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Impacts of Sulfur and Microalgae Co-fertilization on Saline-alkaline Soil of Sunflower Field in Hetao Irrigation District

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Abstract [Objectives] To evaluate the impacts of the elemental sulfur (S⁰) and micro-algae (MA) co-fertilization on saline-alkaline soil of sunflower field in the Hetao Irrigation District (HID). [Methods] The greenhouse pot experiment was conducted with four treatments: control (CK), single S⁰ fertilization (S), single MA fertilization (A), and S⁰ and MA co-fertilization (SA) for comparing the selected soil properties and sunflower plant heights and weights in different treatments. [Results] The results showed that the mean soil organic matter (SOM) under the SA (25.08 g/kg) was significantly higher than that for the CK (20.59 g/kg), S (22.47 g/kg), and A (22.95 g/kg). The mean pH under the SA (7.75) was significantly lower than that for the CK (8.14), S (7.82), and A (7.96). The mean soil exchangeable Na⁺ concentration under the SA was significantly lower than that for the S. The mean soil electrical conductivity (EC) under the SA was 9.76% lower than that for the S. The means of Cl⁻ (1.22 g/kg) and SO₄²⁻ (1.90 g/kg) in soil under the SA were lower than that for the S (1.30, 2.06 g/kg) and A (1.31, 1.97 g/kg), respectively. For plant height 3 at the late stage of plant growth, the mean plant height 3 under the SA (89.00 cm) was higher than that of the CK (69.60 cm) and A (74.33 cm). The total weights of the fresh sunflower heads, fresh stems, and dry seeds under the SA were higher than that for the CK, S, and A. [Conclusions] In conclusion, the S⁰ and MA co-fertilization had positive effects on improving saline-alkaline soils, the soil under the S⁰ and MA co-fertilization could be better conditions for promoting sunflower growth than that for the S, Z, and CK, and thereby the S⁰ and MA co-fertilization could be a new idea to improve saline-alkaline soil in the cold and arid regions.

Key words Sulfur and microalgae co-fertilization, Sunflower, Saline-alkaline soil, Hetao Irrigation District (HID)

1 Introduction

Hetao Irrigation District (HID) (40° 12' – 41° 20' N, 106° 10' – 109° 30' E; altitude above sea level: 1 007 – 1 015 m) in Inner Mongolia belongs to the cold and arid region, with about 680 000 ha of irrigated farmland. The soil is classified as irrigated warped soil by the China Soil System Classification. The soil is alkaline, mean pH > 8.0, with inter-annual periodic secondary salinization and low organic matter (SOM) content^[1]. Sunflower (*Helianthus annuus* L.) is cultivated in the low-yield saline-alkaline lands in the HID, which accounts for about 1/3 of the total arable area in the HID. The mean yield from 2000 to 2018 was only 2 370 kg/ha^[2]. The previous studies on improvement of low-yield saline-alkaline lands were mainly to solve the problems of the high soil salt contents from the perspective of water and salt transport. However, little is known the studies for improving the low-yield arable lands

from the perspective of reducing soil pH and alkalization.

Elemental sulfur (S⁰) can reduce soil pH and soil exchangeable sodium (Na) percentage (ESP)^[3-6], improve nitrogen (N) availability^[7], reduce nitrate nitrogen (NO₃⁻-N) leaching, but likely result in sulfate (SO₄²⁻) enrichment^[8-5]; whereas microalgae (MA) can reduce SO₄²⁻^[9], enhance SOM and N nutrients^[10]. The blue-green algae (cyanobacteria), combined with salt-tolerant plants, can remove more salt from the soil^[11]. The main role of cyanobacteria is to reconstruct the soil microbiota^[11] and absorb Na⁺ in the soil^[12]. The S⁰ and MA have a certain synergistic and complementary effects on the improvement of saline-alkaline soils (see our previous study^[13]). Therefore, the combined application of S⁰ and MA could be an idea for the improvement of saline-alkaline lands.

The objective of this study was to evaluate the impacts of the S⁰ and MA co-fertilization on the selected soil properties [SOM, cation exchange capacity (CEC), NO₃⁻-N, available phosphorus (AP), pH, ESP, exchangeable Na⁺ (ENa⁺), electrical conductivity (EC), K⁺, Na⁺, Ca²⁺, Mg²⁺, Cl⁻, SO₄²⁻ and HCO₃⁻] in the HID through establishing a greenhouse pot experiment.

2 Materials and methods

2.1 Experiential design This experiment was conducted in the greenhouse of Sanrui Agricultural Technology Co., Ltd. from the winter of 2021 to the spring of 2022. The experimental design was

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a sunflower potting experiment with 4 treatments combined with S⁰ fertilizer and MA biofertilizer (the main components include two species of cyanobacteria and one species of chlorophyta), each treatment had 6 replicates. Treatment 1 was control (C; no application of S⁰ and MA); Treatment 2 was the application of microalgae biofertilizer (A; 1.8 mL/kg soil); Treatment 3 was the application of elemental sulfur fertilizer (S; 2.65 g/kg soil); Treatment 4 was that S (2.65 g/kg soil) and A (1.8 mL/kg soil) were applied together (SA). S⁰ fertilizer was mixed with the prepared soil, then potted the soil. Three days later, sunflowers were planted in the pots. MA biofertilizer as liquid fertilizer was applied immediately after planting and irrigating. The experimental crop was sunflower (variety: SH361).

The test soil was collected from the sunflower field (108°11'25.18" E, 41° 2' 50.13" N) located in Kwaiboyuan, Wuyuan, Bayannur, Inner Mongolia. The soil was classified as irrigated warped soil with silt clay texture, pH of 8.16, EC of 0.714 mS/cm, SOM of 12.86 g/kg, exchangeable Na⁺ of 3.03 cmol/kg, CEC of 12.11 cmol/kg, ESP of 25.00%. After adding organic substrate (10% of the soil) and compound fertilizer (N : P : K = 18 : 18 : 18), a blender was used for mixing them. Then, 10.5 kg of the mixed soil was weighed and put into a pot with a height of 25 cm and a diameter of 20 cm. A total of 24 pots were prepared. On December 7, 2021, sunflower seeds were planted in the 24 pots after which watered as needed and weeded in time. Only one sunflower plant in each pot was kept after removing the excess seedlings. All pots were harvested on April 3, 2022.

2.2 Data measurements Sunflower plant heights were measured on January 15, February 10, and March 3, 2022, respectively. The sunflower stalks above the ground and seeds were weighed on April 3, 2022. Soil samples were collected on March 4, 2022. The SOM, NO₃⁻-N, AP, pH, ESP, ENa⁺, EC, K⁺, Na⁺, Ca²⁺, Mg²⁺, Cl⁻, SO₄²⁻, and HCO₃⁻ were measured by the laboratory of the Inner Mongolia Hengtai Testing Technology Co., Ltd. (Bayannur, Inner Mongolia). All of the testing methods

were the national standardized methods including *Analysis of Water-Soluble Salts in Forest Soils* (LY/T1251-1999); *Determination of Forest Soil Organic Matter and Calculation of Carbon-Nitrogen Ratio* (LY/T1237-1999); *Determination of Nitrate Nitrogen in Soil by Ultraviolet Spectrophotometry* (GB/T32737-2016); *Determination of Forest Soil pH* (LY/T1239-1999); *Soil Testing Part 5: Determination of Cation Exchange in Calcareous Soils* (NY/T1121.5-2006); *Determination of Exchange Sodium in Alkalinizing Soil* (LY/T1248-1999); *Calculation of Soil Exchangeable Sodium Percentage* (LY/T1249-1999); *Phosphorus Determination Methods of Forest Soils* (LY/T 1232-2015).

2.3 Statistical analysis The PROC GLIMMIX method (mixed model) in SAS 9.4 statistical analysis software was used to compare the differences in the select soil properties and sunflower plant heights under different treatments. The significance level $\alpha = 0.05$. Excel 2019 was used for processing data and preparing tables.

3 Results

3.1 SOM, CEC, NO₃⁻-N, and AP The data of SOM, CEC, and NO₃⁻-N under different treatments are presented in Table 1. The mean SOM (25.08 g/kg) under the SA was significantly higher than that for the CK (20.59 g/kg) and S (22.47 g/kg). The mean CEC (14.71 cmol/kg) under the SA was significantly higher than that of CK (13.85 cmol/kg). NO₃⁻-N (194.0 mg/kg) under the SA was significantly higher than that of A (152.80 mg/kg) and significantly lower than that of CK (231.97 mg/kg). The means of AP under different treatments were not significantly different (Table 1).

3.2 Soil pH, ESP, ENa, and EC The results of soil pH, ESP, and EC under different treatments are presented in Table 1. The mean pH (7.75) under the SA was significantly lower than that of CK (8.14), A (7.96), and S (7.82). There were no significant differences in means of ESP and ENa under different treatments. The mean EC under the S (2.05 mS/cm) was significantly higher than that for the CK (1.78 mS/cm) (Table 1).

Table 1 Means of SOM, CEC, NO₃⁻-N, AP, pH, ESP, ENa⁺, and EC under different treatments in the pots planted a sunflower in the greenhouse

Treatments [†]	SOM g/kg	CEC cmol/kg	NO ₃ ⁻ -N mg/kg	AP mg/kg	pH	ESP %	ENa ⁺ cmol/kg	EC mS/cm
CK	20.59 ^{b‡}	13.85 ^b	231.97 ^a	57.45 ^a	8.14 ^a	11.16 ^a	1.54 ^a	1.78 ^b
A	22.95 ^{ab}	14.67 ^a	152.80 ^c	57.72 ^a	7.96 ^b	10.09 ^a	1.46 ^a	1.92 ^{ab}
S	22.47 ^b	14.13 ^{ab}	217.82 ^{ab}	56.49 ^a	7.82 ^c	10.00 ^a	1.41 ^a	2.05 ^a
SA	25.08 ^a	14.71 ^a	194.08 ^b	54.39 ^a	7.75 ^d	10.26 ^a	1.51 ^a	1.85 ^{ab}
	Analysis of variance ($P > F$)							
	0.008	0.04	<0.001	0.71	<0.001	0.85	0.92	0.11

Note: [†] CK refers to control; A denotes microalgae biofertilization; S represents elemental sulfur fertilization; SA refers to combined S and A fertilization; [‡] Different lowercase letters indicate significant differences in the soil properties under different treatments ($\alpha = 0.05$). The same below tables.

3.3 Soil ions Data of soil ions K⁺, Na⁺, Ca²⁺, Mg²⁺, Cl⁻, SO₄²⁻, and HCO₃⁻ under different treatments are presented in Table 2. The K⁺ under the SA (0.154 g/kg) was significantly higher than that for the CK (0.131 g/kg). The Na⁺ under the SA (1.08 g/kg) was significantly lower than that of the S (1.18 g/kg).

The Ca²⁺ under the SA and S (0.53 and 0.54 g/kg, respectively) was significantly higher than that of CK (0.45 g/kg). However, The Mg²⁺ under the S and A (0.36 and 0.356 g/kg, respectively) was significantly higher than that of the CK (0.299 g/kg) (Table 2).

There were no significant differences in soil anions Cl^- and SO_4^{2-} under different treatments (Table 2). However, under the four treatments, the mean SO_4^{2-} content under the S was highest (2.06 g/kg), while SO_4^{2-} content under the SA (1.90 g/kg) was

the same as that under the control (1.90 g/kg), lower than that for the A (1.97 g/kg). The HCO_3^- under the S (0.18 g/kg) was significantly lower than that of CK (0.25 g/kg), A (0.22 g/kg), and SA (0.23 g/kg) (Table 2).

Table 2 Means of soil ions K^+ , Na^+ , Ca^{2+} , Mg^{2+} , Cl^- , SO_4^{2-} , and HCO_3^- under different treatments in the pots planted a sunflower in the greenhouse

Treatment [†]	K^+	Na^+	Ca^{2+}	Mg^{2+}	Cl^-	SO_4^{2-}	HCO_3^-
CK	0.131 ^{b‡}	0.99 ^c	0.45 ^b	0.299 ^b	1.18 ^a	1.90 ^a	0.25 ^a
A	0.149 ^{ab}	1.11 ^{ab}	0.51 ^{ab}	0.356 ^a	1.31 ^a	1.97 ^a	0.22 ^b
S	0.152 ^{ab}	1.18 ^a	0.54 ^a	0.360 ^a	1.30 ^a	2.06 ^a	0.18 ^c
SA	0.154 ^a	1.08 ^{bc}	0.53 ^a	0.323 ^{ab}	1.22 ^a	1.90 ^a	0.23 ^{ab}
Analysis of variance ($P > F$)							
	0.09	0.0059	0.07	0.11	0.389	0.426	<0.001

3.4 Sunflower height and weight Results of sunflower heights under different treatments are presented in Table 3. The plant height 1 under the SA, S, and A (30.75, 31.25, and 27.33 cm, respectively) was significantly higher than that of CK (19.67 cm). The plant height 2 under the SA and S (60.33 and 62.85 cm, respectively) was significantly higher than that of the A (50.25 cm) and CK (37.00 cm). The plant height 3 under the SA and S (89.00 and 87.00 cm, respectively) were significantly higher than those of the A (74.33 cm) and CK (69.60 cm) (Table 3).

Table 3 Means of sunflower heights under different treatments in the pots planted a sunflower in the greenhouse

Treatments [†]	Height 1	Height 2	Height 3
CK	19.67 ^{b‡}	37.00 ^c	69.60 ^b
A	27.33 ^a	50.25 ^b	74.33 ^b
S	31.25 ^a	62.85 ^a	87.00 ^a
SA	30.75 ^a	60.33 ^a	89.00 ^a
Analysis of variance ($P > F$)			
	<0.001	<0.001	<0.001

The total weight of sunflower head (fresh), stem (fresh), and seed (dry) under different treatments are presented in Table 4. The total weights of the sunflower head, stem, and seed under the SA and S were drastically higher than those of A and CK, and the total weights of the head, stem, and seed under the A were lower than that of CK (Table 4).

Table 4 Total weights of sunflower head (fresh), stem (fresh), and seed (dry) in the 6 pots under different treatments

Treatments [†]	Height 1	Height 2	Height 3
Treatments [†]	Head (fresh) //g	Stem (fresh)	Seed (dry)
CK	225.0	242.8	20.7
A	178.2	193.6	19.3
S	236.3	260.0	32.7
SA	241.8	261.8	33.1

4 Discussion

4.1 Impacts of the combined application of S^0 -MA fertilizers on SOM, CEC, NO_3^- -N, and AP The findings in this study showed that the SOM under the combined S^0 -MA application was

significantly higher than that of the CK and S^0 application, and also higher than that of the MA fertilization, indicating that the combined S^0 -MA application can increase SOM (Table 1). This is mainly because MA fertilizer is more effective at photosynthesis under low soil pH conditions due to the S^0 application (Table 1) (MAs have suitable pH values of about 6–8)^[9], thereby increasing soil SOM content. The CEC under the combined S^0 -MA application was significantly higher than that of the CK (Table 1). This likely is because the SOM under the SA was significantly higher than that for the CK (Table 1). The soil NO_3^- -N under the application of MA alone was significantly lower than that of the CK and S^0 -MA (Table 2), indicating that a large amount of NO_3^- -N can be consumed by the microalgae alone^[9], and if S^0 is combined with microalgae, the consumption of NO_3^- -N by the microalgae can be reduced. The means of AP under the SA, S, A, and CK were not significantly different (Table 1). However, the means of sunflower height 2 and 3 and total weight under the A and CK were significantly lower than that for the SA and S (Tables 3 and 4), indicating that the AP uptake of sunflower under the SA and S could be substantially higher than that for the A and CK, consequently, the soil AP under the SA and S could be significantly higher than that for the A and CK.

4.2 Impacts of the combined application of S^0 -MA fertilizer on soil saline-alkaline properties

The results of this study showed that the pH under the combined application of S^0 -MA was significantly lower than that of the CK, S^0 fertilizer, and MA fertilizer (Table 1). S^0 fertilizer can reduce soil pH because S^0 is oxidized by microorganisms to produce SO_4^{2-} and H^+ , which in turn reduces soil pH^[3–5]. MA fertilizer can significantly reduce soil pH in some places, but not in other places^[12–15]. However, the combined application of S^0 -MA can significantly reduce the soil pH (Table 1), indicating that the combined application of S^0 -MA can effectively offset the fluctuation of soil pH affected by a single application of MA fertilizer, compared with a single application of microalgae. The combined application of S^0 -MA did not significantly affect soil ESP (Table 1), but the mean ESP under the SA

was 8.07% lower than that of the CK. S⁰ application alone can significantly increase soil conductivity (Table 1), indicating that S⁰ increases soil salinity. This is mainly because S⁰ is oxidized by microorganisms to produce SO₄²⁻ and H⁺ [3-5], thus forming more sulfates. However, the EC under the combined S⁰-MA application was 9.76% lower than that of the S⁰ fertilizer alone (Table 2), mainly because cyanobacteria in the MA fertilizer can reduce the Na⁺ concentration in soil [12].

4.3 Impacts of the combined application of S⁰-MA fertilizers on soil ions The observations in this study showed that the soil Na⁺ concentration under the combined application of S⁰-MA was significantly lower than that of S⁰ fertilizer (Table 2). This is mainly because the cyanobacteria in MA fertilizer could adsorb Na⁺ concentration in soil [12]. However, there were no significant differences between the concentrations of K⁺, Ca²⁺, and Mg²⁺ under the combined application of S⁰-MA and the concentrations of three ions under the single S⁰ application and MA, but the concentrations of K⁺, Ca²⁺, and Mg²⁺ under these three treatments were significantly higher than those under the CK (Table 2). This indicates that the S⁰-MA, S⁰, and MA application may increase the concentration of soil K⁺, Ca²⁺, and Mg²⁺, increasing soil total salt. The increase of soil total salt content by S⁰ fertilizer has previously been discussed, and the reasons for the increase of soil K⁺, Ca²⁺, and Mg²⁺ by single MA application are not clear, and further research is needed.

Although there were no significant differences between the Cl⁻ and SO₄²⁻ under the combined S⁰-MA application and the single application of S⁰, MA, and CK, the Cl⁻ and SO₄²⁻ under the combined S⁰-MA application were lower than that of S⁰ and MA alone, and were almost equal to that of the CK (Table 3). This indicates that the combined S⁰-MA application can offset some Cl⁻ and SO₄²⁻ increased by the S⁰ and MA alone, and the mechanism needs to further be investigated. However, sulfur is an element necessary for the growth of microalgae and is consumed by microalgae in the form of SO₄²⁻ [9], so microalgae necessarily consume part of the soil SO₄²⁻ increased due to S⁰ fertilization. HCO₃⁻ under the combined S⁰-MA application was significantly higher than that of the S⁰ alone (Table 2), and the reasons for this need to further be revealed.

4.4 Impacts of the combined application of S⁰-MA fertilizers on sunflower height and weight The height1 of sunflower plants under the combined S⁰-MA application, single S⁰ application, and single MA application (sunflower seedling stage) was significantly higher than that of the CK. However, in the late growth stage of sunflowers, the plant heights 2 and 3 under the S⁰-MA and S⁰ were significantly higher than that for the MA and CK (Table 3). The distribution of the weight of sunflower head, stem, and seed under different treatments was similar to the heights (Table 4). The main reason for the findings could be that the combination of S⁰-MA and the sulfur alone can significantly reduce soil pH (Table 1).

Moreover, compared with the S⁰ application alone, the combined S⁰-MA application can significantly reduce soil pH (Table 1) and Na⁺ content (Table 2), significantly increase SOM (Table 1) and HCO₃⁻ (Table 2), reduce Cl⁻ and SO₄²⁻ content (Table 2) and EC (Table 1). These indicate that the soil under the combined S⁰-MA application has better conditions for crop growth.

5 Conclusions

Based on the above results and discussion, we reached the following conclusions. (i) The combined application of the elemental sulfur and microalgae fertilizers has better effects on the SOM, NO₃⁻-N, AP, CEC, pH, crop height1-3, and sunflower weights, but significantly increased K⁺ and Ca²⁺ (did not impact the Na⁺, Mg²⁺, Cl⁻, SO₄²⁻, and HCO₃⁻), compared with the control. (ii) The combined application of the elemental sulfur and microalgae fertilizers has better effects on SOM, pH, and Na⁺, but increased HCO₃⁻, compared with the single sulfur. (iii) The combined application of the elemental sulfur and microalgae fertilizers has better effects on NO₃⁻-N, AP, pH, height 2 – 3, and weights, compared with the single microalgae. Therefore, the combined sulfur-microalgae application can provide a better soil environment for crop growth, indicating that the combined sulfur-microalgae application could be used as a new way for improving saline-alkaline farmlands in the cold and arid regions.

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According to the regression equation, it can be found that the highest yield fertilization amount was 8.75 kg of pure nitrogen, corresponding to a yield of 299.42 kg. Combined with fertilizer

price of 5 yuan/kg of pure nitrogen and peanut price of 6 yuan/kg, the amount of fertilizer applied for the best economic yield was 7.68 kg, corresponding to a yield of 279.22 kg (Table 5).

Table 5 Economic benefit analysis of optimal nitrogen application rate experiment in summer peanut

Treatment	Fertilizer cost yuan/ha	Peanut yield		Net income		
		Yield//kg/ha	Unit price//yuan	Income //yuan/ha	yuan/ha	Rank
Optimized nitrogen plot	1 215	4 233.15	6	24 183.9	25 398.9	1
130% Optimized nitrogen plot	1 365	4 104.15	6	23 259.9	24 624.9	2
70% Optimized nitrogen plot	1 065	3 705.00	6	21 165.0	22 230.0	3
No-nitrogen plot	690	2 801.70	6	16 120.2	16 810.2	4

4 Conclusions

The experimental results indicate that for the blue-black soil field with higher fertility level, the optimum economic nitrogen fertilization rate was 115.2 – 131.25 kg/ha, and the highest fertilization rate was 131.25 kg/ha on the basis of 70 kg/ha of phosphorus and potassium fertilizer application. When the nitrogen fertilizer application rate exceeded the optimal economic fertilization rate, although there was still room for a small increase in peanut yield, the economic benefits declined instead. When the nitrogen fertilizer application rate exceeded the maximum fertilization rate, the peanut yield would show a downward trend, and the economic benefit would be further decreased^[4-5]. Combined with local soil fertility conditions, rational application of nitrogen fertilizer can not only realize the simultaneous increase of summer peanut yield and benefits, but also reduce the waste of fertilizer resources and environmental pollution^[6].

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