# EFFECT OF HUMIC ACID COMBINED WITH FERTILIZER ON THE IMPROVEMENT OF SALINE-ALKALI LAND AND COTTON GROWTH

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**Abstract.** The effect of humic acid combined with fertilizer is an important basis for improving the soil's ecological environment. Therefore, this study is aimed at investigating the effect of the combined application of humic acid and fertilizer on the physicochemical properties of saline-alkali soil, cotton growth, and nutrient utilization in an arid area. The random field block test method was adopted, and biochemical humic acid (BHA), ammoniated humic acid (AHA), and microbial activated humic acid (MHA) were used as experimental materials. Soil pH, electrical conductivity (EC), water-soluble cation content, soluble nitrogen content, available phosphorus content, cotton biomass, and yield were determined. The results showed that humic acid combined with fertilizer had no obvious effect on the pH of the salinized soil. However, the addition of three types of humic acid showed a certain reduction effect on soil EC, water-soluble Na<sup>+</sup> and K<sup>+</sup> content, and sodium adsorption ratio (SAR), affecting the soil depth of 0-40 cm. In addition, the content of available phosphorus in soil could be increased by adding three humic acids, among which MHA was the best. The addition of humic acid could significantly improve the yield of cotton, but there was no significant difference between the three.

**Keywords:** biochemical humic acid, ammoniated humic acid, microbial activated humic acid, soil fertility, cotton yield

#### Introduction

Cotton cultivation in Xinjiang plays an important role in China's cotton production (Yang et al., 2019). In 2019, the area sown with cotton in Xinjiang was 2540.5 thousand hectares, and its total cotton output was 5.889 million tons, accounting for 76.1% and 84.9% of the national total, respectively (Li et al., 2020). Due to scarce rainfall and intense evaporation, 37.72% of cultivated land in Xinjiang is endangered by saline-alkali conditions (Xiao et al., 2019). Excessive salt ions and alkaline environment in saline-alkali soil cause the loss of nitrogen fertilizer volatilization, leading to cotton nutritional disorders, and thus affecting the growth and yield of cotton (He et al., 2020). Therefore, the fertilization of cotton in saline-alkali land should be determined not only based on the nutrient demand of cotton, but also the amelioration of saline-alkali land and the improvement of soil fertility.

Humic acid is a kind of organic polymer active material, which has been widely used in many fields (Ciarkowska et al., 2017; Dehghani et al., 2018; Xie et al., 2019). Studies have shown that humic acid itself can be used as an organic fertilizer to increase soil fertility (Zhang et al., 2019a). Fawzy et al. (2010) found that increasing humic acid application could improve the vegetative growth of bean plants and improve the yield and quality of beans. Fan et al. (2014) pointed out that the chlorophyll content and

chloroplast ultrastructure of chrysanthemum were significantly improved after applying humic acid on the leaves compared with that after applying common chemical fertilizer. Selladurai and Purakayastha (2016) found that there was no significant difference between humic acid and fertilizer in yield, N, P, K content, absorption, and utilization efficiency. Li et al. (2019) showed that humic acid increased soil nutrient content, including total nitrogen, total phosphorus, total potassium, available nitrogen, available phosphorus, available potassium, and organic matter, showing the greatest effect in the third year. As a soil conditioner, humic acid can also change the composition of soil ions (Abe et al., 2011), promote the formation of soil aggregates (Liu et al., 2019), increase soil permeability (Khaled and Fawy, 2011), and accelerate the leaching and irrigation of soil surface salts and alkalis (Zhao et al., 2017). Humic acid can also activate soil phosphorus, improve the supply of phosphorus in saline-alkali soil, and promote the absorption and utilization of phosphorus by crops (He et al., 2011; Ifansyah, 2014). In addition, humic acid can regulate root morphogenesis and promote osmotic regulation of substance accumulation and nutrient absorption of crops (Asli and Neumann, 2010; Jindo et al., 2012).

The above studies indicate that humic acid plays a positive role in ameliorating saline-alkali soil and improving nutrient utilization efficiency. However, at present, the sources of humic acid are complex, and different sources of humic acid have great differences in chemical composition, structure, and properties, and their improvement effects on different types of saline-alkali soil are not the same (Erhayem and Sohn, 2014; Türkmen et al., 2004). The effects of different humic acids and chemical fertilizers on the improvement of saline-alkali land and crop growth are less studied. Therefore, 3 kinds of humic acids from different sources were used as the material in this study. Through field experiments, the effects of humic acids combined with fertilizers on soil physicochemical properties, cotton growth, and nutrient utilization in Xinjiang saline-alkali land were discussed, so as to clarify the application effects of different humic acids combined with fertilizers in saline-alkali land in inland arid areas.

#### Materials and methods

#### The study area and test materials

The experiment was conducted in Baotouhu Farm (41°39'N, 85°48'E) in Korla, Xinjiang, China in 2016. This region is a typical inland arid climate type, with annual precipitation of 53.5-62.5 mm, evaporation of 2270-2785 mm, average temperature of 11.2 °C, annual sunshine number of 3035.7 h, wind speed of 2.36 m·s<sup>-1</sup>, and frost-free period of 145-242 days. Before the experiment, the physical and chemical properties of the initial soil were determined. The soil in the study area (0-40 cm) was sandy loam (64.27% sand, 32.83% silt, 2.9% clay), soil bulk density was 1.70 g·cm<sup>-3</sup>, saturated water content was 0.398 cm<sup>3</sup> cm<sup>-3</sup>, wilting coefficient was 0.050 cm<sup>3</sup>·cm<sup>-3</sup>, field water capacity was 0.196 cm<sup>3</sup>·cm<sup>-3</sup>, organic matter content was 10.7 g kg<sup>-1</sup>, total nitrogen was 0.61 g·kg<sup>-1</sup> <sup>1</sup>, available phosphorus was 18.64 mg·kg<sup>-1</sup>, available potassium was 233 mg·kg<sup>-1</sup>, pH was 8.27, and electroconductibility (EC) was 5.47 ds·m<sup>-1</sup>. The tested cotton variety was No. 43 of Zhonglu Institute. The tested fertilizer urea (N, 46.4%) and superphosphate (P<sub>2</sub>O<sub>5</sub>, 14%) was purchased from the market. The 3 humic acids were all provided by Beijing Aojia Ecological Agriculture Co., LTD., including biochemical humic acid (BHA), ammoniated humic acid (AHA), and microbial activated humic acid (MHA). The basic physicochemical properties of the 3 humic acids are shown in *Table 1*.

**Table 1.** Basic physicochemical properties of humic acid material

Tested material	Organic matter (%)	Humic acid (%)	Total nitrogen (g·kg <sup>-1</sup> )	Total phosphorus (g·kg <sup>-1</sup> )	Total potassium (g·kg <sup>-1</sup> )	pН
ВНА	70	40	1.8	11.8	19.8	9.72
AHA	50	40	47.1	11.9	0.9	9.89
MHA	50	40	25.1	16.2	2.2	8.35

#### Experimental design

Using the field test method, 6 treatments were set as follows:

- 1. -N (only phosphorus fertilizer, no nitrogen fertilizer)
- 2. -P (only nitrogen fertilizer, no phosphorus fertilizer)
- 3. NP (nitrogen and phosphate fertilizer)
- 4. BHA + NP (BHA combined with nitrogen and phosphate fertilizer)
- 5. AHA + NP (AHA combined with nitrogen and phosphorus fertilizer)
- 6. MHA + NP (MHA combined with nitrogen and phosphate fertilizer)

Each treatment area was 30 m<sup>2</sup> (6 m long and 5 m wide), with 3 replicates, arranged in random groups. A one-time application of urea (420 kg·hm<sup>-2</sup>) and superphosphate (643 kg·hm<sup>-2</sup>) was as base fertilizer, and three humic acids were applied at 1500 kg·hm<sup>-2</sup>. Considering the high content of available potassium in the test soil, potassium fertilizer was not applied in the field.

Cotton was planted by drip irrigation under the film. The experimental field was sown on April 20, 2016, with a film and two pipes arranged in four rows. The plant spacing was 10 cm, the narrow row spacing was 20 cm, the wide row spacing was 50 cm, and the film spacing was 40 cm. The prevention and control of diseases, pests, and weeds during cotton growth and water management were equivalent to conventional production fields. The irrigation quota of cotton growth period was 5250 m<sup>3</sup>·hm<sup>-2</sup>, the irrigation period was 7-10 days, and the total irrigation time was 10 times. Aphids and red spiders were prevented at stage 14 in BBCH scale and cotton bollworms were prevented at stage 61 in BBCH scale for cotton.

#### Investigate items and methods

The chlorophyll content of functional leaves was measured by SPAD-50-Puls portable chlorophyll meter at stages 56 and 75 in BBCH scale for cotton. At stage 105 in BBCH scale, three uniformly robust fields with an area of 6.67 m² were selected, the number of bolls with a diameter greater than 2 cm were recorded, 100 bolls were selected to be weighed in each test area, so as to calculate the cotton yield (Liang et al., 2019). Four plants of cotton were taken in each experimental plot, and the root, stem, leaf, and bell were determined by the drying method (Tan et al., 2018). The dried cotton plants were crushed for the determination of nitrogen and phosphorus content. The plant samples were digested with concentrated H<sub>2</sub>SO<sub>4</sub>-H<sub>2</sub>O<sub>2</sub>, total nitrogen was determined by the Kjeldahl method, and total phosphorus was determined by the vanadium-molybdenum yellow colorimetric method (Sudiarto et al., 2019). Soil samples (100 g, three replicates) were taken from soil layers of 0-10 cm, 10-20 cm, and 20-40 cm respectively to determine the contents of pH, EC, the contents of water-soluble K<sup>+</sup>, Na<sup>+</sup>,

Ca<sup>2+</sup>, and Mg<sup>2+</sup>, NO<sub>3</sub>-N, NH<sub>4</sub>+-N, and soluble total nitrogen (TSN) as well as available phosphorus. Soil pH was determined by pH meter method, soil electroconductibility (EC) was determined by electroconductibility meter method, water-soluble Ca<sup>2+</sup> and Mg<sup>2+</sup> were determined by atomic absorption spectrophotometry method, and water-soluble K<sup>+</sup> and Na<sup>+</sup> were determined by flame photometry method (Sharpley, 1991). NO<sub>3</sub>-N was determined by UV spectrophotometer method, NH<sub>4</sub>+-N by indophenol blue colorimetric method, and TSN by alkaline potassium persulfate oxidation method (Margot et al., 1994). The content of available phosphorus in soil was determined by 0.5 mol·L<sup>-1</sup> NaHCO<sub>3</sub> extraction and molybdenum-antimony resistance colorimetric method (Zhang et al., 2019b).

#### Data processing and analysis

Microsoft Excel 2016 and SPSS 22.0 statistical software were used for data processing and statistical analysis, and the least significant difference (LSD) method ( $\alpha = 0.05$ ) was used for multiple comparison.

Xing et al. (2010) proposed the calculation method of soil soluble organic nitrogen (SON):

$$SON = TSN - (NH_4^+ - N + NO_3^- - N)$$
 (Eq.1)

where TSN is soil soluble total nitrogen.

Mahdy (2011) used the calculation formula of soil sodium adsorption ratio (SAR):

$$SAR = \frac{\left[Na^{+}\right]}{\sqrt{\frac{\left[Ca^{2+}\right] + \left[Mg^{2+}\right]}{2}}}$$
 (Eq.2)

Zhang et al. (2016) and Wang et al. (2019) proposed the calculation method of apparent availability of nitrogen (phosphorus) fertilizer, nitrogen (phosphorus) agronomic efficiency, and nitrogen (phosphorus) partial productivity.

$$AAN = \frac{NUF - NUD}{NUA} \times 100\%$$
 (Eq.3)

$$AAP = \frac{PUF - PUD}{PUA} \times 100\%$$
 (Eq.4)

$$NAE = \frac{Y_F - Y_{DN}}{NUA}$$
 (Eq.5)

$$PAE = \frac{Y_F - Y_{DP}}{PUA}$$
 (Eq.6)

$$NPP = \frac{Y_F}{NUA}$$
 (Eq.7)

$$PPP = \frac{Y_F}{PUA}$$
 (Eq.8)

where AAN and AAP represent the apparent availability of nitrogen (phosphorus) fertilizer (%), NUF and PUF represent the amounts of nitrogen (phosphorus) uptake in the fertilized area (kg), NUD and PUD represent the amounts of nitrogen (phosphorus) uptake in nitrogen (phosphorus) deficient area (kg), and NUA and PUA represent the amounts of nitrogen (phosphorus) nutrient application (kg). NAE and PAE represent nitrogen (phosphorus) agronomic efficiency (kg·kg<sup>-1</sup>), Y<sub>F</sub> represents fertilized area yield, Y<sub>DN</sub> and Y<sub>DP</sub> represent nitrogen (phosphorus) deficient area yields. NPP and PPP represent nitrogen (phosphorus) partial productivity (kg·kg<sup>-1</sup>).

#### Results

#### The effect of humic acid combined with fertilizer on soil pH

The pH value directly affects many chemical reactions in the soil. In 0-40 cm soil layer, pH value showed a trend of gradual increase with soil depth (*Fig. 1*). Compared with the control group (NP) without humic acid, the application of 3 kinds of humic acid materials had no significant effect on the pH value of the soil at 0-10 cm, 10-20 cm, and 20-40 cm soil layers. However, compared with the three humic acid materials, the pH value of the soil treated with BHA in the 0-10cm soil layer was lower than that of AHA and MHA (*Fig.1A*), the pH value of the soil treated with MHA in the 10-20 cm soil layer was lower than that of AHA (*Fig.1B*), and there was no significant difference between the three test materials in the 20-40 cm soil layer (*Fig. 1C*).

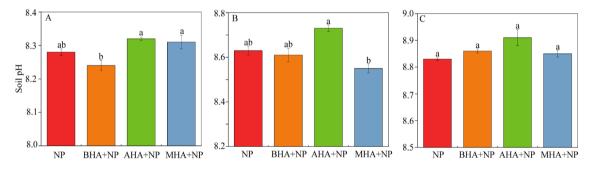


Figure 1. Influence of humic acid combined with fertilizer on soil pH. A, B, and C represent soil layers 0-10 cm, 10-20 cm, and 20-40 cm, respectively. "NP" represents nitrogen and phosphate fertilizer, "BHA + NP" represents biochemical humic acid combined with nitrogen and phosphate fertilizer, "AHA + NP" represents ammoniated humic acid combined with nitrogen and phosphorus fertilizer), and "MHA + NP" represents microbial activated humic acid combined with nitrogen and phosphate fertilizer. The error lines represent the standard variance, and different lowercase letters represent significant differences among treatments in the same soil layer (P < 0.05)

#### The effect of humic acid combined with fertilizer on soil electric conductivity

As a saline-alkali soil improvement agent, humic acid had a certain effect on electroconductibility (EC) of 0-40 cm soil layer (Fig. 2). Compared with control (NP), the EC values of 0-10 cm soil layer treated by BHA, AHA and MHA decreased by

27.4%, 26.3%, and 21.4% respectively (*Fig. 2A*), the EC values of 10-20 soil layer decreased by 23.8%, 21.9%, and 12.7% respectively (*Fig. 2B*), and the EC values of 20-40 cm soil layer decreased by 20.9%, 131%, and 8.8% respectively (*Fig. 2C*). Compared with NP, BHA in the three soil layers all showed significant differences, while AHA and MAH only showed significant differences in the 0-10 cm soil layer.

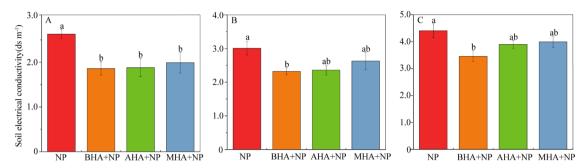


Figure 2. Influence of humic acid combined with fertilizer on soil electric conductivity. A, B, and C represent soil layers 0-10 cm, 10-20 cm, and 20-40 cm, respectively. "NP" represents nitrogen and phosphate fertilizer, "BHA + NP" represents biochemical humic acid combined with nitrogen and phosphate fertilizer, "AHA + NP" represents ammoniated humic acid combined with nitrogen and phosphorus fertilizer), and "MHA + NP" represents microbial activated humic acid combined with nitrogen and phosphate fertilizer. The error lines represent the standard variance, and different lowercase letters represent significant differences among treatments in the same soil layer (P < 0.05)

#### The effect of humic acid combined with fertilizer on soil cation composition and SAR

Soil salinization is the process of adsorption of sodium ions by soil colloid and the exchange of cations between the solid phase and liquid phase, and sodium adsorption ratio (SAR) is an important indicator of soil alkalinization. Compared with NP, the combination of humic acid and fertilizer had no significant effect on the content of water-soluble Ca<sup>2+</sup> and Mg<sup>2+</sup> in the 0-40 cm soil layer, but reduced the content of water-soluble K<sup>+</sup> and Na<sup>+</sup> by 5.5-40.0% and 12.8-22.0%, respectively. Among the three humic acid materials, the content of water-soluble Ca<sup>2+</sup> and Mg<sup>2+</sup> in the 0-10 cm soil layer treated by MHA was significantly higher than that of BHA and AHA, and there was no significant difference in the soil layer below 10 cm. The content of water-soluble K<sup>+</sup> in the 20-40 cm soil layer treated by AHA was significantly higher than that of BHA and MHA. However, there was no significant difference in the content of water-soluble Na<sup>+</sup> among the three materials in any soil layer. The combination of three humic acids and fertilizers can significantly reduce the SAR of 0-40 cm soil layer by 7.7-22.7%. However, there was no significant difference between different humic acid materials (*Table 2*).

#### The effect of humic acid combined with fertilizer on soil soluble nitrogen content

Soil inorganic nitrogen mainly includes nitrate nitrogen (NO<sub>3</sub><sup>-</sup>-N) and ammonium nitrogen (NH<sub>4</sub><sup>+</sup>-N). Compared with NP, the content of NO<sub>3</sub><sup>-</sup>-N in the 0-40 cm soil layer at the maturity stage of cotton treated with BHA, AHA and MHA decreased by 9.9-28.1%, 18.0-78.6% and 37.6-66.2%, respectively, especially the AHA and MHA humic acid treatment showed the greatest reduction (*Table 3*). The influence of NH4 + -N

content in soil treated with MHA was not significant, but the NH<sub>4</sub><sup>+</sup>-N content in soil treated with MHA was significantly increased in 0-10 cm and 20-40 cm soil layers (*Table 3*).

**Table 2.** Effects of humic acid combined with fertilizer on soil water-soluble cation composition and SAR

Soil lover (em)	Treatment		CAD			
Soil layer (cm)	Treatment	<b>K</b> <sup>+</sup>	Na <sup>+</sup>	Ca <sup>2+</sup>	$\mathrm{Mg}^{2+}$	SAR
	NP	6.16a	20.86a	4.9ab	1.68ab	11.5a
0-10	BHA + NP	5.60ab	18.20b	4.34b	1.54b	10.61b
0-10	AHA + NP	5.18b	16.80b	4.20b	1.40b	10.04b
	MHA + NP	4.90b	16.80b	5.32a	1.82a	8.89b
	NP	4.90a	45.50a	5.04a	1.96a	24.32a
10-20	BHA + NP	2.94b	38.92b	4.76a	1.96a	21.23b
10-20	AHA + NP	3.78b	37.66b	4.62a	2.10a	20.54b
	MHA + NP	4.06b	38.36b	4.76a	1.96a	20.93b
	NP	3.64a	61.18a	6.44a	2.10a	29.61a
20-40	BHA + NP	3.08b	52.64b	6.02a	2.24a	25.90b
20-40	AHA + NP	3.44a	53.20b	6.16a	2.52a	25.54b
	MHA + NP	3.08b	47.74b	6.30a	2.38a	22.92b

<sup>&</sup>quot;NP" represents nitrogen and phosphate fertilizer, "BHA + NP" represents biochemical humic acid combined with nitrogen and phosphate fertilizer, "AHA + NP" represents ammoniated humic acid combined with nitrogen and phosphorus fertilizer), and "MHA + NP" represents microbial activated humic acid combined with nitrogen and phosphate fertilizer. Different lowercase letters represent significant differences among treatments in the same soil layer (P < 0.05)

**Table 3.** Effects of humic acid combined with fertilizer on soil soluble nitrogen content  $(mg \cdot kg^{-1})$ 

Soil layer (cm)	Treatment	NH <sub>4</sub> <sup>+</sup> -N	NO <sub>3</sub> -N	SON	TSN
	NP	1.68b	7.52a	9.03b	17.42b
0-10	BHA + NP	2.24ab	6.76ab	10.18b	19.42b
0-10	AHA + NP	2.03ab	6.15b	14.54a	22.89a
	MHA + NP	2.41a	4.68c	14.70a	22.33a
	NP	1.79ab	6.13a	9.87b	18.72a
10-20	BHA + NP	1.41b	6.41b	11.68a	18.89a
10-20	AHA + NP	2.08a	1.83c	7.45b	11.76c
	MHA + NP	1.93a	2.07c	12.13b	14.73d
	NP	1.27bc	13.14a	7.44b	21.51a
20-40	BHA + NP	1.15c	10.54b	9.63a	21.58a
∠ <del>∪-4</del> ∪	AHA + NP	1.57b	2.81d	6.98b	12.39c
	MHA + NP	1.78a	5.12c	9.75a	15.76d

SON and TSN represent soil soluble organic nitrogen and soluble total nitrogen respectively. "NP" represents nitrogen and phosphate fertilizer, "BHA + NP" represents biochemical humic acid combined with nitrogen and phosphate fertilizer, "AHA + NP" represents ammoniated humic acid combined with nitrogen and phosphorus fertilizer), and "MHA + NP" represents microbial activated humic acid combined with nitrogen and phosphate fertilizer. Different lowercase letters represent significant differences among treatments in the same soil layer (P < 0.05)

The analysis results of soluble organic nitrogen (SON) showed that the SON content in the 10-20 cm and 20-40 cm soil layers treated with BHA was significantly higher than NP content, while that in the 0-10 cm soil layer treated with AHA was significantly higher than NP content, and that in the 0-10 cm and 20-40 cm soil layers treated with MHA was significantly higher than NP content (*Table 3*). Thus, the application of humic acid has the effect of increasing SON content, and the depth of BHA and MHA in the season of application reaches 40 cm soil layer, while AHA only has a significant effect at 0-10 cm soil layer.

Soil soluble total nitrogen (TSN) is the sum of  $NO_3$ -N,  $NH_4$ +-N, and SON. The TSN content of BHA and NP in the 0-40 cm soil layer was not significantly different, while the TSN content of AHA and MHA in the 0-10 cm soil layer was significantly higher than NP, and the TSN content of AHA and MHA in the 0-10 cm soil layer was significantly lower than NP. Among the three humic acids, BHA in 0-10 cm soil layer was lower than AHA and MHA, and there is no significant difference between the latter two. The TSN content in the 10-40 cm soil layer was manifested as BHA > MHA > AHA, and the difference between the three treatments was significant.

### The effect of humic acid combined with fertilizer on soil available phosphorus content

The content of available phosphorus in 0-40 cm soil significantly decreased with the increase of soil depth (*Fig. 3*). The combination of humic acid and fertilizer significantly increased the available phosphorus content in soil layers 0-10 cm and 20-40 cm by 17.9-73.4%, and 62.3-98.4%, respectively (*Fig. 3A* and *C*), but the available phosphorus content in soil layers 10-20 cm showed no obvious advantage (*Fig. 3B*). The content of available phosphorus in soil under MHA treatment was the highest in all 3 soil layers and was significantly higher in 0-10 cm soil layer than other treatments.

## The effect of humic acid combined with fertilizer on chlorophyll content and biomass of cotton

No application of nitrogen fertilizer or phosphorus fertilizer had no significant effect on the chlorophyll content of cotton functional leaves at the flowering and boll period, but it significantly reduced the total biomass of cotton. The combination of humic acid and fertilizer effectively increased the chlorophyll content of functional leaves by 6.7-8.4% compared with NP, but the difference between the three humic acid materials was not significant. In addition, the combined application of BHA, AHA, and MHA increased the total biomass of cotton by 9.2%, 19.3%, and 11.6%, respectively, compared with NP (*Table 4*). The analysis of biomass distribution of different parts of cotton plants showed that nitrogen deficiency mainly affected the biomass of leaves, stems, and bolls, phosphorus deficiency mainly affected the biomass of leaves and bolls, and humic acid combined with nitrogen and phosphorus mainly promoted the biomass of leaves and bolls.

#### The effect of humic acid combined with fertilizer on yield and components of cotton

Under the condition of soil fertility, only nitrogen or phosphorus fertilizer could significantly reduce cotton yield. Compared with the application of nitrogen and phosphorus fertilizer (NP), the yield of cotton increased by 15.9%, 15.9%, and 14.1% respectively with the addition of BHA, AHA, and MHA. However, the yield of cotton among the three humic acid treatments was almost the same (*Table 5*). Through the analysis

of the components of cotton yield, it was found that the deficiency of nitrogen supply in saline-alkali land had a significant effect on the number of effective bolls per plant and the weight of a single boll. The deficiency of phosphorus supply mainly affected the weight of single boll. The increase of cotton yield with BHA and AHA was mainly due to the increase of the number of effective bolls per plant, while the combination of MHA and chemical fertilizer had a significant effect on the weight of a single boll.

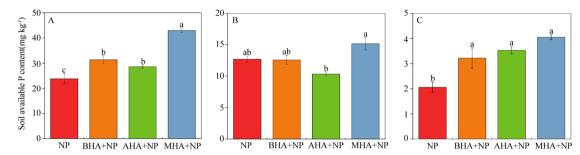


Figure 3. Influence of humic acid combined with fertilizer on soil available phosphorus content. A, B and C represent soil layers 0-10 cm, 10-20 cm and 20-40 cm, respectively. "NP" represents nitrogen and phosphate fertilizer, "BHA + NP" represents biochemical humic acid combined with nitrogen and phosphate fertilizer, "AHA + NP" represents ammoniated humic acid combined with nitrogen and phosphorus fertilizer), and "MHA + NP" represents microbial activated humic acid combined with nitrogen and phosphate fertilizer. The error lines represent the standard variance, and different lowercase letters represent significant differences among treatments in the same soil layer (P < 0.05)

**Table 4.** Effects of humic acid combined with fertilizer on chlorophyll content and biomass of cotton

Two oders and	SPAD	Biomass (t hm <sup>-2</sup> )					
Treatment		Root	Stem	Leaf	boll	<b>Total biomass</b>	
-N	54.75b	0.62ab	3.91c	7.21c	14.29d	26.03c	
-P	56.84ab	0.64ab	4.46b	6.81c	15.38c	27.29c	
NP	55.65b	0.69a	4.10bc	8.41b	19.23b	32.43b	
BHA + NP	60.34a	0.71a	4.23b	9.12ab	21.35a	35.41ab	
AHA + NP	59.76a	0.59b	5.48a	11.73a	20.88a	38.68a	
MHA + NP	59.39a	0.69a	4.40b	10.55a	20.55a	36.19ab	

<sup>&</sup>quot;-N" represents only phosphorus fertilizer, no nitrogen fertilizer, "-P" represents only nitrogen fertilizer, no phosphorus fertilizer, "NP" represents nitrogen and phosphate fertilizer, "BHA + NP" represents biochemical humic acid combined with nitrogen and phosphate fertilizer, "AHA + NP" represents ammoniated humic acid combined with nitrogen and phosphorus fertilizer), and "MHA + NP" represents microbial activated humic acid combined with nitrogen and phosphate fertilizer. Different lowercase letters represent significant differences among treatments in the same soil layer (P < 0.05)

# The effect of humic acid combined with fertilizer on absorption and utilization of nitrogen and phosphorus in cotton

The nitrogen and phosphorus utilization rates of cotton with combined application of humic acid and fertilizer increased by 42.0-105.8% and 52.7-112.7%, respectively, with significant differences. Among the three tested humic acid materials,

AHA > MHA > BHA (*Table 6*). Compared with NP, the agronomic efficiency of nitrogen and phosphorus increased by 29.6-51.9% and 47.2-71.7%, respectively, after the combination of humic acid and fertilizer. The agronomic efficiency of BHA combined with fertilizer was the highest, which indicated that the application of BHA was more conducive to the improvement of unit nutrient yield. The partial productivity of nitrogen and phosphate fertilizer treated with humic acid was significantly higher than that of the control group, but there was no significant difference among the three humic acid materials.

Treatment	Yield com	Viold (leg hm-2)		
Treatment	Effective bolls per plant	Single boll weight (g)	Yield (kg hm <sup>-2</sup> )	
-N	5.87d	5.93d	4212d	
-P	6.96cd	6.04d	4623c	
NP	7.39bc	6.50b	5387b	
BHA + NP	9.15a	6.23c	6241a	
AHA + NP	8.61a	6.21c	6243a	
MHA + NP	8.46ab	6.70a	6147a	

**Table 5.** Effects of humic acid combined with fertilizer on yield and components of cotton

<sup>&</sup>quot;-N" represents only phosphorus fertilizer, no nitrogen fertilizer, "-P" represents only nitrogen fertilizer, no phosphorus fertilizer, "NP" represents nitrogen and phosphate fertilizer, "BHA + NP" represents biochemical humic acid combined with nitrogen and phosphate fertilizer, "AHA + NP" represents ammoniated humic acid combined with nitrogen and phosphorus fertilizer), and "MHA + NP" represents microbial activated humic acid combined with nitrogen and phosphate fertilizer. Different lowercase letters represent significant differences among treatments in the same soil layer (P < 0.05)

Treatment	Nutrient apparent use efficiency (%)		Agronomic efficiency of fertilizer (kg kg <sup>-1</sup> )		Partial fertilizer productivity (kg kg <sup>-1</sup> )			
	N	P	N	P	N	P		
NP	13.8c	11.0c	8.1c	12.7c	38.3b	83.2b		
BHA + NP	19.6b	16.8b	12.3a	21.8a	44.2a	96.5a		
AHA + NP	28.4a	23.4a	11.2ab	19.8a	44.3a	96.3a		
MHA + NP	20.8b	21.7a	10.5b	18.3b	43.5a	94.8a		

Table 6. Effects of humic acid combined with fertilizer on cotton nutrient utilization

"NP" represents nitrogen and phosphate fertilizer, "BHA + NP" represents biochemical humic acid combined with nitrogen and phosphate fertilizer, "AHA + NP" represents ammoniated humic acid combined with nitrogen and phosphorus fertilizer), and "MHA + NP" represents microbial activated humic acid combined with nitrogen and phosphate fertilizer. Different lowercase letters represent significant differences among treatments in the same soil layer (P < 0.05)

#### **Discussion**

# The combination of humic acid and fertilizer can improve soil physicochemical properties

According to some analyses, because humic acid is an organic acid, it can combine with cations in the soil to form humate, and then form a buffer system of mutual transformation between humic acid and humate, so it can play a role in regulating soil pH (Boguta et al., 2019). However, in our study, the effect of the three humic acid

materials on soil pH was not significant (Fig. 1), which may be related to the high pH value and of the short period of humic acid materials in this test, or the application of humic acid materials may increase the buffer strength of the soil (Zeng et al., 2013).

Our results showed that the application of the three humic acid materials had a significant reduction in soil EC value, water-soluble Na<sup>+</sup> and K<sup>+</sup> content, and SAR (Fig. 2; Table 2), which were similar to the research results of Wang et al. (2016). The reason is that humic acid is a kind of colloid with negative electricity, which can absorb the univalent cation, promote the formation of soil aggregates and increase the permeability of the soil, and accelerating the leaching of salt and alkali on the surface of the soil (Zhao et al., 2017). It is also possible that the hydroxyl and carboxyl functional groups of humic acid condense with calcium ions in the soil, forming soil aggregates through the physiological action of crop roots, thus improving the soil structure and facilitating the leaching of salts (Yonebayashi and Hattori, 1988). In this study, in the 0-40 cm soil layer, the EC value treated with BHA was lower than that of AHA and MHA (Fig. 2), which may be related to the fact that BHA is biochemical humic acid, with more active groups and large exchange capacity of salt ions (Zhang and Zhang, 2014). There were no significant differences among the three humic acid materials in the influence of soil water-soluble Na<sup>+</sup> and K<sup>+</sup> content and SAR (Table 2). Compared with NP, the contents of water-soluble Ca<sup>2+</sup> and Mg<sup>2+</sup> in soil treated with three humic acids were not significantly different, but the contents of water-soluble Ca<sup>2+</sup> and Mg<sup>2+</sup> in 0-10 cm soil layer treated with MHA was significantly higher than that of BHA and AHA (*Table 2*), and the reasons remain to be further analyzed.

Through the analysis of the differences of the nitrogen content of various forms in the soil, it was found that the content of NO<sub>3</sub>-N in the soil layer 0-40 cm after humic acid application significantly decreased, while the content of NH<sub>4</sub><sup>+</sup>-N and SON increased (Table 3). The content of TSN increased in 0-10 cm soil layer with the humic acid application, but it was lower in the soil layer below 10 cm than that in the control, which was similar to the results of Tan et al. (1972). The influence of humic acid on the form of soil nitrogen may be related to the that the carboxyl group and phenolic hydroxyl group of humic acid can react with the amide group of urea to form humic acid urea complex, and the carboxyl group of humic acid can combine with ammonium to form ammonium humate (Aquino et al., 2011). It is also possible that humic acid can stimulate root growth and promote the uptake of nitrogen by crops (Jindo et al., 2012). In farmland, soluble organic nitrogen mainly comes from the humification of organic matter and microbial decomposition (Zang et al., 2000). Humic acid, as an organic modifier, increases the content of soil organic matter on the one hand (Jiménez-González et al., 2019), and on the other hand provides more carbon sources for microorganisms, which has a stimulating effect on the decomposition of soil organic matter (Singh et al., 2019), which may also be the reason why humic acid application improves the content of soil soluble organic nitrogen (Xing et al., 2010). Among the three humic acid materials, mineral humic acid had the best effect in reducing the content of NO<sub>3</sub>-N in soil and increasing the content of NH<sub>4</sub>+-N. The effect of BHA and MHA on increasing the content of SON was as deep as 40 cm soil layer in the application season, while the effect of AHA was only significant in 0-10 cm soil layer. There was no significant difference in the TSN content between BHA and NP in 0-40 cm soil layer, while the TSN content of AHA and MHA in 0-10 cm soil layer was significantly higher than that of NP, and that in soil layer below 10 cm is significantly lower than that in NP (Table 3). The results of the significant increase of TSN content of mineral source humic acid in 0-10 cm soil layer were mainly due to the increase of SON content, while the results of the decrease of TSN in 10-20 cm and 20-40 cm soil layer were mainly due to the decrease of NO<sub>3</sub>-N content. The decrease of NO<sub>3</sub>-N content is beneficial to the reduction of nitrogen leaching loss and the alleviation of groundwater pollution. The increase of NH<sub>4</sub>+-N and SON content is beneficial to the fixation of soil nitrogen and has a positive effect on the satisfaction of nitrogen nutrition demand in the later growth stage of cotton. It can be seen that the effect of mineral humic acid on nitrogenous fertilizer is better than that of biochemical humic acid.

Relevant research results showed that after humic acid was applied into the soil, it could combine with Ca<sup>2+</sup> in the soil and reduce Ca-P precipitation (Perassi and Borgnino, 2014). The hydroxyl functional group of humic acid can react with phosphoric acid to form water-soluble phosphorus humic acid and humic acid ammonium phosphorus (Cimrin and Yilmaz, 2005). Wang et al. (2020) found that the available phosphorus content in the soil treated with additional humic acid was significantly higher than that treated with phosphate fertilizer alone, and the fixation rate of soil to phosphorus was reduced. This study also drew a similar conclusion that humic acid could improve the content of soil available phosphorus to a certain extent, and the effect of MHA was the most significant (*Table 4*).

The three humic acid materials all showed a certain effect on the improvement of saline-alkali soil, but the influence degree of different materials on the physical and chemical properties of soil was different. The reason for the difference is that humic acid is a kind of mixture composed of various compounds with different molecular weights. Humic acid raw materials from different origins and different sources have different processing and activation technologies, and their active humic acid content, functional group type, quantity, and activity are different (Wang et al., 2016). In addition, it also may be that the contents of organic matter, total nitrogen, and total potassium of the three humic acid materials vary greatly (*Table 1*), even though the availability of the materials as the total nutrients is limited in season, which led to the different effects of the materials on the physical and chemical properties of saline and alkaline land.

# The combination of humic acid and fertilizer promote the growth and development of plants, and increase the yield and nutrient absorption and utilization of cotton

Humic acid can obviously promote the absorption of N, P, and K nutrients in plants, effectively improve the agronomic traits of crops, increase the yield of crops, and promote the absorption and utilization of nutrients in crop plants (Fawzy et al., 2010). In our study, the synergistic effect of humic acid was mainly reflected in the significant improvement of chlorophyll content, biomass, yield, apparent fertilizer utilization rate, and agronomic efficiency of cotton. The increase of chlorophyll content may be related to the application of humic acid promoting the absorption of Mg<sup>2+</sup> or Fe<sup>2+</sup> (Mackowiak et al., 2001). This study showed that different humic acid materials could improve cotton biomass in different degrees under the same nutrient input, and AHA treatment reached a significant level, which was similar to the results (Atiyeh et al., 2002). This may be because mineral humic acid can promote the activities of polyphenol oxidase, catalase, and invertase, etc. The enhancement of the enzyme activity speeds up the metabolic process of cotton, thus promoting the growth of cotton (Arancon et al., 2006). Compared with NP, the yield of cotton treated with additional humic acid increased by 14.1-15.9%, but there was no significant difference between the three humic acid

materials on the yield (*Table 5*). Compared with NP, the utilization rate of nitrogen and phosphorus fertilizer in cotton treated with humic acid increased by 42.0-104.2% and 11.7-55.0%, respectively, and the AHA treatment had the best effect of increasing the utilization rate of nitrogen and phosphorus fertilizer (*Table 6*). The combination of humic acid and fertilizer can improve yield and nutrient utilization. On the one hand, it is related to the improvement of physical and chemical properties of saline-alkali soil by humic acid, which reduces the harm of saline-alkali to crops and creates a more favorable soil environment for the growth of cotton. In addition, humic acid has the function of activating nitrogen and phosphorus and improves the availability of nitrogen and phosphorus (Allison, 2006; Khaled and Fawy, 2011). On the other hand, it may be related to the fact that humic acid can stimulate root growth and development of crops, increase root length, root mass, and root activity, and thus contribute to the improvement of root absorption capacity (Hatami et al., 2018; Jindo et al., 2012).

#### **Conclusions**

The effect of a combination of humic acid and fertilizer on saline-alkali land improvement is remarkable. Compared with single fertilizer application, electrical conductivity (EC), water soluble Na<sup>+</sup> and K<sup>+</sup>, content and SAR of soil treated with additional humic acid decreased significantly. BHA had the best effect on reducing soil EC, but there was no significant difference among the three humic acid materials in reducing the content of Na<sup>+</sup> and K<sup>+</sup> and the effect of SAR. The content of soluble organic nitrogen in soil could be improved to some extent by applying humic acid, and the BHA and MHA acted at a depth of 40 cm. The content of available phosphorus in soil could be significantly increased by 18.7-101.5% by applying humic acid, among which the MHA effect was the best. The effect of three humic acid materials on the growth and yield of cotton was not obvious but compared with the control treatment with the same nutrient fertilizer. The chlorophyll content and biomass of flowering and boll stage functional leaves of cotton with additional humic acid were significantly increased, the cotton yield increased by 15%, and cotton nitrogen and phosphorus fertilizer use efficiency increased by 42.0-105.8% and 52.7-112.7%, respectively. To sum up, the application of different humic acid materials on saline-alkali land has different effects on soil physical and chemical properties, but the overall effect of improving saline-alkali land and increasing cotton yield is significant. However, due to the short period of this experiment, the medium and long-term effects of different humic acids on soil physical and chemical properties and crop growth need to be further studied.

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