China's largest cotton producing area. Therefore, cotton straw is very abundant there. Recent studies have shown that straw return and carbonized straw return may be effective ways to improve saline soil properties in arid and semi-arid areas.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2102_957974

Crop straw is rich in nutrients such as nitrogen, phosphorus, and potassium. Previous studies have shown that straw return has a positive effect on soil structure (Huang et al., 2017; Meng et al., 2019) and water retention capacity (Zhang et al., 2021). Besides, straw return can also increase soil total nitrogen (Yin et al., 2018), available phosphorus, and potassium contents (Huang et al., 2012; Yan et al., 2016; He et al., 2017), and application of chemical fertilizers together with straw return can more significantly increase soil available potassium and organic matter contents (Wang et al., 2015). More importantly, some studies have found that straw return could effectively alleviate salt stress, regulate the oxidative stress responses in crops (Ali et al., 2017), and enhance crop salt tolerance (Zhang et al., 2019). For example, Ren et al. (2021) showed that straw return could reduce soil salt and malondialdehyde contents in crops, increase soluble sugar content, and promote crop growth.

Biochar is a carbon-rich product produced by high-temperature pyrolysis of biomass under anoxic or anaerobic conditions. It has the characteristics of large specific surface area, light weight, and low density (Pan et al., 2021). As a stable carbon source, biochar can significantly increase the organic matter content (Zhang et al., 2019), promote the transformation of soil nitrogen (Moradi et al., 2019), and ensure the retention of C and N in salinized soil (Wang et al., 2015). Biochar can be used as a phosphorus carrier in salinized soil. Through the adsorption and desorption process of biochar to phosphorus, it can maintain the phosphorus content suitable for plant growth (Yang et al., 2022). Studies have found that biochar can alleviate oxidative stress in crops caused by high soil salinity (Kul et al., 2021; Egamberdieva et al., 2022), improve crop root and leaf morphology (El Nahhas et al., 2021), and increase photosynthetic rate and nutrient absorption (Zhao et al., 2020). Gou et al. (2013) found that application of biochar into salinized soil could significantly reduce the relative electrical conductivity (REC) of crop leaves and roots, and promote physiological stability in crops (Ren et al., 2021). Farhangi-Abriz et al. (2017) showed that application of biochar significantly increased the chlorophyll content and biomass of legumes under salt stress.

At present, most researches mainly focuses on the application of straw or biochar alone to achieve the purpose of improving salinized soils. There are few reports on the effects of straw and straw-derived biochar with application rates equal to the same amount of carbon on the water, salt, and nutrient status of salinized soil. In addition, the effect of straw return and biochar application on organic osmolytes in cotton under different soil salinities is also not clear. Therefore, in this study, soil column simulation experiments were conducted to explore the effects of straw and biochar with application rates equal to the same amount of carbon on the water, salt, and nutrient contents of soils with different salinities, and changes in cotton physiology were also analyzed. We hypothesized that (1) straw return and biochar application might reduce soil salinity and increase nutrient contents. (2) In the soils with different salinities, the application of different amounts of carbon in the ways of straw and biochar might have different effects on cotton physiology, but salt stress might be alleviated with the increase of the application rate. This study will deepen our understanding of the use of cotton straw to improve saline soil in arid regions.

Materials and methods

Experimental materials

Soil column experiments were carried out at the Experimental Station of Shihezi University in Xinjiang Province, China (86°3′N, 44°18′E) in 2020. The saline-alkali soils of different salinities were prepared by mixing the saline-alkali soil collected from a salinized wasteland in Beiwucha in Manas County, Xinjiang (soil salinity (0-30 cm soil layer): 20-30 g/kg) and the soil collected from a nearby cotton field (continuous cotton cropping > 10 years) (soil salinity: 1-1.5 g/kg) in different proportions. The soil mixture is rich in chloride and sulfate, with main salt ions of Na^+, Ca^{2+}, K^+, SO_4^{2-}, and Cl^-. Cotton straw, with total nitrogen content of 0.85%, total phosphorus content of 0.46%, total potassium content of 2.11%, organic carbon content of 48.76%, and pH value of 6.95, was collected from local cotton fields. Biochar, with total nitrogen content of 1.32%, total phosphorus content of 0.75%, total potassium content of 1.43%, organic carbon content of 65.35%, and pH value of 9.43, was prepared by oxygen-limited pyrolysis of cotton straw at the lower limit of 450 ℃ for 6 h.

There were two factors in the experiment. One was the carbon application rate. Cotton straw and carbonized cotton straw with the same amount of carbon were applied in two application rates, including straw applications of 6 $t \cdot hm^{-2}$ (C1) and 12 $t \cdot hm^{-2}$ (C2), biochar applications of 2.25 $t \cdot hm^{-2}$ (B1) and 4.5 $t \cdot hm^{-2}$ (B2), and a control group (CK) without straw and biochar application. The carbon content in C1 and C2 were equal to that in B1 and B2, respectively. The other was the soil salinity. Soils were mixed in different proportions to prepare soils of three salinities: 1.5 g/kg (S1), 5 g/kg (S2), and 10 g/kg (S3). Straw and biochar were fully mixed with the soils before the test. Soil column was 36 cm in diameter and 80 cm in height, and the bottom was closed to avoid leaching of water, salt, and nutrients. The soils were transferred into the soil columns according to the target soil bulk density (1.42 g/cm^3), and then buried in the field flush with the surface. There were 15 treatments totally (*Tables 1-3*, *Figure 1*), and each had 3 replicates.

Table 1. *Physical and chemical properties of soils*

Soil type	pH	TN content	Total salt content	SOM content	AP content	AK content	Types of saline soil
		(g/kg)	(g/kg)	(g/kg)	(mg/kg)	(mg/kg)	
Soil from a saline wasteland	8.67	0.41	24.35	8.23	9.86	149.85	Chloride-sulfate type
Soil from a cotton field	8.1	0.65	1.5	14.57	22.26	232.67	Chloride-sulfate type

Note: TN: Total nitrogen content; SOM: Organic matter content; AP: Available phosphorus content; AK: Available potassium content

Table 2. *Classification of salinized soil (Farhangi-Abriz et al., 2017)*

Grade	Non-salinized soil	Slightly salinized soil	Moderately salinized soil	Salinized soil	Severely salinized soil
Total salt content /(g/kg)	< 3	3 ~ 6	6 ~ 10	10 ~ 20	>20
Plant growth status	Normal	Slightly inhibited	Moderately inhibited	Severely inhibited	Death

Drip irrigation and plastic mulching were employed in cotton planting. Cotton (variety Xinluzao 43) was sown on April 18, 2020. Four rows were sown under one film (66 cm), and one drip irrigation tape was laid between two rows. The plant spacing was 11 cm. Four cotton plants were planted in each soil column. The column spacing was 10 cm. Ten times of irrigation were carried out during the growth period of cotton (5400 m^3/hm^2 totally), and the irrigation amount was controlled by water meter. Superphosphate (P_2O_5, 46%) of 105 $kg \cdot hm^{-2}$ and potassium sulfate (K_2O, 50%) of 75 $kg \cdot hm^{-2}$ were basally applied. The application rate of urea (total nitrogen ≥46.2%) was 360 $kg \cdot hm^{-2}$, of which 20% was basally applied, and 80% was topdressed with irrigation water. Other agricultural management measures were consistent with local practice.

Table 3. *Experimental design*

Soil salinity	Treatment description	Treatment
1.5 g/kg (S1)	No application of cotton straw and biochar	S1CK
	Cotton straw of 6 $t \cdot hm^{-2}$ was applied to the soil	S1C1
	Cotton straw of 12 $t \cdot hm^{-2}$ was applied to the soil	S1C2
	Biochar of 2.25 $t \cdot hm^{-2}$ was applied to the soil	S1B1
	Biochar of 4.5 $t \cdot hm^{-2}$ was applied to the soil	S1B2
5 g/kg (S2)	No application of cotton straw and biochar	S2CK
	Cotton straw of 6 $t \cdot hm^{-2}$ was applied to the soil	S2C1
	Cotton straw of 12 $t \cdot hm^{-2}$ was applied to the soil	S2C2
	Biochar of 2.25 $t \cdot hm^{-2}$ was applied to the soil	S2B1
	Biochar of 4.5 $t \cdot hm^{-2}$ was applied to the soil	S2B2
10 g/kg (S3)	No application of cotton straw and biochar	S3CK
	Cotton straw of 6 $t \cdot hm^{-2}$ was applied to the soil	S3C1
	Cotton straw of 12 $t \cdot hm^{-2}$ was applied to the soil	S3C2
	Biochar of 2.25 $t \cdot hm^{-2}$ was applied to the soil	S3B1
	Biochar of 4.5 $t \cdot hm^{-2}$ was applied to the soil	S3B2

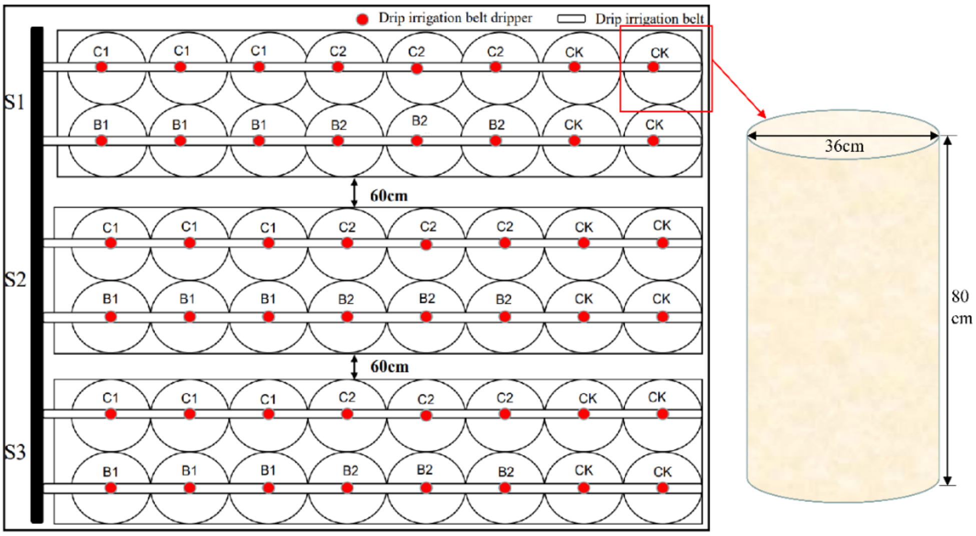

Figure 1. *Schematic diagram of soil column test design*

Test indexes

Determination of soil indexes

The 0-40 cm soil layer was collected during the boll opening stage of cotton, and soil physical and chemical properties was determined with reference to *Analysis of Soil Agrochemicals* (Lu, 1999). Soil pH was measured with a pHS-3C pH meter (Shilu

Instrument Co. Ltd, Shanghai, China). Soil water content (SWC) was determined by the drying method, and soil conductivity ($EC_{1:5}$) was determined by the conductivity meter (METTLER TOLEDO, Shanghai) (water: soil = 5: 1). The available phosphorus (AP) content was determined by the molybdenum antimony anti-colorimetric method, the available potassium (AK) content was determined by the flame photometer method, the soil total nitrogen (TN) content was determined by the Kjeldahl method, and the soil organic matter (SOM) content was determined by the potassium dichromate titration method.

After cotton harvest, the soils of the 0-40 cm layer were sampled from each treatment using soil drills. There were three sampling points for each treatment, and about 300 g of soil was sampled from each sampling point. A total of 45 soil samples were finally obtained. To prevent evaporation-induced water loss, soil samples were put into sealed bags, stored in ice boxes, and taken back to the lab. Part of the fresh soil was used to determine the soil water content according to the method of Lu (1999), and the other part was used to determine soil salinity, nutrient content, and pH after air-drying, grinding, and passing through a 2 mm sieve. Soil pH was determined by using a pH meter (pHS-3C, Shilu Instrument Co. Ltd, Shanghai, China), and soil electrical conductivity (EC) was determined by using a conductivity meter (Mettler Toledo, Shanghai, China) (water: soil=5: 1). Besides, soil available phosphorus content (AP) and organic matter content (SOM) were determined by the methods of Lu (1999), soil available potassium content (AK) was determined by using a flame photometer (M410, Sherwood, England) (Bao, 2000), and soil total nitrogen content (TN) was determined by Kjeldahl method (Bao, 2000).

The fitted relationship between soil conductivity and soil salt content was used to characterize soil salinity (Wang, 2014).

$$SCC = 3.51 \times EC_{1:5} + 0.38 \ (R^2=0.9517) \qquad \text{(Eq.1)}$$

where SCC is the soil salt content ($g \cdot kg^{-1}$) and $EC_{1:5}$ is the soil conductivity ($mS \cdot cm^{-1}$).

Determination of physiological indicators of cotton

Three cotton plants were collected from each treatment at the budding, flowering, and boll-forming stages, transferred in ice box, and brought back to the laboratory to determine the physiological indicators of cotton. The contents of chlorophyll a (Chl a) and chlorophyll b (Chl b) in cotton leaves were determined by spectrophotometry (Li et al., 2016), the malondialdehyde content (MDA) in cotton leaves (LMDA) and root (RMDA) were determined by thiobarbituric acid method (Li et al., 2016), and the soluble sugar (SS) content in cotton leaves (LSS) and root (RSS) were determined by anthrone colorimetry (Li et al., 2016). The relative electrical conductivity (REC) of cotton leaves (LRC) and root (RRC) were determined by soaking and boiling method (Li et al., 2016).

$$\text{Relative electrical conductivity} = L1 - L0/L2 - L0 \qquad \text{(Eq.2)}$$

where L0 is the background conductivity, L1 is the conductance value of extravasate before a water bath at 100 °C, L2 is the conductance value of extravasate after the water bath at 100 °C.

Data analysis and processing

Data processing and two-way ANOVA were completed using Excel 2010 (Microsoft, USA) and SPSS 19.0 (SPSS Inc., Chicago, USA), and Duncan's multiple comparisons test was performed to determine the significance of differences at $p < 0.05$. Principal component analysis (PCA) and graphing were completed using Origin 2018 (Northampton, MA, USA).

Results

Effects of straw return and biochar application on the water content, pH, and electrical conductivity of soils with different salinities

There was no difference in soil pH between C1 (S1C1, S2C1, and S3C1) treatment and CK (S1CK, S2CK, and S3CK) and between C2 (S1C2, S2C2, and S3C2) treatments and CK ($p > 0.05$). There was also no difference in soil pH between C2 treatments and C1 treatments and between B1 treatments and B2 treatments ($p > 0.05$).

The higher the soil salinity, the higher the soil water content (*Figure 2*). Compared with CK, straw return (C1 and C2 treatments) and biochar application (B1 and B2 treatments) increased the water content of soils with different salinities. Soil water contents in B2 (S1B2, S2B2, and S3B2) treatments were the highest, which were 24.19%, 19.01%, and 24.19% higher than that in the CK, respectively ($p < 0.05$). Besides, soil water content was higher in S1B2 and S2B2 treatments than in S1C1, S1C2, S2C1, and S2C2 treatments ($p < 0.05$). There was no difference between S3B2 and S3C1 treatments and between S3B2 and S3C2 treatments ($p > 0.05$).

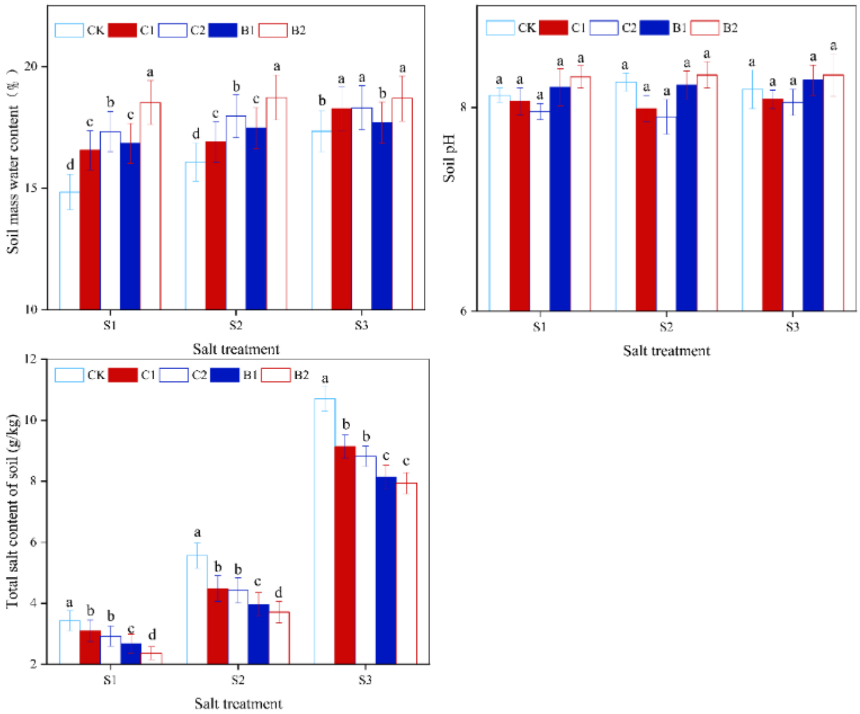

Figure 2. *Effects of straw return and biochar application on the physical and chemical properties of soils with different salinities. Bars represent the standard deviation of the mean (n = 3). Different lowercase letters indicate significant differences between different treatments (p < 0.05)*

Compared with CK, straw return and biochar application significantly decreased soil salinity ($p < 0.05$) (*Figure 2*). At the same soil salinity level, the soil salinity was lower in biochar application (B) treatments than in straw return (C) treatments ($p < 0.05$). Besides, there was no difference between the two straw return treatments ($p > 0.05$). The soil salinity was lower in S1B2 and S2B2 treatments than in S1B1 and S2B1 treatments ($p < 0.05$). There was no difference in soil salinity between S3B1 and S3B2 treatments ($p > 0.05$).

Changes in soil nutrient contents of soils with different salinities after straw return and biochar application

At the same soil salinity level, the contents of SOM, AP, and AK in B and C treatments were higher than those in the CK ($p < 0.05$), and those in B2 treatments were the highest. Besides, the contents of TN, SOM, AP, and AK in C1 and B1 treatments were lower than those in C2 and B2 treatments, respectively ($p < 0.05$).

There was no difference in the contents of SOM and AP between S1C1 and S1B1 treatments and between S2C1 and S2B1 treatments ($p > 0.05$), but the contents of SOM and AP in S1B2 and S2B2 treatments were higher than those in S1C2 and S2C2 treatments ($p < 0.05$). Besides, there was no difference in the contents of SOM and AP between S3B2 and S3C2 treatments ($p > 0.05$). The soil AK content in S2B1 and S3B1 treatments were higher than that in S2C1 and S3C1 treatments ($p < 0.05$), respectively, and there was no difference between S3B1 and S3C1 treatments ($p > 0.05$). The soil TN content were higher in S2B1, S2B2, S3B1, and S3B2 treatments than in the S2C1, S2C2, S3C1, and S3C2 treatments ($p < 0.05$). There was no difference in soil TN content between S1C2 and S1B2 treatments ($p > 0.05$) (*Table 4*).

Table 4. *Effects of straw return and biochar application on nutrient contents in soils with different salinities*

Treatment		TN content (g/kg)	SOM content (g/kg)	AP content (mg/kg)	AK content (mg/kg)
S1	CK	0.59±0.13e	14.07±0.34gh	28.83±1.71gh	276.75±18.88h
	C1	0.67±0.16c	15.08±0.69e	32.89±1.16e	307.14±14.95e
	C2	0.72±0.13a	17.34±0.55b	36.28±1.35b	355.36±18.23b
	B1	0.69±0.13b	15.05±0.46e	34.65±1.22e	326.21±13.31cd
	B2	0.73±0.16a	18.44±0.33a	37.67±1.54a	375.34±11.76a
S2	CK	0.50±0.11i	12.90±0.60i	27.75±1.64i	261.08±14.64i
	C1	0.51±0.13h	13.63±0.43h	31.67±1.31h	286.12±13.68gh
	C2	0.57±0.09f	15.93±0.25d	34.82±1.21d	336.16±15.56c
	B1	0.54±0.12g	13.85±0.52h	32.21±1.43h	302.61±10.36e
	B2	0.64±0.11d	16.75±0.51c	36.29±1.45c	367.66±10.69a
S3	CK	0.41±0.12k	11.17±0.41j	27.02±1.65j	262.81±11.84i
	C1	0.44±0.13k	12.94±0.46i	31.11±1.13i	274.98±13.71h
	C2	0.52±0.07h	14.52±0.65fg	32.10±1.28fg	296.70±12.45fg
	B1	0.48±0.09j	12.90±0.51i	30.32±1.16i	285.94±15.69gh
	B2	0.57±0.11f	14.95±0.62ef	34.31±1.39ef	322.12±15.39d
Significance	Salt treatment (ST)	*	**	**	**
	Material treatment (BT)	*	**	**	**
(*p*-value)	Salt× Material treatment STxBT	*	**	**	**

Notes: TN, Total nitrogen content; SOM, Organic matter content; AP, Available phosphorus content; AK: Available potassium content; All values are means ± SD (n = 3). Different lowercase letters represent significant differences between treatments at $p < 0.05$

Effect of straw return and biochar application on the physiological characteristics of cotton in key growth periods

In the growth period, the contents of Chl a and b increased first and then decreased in boll-forming stage, with the highest occurring in flowering stage. The contents of Chl a and b in B1, B2, C1, and C2 treatments were higher than those in the CK ($p < 0.05$). With the increase of the application rates of straw and biochar, the contents of Chl a and b increased ($p < 0.05$), and the contents of Chl a and b in B2 and B1 treatments were higher than those in C2 and C1 treatments ($p < 0.05$), respectively. The higher the soil salinity, the lower the contents of Chl a and b in each growth stage. The contents of Chl a and b in S1C1, S1C2, S2C1, S2C2, S3C2, S1B1, S1B2, S2B1, S2B2, and S3B2 treatments were higher than those in CK ($p < 0.05$), but there was no difference between S3B1 treatment and CK and between S3C1 treatment and CK ($p > 0.05$) (*Figure 3*).

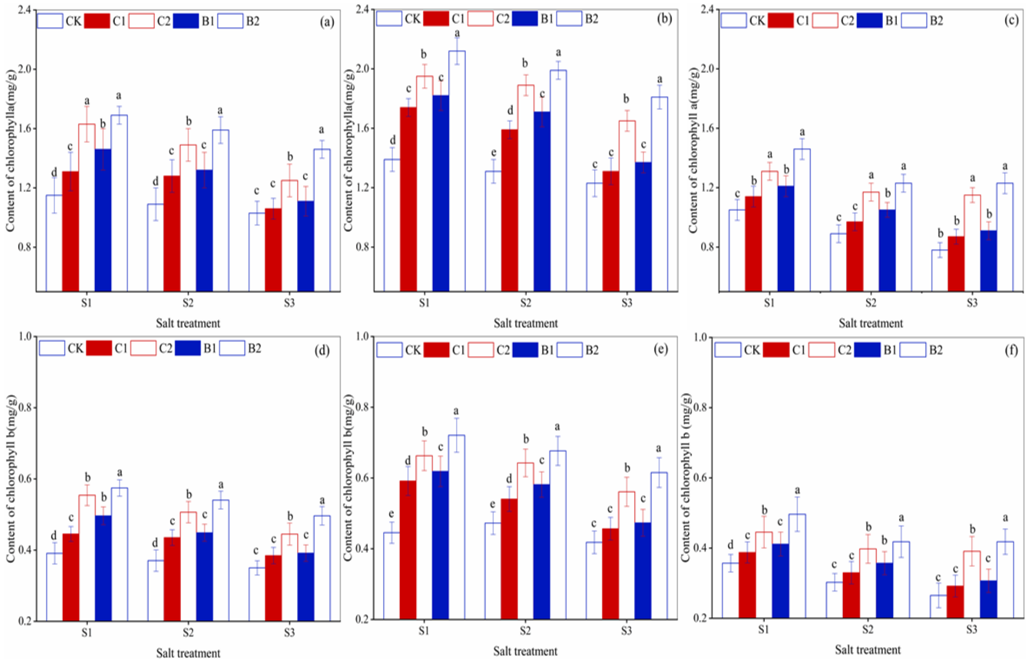

Figure 3. *Effect of straw return and biochar application on cotton leaf chlorophyll content in the key growth stages. Bars represent the standard deviation of the mean (n = 3). Note: (a), Chl a content at the bud stage; (b), Chl a content at the flowering stage; (c), Chl a content at the boll-forming stage (d), Chl b content at the bud stage; (e), Chl b content at the flowering stage; (f), Chl b content at the boll-forming stage. Different lowercase letters indicate significant differences between different treatments (p < 0.05)*

The REC of cotton leaves and root gradually decreased in the growth period, and the REC of root was higher than that of leaves (*Fig. 4*). Compared with CK, straw return and biochar application significantly reduced the REC of cotton leaves and root under the three salinity levels, and the REC of leaves and root decreased more significantly with the increase of straw and biochar application rates. The effect of B1 and B2 treatments on reducing the REC of leaves and root were better than that of C1 and C2 treatments, respectively. The REC in S1C1, S1C2, S2C1, S2C2, S3C2, S1B1, S1B2, S2B1, S2B2, and S3B2 treatments was higher than that in the CK ($p < 0.05$), but there was no difference between S3B1 treatment and CK and between S3C1 treatment and CK ($p > 0.05$).

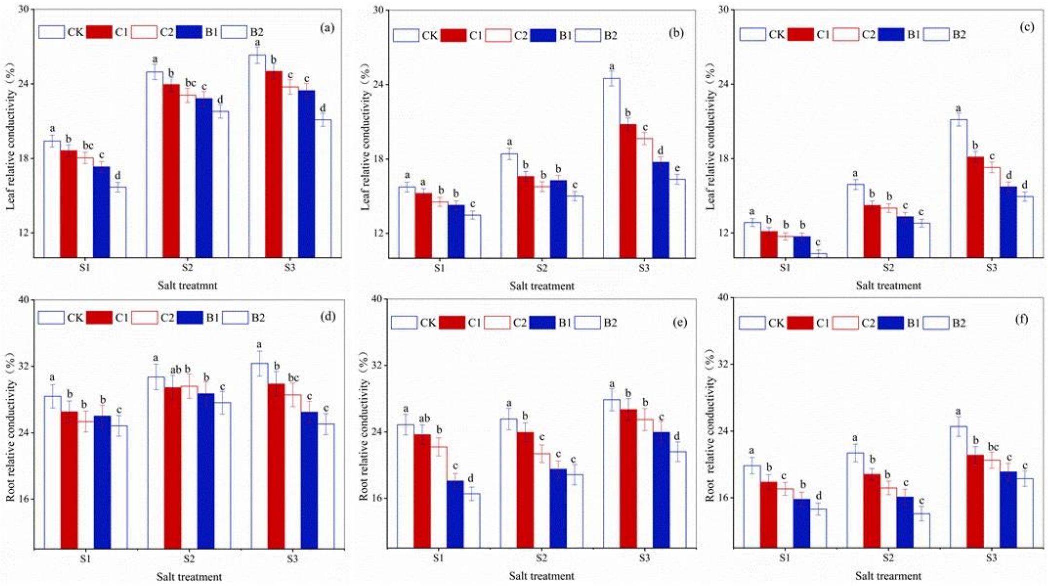

__Figure 4.__ Effect of straw return and biochar application on the relative conductivity in the key growth periods of cotton. (a), Leaf relative conductivity at the bud stage; (b), Leaf relative conductivity at the flowering stage; (c), Leaf relative conductivity at the boll-forming stage; (d), Root relative conductivity at the bud stage; (e), Root relative conductivity at the flowering stage; (f), Root relative conductivity at the boll-forming stage. Bars represent the standard deviation of the mean (n = 3). Different lowercase letters indicate significant differences between treatments (p < 0.05)

The MDA content in cotton leaves and root gradually decreased in the growth period, while the content of soluble sugar showed an increasing trend (*Table 5*). The contents of LMDA and RMDA in S1C1, S1C2, S2C1, S2C2, S3C2, S1B1, S1B2, S2B1, S2B2, and S3B2 treatments were lower than those in CK ($p < 0.05$), while the contents of LSS and RSS showed an opposite trend ($p < 0.05$). There was no difference in the contents of MDA and soluble sugar between S3C1 treatment and CK and between S3B1 treatment and CK ($p > 0.05$). With the increase of biochar application rate, the MDA content decreased while the content of soluble sugar increased. The contents of LMDA and RMDA in B2 treatments were lower than those in B1 treatments ($p < 0.05$), while the contents of LSS and RSS were higher than those in B1 treatments ($p < 0.05$). The effect of biochar application was better than that of straw return. The MDA content in B1 and B2 treatments was lower than that in C1 ($p > 0.05$) and C2 ($p < 0.05$) treatments, respectively, while the soluble sugar content in B1 and B2 treatments was higher than that in C1 ($p > 0.05$) and C2 ($p < 0.05$) treatments, respectively. Besides, the MDA and soluble sugar contents of cotton leaves and root increased with the increase of soil salinity

PCA analysis of soil chemical indexes, nutrient contents, and cotton physiological indicators at different soil salinity levels

The average contribution rates of PC1 in S1 (S1C1, S1C2, S1B1, and S1B2), S2 (S2C1, S2C2, S2B1, and S2B2), and S3 (S3C1, S3C2, S3B1, and S3B2) treatments were 86.3%, 84.6%, and 81.7%, respectively.

Table 5. *Effects of straw return and biochar application on the malondialdehyde and soluble sugar contents of cotton in key growth stages*

.		Leaf MDA content （mg/kg）			Root MDA content （mg/kg）			Leaf soluble sugar content （mg/kg）			Root soluble sugar content （mg/kg）		
Treatment	**Growth period** **Treatment**	**Bud stage**	**Flowering stage**	**Boll-forming stage**	**Bud stage**	**Flowering stage**	**Boll-forming stage**	**Bud stage**	**Flowering stage**	**Boll-forming stage**	**Bud stage**	**Flowering stage**	**Boll-forming stage**
S1	CK	54.43± 1.09Aa	48.99± 0.98Ba	41.6± 0.83Ca	48.39± 0.97Ae	41.61± 0.83Ba	32.62± 0.65Ca	4.35± 0.11Ce	5.33± 0.13Bd	6.58± 0.14Ad	5.04± 0.10Bd	5.86± 0.12Be	7.58± 0.15Ad
	C1	52.25± 1.04Ab	47.03± 0.94Bb	38.38± 0.77Cb	45.74± 0.91Ad	36.12± 0.72Bb	30.37± 0.61Cb	4.89± 0.12Cd	5.86± 0.15Bc	7.39± 0.18Ac	5.14± 0.11Cd	6.23± 0.12Bd	9.01± 0.18Ac
	C2	46.75± 0.92Ac	45.63± 0.85Ac	34.08± 0.68Bc	38.38± 0.77Ab	25.64± 0.51Bd	26.05± 0.52Cd	5.82± 0.13Cb	6.82± 0.17Bb	8.38± 0.21Aab	6.04± 0.13Cb	8.18± 0.16Bb	10.68± 0.21Aa
	B1	49.76± 1.00Ab	44.78± 0.91Bbc	37.31± 0.75Cb	43.32± 0.87Ac	29.35± 0.59Bc	27.54± 0.55Cc	5.38± 0.13Bc	6.12± 0.15Bc	8.04± 0.20Ab	5.55± 0.12Cc	7.56± 0.15Bc	10.24± 0.20Ab
	B2	41.5± 0.83Ad	40.75± 0.81Ad	33.22± 0.61Bd	34.9± 0.69Aa	22.06± 0.44Be	24.95± 0.46Ce	6.29± 0.16Ca	7.31± 0.18Ba	8.67± 0.22Aa	7.24± 0.14Ca	8.58± 0.17Ba	10.73± 0.21Aa
S2	CK	59.35± 1.19Aa	53.43± 1.07Ba	51.51± 1.03Ca	66.56± 1.33Ad	64.19± 1.28Ad	62.56± 1.25Ae	6.26± 0.16Cd	8.63± 0.21Bd	10.49± 0.22Ab	8.32± 0.17Bc	9.43± 0.19Bc	11.09± 0.12Ac
	C1	56.14± 1.12Ab	52.33± 1.05Bab	42.01± 0.84Cb	60.10± 1.20Ac	57.87± 1.16Bc	55.79± 1.12Cd	7.43± 0.18Cc	8.96± 0.23Bcd	11.73± 0.24Aa	9.48± 0.19Cb	10.61± 0.21Bb	11.55± 0.13Ab
	C2	52.96± 1.01Ac	50.37± 1.01Ac	36.34± 0.73Bc	56.93± 1.12Ab	51.42± 1.03Bb	47.95± 0.96Cb	8.64± 0.21Cb	9.83± 0.24Bb	11.49± 0.26Aa	9.76± 0.20Bb	10.95± 0.22Bab	11.74± 0.14Aab
	B1	53.93± 1.08Ab	51.14± 1.02Bbc	38.49± 0.77Cb	58.45± 1.17Abc	55.93± 1.12Cbc	51.79± 1.04Cc	7.93± 0.20Ac	9.23± 0.23Bc	11.39± 0.24Ba	9.52± 0.19Ab	10.87± 0.22Bb	11.59± 0.13Cb
	B2	50.43± 0.96Ad	48.99± 0.98Ad	31.39± 0.63Bd	53.88± 1.08Aa	48.61± 0.97Ba	44.64± 0.89Ca	8.96± 0.22Ca	10.64± 0.26Ba	11.62± 0.25Aa	10.12± 0.21Ca	11.23± 0.23Ba	12.03± 0.14Aa

.		**Leaf MDA content**（mg/kg）			**Root MDA content**（mg/kg）			**Leaf soluble sugar content**（mg/kg）			**Root soluble sugar content**（mg/kg）		
S3	CK	79.09±1.58Aa	72.61±1.53Ba	65.95±1.38Ca	81.64±1.11Aa	74.45±1.24Ba	67.13±1.16Ca	8.14±0.20Cc	10.21±0.25Bc	12.78±0.32Ac	10.71±0.21Bc	11.02±0.22Bc	12.78±0.26Ac
	C1	77.89±1.44Aa	70.38±1.27Ba	59.04±1.14Ca	79.14±0.91Aa	72.06±1.16Ba	65.74±1.15Ca	8.21±0.21Cc	10.57±0.26Bc	13.12±0.34Ac	11.09±0.22Bc	11.47±0.23Bc	13.05±0.27Ac
	C2	72.56±1.40Ab	66.29±1.32Bb	55.16±1.07Cb	73.85±0.75Ab	65.38±0.91Bb	58.71±0.90Cb	9.31±0.23Cb	11.81±0.31Bb	14.75±0.36Ab	12.39±0.27Bb	12.48±0.25Bb	13.96±0.28Ab
	B1	76.15±1.31Aa	69.98±1.25Ba	64.68±1.10Ca	78.92±0.85Aa	71.77±1.03Ba	64.61±0.97Ca	8.37±0.21Cc	10.71±0.31Bc	14.24±0.36Ac	11.27±0.26Bc	11.48±0.24Bc	13.17±0.28Ac
	B2	68.16±1.22Ac	62.35±1.19Bc	56.42±0.99Cb	69.75±0.68Ac	62.93±0.79Bc	56.96±0.81Cb	10.03±0.26Ca	12.64±0.34Ba	15.35±0.37Aa	12.81±0.26Ba	13.07±0.27Ba	14.68±0.28Aa
Significance	Salt treatment (ST)	**	**	**	**	**	**	**	**	**	**	**	**
	Material treatment (BT)	**	**	**	**	**	**	**	**	**	**	**	**
(p-value)	Salt ×Material treatment ST×BT	**	**	**	**	**	**	**	**	**	**	**	**

Notes: The data are mean ± SD (standard deviation) (n =3). Different lowercase letters indicate significant differences between treatments at $p < 0.05$. Different uppercase letters indicate significant differences between different growth periods at $p < 0.05$

DOI: http://dx.doi.org/10.15666/aeer/2102_957974

Therefore, at the same soil salinity level, straw return and biochar application were the main factors influencing soil physicochemical properties, nutrient contents, and cotton physiology (*Figure 5*). S1 had a significant effect on AP, AK, TN, Chl a, Chl b, RSS, SWC, AK, AP, LSS, RSS, Chl a, and Chl b (*Fig. 5a*). S2 had a significant effect on SWC, AK, SOM, and RSS (*Fig. 5b*). S3 had a significant effect on SWC, AK, AP, LSS, RSS, Chl a, and Chl b (*Fig. 5c*). PC1 was positively correlated with SWC, TN, AP, AK, SOM, Chl a, and Chl b, and negatively correlated with pH, LMDA, RMDA, LRC, RRC, and EC. pH was positively correlated with LMDA, RMDA, LRC, and RRC, and negatively correlated with Chl a, Chl b, LSS, RSS, TN, AK, AP, and SOM. SWC was positively correlated with Chl a, Chl b, TN, AK, AP, and SOM, and negatively correlated with LMDA, RMDA, LRC, and RRC. EC was positively correlated with LMDA, RMDA, LRC, and RRC, and negatively correlated with Chl a, Chl b, LSS, RSS, TN, AK, AP, and SOM.

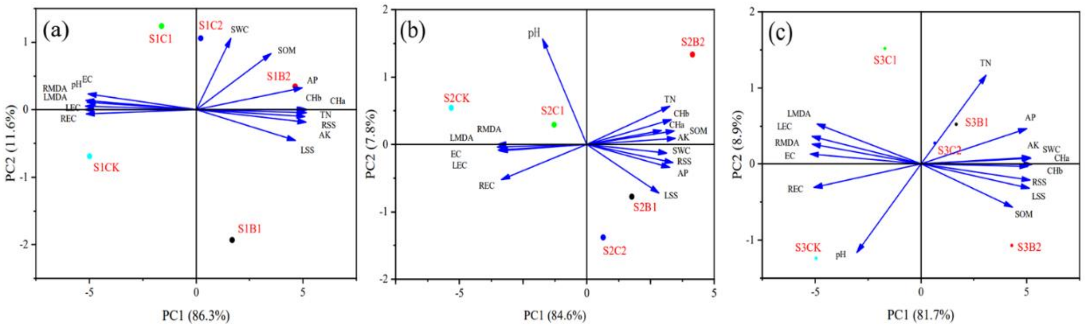

Figure 5. *PCA analysis of physical and chemical properties of soils with different salinities and aboveground physiological response of cotton. Note: (a) Low salt treatment; (b) Moderate salt treatment; (c) High salt treatment*

Discussion

Effects of straw return and biochar application on physicochemical properties of soils with different salinities

Due to high salt content and poor soil structure, saline soil greatly inhibits crop growth. However, straw return and biochar application can improve the physicochemical properties of saline soil, and alleviate the salt stress (Sun et al., 2016). For example, Zhang et al. (2020) found that biochar application and straw return could effectively reduce soil pH. In this study, straw return reduced soil pH to different degrees. It may be due to the release of K^+ during the decomposition process of straw, which indirectly reduces soil Na^+ content (Li et al., 2014). Besides, organic acids are produced during the decomposition process in salinized soils (Chen et al., 2020). However, the soil pH in this study had no difference. This may be due to the high soil pH in Xinjiang. In this study, biochar application increased the pH value of salinized soils. It may be due to that the alkaline functional groups on the surface of biochar increase soil pH through adsorption and substitution (Yu et al., 2019). Yue et al. (2015) and Han et al. (2021) showed that straw return and biochar application to salinized soil could increase soil water content, reduce soil salinity, and accelerate the desalination process. In this study, straw return and biochar application increased soil water content and decreased soil salinity. It may be due to that straw return and biochar application could increase soil porosity and decrease soil

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2102_957974

bulk density, which increase soil water holding capacity and water permeability (Zhao et al., 2014) and promotes salt leaching, thus reducing soil salinity.

Carbon is an important nutrient that affects the growth and yield of cotton. Biochar contains a large amount of unstable carbon, which can significantly enhance soil carbon sequestration capacity. Straw return can also increase soil carbon storage, because organic matter is produced in the decomposition process (Huang et al., 2017; Pan et al., 2021). Studies have shown that continuous straw return and biochar application can significantly increase the organic matter content in the 0-40 cm layer of salinized soil, and the organic matter content increases with the increase of straw and biochar application rates (Su et al., 2019; Hossain et al., 2020). This study showed that straw return and biochar application significantly increased the content of organic matter in salinized soils. It may be due to that straw return and biochar application can increase soil aggregates and regulate the composition and diversity of microbial communities, thus promoting the accumulation of organic matter (Zhao et al., 2019). Besides, a large amount of active organic carbon components such as easily oxidized carbon are produced during the oxidation process of biochar and directly released in the soil (Zhang et al., 2017). Chen et al. (2019) found that biochar could significantly increase the content of AP and AK in paddy soil. Han et al. (2020) found that biochar could significantly increase the TN content in saline-alkali soil. In this study, straw return and biochar application significantly increased the contents of TN, AP, and AK in salinized soils. It may be due to that (1) nutrients are gradually released during decomposition and oxidation of straw and biochar. (2) Straw return and biochar application improve soil physicochemical properties, reducing the leaching of nutrients and the nitrification-denitrification loss of nitrogen. (3) Biochar has a very strong ion exchange capacity. It can affect the content of elements such as Fe and Al in the soil, reduces the fixation of phosphorus and potassium, and increase the content of AP and AK through the strong adsorption of negative charge (Beheshti et al., 2017). In this study, it was also found that with the increase of soil salinity, the effect of straw and biochar on increasing soil nutrients was weakened. It may be due to that the salt content in the soils is too high, and a large amount of Na^+ destroys the soil structure and affects the retention of soil nutrients. On the other hand, the increase of soil salinity could negatively affect the composition, activity, and relative abundance of soil microorganisms (Rath et al., 2015). This decreases the decomposition and oxidation efficiency of straw and biochar (Dahlawi et al., 2018), and weakens the agglomeration of soil particles, resulting in decreased soil nutrient content (Duan et al., 2021).

Effects of straw return and biochar application on the physiology of cotton in salinized soils

Soluble sugar is an important osmotic regulator in plants. It has a positive effect on maintaining the osmotic potential of plant cells, protecting cell membrane structure, and regulating osmotic balance (Jiang et al., 2020). MDA and REC are important indicators of crop stress responses. Studies have shown that the contents of soluble sugar and MDA and REC in leaves increase with the increase of soil salinity (Fang et al., 2021), and straw return and biochar application could significantly reduce those in salt-stressed crop leaves (Shi et al., 2021). In this study, straw return and biochar application increased the content of soluble sugar, while decreased the MDA content and REC in cotton leaves in the soils with different salinities. It indicates that straw return and biochar application could enhance the ability of cotton to synthesize osmotic regulators, eliminate the damage of oxygen free radicals to cotton cell membranes, and maintain the homeostasis inside and outside the cell. Chlorophyll is the material basis for plant photosynthesis (Muhammad et al., 2021). This study showed that with the increase of soil salinity, the chlorophyll content decreased significantly, and the color of leaves became lighter. This may be due to that high salt content leads to the accumulation of excess Na^+ and Cl^-, which increases the activity of chlorophyllase, and destroys the chloroplast structure and the balance between chlorophyll synthesis and degradation, thus reducing the chlorophyll content (Hao et al., 2021). However, it was found that at the same salinity level, the chlorophyll content of cotton increased with the increase of straw and biochar application rates. It may be due to that straw return and biochar application increases the soluble sugar content of leaves and accelerates carbon and nitrogen metabolism (Farhangi-Abriz et al., 2018), thus increasing chlorophyll content.

It is worth noting that straw return and biochar application in this study could significantly increase the chlorophyll and soluble sugar contents, and reduce the REC and MDA content of cotton roots and leaves in the soils with low and moderate salinity. However, in the soil with high salinity, only the high straw and biochar application rate showed a significant effect. It may be due to that under the low application rate, the binding sites of the base ions of straw and biochar decrease, leading to a decrease in the substitution ability of soil base ions and an increase in the absorption of base ions by crops. This ultimately destroys the osmotic balance of cotton cells (Chen et al., 2011). On the other hand, compared with the high application rate, the low application rate of straw and biochar weakens the soil's ability to transform and hold nutrients, reducing crop's nutrient uptake. In this study, the contents of soluble sugar and MDA in cotton root were not significantly different from those in the CK under the low application rate of straw and biochar. It may be due to that under the low application rate of straw and biochar, the high salinity of the soil causes ion poisoning to cotton root, destroys the osmotic balance of root cells, and impairs the absorption and transport of soil nutrients by cotton roots. Besides, under high salt stress, the accumulation of oxygen free radicals and the loss of endolysates in cotton leaves could increase the MDA content and REC, damage cell structure, and affect the normal physiological metabolism of cotton (Abbas et al., 2022).

Conclusion

(1) Cotton straw return and cotton straw-derived biochar application significantly reduced the salt content of soils with different salinities and increased soil water content. Straw return decreased the pH of salinized soils, while biochar application slightly increased the pH of salinized soils.

(2) Straw return and biochar application increased the contents of TN, SOM, AP, and AK in soils with different salinities, and the contents of TN, SOM, AP, and AK increased with the increase of the application rates of straw and biochar (B2 > C2> B1> C1 > CK).

(3) Straw return and biochar application could effectively regulate the organic osmolytes in cotton plants under different soil salinities. Biochar application significantly increased the chlorophyll and soluble sugar contents, and decreased the REC and MDA content of cotton leaves and root. However, the effects of high and low application rates of cotton straw and cotton straw-derived biochar were different in soils with different salinities. That is, both high and low application rates could significantly regulate the organic osmolytes in the key growth period of cotton under moderate and low soil salinities, while only high application rate showed a significant effect under high soil salinity. Therefore, cotton straw and cotton straw-derived biochar could alleviate the

damage of salt stress to the aboveground and underground parts of cotton, and the effects of biochar application was better than those of straw return.

Acknowledgements. This work was supported by the National Natural Science Foundation of China [grant number 42161042] and the Major Scientific Research Projects of Xinjiang Production and Construction Corps [grant number 2020AB018].

Disclosure Statement. The authors declare that they have no conflict of interests.

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