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REGULAR ARTICLE

Addition of HPMA affects seed germination, plant growth and properties of heavy saline-alkali soil in northeastern China: comparison with other agents and determination of the mechanism

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Abstract China has a large area of inland saline-alkali land, equivalent to 40% of the total cultivated land in the country. The principal features of these lands are high salt content, high pH, and poor soil structure with low water infiltration and poor drainage. These conditions effectively prevent the exploitation of such land for agriculture. In this study, we have compared 17 soil conditioning agents for their abilities to promote seed germination and growth under both laboratory and field conditions. One of these, Hydrolyzed Polymaleic Anhydride (HPMA), was identified as a highly effective agent for soil improvement. Laboratory germination experiments and laboratory and field cultivation of a variety of plants both showed that addition of HPMA could significantly increase the germination percentage and plant growth rate. Distinct from other Ca-carrier agents such as gypsum, HPMA increases the dissolution of $CaCO_3$, which is abundant in the calcareous saline-alkali soils. This allows Ca^{2+} in soil solution to displace the over-abundant Na^+ in the soil colloids. This process greatly improves soil properties such as the bulk density, which decreased, and the capillary soil rise height of water and soil water infiltration rate, which increased. Direct SEM and AFM imagery showed flocculent soil precipitation (soil aggregates) after HPMA addition, and a looser structure of those aggregates. The addition of HPMA also reduced the soil pH and EC. These changes in soil chemical and physical properties are a likely explanation for the soil improvement effected by HPMA. The high content of insoluble $CaCO_3$ in saline-alkali land such as that in northeastern China (up to 13%) favors the further exploration of HPMA as an ameliorative agent.

Keywords Saline-alkali land improvement · Seed germination and plant growth rate · Soil pH · Soil EC · Soil physical properties · Atomic force microscope · Scanning electron microscope

Abbreviations

HPMA	Hydrolyzed polymaleic anhydride
PAA	Polyacrylic acid
T225	Acrylic acid-2-hydroxypropyl acrylate copolymer

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AA/AMPS	Acrylic acid-2-acrylamido-2-methylpropane sulfonic acid copolymer
MA-AA	Copolymer of maleic and acylic acid
ATMP	Amino trimethylene phosphonic acid
BHMTPMPA	Bis (hexamethylene triamine penta (methylene phosphonic acid)
HEDP	1-hydroxy ethylidene-1,1-diphosphonic acid
PAPEMP	Polyamino polyether methylene phosphonae
EDTA	Ethylenediaminetetraacetic acid
CA	Citric acid
CC	Citric calcium
NTA	Nitrilotriacetic acid
SOM	Soil organic matter
EC	Electrical conductivity
ESP	Exchangeable sodium percentage
CEC	Cation exchange capacity

Introduction

Throughout the world, about 15% of the total land area has been degraded by soil erosion or physical and chemical degradation, including soil salinization (Wild 2003). In many countries, including China, dry-land salinity far from coastal areas has become a major issue in natural resource management. Because of its implications for food security, it has also become of increasing concern among farmers, scientists and politicians.

Salinity problems are compounded when the affected soils are also alkaline. These areas are characterized by excessive Na_2CO_3 and $NaHCO_3$, salts whose toxicity to plants is even greater than that of NaCl (Abrol et al. 1988; Wang 1993). One of the larger such areas is the Songnen Plain in the central part of northeastern China (43°30′–48°40′ N; 121°30′–127°00′ E). With an area of 17.0×10^6 ha, the Songnen Plain is experiencing rapidly increasing alkalinization and desertification, restricting the economic development of the area and threatening human survival (Lin and Tang 2003; Li et al. 2007; Wang et al. 2009a, b).

As part of efforts toward remediation and reclamation, one aim of soil scientists is to find effective soil conditioners for saline-alkali lands. Until now, gypsum-like Ca^{2+} solid agents and organic/inorganic acids have been the most common chemicals applied to large areas (Sharma and Swarup 1997; Wallace 1998; Amezketa et al. 2005; Tang et al. 2006; Zia et al. 2007). These approaches are grounded in studies such as those of Sharma and Swarup (1997) who compared the effects of pyrites and gypsum on saline-alkali land improvement. Wallace (1998) similarly concluded that a gypsum solution applied in irrigation water provided the most effective soil remediation. Amezketa et al. (2005), on the other hand, preferred sulfuric acid for its rapid reduction of EC and pH and its prevention of soil crusting, although three other gypsum-materials were equally effective. The mechanism for these soil modifications is believed to involve changes in physical properties and cation exchanges between the solution and soil colloids. Gypsum and gypsum-related agents may enhance Ca^{2+} release in the soil solution, and exchange between Na^+ at surface of soil particles and Ca^{2+} in soil solution (Li 2006). Through these effects, they improve soil physical and chemical properties good for plant growth.

With respect to plant responses, however, the effects of these agents are not uniform. Zia et al. (2007), for example, compared H_2SO_4 and gypsum in calcareous saline-alkali soil; while both agents increased the productivity of rice and wheat, they only slightly increased the productivity of Kallar grass (*Leptochloa fusca*). This disparity is significant, as Kallar grass is widely used for reclamation of saline soils (Ahmad et al. 1990).

As an alternative approach, water-soluble polymeric agents have also received attention in remediation field studies. Bouranis (1998) emphasized that polymer agents designed to improve physical properties of soil should promote the formation of soil aggregates and improve the water-holding capacity, for example by increasing the soil porosity and reducing its bulk density. One such agent, developed in the 1950s, consisted mainly of vinyl acetate maleic acid (VAMA), hydrolyzed polyacrylonitrile (HPAN) and isobutylene maleic acid (IBM) (Nelson 1998). This was marketed by Monsanto as “Krilium” (Quastel 1953).

The results of polymer-based amendment studies have been promising. Levy and Ben-Hur (1998), for example, reported that water-soluble polyacrylamide (PAMs) could improve physical properties of soil and increase crop yield in various problem soils including saline land. Zahow and Amrhein (1992) reported that one nonionic and two anionic PAMs, and one cationic guar-derivative polymer were able to improve the quality of saline-alkali soil by improving its capability for water penetration. Tang et al. (2006) concluded that, for rain-fed agriculture, the spreading of dry granular PAM mixed with phosphogypsum was more effective in reducing runoff and erosion in soils varying in texture and sodic condition than phosphogypsum on its own.

Similar to PAMs, hydrolytic polymaleic anhydride (HPMA) is also a water soluble polyelectrolyte. Commonly used as an inhibitor of carbonate scale formation in industrial boilers because of its stability in alkaline waters at temperatures up to 300°C (http://www.thwater.net/03-HPMA.htm), HPMA is considered non-toxic to the environment and to algae (Nabholz et al. 1993), thus making it a candidate for consideration as a soil conditioner. The same can be said of some other water treatment agents, such as the polyacrylics (PAA, T225, AA/AMPS, MA-AA), and poly-phosphonic acids (ATMP, BHMTPMPA, HEDP, PAPEMP). Furthermore, agents such as charcoal, organic acids of woody vinegar, EDTA and cation exchanging resins could change soil alkalinity and salinity and improve saline alkali soil properties (Yang and Wang 2005). Experiments are needed, however, to determine whether these are effective in treating saline-alkali land (He et al. 2008; Wang et al. 2009a, b).

The present study is part of an on-going search for alternative agents for rescuing saline-alkali land. We present, first, a laboratory comparison of the effect of HPMA and 16 other agents on the chemical characteristics of saline-alkali soil and their effects on seed germination. Second, we report laboratory and field studies of the effects of HPMA on soil pH, EC and plant growth. For this, we have selected a wide range of species with previously reported differences in saline-alkali tolerance. Finally, through determination of HPMA-induced changes in soil physical properties, chemical cation exchange, and micro-structural observations of soil colloids and aggregates in laboratory, we consider possible mechanism by which HPMA improves saline-alkali soil.

Materials and methods

Study site and soil characterization Saline-alkali soil for laboratory testing was collected from the top soil (0–30 cm) in typical saline-alkali regions of the Songnen Plain (45°59′55″ N, 124°29′48″ E). As a non-saline loam control, we used dark-brown forest soil (0–30 cm) from the Harbin experimental forest farm of Northeast Forestry University (45°43′6″ N, 126°37′54″ E). Table 1 shows a comparison of the physical and chemical properties of the two soils. In particular, note that soluble Ca^{2+} and Mg^{2+} were much lower in saline-alkali soil than in the non-saline loam control, while Na^+ and K^+ were much higher. The concentration of exchangeable Na^+ was nearly 20 times greater in saline-alkali soil than in the non-saline loam control, and this was responsible for the high exchangeable sodium percentage (ESP) of nearly 40% of ESP for saline-alkali soil. The pH of saline-alkali soil (10) was nearly four units higher than the non-saline loam control.

Comparison of effectiveness of HPMA and 16 other agents based on seed germination tests A total of 17 compounds were tested in this experiment as potential soil remediation agents. Table 2 lists these, along with the dosage used in the various experimental protocols. For the seed germination and growth experiment, each compound was fully mixed with the saline-alkali soil, and distributed among plastic, 2 cm×2 cm×2 cm pots. Soils were sampled for measurement of pH and EC immediately after mixing with the agents; in both cases a water to soil ratio of 5:1 was used. pH was measured with a Sartorius pH meter (PB-10, Shanghai) and

Table 1 Soil properties of saline-alkali soil and non-saline loam control in the present study. Values in the parenthesis are standard deviations (n=6)

	Saline alkali soil	Non-saline loam control
Texture		
Clay (%)	42.3 (9.0)	31.0 (7.1)
Silt (%)	26.7 (6.2)	37.8 (6.1)
Sand (%)	31.0 (8.1)	31.2 (8.7)
Soluble Ca^{2+} (mmol kg^{-1})	1.32 (0.86)	122.3 (15.2)
Soluble Mg^{2+} (mmol kg^{-1})	1.10 (0.78)	35.2 (8.7)
Soluble Na^+&K^+(mmol kg^{-1})	41.5 (33.0)	7.2 (2.8)
Exchangeable Na^+(mmol kg^{-1})	130.4 (81.0)	7.14 (1.69)
CEC (mmol kg^{-1})	329.3 (68.9)	260.9 (60.8)
ESP (%)	38.2 (19.9)	2.94 (1.13)
$CaCO_3$ (%)	13.9 (4.6)	0.47 (1.02)
pH	10.0 (0.4)	6.4 (0.5)
SOM	2.5 (0.4)	5.1 (0.7)

CEC cation exchange capacity, *ESP* exchangeable sodium percentage, *SOM* soil organic matter

Table 2 Agents, content of active ingredient or quality, and dosage used in this study

No	Agents	Content of active ingredient or Quality	Suppliers	Dosage
1	HPMA	50%	Taihe, Shandong	1–42.8 L m^{-3}; 2–25 L m^{-3}; 3–25 L m^{-3} and with 30% silt sand mixture;4–25 L m^{-3}
2	Gypsum	80–90%	Xuelian,Shanxi	1–10 kg.m^{-3}
3	HEDP	50%	Taihe, Shandong	1–42.8 L.m^{-3}
4	T225	28.5%	Taihe, Shandong	1–42.8 L.m^{-3}
5	PAPEMP	45%	Taihe, Shandong	1–42.8 L.m^{-3}
6	MA-AA	50%	Taihe, Shandong	1–42.8 L.m^{-3}
7	ATMP	50%	Taihe, Shandong	1–42.8 L.m^{-3}
8	AA/AMPS	30%	Taihe, Shandong	1–42.8 L.m^{-3}
9	PAA	40%	Taihe, Shandong	1–42.8 L.m^{-3}; 4–25 L. m^{-3}
10	Charcoal	85%	Ruili, Shandong	1–142.9 kg.m^{-3}
11	BHMTPMPA	43–48%	Taihe, Shandong	1–42.8 L.m^{-3}
12	Wood vinegar	10%	Ruili, Shandong	1–42.8 L.m^{-3}
13	EDTA	Analytical reagent	Yixing,Jiangxi	1–4.28 kg.m^{-3}; 4–4.28 kg m^{-3}
14	H^+ Resin	Absorption ≥4 mmol.g^{-1}	Zhengguang, Shanghai	1–42.8 L.m^{-3}; 4–42.8 L m^{-3}
15	CA	Analytical reagent	Runjie, Shanghai	1–4.28 kg.m^{-3};
16	CC	Analytical reagent	Yuancheng, Hubei	1–4.28 kg.m^{-3}
17	NTA	Analytical reagent	Kelun, Guangzhou	1–4.28 kg.m^{-3}

In dosage column, 1—is the dosage used in laboratory seed germination experiment; 2—is the dosage used in laboratory plant cultivation experiment; 3—is the dosage used in field plant cultivation experiment; 4—is the dosage used in laboratory simulation experiment of saline-alkali soil solution

electrical conductivity (EC) with a conductivity meter (DDS-307, Shanghai). Non-saline loam and untreated saline-alkali soil were used as controls.

Thirty healthy seeds were planted in each of three pots as replicates for each treatment. To compare the effects of these compounds, the germination percentage and seedling growth of Chinese cabbage (*Brassica oleracea* Dongnong 906) were measured together with soil properties (soil EC, pH). Chinese cabbage was selected because of its low saline-alkali tolerance and sensitivity to changes in saline-alkali soil properties (Yang et al. 2003). Seeds obtained from the College of Horticulture, Northeast Agricultural University were first selected by floating on water, and then soaked at 30°C in distilled water for 25 min. They were then disinfected in 1% $KMnO_4$ solution for 15 min and rinsed with distilled water prior to sowing. Seedlings were germinated in a greenhouse with temperatures between 25°C and 30°C and relative humidity from 50% to 80%. The plants was watered daily and germinating seeds were counted. Seeds were considered to have germinated when the cotyledons were fully expanded and roots were established. The observations were completed 11 days after sowing, at which time germination percentage was calculated, and seedling fresh biomass, and lengths of roots and shoots were measured.

Laboratory and field cultivation of plants in saline-alkali soil with HPMA addition To test the effect of HPMA on saline alkali soil properties, a total of 13 species were selected for laboratory (greenhouse or growth chambers) and field cultivation (Table 3). The species differ both in their growth forms and life history strategies, and in their tolerance of soil salinity and alkalinity (Fu 1995; Liu et al. 2006; Sun et al. 2006). The test group included four species of vegetables (Chinese cabbage, potato, spinach and radish), four major cash crops (sorghum, sugar beet, sunflower and flax), three annual or perennial forbs (*Iva xanthifolia*, *Catharanthus roseus* and *Ambrosia trifida*) and two woody species [sea buckthorn (*Hippophae rhamnoides*) and poplar (*Populus x xiaohei*)]. Seven of the 13 species are considered to be poorly tolerant of saline-alkali conditions; the other five are highly tolerant (Table 3).

To test the effect of HPMA on growth, eight species were cultivated in large soil boxes (40 cm× 30 cm×60 cm) under controlled conditions using soils and HPMA mixes identical to those in the germination study. There were three replicates for each treatment. During the experiment, six of the species (Sorghum, *Iva xanthifolia*, Sugar beet, Potato, Sunflower, Flax—Table 3) were germinated

Table 3 Plant species used in this study and their functional characteristics and previously reported saline-alkali tolerance

No	Species name	Functional group	Saline-alkali tolerance	Note
1	Chinese cabbage (*Brassica oleracea* L. *"Dongnong 906"*)	Vegetable	Low	1, 3
2	Sorghum (*Sorghum vulgare* Pers.)	Crop	Low	2
3	*Iva xanthifolia* Nutt.	Annual forb	Low	2
4	Sugar beet (*Beta vulgaris* L. "TC701")	Crop	High	2
5	Potato (*Solanum tuberosum* L. "MLS303")	Vegetable	Low	2
6	Sunflower (*Helianthus annuus* L.)	Crop	High	2
7	Flax (*Linum perenne* L.)	Crop	High	2
8	Sea buckthorn (*Hippophae rhamnoides* L.)	Woody shrub	High	2
9	poplar (*Populus* × *xiaohei* T. S. Hwang et Liang)	Tree seedling	High	2, 3
10	Spinach (*Spinacia oleracea* L.)	Vegetable	Low	3
11	Radish (*Raphanus sativus* L.)	Vegetable	Low	3
12	*Catharanthus roseus* (L.) G. Don	Perennial forb	Low	3
13	*Ambrosia trifida* L.	Annual forb	Low	3

1—species used in the seed germination experiment; 2—species used in growth chamber/greenhouse plant cultivation experiment; 3—species used in field plant cultivation experiment

directly in treated saline-alkali soil and untreated control soil in growth chambers (ZPW400, Dongtuo, Harbin). Chamber conditions were: temperature—25°C, photoperiod—12 h at 150 μmol.m^{-2} s^{-1}, relative humidity—approximately 50%. In addition, 1 year old seedlings of sea buckthorn were transplanted into the treated saline-alkali soil and untreated control in the same growth chamber. One year old seedlings of poplar were transplanted and cultivated in under greenhouse conditions under natural light (February to April, 2008); air temperature ranged from 18°C to 25°C and humidity was 30–60%. In both growth environments, plants were watered as needed at 3–7 day intervals. Soil pH and EC were measured after 1 month of treatment. Growth in height, diameter or shoot length were used as the criteria for evaluating the effectiveness of HPMA treatments.

Field tests of the effect of HPMA on saline-alkali soil were carried out at the site from which saline-alkali soil was collected for the greenhouse studies. This strongly saline-alkali site was degraded to the point of having no live plant cover. Three replicate 1 m×1 m plots were excavated to a depth of 30 cm (80 cm for poplar) and the soil was removed. A portion of the excavated soil (0.7 m^3) was mixed with 0.3 m^3 fine sand, and 25 L HPMA, and the treated soil was returned to the trenches. Untreated soil was returned to a control trench. Six species differing in saline-alkali tolerance were planted in each plot (see Table 3, note 3). Planting included seeds of three vegetables (Chinese cabbage, spinach and radish), seedlings of two forbs (*Catharanthus roseus* and *Ambrosia trifida*), and 1-year seedlings of poplar. The non-saline loam control used in the greenhouse studies was not available for the field cultivation test.

During the field tests, irrigation was done at intervals of about 5–15 days when the soil was visibly dry as more sophisticated monitoring equipment was not available. The experiment lasted from late June to September, during which the mean daily air temperature was 20.1°C (SD=3.5°C). The height of each plant was measured eight times in order to quantify the effect by HPMA addition. The best-fit straight line relating height and day after planting was used to model plant growth rates. From the planting of seeds and seedlings to the end of this experiment, the soil was sampled at intervals of ca. 2 weeks for pH and EC measurements, and the mean values were used to compare growth and soil chemical changes.

Mechanistic analysis of the effect of the HPMA soil treatment To determine in more detail the effects HPMA on the saline-alkali soil and its possible underlying mechanism, we measured soil physical and chemical properties associated with the large box, greenhouse cultivation study. For the measurement of soil bulk density and porosity, two 100 cm^3 intact soil samples were collected 1 month after planting of each species. One of these was air-dried for bulk density measurement, and the other was used to measure the soil porosity according to the method described by Lao (1988) and Wang et al. (2008). For each treatment (saline-alkali soil, non-saline loam soil or saline-alkali soil with HPMA addition), approximately 1 kg of air-dried soil (composite samples from each box) was collected for the determination of the soil capillary water rise height and the water infiltration rate as described in Liu (1982), Lao (1988) and Yi (2009). Briefly, after grinding and sieving through a 0.2 mm mesh sieve, the soil samples were loaded into a 0.5 m transparent PVC tube (inner diameter 0.8 cm) which was held vertically with its bottom in water (1 cm). The height to which water rose in the tube was taken as the soil capillary water rise height. To determine the infiltration rate of water into the soil, soil samples were loaded into 0.5 m transparent PVC tube (inner diameters 5 cm). Five cm of water pressure were applied and the water infiltration distance was recorded with time. The infiltration rate into unsaturated soil (IR) was computed as $IR = V/(S*T)$,where V is water volume of infiltration (cm^3), S is the cross sectional area of the tube (19.625 cm^2) and T is elapsed time (minute). Measurements were conducted over periods of 1–10 min; for the non-saline loam soil, infiltration was very fast, while for the saline alkali soil, it was much slower. Mean values for infiltration rates were determined for three replicate trials for each soil.

To visualize the effect of HPMA on saline alkali soil, two sets of experiment were conducted. In the first, after fully mixing the HPMA and the saline-alkali soil, 1:5 soil:water solutions were made for treated and untreated soils. These were shaken for 5 min and allowed to stand for 5 h before photographing. In the second experiment, untreated saline-alkali soil was extracted and the supernatant was divided into two aliquots before HPMA addition to one. After an additional 2.5 h, the treated and untreated samples were visually inspected and photographed.

HPMA treatment effects were also examined using scanning electron microscopy (SEM) and atomic force microscopy (AFM) using methods revised from Gerin and Dufrene (2003) and Citeau et al. (2006). For SEM, about 5 g of dried soil was fully dispersed in 100 ml of pure water in a 250 ml beaker, and left to stand undisturbed for at least 5 h. The supernatant was partitioned into two tubes, and HPMA was added to one (adjusted to pH=7.0). After an additional 2.5 h, 100 μl of each solution was dispensed to a mica surface and air dried before SEM imaging (Hitachi S-3400 N, Japan at an accelerating voltage of 12.5 kV).

For AFM visualization of nano-sized samples, duplicate soil solutions were prepared (1 g of dried soil dispersed in 100 ml of pure water in a 250 ml beaker). One was used as the saline alkali soil control (pH=10.34) and the other was HPMA treated and pH was adjusted to 6.7. Both suspensions were left to stand undisturbed for at least 48 h. The supernatants were decanted and centrifuged at 12,000 g for 10 min. The resulting precipitates were redispersed in 5 ml pure water and 50 μl of the solution was placed on a mica surface and air dried before AFM imaging. AFM images were obtained in tapping mode using a PicoPlus II AFM system from Molecular Imaging (MI) Corporation (AZ, USA). A NSC15 ultra-sharp silicon cantilever from MikroMasch was used with a nominal spring constant of 40 N/m and curvature radius <10 nm. The cantilever underwent oscillation at 325 kHz. Images were captured at 512×512 pixel resolution. The dimensions were measured manually using the PicoScan 5.3.3 software.

To determine the Ca and Na changes between soil particles and soil solution, soil solutions (1:5 soil: water) were prepared and comparative measurements were conducted with and without addition of HPMA. For soil extraction, saline-alkali soil suspension with or without HPMA addition, solutions were centrifuged at 5,000 g for 10 min before the measurements. For the sediments after centrifugation, soil was first digested with nitric acid, hydrofluoric acid and perchloric acid in a Teflon crucible on an electric hot plate (at approximately 300°C), and the digested solution was diluted to a constant volume using deionized distilled water prior to measurement of Ca and Na (Lao 1988). All the measurement of Ca and Na were carried out using an atomic absorption spectrophotometer (TAS-990, PG General, Beijing, China).

Data analysis All statistical analyses (analysis of variance, regression analysis and post hoc multiple comparisons) of the data were performed using SPSS13.0.

Results

Comparison of cabbage seed germination, growth and soil chemical properties after addition of HPMA and other agents

Seed germination in the non-saline loam soil was 93.3%, and in untreated saline-alkali soil was zero. As shown in Table 4, 11 of the 17 agents tested proved to be effective in promoting seed germination (HPMA, Gypsum, HEDP, T-225, PAPEMP, MA-AA, ATMP, AA/AMPS, PAA, Charcoal, BHMTPMPA), but no seeds germinated following application of the other six agents (wood vinegar, EDTA, H^+ Resin, CA, CC, NTA). Following addition of HPMA, the seed germination percentage was 72.5%, which, although significantly lower than the non-saline loam control (93.3%) ($p<0.05$), was three to seven times higher than all the other effective agents (10% to 27.5%) ($p<0.05$). The biomass of seedlings in soil with the HPMA addition was approximately twice that with gypsum addition ($p<0.05$), but, again, much lower than in the non-saline loam control ($p<0.05$). In the 11 effective agents, the greatest biomass, seedling height and root length (albeit with poor germination) were found in the charcoal addition treatment, at

Table 4 Effectiveness of 17 agents and the combined addition of HPMA and gypsum on improving saline-alkali soil according to soil chemical properties, seed germination and seedling status. Different letters in the same column indicate a significant difference ($p<0.05$). Data in the parenthesis are the standard deviation ($n=3$)

Soil agents	EC (μS cm^{-1})	pH	Germination and seedling growth status			
			Germination percentage (%)	Biomass (mg $ind.^{-1}$)	Seedling height (cm)	Root length (cm)
Control						
Non-saline loam soil	978(26.2)b	6.9(0.1)c	93.3(7.6)e	88.0(14.1)e	3.81(0.65)f	4.25(1.58)d
Saline-alkali soil	2510(272.3)e	10.7(0.2)h	–	–	–	–
Effective agents						
HPMA*	2660(330.2)e	6.1(0.2)b	72.5(4.8)d	43.3(1.26)cd	1.09(0.13)c	2.29(0.69)c
Gypsum*	1570(184.3)c	7.7(0.1)d	27.5(7.5)c	23.3(1.80)b	0.98(0.17)c	0.87(0.085)b
HPMA + Gypsum*	2250(189.2)d	6.3(0.1)b	63.0(9.2)d	52.6(17.0)d	2.77(0.97)e	3.27(1.46)cd
HEDP	1401(128.2)c	5.9(0.3)b	26.7(13.2)c	55.1(24.5)d	2.08(0.67)de	1.88(0.82)bc
T-225	1657(112.6)d	7.9(0.2)d	23.3(9.2)c	58.2(33.7)d	2.68(0.53)e	2.12(0.97)c
PAPEMP	2530(222.4)e	6.0(0.1)b	23.3(23.1)c	40.5(14.6)c	2.13(0.62)e	1.19(0.42)bc
MA-AA	2860(232.1)e	4.5(0.1)a	23.3(20.2)c	42.1(22.7)c	2.68(0.97)e	2.56(1.03)c
ATMP	2290(130.2)e	6.1(0.2)b	23.3(7.5)c	26.6(5.1)b	1.50(0.13)d	0.86(0.19)b
AA/AMPS	1830(93.9)d	7.6(0.1)d	16.7(12.4)bc	48.9(35.2)cd	2.24(1.46)de	1.91(1.08)bc
PAA	1070(59.9)b	6.7(0.1)c	10.0(6.3)b	38.9(6.5)c	0.64(0.12)b	1.01(0.30)b
Charcoal	5700(297.3)f	10.4(0.2)	10.0(6.3)b	64.8(20.2)d	2.41(0.71)e	3.52(1.74)cd
BHMTPMPA	2680(113.5)e	6.1(0.1)b	10.0(9.5)b	37.2(6.6)c	2.37(0.41)e	2.41(0.34)cd
Mean	2386.2(1249.3)	6.8(1.5)	24.2(17.4)	43.5(12.7)	1.89(0.72)	1.87(0.83)
Non-effective agents						
Wood vinegar	978(24.3)b	8.7(0.2)e	–	–	–	–
EDTA	670(33.3)a	9.7(0.2)g	–	–	–	–
H^+ Resin	740(46.5)a	10.0(0.3)g	–	–	–	–
CA	8200(373.2)g	10.2(0.1)g	–	–	–	–
CC	2120(206.4)	9.3(0.2)f	–	–	–	–
NTA	903(59.6)b	9.1(0.1)f	–	–	–	–
Mean	2268.5(2953.9)	9.5(0.6)	–	–	–	–

– indicates no germination in these treatments about 80% of those observed in the non-saline loam control. The lowest biomass and plant sizes were found in the gypsum addition treatment (Table 4).

pH and EC values for untreated saline-alkali soil were 10.7 and 2510 μS cm^{-1}. Addition of the 11 effective agents reduced the soil EC from 2,510 μS cm^{-1} to 2386.2 μS cm^{-1} on average but with large differences between agents. They were also effective in decreasing the soil pH (except for charcoal), from 10.7 for untreated saline-alkali soil to 6.8 on average. Six of the 17 agents were ineffective in reducing either pH or EC (Table 4).

Table 4 also shows the differences between individual and combined additions of gypsum and HPMA. EC value in the saline-alkali soil + HPMA + gypsum treatment were significantly lower than with HPMA addition alone ($p<0.05$), but about 1.5 times higher than with gypsum addition ($p<0.05$). The pH with the combined addition of HPMA and gypsum was not significantly different from those of HPMA addition, but was higher than the gypsum addition ($p<0.05$). Compared with addition of gypsum alone, the combined gypsum and HPMA treatment showed higher seed germination, although it was still significant lower than with HPMA alone. Comparing with the HPMA addition alone, HPMA + gypsum gave a small but insignificant increase in biomass, but much larger increases in seedling height (2.5-fold) and root length (1.4-fold) ($p<0.05$). These changes were even larger when comparing with the gypsum-only addition ($p<0.05$). In all cases, the combined addition gave much lower seed germination, biomass, seedling height and root length than those in the non-saline loam control (Table 4).

Laboratory and field growth of plants cultivated in saline alkali soil treated with HPMA, and changes in soil pH and EC

Figure 1 summarizes the growth of eight species planted in saline-alkali soil, saline-alkali soil with HPMA addition, and non-saline loam control under growth chamber/greenhouse conditions. Although differing in their tolerance to salt, growth of six herbaceous species (*Sorghum dochna*, *Iva xanthifolia*, *Beta vulgaris*, *Solanum tuberosum*, *Helianthus annuus* and *Linus stelleroides*) was increased in HPMA treated soil compared to untreated control saline-alkali soil ($p<0.05$). At the final stage of the experiment, increases in height of 10%, 100%, 40% and 50% were observed in *Sorghum dochna*, *Iva xanthifolia*, *Beta vulgaris* and *Helianthus annuus*, respectively. *Solanum tuberosum* failed to germinate in the saline-alkali soil control, whereas it grew up to 30 cm with addition of HPMA, about 80% of the height in the non-saline loam control. The performance of the two woody species (*Hippophae rhamnoides*, *Populus* × *xiaohei*) was similar to the herbs; the new shoot length of *Hippophae rhamnoides* was about 15% greater than in the saline-alkali control, and the basal diameter of *Populus* × *xiaohei* was about 40% greater than in untreated saline-alkali soil (Fig. 1). In all species, reductions in soil pH by at least one unit and EC by 25–100% were observed after addition of HPMA relative to saline-alkali soil ($p<0.05$). These values were still significantly higher than in non-saline loam ($p<0.05$; Fig. 1).

Table 5 shows the field growth of six species (Chinese cabbage, *Catharanthus roseus*, *Ambrosia trifida*, spinach, radish and poplar) after addition of HPMA to saline-alkali soil. *C. roseus, A. trifida* and poplar were transplanted from laboratory-cultivated seedlings, and the other species were directly germinated from seeds in the field. In contrast to the absence of germination in untreated saline-alkali soil, three species (Chinese cabbage, radish and spinach) germinated in the HPMA treated field plots, and their daily growth rates reached 0.5 mm, 0.9 mm and 0.7 mm, respectively. For the transplanted species, the growth rates in the HPMA treated plots were 1.36, 1.47 and 2.2 times higher than in untreated control soil, respectively (Table 5). For each species, soil pH was reduced by more than one unit and EC was reduced by more than 50%; most of these differences were statistically significant ($p<0.05$) (Table 5).

Changes in soil physical properties after HPMA addition

Addition of HPMA also modified the physical properties of the saline-alkali soil (Figs. 2 and 3). As shown in Fig. 2a, HPMA greatly increased the capillary rise of soil water (about 30 cm). While this is less than in the non-saline loam control (35 cm), it is about 7.5 times more than for saline-alkali soil (4 cm). Surface applied water infiltration rates were constant with time for all the treatments (Fig. 2b), and HPMA significantly increased the rate over that in

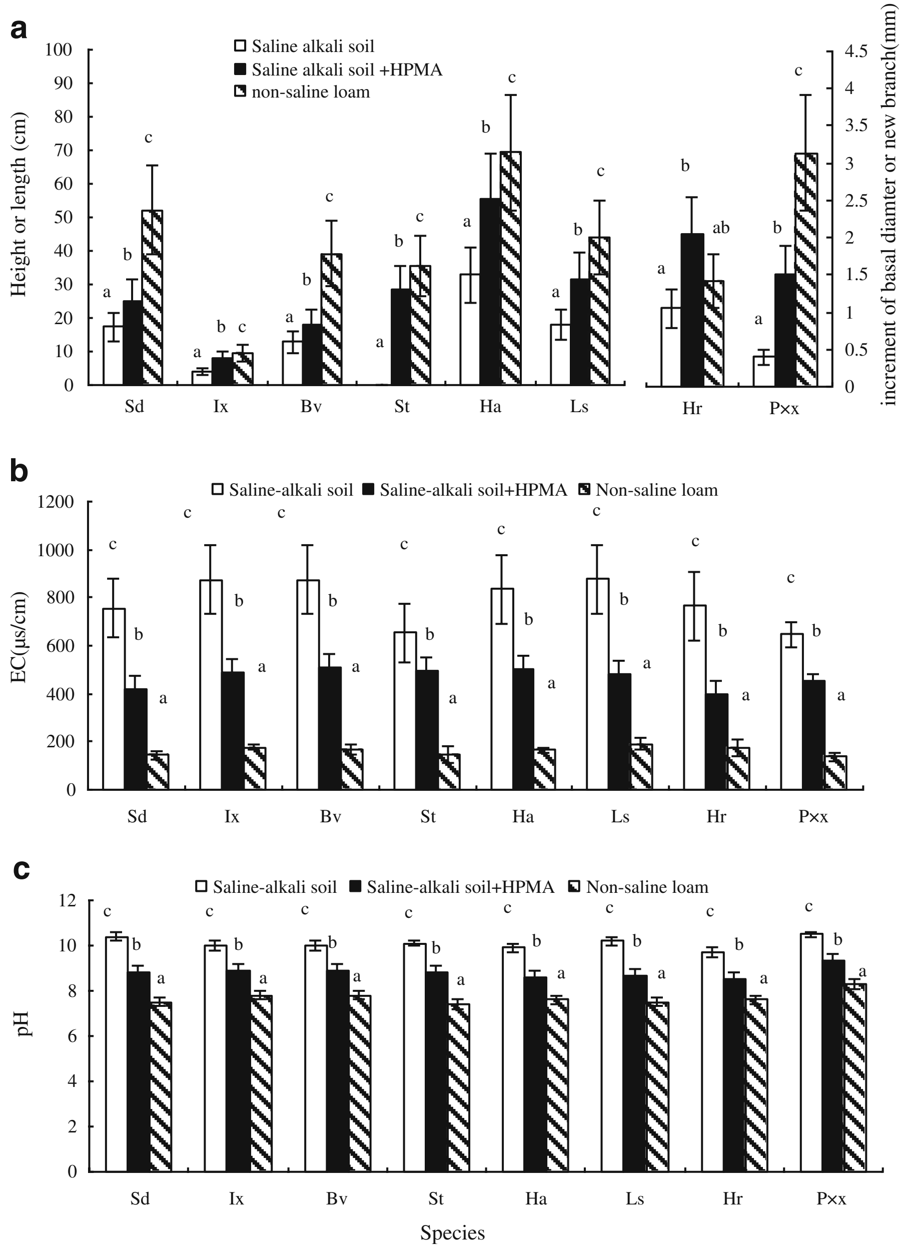


Fig. 1 Effect of HPMA addition on the growth of eight species, soil pH and EC comparing with controls of untreated saline-alkali soil and non-saline loam in the laboratory cultivation experiment. Different letters indicate a significant difference ($p<0.05$). Vertical bars on each column are the standard deviation. The x axis labels are Sd (*Sorghum dochna*), Ix (*Iva xanthifolia*), Bv (*Beta vulgaris*), St (*Solanum tuberosum*), Ha (*Helianthus annuus*), Ls (*Linus stelleroides*), Hr (*Hippophae rhamnoides*), P × x (*Populus* × *xiaohei*), respectively. **a** Growth differences; left y axis is for direct-germinated species, i.e. height of Sd, Ix, Bv, Ha, Ls or shoot length for St in centimeter; right y axis is for transplanted species, i.e., increment of basal diameter for P × x or new branch diameter increment for Hr; **b** soil EC for all the species; **c** soil pH values for all the species

untreated saline-alkali soil. The mean infiltration rate for the treated saline-alkali soil was 0.20 cm min^{-1}, which was five times faster than in the untreated saline-alkali soil control (0.04 cm min^{-1}) although still much lower than for the non-saline loam control (1.89 cm min^{-1}; Fig. 2c). The bulk density of the saline-alkali soil (Fig. 2d) was 1.25 g cm^{-3}, falling to 1.20 g cm^{-3} after the addition of HPMA; again, these values are still significantly higher than those for the non-saline loam control (Fig. 2d). Finally, HPMA addition increased the porosity of the saline-alkali soil to the level of the non-saline loam control (Fig. 2e).

That these changes in porosity and infiltration reflected physical changes in the soil structure associated with HPMA addition was verified by SEM and Atomic Force Microscopy (AFM) imaging. Addition of HPMA to both the saline-alkali soil and to the supernatant of a soil extract solution resulted in flocculent precipitation and clarification of the solution (Fig. 3a, b). SEM and AFM imaging was used to

Table 5 Effect of HPMA on soil pH, EC and the height growth rates of six species in the field. Different letters for the same parameter and species indicate a significant difference ($p<0.05$). Data in parenthesis are the standard deviation ($n=3$)

Species	pH		EC(μs cm^{-1})		Height growth rate (mm day^{-1})	
	Saline-alkali soil control	With HPMA	Saline-alkali soil control	With HPMA	Saline-alkali soil control	With HPMA
Chinese cabbage (*Brassica oleracea* L.*"Dongnong 906"*)	9.8(0.4)a	8.5(0.7)b	439.1(146.9)a	256.5(44.7)b	–	$t<35$ day; 0.50(0.11) $t>35$ day; 0.30(0.12)
Catharanthus roseus	9.9(0.4)a	8.3(0.5)b	745.8(73.9)a	366.0(59.3)b	1.039(0.112) a	1.417(0.181)b
Ambrosia trifida	9.8(0.4)a	8.1(0.7)b	935.7(117.7)a	468.3(132.3)b	3.601(0.650) a	5.306(0.95)b
Spinach(*Spinacia oleracea* L.)	9.9(0.4)a	8.2(0.6)b	862.7(161.5)a	307.6(117.7)b	–	0.90(0.25)
Radish (*Raphanus sativus* L.)	9.8(0.4)a	8.3(0.8)b	862.7(293.0)a	205.4(103.1)b	–	0.74(0.15)
Poplar (*Populus* × *xiaohei* T. S. Hwang et Liang)	9.8(0.2)a	8.7(0.85)a	1958.2(541.3)a	336.8(132.3)b	3.037(0.992) a	6.743(1.435)b

– Seeds did not germinate in these treatments

visualize the structure of the resulting aggregates. SEM imaging of the resulting aggregates showed unevenly-sized particles with sharp edges and corners in the untreated soil (Fig. 3c). In contrast, the addition of HPMA resulted in elimination of nearly all the edges and corners and an overall a much smoother picture (Fig. 3d). Three dimensional differences, as visualized using AFM, revealed that in untreated soil, soil particles were tightly packed together, generally separated by less than 1 nm; the addition of HPMA led to separation of the compact soil into a loosely packed form with large spaces between particles (Fig. 3e, f). In these images, untreated soil particles ranged from 100 nm to 270 nm in height, while HPMA addition reduced the sizes to between about 10 nm to 100 nm.

Discussion

China has a large area of inland saline-alkali land, approximately 8.1 MHa, equivalent to 40% of the total cultivated land in the country (Abrol et al. 1988; Wang 1993). The principal features of these lands are high salt content and high pH, and poor soil structure with low water infiltration and poor drainage. These conditions effectively prevent the exploitation of such land for agriculture. From the standpoint of food security, soil and land rehabilitation in these areas is critical (Wang et al. 2009a, b). A similar statement can be made about saline-alkali lands throughout the world.

Over the years and in different areas, a wide variety of natural and synthetic agents have been used as soil conditioners in these efforts. In this study, we compared 17 agents in seed germination studies, finding that HPMA addition treatment was the most effective soil conditioner, leading to 2.5 times greater germination than gypsum addition, and even greater differences compared to other agents effective in allowing germination, i.e PAA, MA-AA and charcoal. HPMA was particularly effective in reducing soil pH (Table 4). The effect on pH and EC of the other agents tested was much lower than that of HPMA, and this was also visible in the seed germination rate. However, with respect to seedling growth (seedling biomass, seedling length and root length), all 11 effective agents effected a large improvement over untreated controls. In this case, HPMA gave no marked improvement over the others (Table 4). Mixed addition of some agents, such as PAM and gypsum, had a positive effect relative to their separate additions (Tang et al. 2006). However, the joint addition of HPMA and gypsum did not give better seed germination than the HPMA addition alone (Table 4), indicating that there would be nothing to gain by combining gypsum and HPMA applications.

To determine the effect of HPMA addition on the growth of different plants, various plants were cultivated in the laboratory and the field, and biomass accumulation over the full growth period was used to assess soil improvement (c.f. Levy and Ben-Hur 1998; Zia et al. 2007). Relative to untreated saline-alkali land and non-saline loam controls, addition of

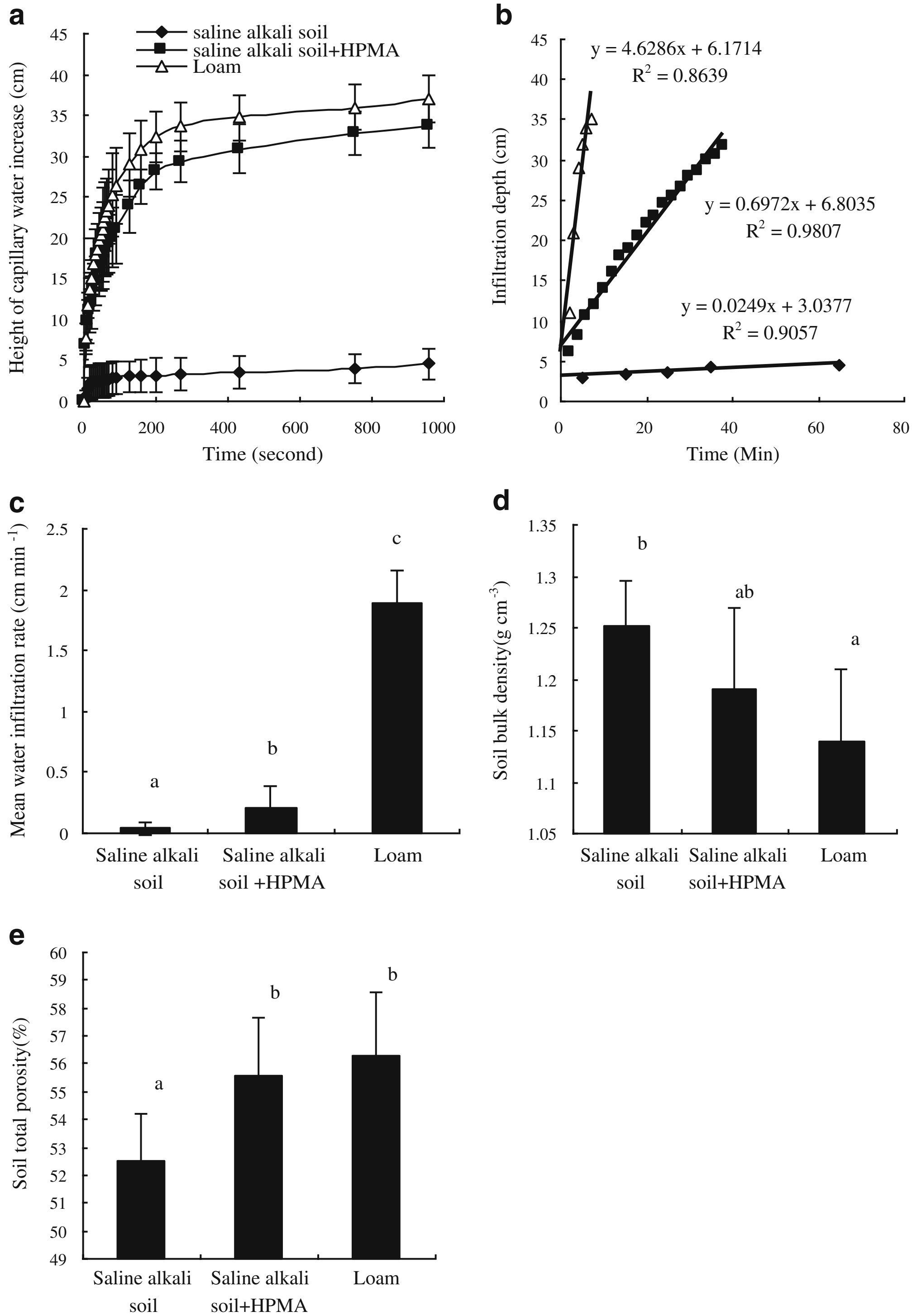
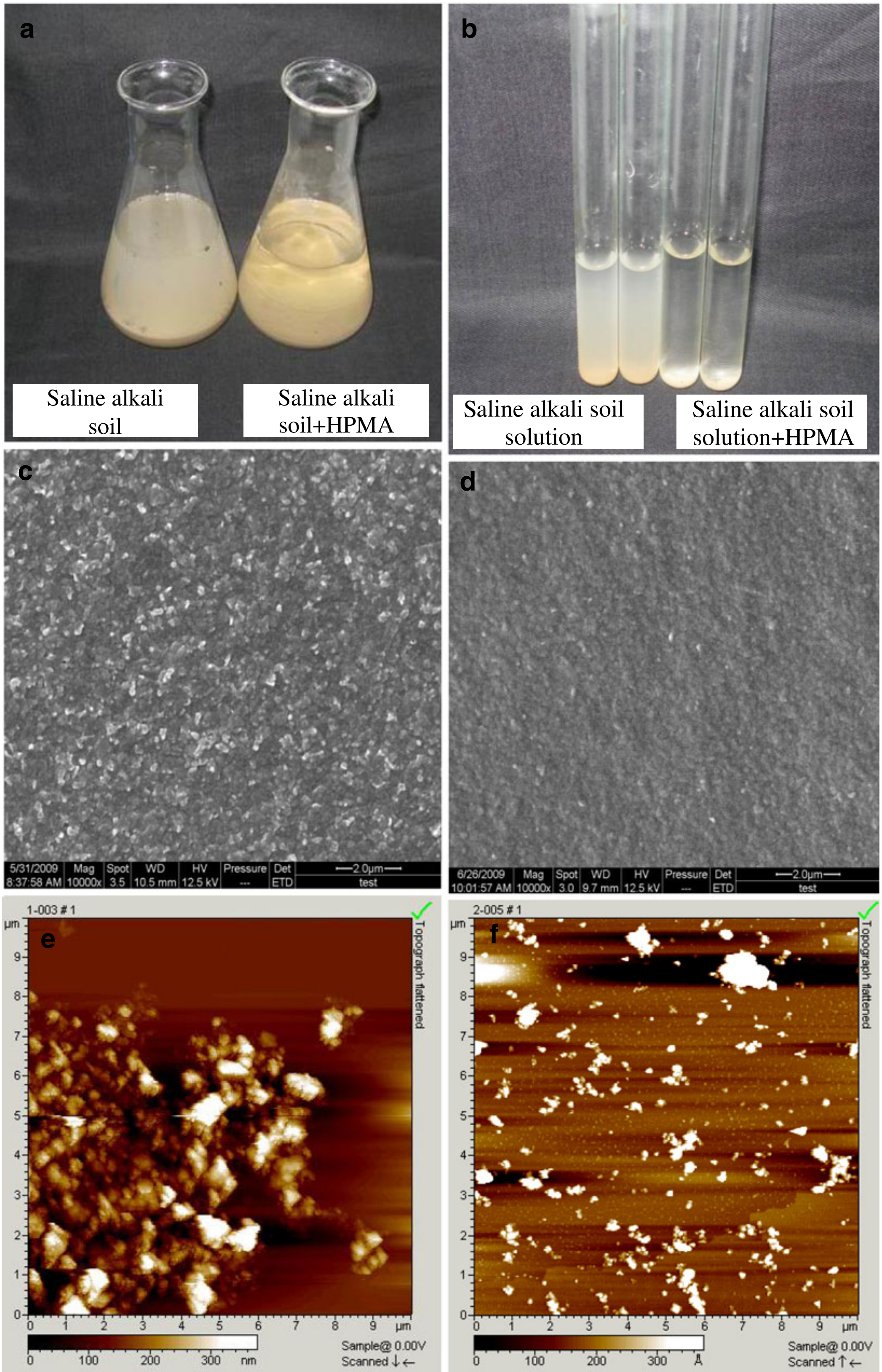


Fig. 2 Soil physical changes after HPMA addition and comparison with the controls of untreated saline-alkali soil and non-saline loam during the laboratory cultivation experiment. Different letters indicate a significant difference ($p<0.05$). Vertical bars indicate standard deviations. **a** Height of capillary water increase; **b** water infiltration depth; **c** Mean infiltration rate of water; **d** Soil bulk density; **e** Soil total porosity


a
Saline alkali soil
Saline alkali soil+HPMA
b
Saline alkali soil solution
Saline alkali soil solution+HPMA
c
5/31/2009 8:37:58 AM | Mag 10000x | Spot 3.5 | WD 10.5 mm | HV 12.5 kV | Pressure --- | Det ETD | 2.0μm test
d
6/26/2009 10:01:57 AM | Mag 10000x | Spot 3.0 | WD 9.7 mm | HV 12.5 kV | Pressure --- | Det ETD | 2.0μm test
1-003 # 1
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◀ **Fig. 3** Comparisons of HPMA treated and non-treated saline-alkali soil samples. **a** visual difference of solutions made by HPMA-treated and non-treated saline alkali soil; b visual difference of saline-alkali soil supernatant with and without HPMA addition; **c** SEM image for saline-alkali soil sample without HPMA addition; **d** SEM image for saline-alkali soil sample with HPMA addition; **e** AFM image for saline-alkali soil sample without HPMA addition; **f** AFM image for saline-alkali soil sample with HPMA addition. AFM images are shown at the original 512×512 resolution

HPMA stimulated the growth of all eight species in laboratory tests (Fig. 1). Addition of HPMA in the field also greatly increased the growth rate of the six species tested (Table 5). Furthermore, the soil pH and EC in both laboratory and field cultivation decreased markedly with HPMA treatment (Fig. 1; Table 5). These results all suggest that, in the seed germination tests and in plant cultivation in laboratory and field, HPMA can be an effective agent for saline-alkali land reclamation.

Application of effective chemical agents is a practical way to improve soil quality since it is highly effective and involves only small dosage in field practice (Wallace and Terry 1998; Long and Zheng 2000). In the seed germination test, the amount of HPMA was 1.8% (42.8 L m^{-3}) on a dry mass basis, whereas in the laboratory and in field cultivation of different plants, the dosage was 1.05% (25 L m^{-3}); much lower than in the seed germination test. Similarly, the other effective agents (see Table 2) were in the same range as HPMA. These dosages of HPMA and other effective agents were similar to those reported previously. For example, Nelson (1998) reported that concentrations of 20 g of VAMA, HPAN per kg of dry soil (2.0%) significantly improved aggregate stability, especially when dry conditioner powder or granular conditioner was thoroughly mixed with nearly dry soil; successful applications and favorable yield increases were reported in several alkaline and saline soils (Nelson 1998).

Two types of chemical agent frequently used at present are gypsum-related agents (Li 2006) and polymer-related agents (Levy and Ben-Hur 1998; Tang et al. 2006) and reviews on their functional mechanism for improving problem soils will facilitate the understanding of our results. The mechanism for the gypsum agents is Ca^{2+} release in soil solution, followed by exchange between Na^{+} at the surface of soil particles and Ca^{2+} in soil solution (Li 2006). Together, these changes improve soil physical and chemical properties that promote plant growth. The benefits of polymer-related agents, on the other hand, generally derive more from their ability to enhance soil physical properties such as soil aggregates, with less significant effects on cation exchange properties (Bouranis 1998). Other studies also emphasized this point. For example, Levy and Ben-Hur (1998) found that PAMs could reduce soil loss at different ESP levels via the formation of more stable soil aggregates and improvement of soil water-holding capacity (see also Nelson 1998). Similarly, Tang et al. (2006) reported that spreading of dry granular PAM mixed with phosphogypsum could dramatically increase infiltration rate and decrease in runoff rate.

The link between changes in the soil properties and the improved germination and growth of plants remains, however, unclear (He et al. 2008; Wang et al. 2009a, b). We sought, therefore, to determine whether the soil improvements upon adding HPMA were related to changes in soil physical properties as well as to replacement of Ca^{2+} and Na^{+} on soil particles. As shown in Fig. 2, several physical characteristics of the soil were significantly improved by the addition of HPMA: the capillary water rise and infiltration rate increased about tenfold, soil bulk density decreased, and soil total porosity increased.

Together these indicate that a much better physical structure has been generated in the reclaimed saline-alkali soils. We hypothesized that these changes might reflect alterations in the sizes of soil particles and their internal structure (c.f. Li 2006) as it has been previously reported that saline-alkali clays in north-eastern China have many smaller particles which tend to be more tightly packed with very small pore spaces (HLJTR 1993). The changes would be visible as, with HPMA addition, loose and flocculent soil aggregates replace the overlapped and compacted soil particles in saline-alkali soil. Accelerating the formation of larger soil aggregates is one way to improve soil physical properties (Bouranis 1998). The microscopic and digital images in this study clearly showed the dramatic changes after HPMA addition (Fig. 3) as HPMA addition resulted in loose flocculent soil precipitations from the turbid and non-transparent muddy solution.

Moreover, the internal structure of these soil aggregates was much smoother than those in the untreated soils. This was visualized using AFM which, as discussed by Gerin and Dufrene (2003), produces a three dimensional view of the materials surface, reflecting even Van der Waals' forces between particles. In this study, the structure of the flocculent precipitation after HPMA addition reflected the looser soil structure characterized by smaller soil particle size and larger distance between particles comparing with saline-alkali control.

From the chemical standpoint, soil physical changes might be due to the replacement of the abundant Na^+ by Ca^{2+} at the surface of soil colloids: high soil Na^+ is associated with compact soils of low porosity, while Ca^{2+} occupation of exchange sites has the opposite effect (Li 2006). The results of the analyses in Table 6 support this: addition of HPMA to a solution of saline-alkali soil yielded much larger increases in Ca (>6.1 times) than Na concentration (<2.2-fold increase). Changes in the soil colloidal fraction (the sediments of the saline-alkali soil solution) were the opposite: the change in Ca was small (decrease by 19% of the control), while the change in Na was larger, at 29% (Table 6). Simulation tests using single and mixed component solutions showed that addition of HPMA increased the Ca concentration by a factor of 23 in the $CaCO_3$ solution ($p<0.05$), with no significant effects in solutions without Ca (data not shown here). Such promotion in the dissolving of the insoluble $CaCO_3$ abundant in the saline-alkali lands (HLJTR 1993) may favor the exchange between overloaded Na^+ in soil colloids and Ca^{2+} in soil solution. A recent study by Liu et al. (2010) supports this finding. Their X-ray diffraction (XRD) spectra showed the peak for the (104) planes of calcite ($CaCO_3$ in chemical composition) disappeared after HPMA treatment. A further X-ray photoelectron spectrum (XPS) showed that Ca nearly disappears with HPMA treatment, which indicates that the insoluble $CaCO_3$ within soil particles had been dissolved by HPMA addition. Our finding manifests that exchanges between Ca^{2+} and Na^+ after the addition of HPMA to saline-alkali soil is probably how this agent acts to improve soil conditions.

Table 6 Changes in Ca, Na of saline-alkali soil solution and its soil sediments with and without HPMA addition. Different letters before and after HPMA addition indicate a significant difference ($p<0.05$). Data in parenthesis are the standard deviation ($n=3$)

Treatment	Ca Content	Na content
Saline-alkali soil solution		
+water(control)	41.50(4.10)a	0.47(0.03)a
+water + HPMA	256.50(21.5)b	1.03(0.12)b
HPMA addition/ control	6.18	2.19
Saline-alkali soil sediments after filtration[a]		
+water(control)	3.05(0.25)a	20.75(2.12)a
+water + HPMA	2.47(0.21)b	14.75(1.21)b
HPMA addition/ control	0.81	0.71

[a] Unit for Ca is $\mu g\ g^{-1}$ dry soil and for Na is $mg\ g^{-1}$ dry soil. The Ca and Na unit for all others are $mg\ L^{-1}$ and $g\ L^{-1}$, respectively

Saline-alkali land in northeastern China is overabundant in $NaHCO_3$ and Na_2CO_3 (>90% of total salt content), and the soil has an ESP of nearly 40% (Table 1). Moreover, the Ca^{2+} concentration in the saline-alkali soil solution is only 2 to 3 mmol kg^{-1} soil. In contrast, values of 80 mmol kg^{-1}, 150 mmol kg^{-1} and 250 mmol kg^{-1} are observed in brown coniferous forest soil, dark forest soil and black soil in the same region, respectively (Table 1 and HLJTR 1993). However, the saline-alkali soil is abundant in insoluble $CaCO_3$, at over 13% (Table 1). Mineral content analysis of soil samples following ignition has also shown a CaO content of up to 8%, whereas the CaO content in brown coniferous forest soil, dark brown forest soil and black soil in the same region of China were only 0.8%, 1.7% and 1.0%, respectively (HLJTR 1993). Highly insoluble $CaCO_3$, with less Ca^{2+} in soil solution contributes significantly to the ability of HPMA to improve saline-alkali land. As suggested by Table 6, addition of HPMA to soil abundant in insoluble $CaCO_3$ may enhance chemical cation exchange reactions between excess Na^+ and Ca^{2+} on soil particles, leading to the change of sodium-hydrophilic soil colloids into calcium-hydrophobic soil colloids. Physically, the dense and compact saline-alkali soil is transformed into a loose and poriferous soil (Figs. 2 and 3), which could benefit the growth of a diverse variety of plants (Nelson 1998; Long and Zheng 2000).

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