Effects of green manure cultivation for aboveground carbon store and returning to the field to ameliorate soil quality in saline alkali soil

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Abstract

The utilization of saline-alkali land together with the consideration of the productive value (improving soil productivity) and ecological value (increasing carbon store ability) has rarely been reported. We conducted a field experiment to investigate the impact of green manure cultivation for aboveground carbon (C) store and then returning this to field to improve soil quality in saline alkali soil. The biomass in *Lolium multiflorum* cultivation treatment was significantly ($P < 0.05$) higher than that of *Medicago sativa* and *Brassica campestris* cultivation. A similar tendency was observed in aboveground C store. Green manure cultivation resulted in largely different physicochemical properties at time of harvest. Returning the green manure to the field could significantly ($P < 0.001$) improve soil fertility. Moreover, the soil fertility index of *Lolium multiflorum* treatment was significantly ($P < 0.05$) enhanced by 55.56% and 33.33%, as compared with *Medicago sativa* and *Brassica campestris* treatments. Based on PLS-PM analysis, both fast-changing soil properties and biomass exhibited the greatest positive impacts (0.72 of the total effects) on soil fertility improvement after aboveground returning to the field. Our research provides evidence that *Lolium multiflorum* is the best potential variety to improve saline alkali soil fertility. Additionally, green manure cultivation in saline-alkali soil is an important way to store carbon in plants, then returning to the field is a feasible approach to improve saline alkali soil quality, which is beneficial for the green and sustainable development of saline-alkali agriculture.

INTRODUCTION

Saline-alkali soil is widely distributed, and is generally characterized by high salt content, poor structure and nutrients, suppresses plant growth, even causes plant death and seriously threatens agricultural production[1,2]. Saline-alkali stress is the most significant factor influencing the sustainable development of agriculture, which urgently needs improvement and utilization[3,4]. Extensive studies have been carried out on salinized soil from different perspectives and progress has been made[5,6]. In general, the improvements of saline-alkali land mainly include physical, chemical and biological measures[7–9]. The physical improvements, which are generally based on irrigation or soil change, require a large amount of capital investment, thus are not suitable for large-scale promotion. Chemical improvement measures[10], usually using gypsum, zeolite, sulfuric acid, citric acid and other chemicals to offset the salt in soil, can achieve enhancement effects in the short term. However, the inorganic amendments are complex and costly, and can also cause secondary pollution to the water and soil environments. Biological fertility improvement measures are mainly adopted to reduce soil salt content by planting highly salt-tolerant plants, which are characterized by strong operability, sustainable development and broad application prospects[11].

The cultivation of salt-tolerant green manure, with both productive and ecological value, is considered to be an effective measure for utilization and sustainable development of saline-alkali land[12]. For productive value, green manure cultivation could largely improve the buffering of alkaline soil, prevent dramatic changes in soil pH value due to excessive alkaline substances, and reduce the accumulation of soluble salt in the soil surface. Besides, the huge plant roots can effectively improve the soil structure, enhance the ability to retain water and fertilizer and improve yields[13]. A large number of studies have demonstrated the advantages of planting forage legumes on improving soil properties[14–16]. For ecological value, the introducing of salt-tolerant plants is a good approach to develop the carbon (C) sequestration potential of saline-alkali land, which can rapidly increase the vegetation cover on the surface, improve greenhouse gas absorption and store up aboveground C, contributing to modification of the global C cycle[17].

In recent decades, more attention has been paid to the cultivation of saline-tolerant forage species. Alfalfa, ryegrass, forage rape, sweet sorghum and sesbania are the potential forages that grow in saline soil[18,19]. *Brassica campestris* can accumulate more soluble sugars, amino acids and other similar compounds to improve the concentration of cell fluid, thus normally absorbing water and nutrients from the saline soil solution with higher concentration and avoiding salt-alkali damage[2]. Previous studies have demonstrated that *Medicago sativa*, an excellent perennial leguminous forage, has strong adaptability and can withstand a certain degree of saline-alkali stress[20]. Italian ryegrass has a large and developed root system, along with strong reproduction, wide distribution, rich

germplasm resources, and good stress resistance[21]. Feng et al.[22] investigated the salt tolerance ability of two Italian ryegrass cultivars, and demonstrated that high salt tolerance was partly due to the prevention of plants from ionic homeostasis disruption. Therefore, it is of great significance to improve soil quality and agricultural productivity by using salt-tolerant green manure in saline-alkali land.

It is well known that utilization of green manure, to a large extent, is helpful for reducing the dependence on mineral fertilizers[23,24]. The green manure returned to the field contributes to the generation of a good growth environment, with increase of soil organic matter and soil nutrient content, as well as improvement of soil aggregate structure, soil microbial community, and micro-ecological environment[25,26]. Accumulated evidence has demonstrated that green manure application could increase soil nutrient cycling and utilization efficiency, depending on the positive effects of soil microbes in the decomposition process[27,28]. Mwafulirwa et al.[29] reported that ryegrass shoot residue addition resulted in higher residue C mineralization rates, accelerated soil microbial activity, and increased soil organic matter priming, as compared with that of root residues, particularly in the early days. Understanding the impacts of different green manure returning to the field on saline alkali soil quality improvement is of benefit to the sustainability of agricultural production.

Soil fertility quality is an essential component of soil quality, which directly affects plant growth, agricultural production structure, distribution and benefits[30]. Appropriate evaluation methods and appropriate soil fertility indicators should be the most important considerations, which have a significant impact on soil fertility quality outcomes[31]. The calculation of soil fertility quality index is the core issue of soil quality evaluation, which is a widely employed way to evaluate the relationship between certain soil factors and soil productivity using fuzzy mathematical methods[32,33]. In this study, we employed different green manure species (*Medicago sativa*, *Lolium multiflorum* and *Brassica campestris*) with eight varieties, using a field experiment, aiming to measure their effects of cultivation for aboveground carbon store and returning to the field to improve saline alkali soil quality.

MATERIALS AND METHODS

Green manure materials

We employed four *Medicago sativa* varieties (Aurora, Sanditi, Eureka+, Sardi), *Lolium multiflorum*, and three *Brassica campestris* varieties (Huayouza 82, Huayouza 158, Huayouza 62) in our experiment. The *Medicago sativa* and *Lolium multiflorum* seeds were provided by Zhengzhou kaiyuan Grass Industry Technology Co., Ltd (China), and *Brassica campestris* seeds were provided by Zhengzhou Huafeng Grass Industry Technology Co., Ltd (China), with all germination rate > 85%.

Field site description

Our field experiment was carried out in Tiaozini, Dongtai, Jiangsu Province, China (32°51' N, 120°56' E). This region is a transition zone between subtropical and warm temperate zone with distinct seasons. The average annual temperature in this area is 15 °C, with the coldest month being January (mean monthly temperature 0.8 °C). July is the hottest month with an average monthly temperature of 27 °C. The average annual rainfall is 1,061 mm. The annual rainfall from June to September accounts for 63% of the whole year. The soil properties were as follows: pH 8.20, salt content 1.83 ‰, soil organic matter 7.92 g·kg^{-1}, total N 0.48 g·kg^{-1}, total P 0.65 g·kg^{-1}, total K 18.76 g·kg^{-1}, soil available N 45.92 mg·kg^{-1}, available P 18.31 mg·kg^{-1}, available K 187.07 mg·kg^{-1}, Ca^{2+} content 41.84 g·kg^{-1}, Mg^{2+} content 13.64 g·kg^{-1}.

Experimental design

The field experiment, with a random design, included eight green manure varieties. We defined each variety as a treatment and a non-cultivation as control (CK). Each treatment had three replicates (plots), and the size of each plot was 2 m × 2 m. The cultivation was performed in late October, 2021. The sowing density of *Medicago sativa*, *Lolium multiflorum* and *Brassica campestris* was 2.5 g·m^{-2}, 2.5 g·m^{-2} and 1 g·m^{-2}, respectively. The sowing depth was 3 cm and row spacing was 25 cm. No fertilization was applied during the growth of green manure. Irrigation and weeding were the same as in routine management.

Collection of aboveground and soil samples

The green manure biomass was recorded at harvest (May 5, 2022). We took random subplots (0.5 × 0.5 m) within each plot and destructively harvested, and the shoots were cut at the soil surface. At the same time, we collected soil samples. Five cores (5 cm diameter and 0−15 cm depth), on the rows, were randomly sampled and sufficiently mixed to yield one representative sample. After sampling, the rest of the green manure in each plot were crushed and returned to the field with the returning depth of 20 cm. Thirty days later, soil samples were collected as previously. All collected soil samples (54 samples in total: 9 treatments × 3 replications × 2) were sieved (2.0 mm mesh) and homogenized for soil physicochemical property analysis.

Determination of aboveground samples

The collected green manure aboveground samples were oven dried at 65 °C for 72 h to a constant weight before weighing. The shoot dry weights were expressed as total aboveground biomass per m². Then the dry shoots were ground through 20 mesh in a Wiley mill. The prepared aboveground samples were measured by an Elementar Analyzer (Vario EL III, Germany) for total carbon (C).

Determination of soil physicochemical properties and soil fertility evaluation

Soil pH was determined with soil-water slurry (1:5, w/v) by a PB-10 pH meter (Sartorius, Germany). Soil electrical conductivity (EC) was measured by a conductivity meter (B-173; HORIBA, Kyoto, Japan). Soil organic C (SOC) was measured by an Elementar Analyzer (Vario EL III, Germany). The total N was determined using a Kjeltec Analyser (FOSS Tecator, Hoganas, Sweden). The determination of soil available nitrogen (N) was measured according to Shi[34]. The soil fertility evaluation was calculated according to previously described methods[35,36].

Statistical analyses

The data in our study were log-transformed when necessary to meet the criteria for a normal distribution. We employed SPSS 22.0 (IBM, Armonk, NY, USA) software for statistical analysis of all parameters. The data from each treatment were analyzed using one-way analysis of variance (ANOVA), and Duncan's multiple range tests ($P < 0.05$) were performed for

multiple comparisons. The Mann-Whitney U test method was used to test soil fertility index differences between non-cultivation and cultivation groups after returning to the field.

For soil fertility evaluation, we employed five soil fertility evaluation parameters, including pH, EC, SOM, TN and AN. Then we chose appropriate function curves and turning points to determine values of each soil fertility parameter, according to our data characteristics. The 'optimum' curve equation is employed for pH and EC, while the 'more is better' curve equation is used for SOM, TN and AN[33,35,36]. The equation for the scoring curve as follows:

(a) The 'optimum' curve equation:

$$f(x) = \begin{cases} 0.1 & x \leqslant L, x \geqslant U \\ 0.1 + 0.9(x-L)/(O_1-L) & L < x < O_1 \\ 1.0 & O_1 \leqslant x \leqslant O_2 \\ 1.0 - 0.9(x-O_2)/(U-O_2) & O_2 < x < U \end{cases}$$

(b) The 'more is better' curve equation:

$$f(x) = \begin{cases} 1.0 & x \geqslant U \\ 0.1 + 0.9(x-L)/(U-L) & L < x < U \\ 0.1 & x \leqslant L \end{cases}$$

where x is the monitoring value of the parameter; $f(x)$ is the score of the parameters ranging between 0.1 and 1.0; U and L are the upper and the lower threshold values of the parameters, respectively. O_1 and O_2 are the best values of the variables.

We employed partial least squares path modeling (PLS-PM), based on 'plspm' (1000 bootstraps) package in R software (v.4.0.0), to determine the complex multivariable relationships among green manure varieties, edaphic variables, plant C content, biomass and soil fertility. Then we tested the model architectures from simple to complex (direct and indirect links, previous effects)[37]. Based on the determination coefficient (R^2) of the explained latent variables and goodness of fit (GoF), we selected the corresponding architecture.
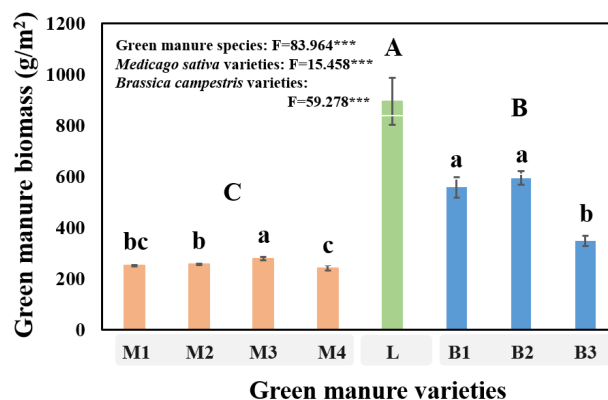
RESULTS

Green manure biomass of different varieties cultivation

There were significant ($P < 0.05$) differences among *Medicago sativa*, *Lolium multiflorum* and *Brassica campestris* cultivation treatments for biomass at harvest (Fig. 1). In total, the largest green manure biomass was observed in *Lolium multiflorum* cultivation treatment (895.11 g·m⁻²). *Lolium multiflorum* cultivation treatment increased the biomass by 2.48 and 0.79 times compared with *Medicago sativa* and *Brassica campestris* cultivation treatments, respectively. Additionally, we found significant ($P < 0.05$) differences among the varieties of each green manure. For *Medicago sativa*, the biomass of Eureka⁺ was significantly ($P < 0.05$) increased by 10.38%, 8.29% and 14.88%, compared with that of Aurora, Sanditi, Sardi, respectively. For the biomass of *Brassica campestris*, there was no significant difference between Huayouza 82 and Huayouza 158, while they were both significantly ($P < 0.05$) higher than that of Huayouza 62, with increasing by 60.66% and 71.29%, respectively.

Green manure carbon sequestration of different varieties cultivation

The carbon content was lowest in *Medicago sativa* cultivation treatment (401.58 g·kg⁻¹), however, no significant difference was observed among *Medicago sativa*, *Lolium multiflorum* and *Brassica campestris* cultivation treatments (Fig. 2a). For

Fig. 1 The green manure biomass of different varieties cultivation at harvest in saline alkali soil field experiment. 'M1, M2, M3 and M4' represent Aurora, Sanditi, Eureka⁺, Sardi, respectively, which all belong to *Medicago sativa*. 'L' refers to *Lolium multiflorum*. 'B1, B2 and B3' represent Huayouza 82, Huayouza 158, Huayouza 62, respectively, which all belong to *Brassica campestris*. One-way analysis of variance (ANOVA) was used to assess the differences among treatments. Bars represent the mean values of three replicates ± SD. Values that do not share the same lower case letter are significantly different ($P < 0.05$) among green manure varieties. Values that do not share the same uppercase letter are significantly different ($P < 0.05$) at different green manure species (*Medicago sativa*, *Lolium multiflorum*, *Brassica campestris*). *** refers to $P < 0.001$.

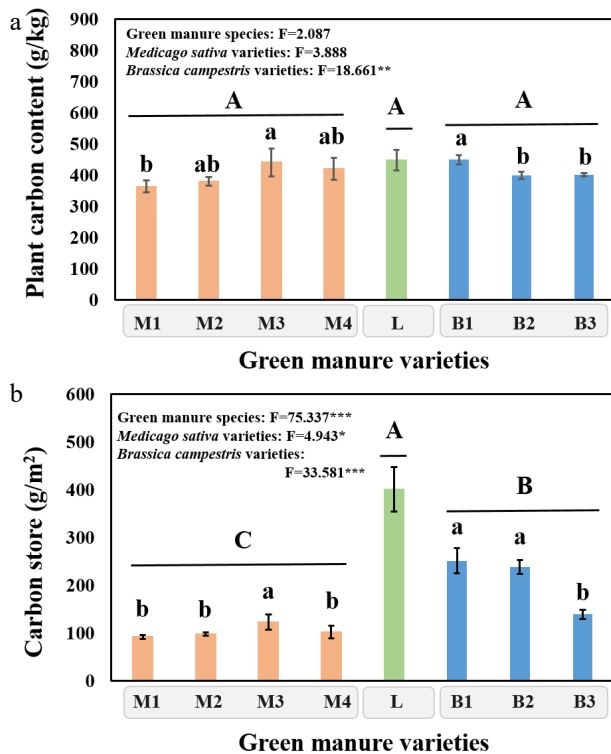*Medicago sativa*, the carbon content of Aurora (364.25 g kg⁻¹) was significantly ($P < 0.05$) lower than that of Eureka⁺. For *Brassica campestris*, the carbon content of Huayouza 82 was significantly ($P < 0.05$) higher than that of Huayouza 158 and Huayouza 62 by 12.55% and 12.09%, respectively. In addition, different green manure cultivation led to a significant ($P < 0.05$) difference in aboveground carbon store (Fig. 2b). The carbon store in *Lolium multiflorum* cultivation (400.58 g·m⁻²) was significantly ($P < 0.05$) higher than that of *Medicago sativa* and *Brassica campestris* cultivation treatments by 2.87 and 0.91 times, respectively. For *Medicago sativa*, the carbon store of Eureka⁺ was significantly ($P < 0.05$) higher by 33.88%, 25.71% and 20.36% than that of Aurora, Sanditi and Sardi, respectively. Additionally, for *Brassica campestris*, the carbon store of Huayouza 62 (139.41 g·m⁻²) was largely significantly ($P < 0.05$) lower than that of Huayouza 82 (251.31 g·m⁻²) and Huayouza 158 (237.78 g·m⁻²), respectively.

Soil physicochemical properties in different cultivation treatment at green manure harvest

The green manure cultivation generated significantly different physicochemical properties in saline alkali soil (Table 1, Supplemental Table S1). The cultivation of *Medicago sativa* (Aurora, Sanditi, Eureka⁺) and *Lolium multiflorum* resulted in a significant ($P < 0.05$) lower pH by 6.32%, 3.67%, 1.69% and 4.03% than that of CK. Moreover, compared with CK, the EC in *Medicago sativa* (except for Sanditi) and *Brassica campestris* were also significantly ($P < 0.05$) declined. For soil organic matter, only Eureka⁺ and Huayouza 62 were significantly ($P < 0.05$) higher than that of CK. Additionally, soil total N in Sardi and Huayouza 62 were significantly ($P < 0.05$) lower than that of CK. For available N, Aurora, Sanditi, Sardi and *Lolium multiflorum* were significantly ($P < 0.05$) higher by 33.08%, 27.53%, 29.70%, 38.49%, when compared with CK.

Variation of soil physicochemical properties in different treatment after green manure returning to field for 30 days

The soil properties were measured after green manure was returned to the saline alkali soil field for 30 d. In general, the soil

properties after green manure return (Table 2, Supplemental Table S2) were generally improved when compared with previous (Table 1). The returning of *Medicago sativa* (only Sardi), *Lolium multiflorum* and *Brassica campestris* (Huayouza 82, Huayouza 158, Huayouza 62) had significant ($P < 0.05$) reduction effects on soil pH, which reduced by 2.04%, 8.30%, 4.90%, 4.77% and 4.17%, respectively, when compared to no-returning (CK). Moreover, as compared with CK, only returning of Sanditi and Eureka$^+$ significantly ($P < 0.05$) decreased soil EC content. In addition, the green manure return led to significant ($P < 0.05$) improvement on soil organic matter. Similarly, significant ($P < 0.05$) promoting effects on soil total N were also observed, expect for Sanditi, as compared to CK. The soil available N in Sardi, *Lolium multiflorum*, and *Brassica campestris* (Huayouza 82) were significantly ($P < 0.05$) increased by 30.55%, 57.24%, 23.22% than that of CK.

Effects of different returning treatment to improve soil quality in saline alkali field experiment

The descriptions of detailed scoring function values and weights assigned to the selected soil fertility parameters are available in Table 3. The weights of pH, EC, soil organic matter, total N and available N were 0.25, 0.23. 0.07, 0.23 and 0.22, respectively. Based on these, the soil fertility index in each returning treatment was calculated. As shown in Fig. 3, we observed significant ($P < 0.001$) difference between the non-cultivation and cultivation group. In the cultivation group, returning *Lolium multiflorum* to the field had the best soil fertility enhancing effect (0.56), which significantly ($P < 0.05$) improved soil fertility by 55.56% and 33.33% compared with *Medicago sativa* and *Brassica campestris*. For *Medicago sativa*, the soil fertility index of Sardi was significantly ($P < 0.05$) higher than that of the other three varieties. However, there was no significant difference among *Brassica campestris* varieties.

Contributions of green manure varieties, soil physicochemical properties, plant C, green manure biomass on soil fertility improvement

PLS-PM analysis was employed to identify direct and indirect effects of different green manure cultivation and returning to the field on saline alkali soil fertility improvement (Fig. 4a). Green manure varieties significantly ($P < 0.05$) positively affected biomass (0.76 of the direct effects) and then positively ($P < 0.001$) affected soil fertility (0.72). Similarly, green manure varieties significantly ($P < 0.001$) positively affected slow-changing soil properties (0.78), then positively ($P < 0.05$)

Fig. 2 (a) Carbon content and (b) carbon store of different varieties cultivated at harvest in saline alkali soil field experiment. 'M1, M2, M3 and M4' represent Aurora, Sanditi, Eureka$^+$, Sardi, respectively, which all belong to *Medicago sativa*. 'L' refers to *Lolium multiflorum*. 'B1, B2 and B3' represent Huayouza 82, Huayouza 158, Huayouza 62, respectively, which all belong to *Brassica campestris*. One-way analysis of variance (ANOVA) was used to assess the differences among treatments. Bars represent the mean values of three replicates ± SD. Values that do not share the same lower case letter are significantly different ($P < 0.05$) among green manure varieties. Values that do not share the same uppercase letter are significantly different ($P < 0.05$) at different green manure species (*Medicago sativa*, *Lolium multiflorum*, *Brassica campestris*). * refers to $P < 0.05$; ** refers to $P < 0.01$; *** refers to $P < 0.001$.

Table 1. Soil properties measured under different treatment at green manure harvest in saline alkali soil field experiment.

Green manure cultivation	Treatment	pH	EC (us·cm^{-1})	Soil organic matter (g·kg^{-1})	Total N (g·kg^{-1})	Available N (mg·kg^{-1})
No-cultivation	CK	9.04 ± 0.16abc	496.33 ± 19.50b	5.27 ± 0.14bc	0.58 ± 0.05abc	38.76 ± 2.03bc
Medicago sativa	M1	8.50 ± 0.13f	429.00 ± 26.21cd	4.41 ± 0.78c	0.58 ± 0.01ab	51.58 ± 2.66a
	M2	8.72 ± 0.02e	470.33 ± 13.32bc	5.11 ± 0.42bc	0.54 ± 0.01cd	49.43 ± 4.35a
	M3	8.89 ± 0.03d	356.67 ± 6.43ef	5.63 ± 0.66a	0.54 ± 0.02bcd	41.89 ± 4.57b
	M4	8.93 ± 0.03cd	391.00 ± 4.36de	4.33 ± 0.24c	0.53 ± 0.02d	50.27 ± 1.61a
Lolium multiflorum	L	8.69 ± 0.04e	567.67 ± 52.88a	5.33 ± 0.46bc	0.60 ± 0.01a	53.68 ± 3.79a
Brassica campestris	B1	9.02 ± 0.04bc	363.00 ± 26.91ef	5.13 ± 0.11bc	0.51 ± 0.01de	37.53 ± 3.16bc
	B2	9.11 ± 0.02ab	330.67 ± 12.66f	5.07 ± 1.32bc	0.51 ± 0.02de	38.87 ± 2.33bc
	B3	9.17 ± 0.01a	328.00 ± 28.69f	6.00 ± 0.21a	0.49 ± 0.01e	34.97 ± 5.50c

CK: no cultivation. 'M1, M2, M3 and M4' represent Aurora, Sanditi, Eureka$^+$, Sardi, respectively, which all belong to *Medicago sativa*. 'L' refers to *Lolium multiflorum*. 'B1, B2 and B3' represent Huayouza 82, Huayouza 158, Huayouza 62, respectively, which all belong to *Brassica campestris*. Data are the mean values of three replicates. Numbers followed by '±' are the standard deviations (SDs). Within a column, values that do not share the same letter are significantly different ($P < 0.05$).

Table 2. Soil properties measured under different treatment after green manure return to saline alkali soil field for 30 d.

Green manure return	Treatment	pH	EC (us·cm⁻¹)	Soil organic matter (g·kg⁻¹)	Total N (g·kg⁻¹)	Available N (mg·kg⁻¹)
No-returning	CK	9.00 ± 0.14ab	591.00 ± 56.45bcd	5.84 ± 0.74b	0.52 ± 0.01c	39.80 ± 1.88d
Medicago sativa	M1	8.89 ± 0.07bc	536.33 ± 32.59d	7.92 ± 1.24a	0.60 ± 0.01b	41.99 ± 4.37cd
	M2	9.11 ± 0.03a	407.33 ± 43.25e	8.55 ± 0.80a	0.54 ± 0.05c	45.70 ± 4.68bcd
	M3	9.00 ± 0.02ab	438.33 ± 49.08e	8.36 ± 0.59a	0.60 ± 0.02b	47.24 ± 4.26bcd
	M4	8.82 ± 0.02c	549.67 ± 15.28cd	7.76 ± 0.55a	0.63 ± 0.02b	51.96 ± 1.87b
Lolium multiflorum	L	8.31 ± 0.12e	519.00 ± 14.53d	7.72 ± 0.88a	0.71 ± 0.02a	62.58 ± 5.98a
Brassica campestris	B1	8.58 ± 0.05d	621.67 ± 5.13bc	7.75 ± 0.44a	0.62 ± 0.02b	49.04 ± 8.57bc
	B2	8.59 ± 0.07d	701.33 ± 71.44a	7.30 ± 0.46a	0.61 ± 0.01b	42.43 ± 3.22cd
	B3	8.64 ± 0.01d	627.67 ± 35.53b	7.61 ± 0.28a	0.68 ± 0.03a	48.57 ± 3.73bcd

CK: no cultivation. 'M1, M2, M3 and M4' represent Aurora, Sanditi, Eureka⁺, Sardi, respectively, which all belong to *Medicago sativa*. 'L' refers to *Lolium multiflorum*. 'B1, B2 and B3' represent Huayouza 82, Huayouza 158, Huayouza 62, respectively, which all belong to *Brassica campestris*. Data are the mean values of three replicates. Numbers followed by '±' are the standard deviations (SDs). Within a column, values that do not share the same letter are significantly different ($P < 0.05$).

Table 3. Scoring function values and weights assigned to selected soil fertility parameters.

		pH (H₂O)	EC (us·cm⁻¹)	Soil organic matter (SOM, g·kg⁻¹)	Total nitrogen (TN, g·kg⁻¹)	Available nitrogen (AN, mg·kg⁻¹)
Scoring curve#		a	a	b	b	b
Turning point	U	9	1,500	15	1.2	120
	L	6.0	100	5	0.5	30
	O1	6.5	300			
	O2	8	400			
weight		0.25	0.23	0.07	0.23	0.22

'a' Refers to the 'optimum' curve equation; 'b' refers to the 'more is better' curve equation.
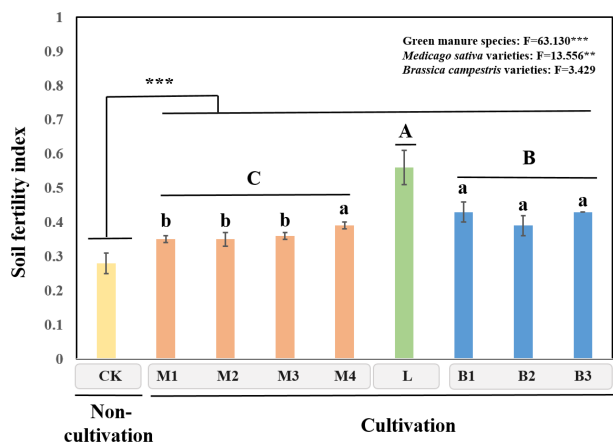
Fig. 3 Soil fertility index of different treatment that returning to the field in saline alkali soil experiment. One-way analysis of variance (ANOVA) was used to assess the differences among treatments. Bars represent the mean values of three replicates ± SD. Values that do not share the same lower case letter are significantly different ($P < 0.05$) among green manure varieties. Values that do not share the same uppercase letter are significantly different ($P < 0.05$) at different green manure species (*Medicago sativa*, *Lolium multiflorum*, *Brassica campestris*). ** refers to $P < 0.01$; *** refers to $P < 0.001$.

affected plant C (0.81), followed with affecting biomass (0.55), and finally affected soil fertility. Furthermore, we observed fast-changing soil properties could significantly ($P < 0.01$) and directly affect biomass (0.91), while slow-changing soil properties had no significant effect on biomass.

Overall, we found that all variates (green manure varieties, slow-changing soil properties, fast-changing soil properties, plant C and biomass) had positive impacts on soil fertility improvement. Among these, both fast-changing soil properties and biomass exhibited the greatest positive impacts (0.72 of the total effects). In addition, the total effects of fast-changing soil properties (0.552) was greater than slow-changing soil properties (0.55). Plant C also largely contribute to saline alkali soil fertility improvement (0.60). Green manure varieties had the lowest but also positive impact (0.07) (Fig. 4b).

DISCUSSION

Previous studies[38–41] have demonstrated that green manure, with positive effects on soil improvement, is a main and feasible approach for sustainable crop production. In the present study, we conducted green manure varieties cultivation experiments in saline-alkali soil. Here we observed that the biomass of different salt-tolerant green manure was significantly different, and there were also certain differences among different varieties of the same green manure, which indicated that both green manure species and cultivars contributed to biomass. Son[42] revealed that the biomass of green manure crop was the highest in ryegrass, which was consistent with our result. Monirifar el al.[43] assessed the effects of cultivar on alfalfa yield in saline conditions, and found that cultivar selections could largely influence the yield. Meza et al.[44] also demonstrated that total plant biomass, aboveground biomass, root biomass were all influenced by forage species. These all supported our results. Establishment of plants provides an important opportunity to store C in both plant biomass and the soil to mitigate climate change[45]. Thus, the plant carbon store was closely correlated with biomass and C content. It is a good way to improve carbon sequestration by increasing plant biomass, especially when there is no difference among plant C contents. Besides, biomass also significantly affected the deposition of photosynthetically-fixed C into the plant-soil system[16]. Although aboveground C store largely contribute to the whole carbon pool, it's better to evaluate the aboveground and belowground together. We should pay attention to carbon sequestration from a more holistic perspective in our further research.

Soluble salt content and pH value can reflect the degree of soil salinization, which are important evaluation indexes of saline-alkali soil[46]. Gelaye et al.[47] employed rhodes grass, alfalfa, sudangrass and blue panicgrass to cultivate them in saline field plots, and found they affected pH and mitigated soil

a

b
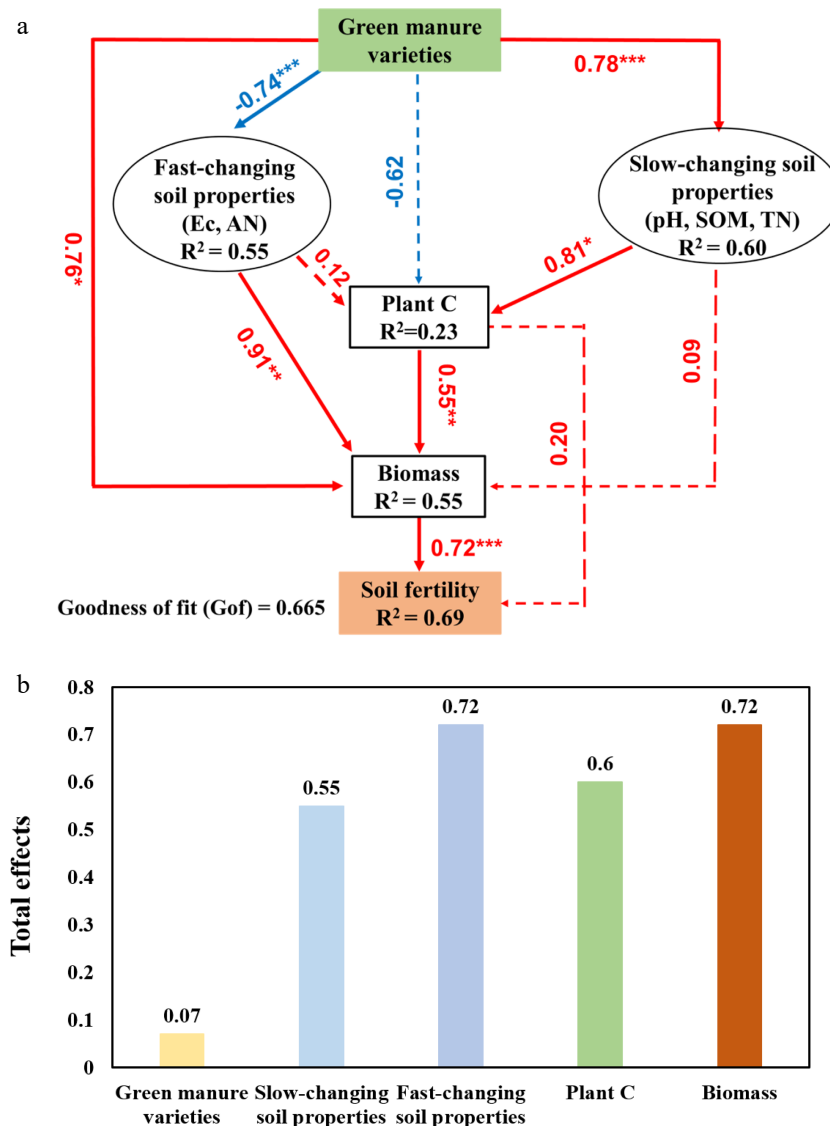
Fig. 4 Cascading relationships of soil fertility with green manure and soil physicochemical properties. (a) Partial least squares path modelling (PLS-PM) disentangling major pathways of the influences of green manure varieties, soil physicochemical properties, plant C, green manure biomass on soil fertility. Red and blue arrows indicate positive and negative flows of causality, respectively. Solid and dashed lines indicate significant (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$) and nonsignificant ($P > 0.05$) levels, respectively. Values on arrows indicate significant standardized path coefficients. R^2 indicates the variance of dependent variable explained by the model. (b) Total effects of soil fertility in the PLSPM models for green manure varieties, fast-changing soil properties, slow-changing soil properties, plant C, and green manure biomass.

salinity and soluble saline ion concentrations. Our study showed that the green manure cultivation reduced soil pH, among which, *Medicago sativa* and *Lolium multiflorum* had a better effect on reducing soil pH than *Brassica campestris*. Additionally, except *Lolium multiflorum*, salt-tolerant plant cultivation could effectively reduce soil EC. The planting of salt-tolerant green manure had an obvious inhibition effect on the surface soil salinity of soil, which might be due to the planting reducing the surface evaporation and keeping the salt in the subsoil or some salt was taken up and carried out of the soil by salt-tolerant grasses. This is consistent with the results obtained by Liu et al.[48] that planting salt-tolerant forage grass can reduce soil salinity and pH value. For soil nutrients, certain cultivation treatments increased soil organic matter (Eureka+, Huayouza 62) and available nitrogen contents (Aurora, Sanditi, Sardi, *Lolium multiflorum*), which was consistent with Jing et

al.[49] that the planting of *M. sativa*, *S. sudanense*, *S. bicolor*, and *P. frumentum* had significant enhancement on soil organic matter and available nutrient contents in inland saline soil. Astier et al.[50] also demonstrated that when vetch and oat were planted, soil organic matter and total N increased. The growth of legumes does not always increase the content of total nitrogen in the soil[25], which supported our result that the total soil nitrogen after *Medicago sativa* and *Brassica campestris* cultivation decreased to different degrees, as compared with non-cultivation, mainly due to the fact that green manure needs to absorb a large amount of a variety of nutrients from soil during its growth and development, and its consumption of nitrogen increases, resulting in a decrease in total nitrogen content.

After the green manure was returned to the saline alkali field, the pH of the majority treatments were decreased, which might

be related to the different dry matter composition of different green manure varieties and their different degradation and transformation rates in soil[51,52]. Cao et al.[53] also found significant reduction in pH was determined under field conditions of 4 years of alfalfa cultivation in salt-affected soils. The content of soil organic matter, total nitrogen and available nitrogen in cultivation treatments were increased to different degrees compared with the control, indicating that returning green manure to the field had an obvious effect on soil fertility improvement. For *Lolium multiflorum* and *Brassica campestris*, their biomass was large, thus the nutrients that carried and were released into the soil were also correspondingly large. For *Medicago sativa*, except the biomass, a large amount of nitrogen was returned to the soil based on the nitrogen fixation effect to increase the nitrogen content in the soil. Previous studies[46,54] demonstrated that a variety of soluble organic matter would be produced in the decomposition process, which could promote the efficient cycling of soil nutrients and regulate the soil nutrient balance. The comprehensive soil fertility quality index is a quantifiable index, which indicates a soils ability to perform specific ecological functions[55]. The difference of soil fertility index among different treatments indicates that returning green manure to the field is a good way to improve saline alkali soil quality. Also, the biomass amount of green manure species largely contributed to the soil fertility improvement during the process of returning to the field. Ma et al.[56] generated a comprehensive evaluation of green manure on soil properties based on a meta-analysis and showed their significant improvement effects on soil quality, which was consistent with our research.

According to our PLS-PM analysis, green manure varieties, slow-changing soil properties, fast-changing soil properties, plant C and biomass make a good explaination for soil fertility improvement after plants are returned to the field. We summarized two ways from the process of cultivation to returning to the field and found plant biomass was the core variate to improve soil fertility. Moreover, plant biomass had the greatest influence on soil fertility, suggesting that the increase of soil fertility mainly depended on the biomass of green fertilizer returned to the field. Thus, we concluded that *Lolium multiflorum* that has a large aboveground biomass was the best potential variety to improve saline alkali soil fertility. Except for plant biomass, fast-changing soil properties, mainly available N, also had an indirect, but large, influence on soil fertility. Feng et al.[57] found that high soil nutrient availability has a positive effects on the alfalfa biomass. Accumulated evidence suggests that fertilization and biomass are strongly correlated in agricultural ecosystems[58]. Zhang et al.[59] demonstrated that N fertilizer application directly promoted ryegrass yield. Therefore, we should pay more attention to improve aboveground biomass to ameliorate soil quality in saline alkali soil in the future.

CONCLUSIONS

We employed different green manure variates to measure the effects of cultivation for aboveground carbon sequestration and returning to the field to ameliorate soil quality in saline alkali soil, based on a field experiment. We determined that plant carbon store was positively correlated with aboveground biomass. Green manure varieties, slow-changing soil

properties, fast-changing soil properties, plant C and biomass all contributed to soil fertility improvement after aboveground returning to the field. The biomass production was a determining factor contributing to soil fertility, and variety with higher biomass production would more effectively improve soil quality in saline alkali soil. Our study can provide crucial theoretical support and a feasible way for green and sustainable development of saline-alkali agriculture.

Conflict of interest

The authors declare that they have no conflict of interest.

Dates

REFERENCES

1. Shao T, Zhao J, Zhu T, Chen M, Wu Y, et al. 2018. Relationship between rhizosphere soil properties and blossom-end rot of tomatoes in coastal saline-alkali land. *Applied Soil Ecology* 127:96−101
2. Li Z, An M, Hong D, Chang D, Wang K, et al. 2022. Transcriptomic and metabolomic analyses reveal the differential regulatory mechanisms of compound material on the responses of *Brassica campestris* to saline and alkaline stresses. *Frontiers in Plant Science* 13:820540
3. De Pascale S, Maggio A, Barbieri G. 2005. Soil salinization affects growth, yield and mineral composition of cauliflower and broccoli. *European Journal of Agronomy* 23:254−64
4. Lu X, Chen Q, Cui X, Abozeid A, Liu Y, et al. 2021. Comparative metabolomics of two saline-alkali tolerant plants *Suaeda glauca* and *Puccinellia tenuiflora* based on GC-MS platform. *Natural Product Research* 35:499−502
5. Bai Y, Zuo W, Shao H, Mei L, Tang B, et al. 2018. Eastern China coastal mudflats: Salt-soil amendment with sewage sludge. *Land Degradation Development* 29:3803−11
6. Sun J, Yang R, Zhu J, Pan Y, Yang M, et al. 2019. Can the increase of irrigation frequency improve the rate of water and salt migration in biochar-amended saline soil? *Journal of Soils and Sediments* 19:4021−30
7. El Hasini S, Halima OI, Azzouzi ME, Douaik A, Azim K, et al. 2022. Organic and inorganic remediation of soils affected by salinity in the Sebkha of Sed El Mesjoune - Marrakech (Morocco). *Soil Tillage Research* 193:153−60
8. Heng T, He X, Yang L, Xu X, Feng Y. 2022. Mechanism of Saline-Alkali land improvement using subsurface pipe and vertical well drainage measures and its response to agricultural soil ecosystem. *Environmental Pollution* 293:118583
9. Li Y, Li G. 2022. Mechanisms of straw biochar's improvement of phosphorus bioavailability in soda saline-alkali soil. *Environmental Science and Pollution Research* 29:47867−72
10. Xiao F, Zhou B, Wang H, Duan M, Feng L. 2022. Effects of different soil amendments on physicochemical property of soda saline-alkali soil and crop yield in Northeast China. *International Journal of Agricultural and Biological Engineering* 15:192−98

11. Wu T, Xu J, Liu J, Guo W, Li X, et al. 2018. Characterization and initial application of endophytic *Bacillus safensis* Strain ZY16 for improving phytoremediation of oil-contaminated saline soils. *Frontiers in Microbiology* 10:991

12. Redondo-Gómez S, Mesa-Marín J, Pérez-Romero JA, López-Jurado J, García-López JV, et al. 2021. Consortia of plant-growth-promoting rhizobacteria isolated from halophytes improve response of eight crops to soil salinization and climate change conditions. *Agronomy* 11:1609

13. Li L, Qiu S, Chen Y, Xu X, Zhao X, et al. 2016. Allocation of photosthestically-fixed carbon in plant and soil during growth of reed (*Phragmites australis*) in two saline soils. *Plant and Soil* 404:277−91

14. Ramos ME, Robles AB, Sánchez-Navarro A, González-Rebollar JL. 2011. Soil responses to different management practices in rainfed orchards in semiarid environments. *Soil Tillage Research* 112:85−91

15. Cui H, Zhou Y, Gu Z, Zhu H, Fu S, et al. 2015. The combined effects of cover crops and symbiotic microbes on phosphatase gene and organic phosphorus hydrolysis in subtropical orchard soils. *Soil Biology and Biochemistry* 82:119−26

16. Qian X, Gu J, Pan H, Zhang K, Sun W, et al. 2015. Effects of living mulches on the soil nutrient contents, enzyme activities, and bacterial community diversities of apple orchard soils. *European Journal of Soil Biology* 70:23−30

17. Ortiz-Ceballos GC, Vargas-Mendoza M, Ortiz-Ceballos AI, Mendoza Briseño M, Ortiz-Hernández G. 2020. Aboveground carbon storage in coffee agroecosystems: The case of the central region of the state of Veracruz in Mexico. *Agronomy* 10:382

18. Díaz FJ, Grattan SR, Reyes JA, de la Roza-Delgado B, Benes SE, et al. 2018. Using saline soil and marginal quality water to produce alfalfa in arid climates. *Agricultural Water Management* 199:11−21

19. Lin J, Hua X, Peng X, Dong B, Yan X. 2019. Germination responses of ryegrass (Annual vs. Perennial) seed to the interactive effects of temperature and salt-alkalistress. *Frontiers in plant science* 9:1458

20. Zhang F, Xu X, Wang G, Wu B, Xiao Y. 2020. *Medicago sativa* and soil microbiome responses to *Trichoderma* as a biofertilizer in alkaline-saline soils. *Applied Soil Ecology* 153:103573

21. Ji HC, Kim KY, Lee SH, Choi GJ, Park NG. 2016. Italian ryegrass. *Indian Journal of Genetics and Plant Breeding* 76(2):228−230

22. Feng QJ, Song SR, Yang Y, Amee M, Chen L, Xie Y. 2021. Comparative physiological and metabolic analyzes of two Italian ryegrass (*Lolium multiflorum*) cultivars with contrasting salinity tolerance. *Physiologia Plantarum* 172:1688−99

23. de Notaris C, Sørensen P, Møller HB, Wahid R, Eriksen J. 2018. Nitrogen fertilizer replacement value of digestates from three green manures. *Nutrient Cycling in Agroecosystems* 112:355−68

24. Zhou W, Ma Q, Wu L, Hu R, Jones DL, et al. 2022. The effect of organic manure or green manure incorporation with reductions in chemical fertilizer on yield-scaled N_2O emissions in a citrus orchard. *Agriculture Ecosystems & Environment* 326:107806

25. Ding T, Yan Z, Zhang W, Duan T. 2021. Green manure crops affected soil chemical Properties and fungal diversity and community of apple orchard in the Loess Plateau of China. *Journal of Soil Science and Plant Nutrition* 21:1089−102

26. Liang K, Wang X, Du Y, Li G, Wei Y, et al. 2022. Effect of legume green manure on yield increases of three major crops in China: A Meta-Analysis. *Agronomy* 12:1753

27. García-Fraile P, Benada O, Cajthaml T, Baldrian P, Lladó S. 2016. *Terracidiphilus gabretensis* gen. nov., sp. nov., an abundant and active forest soil acidobacterium important in organic matter transformation. *Applied and Environmental Microbiology* 82:560−69

28. He H, Li W, Zhang Y, Cheng J, Jia X, et al. 2020. Effects of Italian ryegrass residues as green manure on soil properties and bacterial communities under an Italian ryegrass (*Lolium multiflorum* L.)-rice (*Oryza sativa* L.) rotation. *Soil Tillage Research* 196:104487

29. Mwafulirwa L, Baggs EM, Morley N, Paterson E. 2019. Ryegrass root and shoot residues differentially affect short-term priming of soil organic matter and net soil C-balance. *European Journal of Soil Biology* 93:103096

30. Boudjabi S, Chenchouni H. 2022. Soil fertility indicators and soil stoichiometry in semi-arid steppe rangelands. *CATENA* 210:105910

31. Qi Y, Darilek JL, Huang B, Zhao Y, Sun W, et al. 2009. Evaluating soil quality indices in an agricultural region of Jiangsu Province, China. *Geoderma* 149:325−34

32. Dobermann A. Oberthur T. 1997. Fuzzy mapping of soil fertility—a case study on irrigated riceland in the Philippines. *Geoderma* 77:317−39

33. Glover JD, Reganold JP, Andrews PK. 2000. Systematic method for rating soil quality of conventional, organic, and integrated apple orchards in Washington State. *Agriculture, Ecosystems & Environment* 80:29−45

34. Shi R. 1996. Agricultural Chemistry Analyses of Soils, 2nd Edition. Beijing: China Agricultural Press. pp. 37−39

35. Yan X, Zhang Y, Zhou W, Huang Y, Zhou Q, et al. 2007. Assessment for soil fertility quality of 6 tea bases of Baili tea zone in changsha county. *Research of Agricultural Modernization* 28:354−57

36. Zhang F, Zhou Z, Xiao Y. 2022. Distinct community assembly and co-existence of arbuscular mycorrhizal fungi and diazotrophs across large-scale soil fertility to improve functions in alfalfa cultivation systems. *Environmental Microbiology* 24:5277−91

37. Benitez J, Henseler J, Castillo A, Schuberth F. 2020. How to perform and report an impactful analysis using partial least squares: Guidelines for confirmatory and explanatory IS research. *Information Technology and Management* 57:103168

38. Gao X, Shi D, Lv A, Wang S, Yuan S, et al. 2016. Increase phosphorus availability from the use of alfalfa (*Medicago sativa* L.) green manure in rice (*Oryza sativa* L.) agroecosystem. *Scientific Reports* 6:36981

39. Xie Z, He Y, Tu S, Xu C, Liu G, et al. 2017. Chinese milk vetch improves plant growth, development and ^{15}N recovery in the rice-based rotation system of South China. *Scientific Reports* 7:3577

40. Ding W, Xu X, He P, Ullah S, Zhang J, et al. 2018. Improving yield and nitrogen use efficiency through alternative fertilization options for rice in China: A meta analysis. *Field Crops Research* 227:11−18

41. Lei B, Wang J, Yao H. 2022. Ecological and environmental benefits of planting green manure in paddy fields. *Agriculture* 12:223

42. Lee BJ , Yoon TH , Cho WT , Jun HS, Cho YS. 2013. Effects of green manure cropping on soil biomass-C and soil fertility in green house Soil. *Korea Journal of Organic Agriculture* 21:647−57

43. Monirifar H, Mirmozaffari Roudsari A, Ghassemi S, Tavasolee A. 2020. Harvest time and cultivar effects on growth, physiological traits, yield and quality of alfalfa in saline condition. *International Journal of Plant Production* 14:453−62

44. Meza K, Vanek SJ, Sueldo Y, Olivera E, Ccanto R, et al. 2022. Grass-legume mixtures show potential to increase above- and below-ground biomass production for Andean forage-based fallows. *Agronomy* 12:142

45. Hoogmoeda M, Cunningham SC, Baker PJ, Beringer J, Cavagnaro TR. 2014. Is there more soil carbon under nitrogen-fixing trees than under non-nitrogen-fixing trees in mixed-species restoration plantings? *Agriculture, Ecosystems and Environment* 188:80−84

46. Zhang L, Zhou L, Wei Z, Xu H, Tang Q, et al . 2020. Integrating cover crops with chicken grazing to improve soil nitrogen in rice fields and increase economic output. *Science of the Total Environment Volume* 713:135218

47. Gelaye KK, Zehetner F, Loiskandl W, Klik A. 2019. Comparison of growth of annual crops used for salinity bioremediation in the semi-arid irrigation area. *Plant Soil and Environment* 65:165−71

48. Liu J, Peng S, Faivre-vuillin B, Xu Z, Zhang D, et al. 2008. Erigeron annuus (L.) Pers., as a green manure for ameliorating soil exposed to acid rain in Southern China. *Journal of Soils and Sediments* 8:452−60

49. Jing P, Wang S, Chen Y, Lu W, Ma C. 2017. Adaptation of salt-tolerant forage grasses to saline soil and their ability to improve saline soil utilization in southern Xinjiang region. *Acta Prataculturae Sinica* 26(10):56−63

50. Astier M, Maass JM, Etchevers-Barra JD, Peña JJ, de León González F. 2006. Short-term green manure and tillage management effects on maize yield and soil quality in an Andisol. *Soil Tillage Research* 88:153−59

51. Li H, Dai M, Dai S, Dong X. 2018. Current status and environment impact of direct straw return in China's cropland - A review. *Ecotoxicology and Environmental Safety* 159:293−300

52. Fan W, Wu J, Ahmed S, Hu J, Chen X, et al. 2020. Short-term effects of different straw returning methods on the soil physicochemical properties and quality index in dryland farming in NE China. *Sustainability* 12:2631

53. Cao J, Li X, Kong X, Zed R, Dong L. 2012. Using alfalfa (*Medicago sativa*) to ameliorate salt-affected soils in Yingda irrigation district in Northwest China. *Acta Ecologica Sinica* 32:68−73

54. Wei Z, Zhao Z, Gong Q, Zhai B, Li Z. 2018. Effects of cover crop in an apple orchard on microbial community composition, networks, and potential genes involved with degradation of crop residues in soil. *Biology and Fertility of Soils* 54:743−59

55. Karlen DL, Hurley EG, Andrews SS, Cambardella CA, Meek DW, et al. 2006. Crop rotation effects on soil quality at three northern corn/soybean belt locations. *Agronomy Journal* 98:484−95

56. Ma D, Yin L, Ju W, Li X, Liu X, et al. 2021. Meta-analysis of green manure effects on soil properties and crop yield in northern China. *Field Crops Research* 266:108146

57. Feng Y, Shi Y, Zhao M, Shen H, Xu L, et al. 2022. Yield and quality properties of alfalfa (*Medicago sativa* L.) and their influencing factors in China. *European Journal of Agronomy* 141:126637

58. Mueller ND, Gerber JS, Johnston M, Ray DK, Ramankutty N, et al. 2012. Closing yield gaps through nutrient and water management. *Nature* 490:254−257

59. Zhang F, Che Y, Xiao Y. 2019. Effects of rice straw incorporation and N fertilizer on ryegrass yield, soil quality, and greenhouse gas emissions from paddy soil. *Journal of Soils and Sediments* 19:1053−63