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Fermenting straw reduced salt damage and improved the stability of the bacterial community in a saline–sodic soil

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Abstract

This study aimed to explore the potential of fermenting straw return for remediation of soil salinity. A sealed–pot experiment was used to evaluate four treatments: CK (0 g fermenting rice straw), T1 (120 g fermenting rice straw), T2 (240 g fermenting rice straw), and T3 (360 g fermenting rice straw). Using ^{13}C isotope tracer technique and molecular biological techniques to detect the physical, chemical, and biological properties of saline–sodic soils. The results showed that a small amount of CO_2 was produced upon addition of soda–alkali soil to the soil after straw was applied. Quantitative analysis showed that the proportion of CO_3^{2-} reduction of total CO_3^{2-} was peaked (4.90%) in treatment T3. Concomitantly, under this treatment soil pH, SAR and ESP were reduced, whereas soil porosity and K^+, Ca^{2+}, and Mg^{2+} concentrations, and total nitrogen (TN), SOM, and MBC were increased. PCoA analysis showed that the addition of straw significantly changed the community structure of bacteria in a saline–sodic soil and increased the Chao1 and Shannon indexes. Straw application increased ryegrass shoot and root biomass without allelopathic effects in the saline–sodic soil used. Our results highlighted that rice straw should be collected and artificially decomposed

after rice harvest and then applied for the reclamation of strongly saline–sodic soils in the Songnen Plain and other similar areas.

Keywords：Microbial Diversity; Saline–Sodic Soils; Soil Bacteria; Soil Nutrients; Straw

1. Introduction

Saline soils are widespread in arid and semiarid regions. It has been estimated that, globally, about 955×10^6 ha of arable land suffer from salinity and sodicity [1-2]. Most salinized soils are in an abandoned state because of high salinity, poor structure, and low nutrient content [3]; furthermore, desertification related to soil salinization has intensified year after year. Songnen Plain is the largest area of saline–sodic soils in China, and one of the three largest areas of saline–sodic soils in the world [4]. Therefore, the development and utilization of appropriate strategies for the remediation of saline–sodic lands is not only a matter of paramount importance, it is also a problem that requires urgent attention. The Songjiang river, the Nenjiang river, and many fresh–water lakes in the Songnen Plain provide the conditions for planting rice to reclaim saline–sodic soils after the introduction of irrigation water. Therefore, in recent decades, a large area of rice cultivation has been reclaimed in the Songnen Plain, and surface desalination can be achieved by the combined actions of transverse runoff and longitudinal leaching with irrigation water. It is estimated that the annual yield of rice straw is innumerable. However, only 20.3% of all rice straw produced, is normally returned to the fields after harvest, while 46.6% is burned. The main components of the rice straw after incineration are inorganic salt (i.e. Na_2CO_3, K_2CO_3), which will increase the salt concentration in saline–sodic soils. If the rice straw was effectively returned to the field, it would not only save agricultural resources, but it would reduce environmental pollution as well [5-6].

Straw return to the field is currently, already an important agricultural tillage method, with the technology mainly consisting in either the direct return of crop straw to the field after mechanical comminution or its indirect return by stacking fermentation and animal consumption [7]. However, in recent years, it has been found that straw return to the field has both positive and negative effects on agricultural production. It has been pointed out that straw return to the field hinders mechanical tillage and is not conducive to rice transplanting. Pérez et al. [8] found that hexamethyloxane produced by straw ripening could significantly inhibit the growth of oat roots. At the same time, Weir et al. [9] suggested that phenoglycolic acid and p–hydroxybenzoic acid produced by rice straw decomposition significantly inhibited root growth of rice seedlings. Yu et al. [10] found that straw rotting produced a large number of phenolic acids that acted as allelochemicals inhibiting the normal growth of crops. In contrast, Nyberg et al. [11] found that 70%–90% carbon was released in the form of microbial respiration about 40 days after straw was returned to the field, and large amounts of humus, fulvic acid, humic acid and other substances were produced during the decomposition process, which increased soil organic carbon (SOC) content. Concomitantly, organic acids produced by straw decomposition can activate phosphorus in the soil, and long–term returning of straw to the field can significantly improve the bioavailability of soil phosphorus [12]. Thomsen et al. [13] used a ^{15}N isotope labeling technique and found that each gram of straw can fix 1.0–3.2 mg of N in the soil. Thus, crop straw can provide sufficient carbon and nitrogen for soil microbial activities.

When straw had been returned to the field for approximately 90 days, microorganisms in the topsoil layer propagated rapidly and the number of bacteria increased approximately 1.7 times [14]. Similarly, Kotwica et al. [15] found that the number of bacteria, fungi and actinomycetes increased by 2–6 times after straw mulching. Consistently, Pascual et al. [16] showed that straw application accelerated the formation of soil aggregates by increasing the activity of soil microorganisms. In turn, soil porosity increased and bulk density decreased [17-18].

The positive and negative effects of straw return to the field are largely dependent on the specific test conditions. The main factors limiting the remediation of highly saline–sodic soils in the Songnen Plain are:

1) Predominant anions are CO_3^{2-} and HCO_3^-, which lead to high pH [19];

2) Soils are abandoned for a long time, and the content of organic carbon, N, phosphorus, and other nutrients is low, which limits crop growth [4].

3) Soil porosity in highly dispersed saline–sodic soils is low, and salt in the subsoil easily accumulates in the top layer under the action of capillarity. Based on the foregoing description, we hypothesized that the positive effects of straw return to the field in highly dispersed saline–sodic soils are much greater than the negative effects.

To this end, we have formulated the following hypotheses:

1) Straw can increase soil porosity and improve the structure of saline–sodic soils.

2) The organic acid produced by fermenting straw can react with CO_3^{2-} in saline–sodic soils to reduce the toxic effect of pH on anions in the soil. while, a ^{13}C isotope tracer technique was used to quantitatively analyze the proportion of CO_3^{2-} reduction. Straw return will increase soil nutrient content and the activity of soil microorganisms. It is expected that the results of this experiment will provide a scientific basis for the improvement of saline–sodic soils and the rational utilization of straw in the Songnen Plain.

2. Materials and Methods

2.1. Experimental Design

The soil was selected among typical saline–sodic soil (unreclaimed wasteland soil at depths of 0–20 cm) in Jianping Township, Da'an City, Baicheng City, Jilin Province, in north–eastern China (45°28′13″ N, 124°06′0.7″ E). The basic physical and chemical properties of the soil were as follows: sand=29.0%, silt = 32.2%, clay = 38.8%, pH = 10.5, $EC_{(1:5)}$ = 0.62 mS cm^{-1}, total nitrogen content (TN) = 0.50 g kg^{-1} ; available phosphorus(AP) = 5.20 mg kg^{-1}, soil organic matter = 8.15 g kg^{-1}, exchangeable sodium percentage (ESP) = 62.3%. Experimental soil samples accurately weighing 5.0 kg were placed into the testing device.

The rice straw harvested from paddy field in saline–sodic soil of Jianping Township was delivered to the Institute of Technology of Nanjing Technology University for fermentation and ripening. However, as a field experiment is an open system, which faces great difficulty and uncertainty in attempting to quantitatively analyze the effect of straw ripening, thus, we decided to use the pot–closed–system for this experiment. An amount of fermenting straw was added to simulate the amount of straw returned to the field by local farmers; thus, four experimental treatments were established: CK (0 g pot^{-1} fermenting straw), T_1 (120 g pot^{-1} fermenting straw), T_2 (240 g pot^{-1} fermenting straw), and T_3 (360 g pot^{-1} fermenting straw); four parallel tests. The experimental soils were prepared by accurately weighing of 250 mg $Na_2{}^{13}CO_3$ (^{13}C abundance up to 98%, Shanghai Chemical Research Institute, China) and mixing it evenly with the test soil, the straw and 400 mL distilled water, then the testing device was sealed for 6 months. The test device is shown in Fig. 1. In order to verify the allelopathy of fermenting straw to crop growth, the soil was placed in a square bowl (20 cm ×20 cm ×20 cm) for planting ryegrass after 6 months.

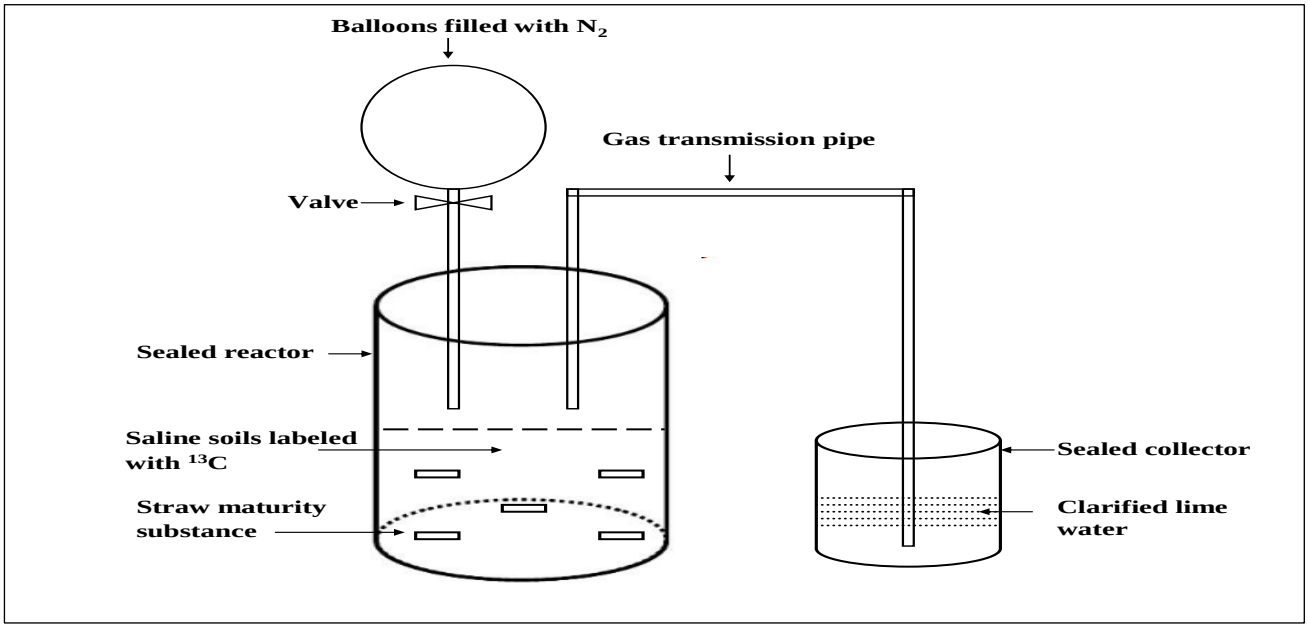


Figure 1: Introduction to test apparatus

2.2. Sampling, measurements, and analyses

After 6 months of the test, the valve on the airtight device was opened to allow N_2 in the balloon to enter the airtight device to discharge excess gas. The discharged gas went through a glass tube into a sufficient amount of clarified lime water (to ensure no HCO_3^{2-} generation). The carbon dioxide produced, precipitated as calcium carbonate by reaction with the clarified lime water that was then filtered with filter paper to collect a sediment. $\delta^{13}C$ in the collected sediment was determined by isotope ratio mass spectrometer (DELTA V Advantage, GRE) in the Stable Isotope Laboratory of the Institute of Environment and Development at the Chinese Academy of Agricultural Sciences. Calculate the proportion of straw to $^{13}CO_3^{2-}$ according to the following formula:

^{13}C abundance in $Ca^{13}CO_3$ precipitate (‰): $R_s = (\delta^{13}C \div 1000 + 1) \times R_{PBD}$, R_{PBD} = 1.078328406; ^{13}C accumulation in $Ca^{13}CO_3$ precipitate (mg): $^{13}C_i = [R_s \div (R_s + 1)] \times C\% \times m$; where C % is carbon content and m is $CaCO_3$ weight.

$Na_2{}^{13}CO_3$ reduction (mg): $D = {}^{13}C_i \times I$, which I is the conversion coefficient and I=8.83;

$Na_2{}^{13}CO_3$ reduction ratio (%): $P = D / 250 \times 100\%$

The soil samples collected from the testing devices were air–dried, crushed, uniformly mixed, and sifted through 2–mm and 1–mm sieves, in preparation for determination of soil pH, $EC_{1:5}$, soil total salt content (TS), and soil bulk density according to methods described by US Salinity Laboratory Staff. Accurately weighed 10 g soil samples were placed in 100 ml glass bottles; 50 ml of water without CO_2 was added, bottles were shaken for 10 min and the resulting solutions filtered. Soil Na^+, K^+, Ca^{2+}, and Mg^{2+} concentrations were measured using a Flame Atomic Absorption Spectrophotometer (4530F, INESA, China). Soil cation exchange capacity (CEC) was calculated after extraction with 0.005 mol L^{-1} EDTA and 1 mol L^{-1} NH_4AC mixed solution. Additionally, exchangeable sodium percentage (ESP) and sodium adsorption ratio (SAR) were calculated as follows [20]:

$$ESP = 100E_{Na} / CEC \qquad SAR = Na^+ / \sqrt{(Ca^+ + Mg^+)/2}$$

Soil total organic carbon (TOC) content and total nitrogen (TN) concentration were determined by an Automatic Carbon and Nitrogen analyzer (Multi N/C 2100, GER). Soil urease, phosphatase, catalase, colyphenol oxidase and invertase enzyme activities were determined by a test kit (Sigma, USA).

Soil samples from the 0–20 cm soil layer were frozen with liquid nitrogen and stored at −40 ℃. Soil DNA solution was extracted from 0.5 g of soil using FastDNA SPIN Kit for Soil Kit (Mpbio, USA). The PCR products from bacteria at 16SV4 were purified by Wizard SV Gel and PCR Clean–Up System kit (Promega, USA). After quality control of the results obtained by Miseq sequencing, the OTU table was obtained by subsequent analysis with the QIIME software. The split_libraries software in QIIME [21] was used to remove the sequences: (1) containing N bases, (2) with a single base repeat greater than 8, (3) with a length less than 200 bp, and then the UCHIME [22] was used to remove chimerism. Using Vsearch (Rognes et al. 2016) to classify high-quality sequences according to 97% similarity and select the sequence with the largest abundance as the representative sequence [23].

Ryegrass (*Lolium perenne L.*) plants were sampled 60 days after planting, washed with distilled water, and separated into root and shoot parts. Root length, and root and shoot fresh weights were measured; then they were oven–dried to constant weight to determine dry matter weight. The allelopathy index was calculated according to the following formula [24]: $RI = 1 - C/T (T \geq C)$, $RI = T/C - 1 (T < C)$, where C is the CK value and T is treatment value, while $RI > 0$ is the promoting effect and $RI < 0$ is the inhibitory effect.

Analysis of variance was performed with the general linear model procedure using SAS (SAS Institute, 2001). Multiple comparisons of means were based on the Least Significant Difference test (LSD) at the 0.05 probability level. PCoA and RDA were performed in Canoco 4.5 software and the importance of soil physicochemical properties on soil salinization indicators was analyzed by Monte Carlo permutation. All charts were drawn by Origin 9.1 (OriginLab, USA) for Windows.

3. Results

3.1. Soil physical and chemical properties

The reduction of $^{13}CO_3^{2-}$ by fermenting straw can be quantitatively analyzed by the isotope tracer technique (Table 1). With the increase of fermenting straw content, $\delta^{13}C$ in the calcium carbonate precipitate showed an upward trend ($P > 0.05$) and the accumulation of ^{13}C in a $Ca^{13}CO_3$ precipitate increased from 0.10 mg in the CK treatment to 1.39 mg in T3 ($P < 0.05$). The neutralization amount of $Na_2{}^{13}CO_3$ in fermenting straw and soda alkali–added soil was calculated based on the amount of ^{13}C that accumulated as calcium carbonate precipitate. We found that $Na_2{}^{13}CO_3$ reduction increased gradually with increasing straw content; thus, it was 3.8 times higher in T3 than in T1, while $Na_2{}^{13}CO_3$ reduction ratio in treatment T3 accounted for the largest proportion (4.90%) of reduced $Na_2{}^{13}CO_3$ among tested treatments. The addition of fermenting straw to soda alkali soil produced a small amount of CO_2 by reacting with CO_3^{2-} in the soil, so as to reduce the CO_3^{2-}content of soda alkali in the soil.

Fermenting rice straw, as a nutrient carrier, can provide a large number of cations for saline–sodic soils. As shown in Table 1, the addition of fermenting straw increased the concentration of Na^+ in the saline–sodic soil used here, but the difference among treatments was not significant ($P > 0.05$). Compared with the CK treatment, K^+ concentration increased by 38.0%, 66.7% and 99.5% in T1, T2, and T3, respectively; similarly, the relative increment of Ca^{2+} and Mg^{2+}concentration compared with the CK treatment ranged 6.6% to 26.3% and 21.7% to 52.2%, respectively. Notably, the effect of adding fermented straw on the concentration of K^+, Ca^{2+} and Mg^{2+} is greater than that on Na^+.

Treatment	$\delta^{13}C$ （%）	$^{13}C_i$ (mg)	D （mg）	P(%)
CK	–8.5±0.45	0.10±0.01 d	0.92±0.12 d	0.37
T1	–7.4±0.36	0.37±0.02 c	3.23±0.35 c	1.29
T2	–7.3±0.87	0.89±0.02 b	7.82±0.62 b	3.13
T3	–6.5±0.56	1.39±0.03 a	12.26±0.74 a	4.9

Note: Values are expressed as means ± standard error. Within each column, different letters indicate statistically significant differences ($P < 0.05$). *13Ci*=13C accumulation in Ca13CO3, *D*= Na213CO3 reduction, *P*= Na213CO3 reduction ratio

Table 1: Reduction of CO_3^{2-} in saline–sodic soil by adding fermenting straw.

		CK	T1	T2	T3
Na^+	(g kg ^{1})	0.347±0.011 a	0.353±0.015 a	0.367±0.008 a	0.378±0.012 a
K^+	(g kg ^{1})	0.021±0.002 d	0.029±0.004 c	0.035±0.003 b	0.042±0.004 a
Ca^{2+}	(g kg ^{1})	0.076±0.004 c	0.081±0.006 b	0.085±0.003 b	0.096±0.004 a
Mg^{2+}	(g kg ^{1})	0.023±0.002 c	0.028±0.003 b	0.033±0.004 ab	0.035±0.004 a
pH		10.37±0.04 a	10.16±0.10 ab	9.73±0.08 b	9.65±0.05 bc
$EC_{1:5}$	(mS cm ^{1})	0.59±0.05 a	0.62±0.04 a	0.65±0.04 a	0.67±0.05 a
Bulk density	(Mg m^{-3})	1.68±0.08 a	1.54±0.09 b	1.49±0.12 c	1.39±0.11 d
Porosity	(%)	43.4±1.1 c	45.0±1.3 b	46.9±1.6 ab	47.7±1.4 a
TN	(g kg ^{1})	0.50±0.09 c	0.63±0.12 b	0.76±0.21 ab	0.84±0.15 a
AN	(Mg kg ^{1})	39.6±4.5 d	58.4±5.6 c	63.8±8.6 b	75.4±7.8 a
AP	(Mg kg ^{1})	5.2±0.6 d	10.8±1.2 c	19.7±3.8 b	27.1±3.6 a
SOM	(g kg ^{1})	8.15±0.8 c	10.65±1.2 b	12.45±0.9 b	15.55±2.4 a

Note：Values are expressed as means ± standard error. Within each column, different letters indicate statistically significant differences ($P < 0.05$).

Table 2: Effect of fermenting straw application on Physical and chemical properties of saline–sodic soil.

The addition of straw can significantly affect the physical and chemical properties of saline–sodic soils (Table 2). Straw fermentation can produce a variety of organic acids; thus, the addition of fermenting straw can significantly reduce the pH of a saline–sodic soil. Further, although straw can carry a large number of salt ions, it does not significantly increase soil EC (P <0.05). However, a significant increment of soil porosity was observed ranging up from 3.5% to 9.9% relative to the CK treatment with straw supply. Therefore, the bulk density of the soil was effectively reduced by straw return.

Low soil nutrient content is one of the main reasons a soda alkali–soil is difficult to use in agriculture. Fermenting straw return significantly improved soil nutrient status (Table 1). Compared with the CK treatment, T1, T2 and T3 treatments increased total nitrogen concentration by 26.0%, 52.0% and 68.0% ($P < 0.05$), and soil alkali–hydrolyzable nitrogen also had a increment from 47.5% to 90.4% ($P < 0.05$) respectively. Similarly, straw decay provided a large amount of

organic matter for the saline–sodic soil; therefore, soil organic matter (SOM) in the three treatments increased by 47.5%, 61.1% and 90.4% respectively ($P < 0.05$). Availability of phosphorus in soda alkali–soils is very low because it is easily fixed and immobilized, while the addition of fermenting straw was able to increase available phosphorus concentration with a ranging from 107.7% to 421.1%, respectively ($P < 0.05$).

The degree of sodicity of a saline–sodic soil can be changed by straw addition (Fig. 2). Soil SAR index decreased only slightly because Ca^{+} and Mg^{+} did not increase significantly. However, a significant increment of ESP was observed ranging from 6.4% to 18.9% relative to the CK treatment with straw supply.

3.2. MBC, MBN, and Bacterial Diversity

The addition of fermenting straw significantly improved microbial activity in a saline–sodic soil (Table 3). We observed that MBC concentration relative to the CK treatment increased by 98.5%, 183.7%, and 265.9% (P <0.05), in T1, T2, and T3, respectively. Similarly, and MBN concentration increased by 74.7%, 122.9% and 156.3% (P <0.05). While, a significant increment of bacteria OUT count was also observed ranged from 30.8% to 61.0% relative to the CK treatment (P <0.05).

The addition of fermenting straw significantly changed the bacterial α– diversity in the saline sodic soil used in these experiments (Fig. 3). The Chao1 index average value of soil bacteria increased from 1629 to 2871 and the Shannon index increased from 8.2 to 9.5, concomitantly to the increase in amount of applied fermenting straw. This indicates that the application of fermenting straw was beneficial to the improvement of microbial stability in the saline–sodic soil used in these experiments.

3.3. Bacterial Community Structure

The distance from the point in the principal coordinate analysis (PCoA) can reflect differences in soil bacterial community structure. PCoA (Fig. 4) showed that the points of the CK treatment were obviously distant from the points of straw return treatments and points of straw application clustered together, which indicated that straw significantly improved the bacterial community structure of the experimental saline–sodic soil used.

Straw return significantly affected soil bacteria community structure **(Figure 5).** The dominant phylum in the saline–sodic soil were *Actinobacteria* (47.4%), *Proteobacteria* (18.6%), *Gemmatimonadetes* (17.6%) *and Bacteroidetes* (9.8%), while the relative abundances of phylum *Proteobacteria, Firmicutes* and *Acidobacteria* increased and the relative abundances of phylum *Actinobacteria* and *Gemmatimonadetes* decreased after with straw addition (Fig. 5a). Similarly, straw application decreased the relative abundances of order *Acidimicrobiales* and *AT425_EubC11*, and increased the relative abundances of orders *Rhodospirillales*, *Bacillales and Clostridiales* **(Figure 5b).** At genus scale, The dominant the microflora in the saline–sodic soil were haloalkaliphilic actinobacterium and bacteria with strong tolerance at low nutrient levels (i.e., *Nitriliruptor*, *Longispora Iamia* and *Sphingomonas*). While, the addition of fermenting straw significantly increased the relative abundance of functional bacteria of decaying organic residue(i.e., *Micromonospora*, *Xanthomonas*), hydrolysis of organic matter(i.e., *Bacillus*, *Chryseolinea*, *Nocardioides*, *Bacteroides*, *Paenibacillus*, *Arthrobacter*), and biological carbon sequestration(i.e., *Streptomyces*, *Gaiella*)**(Figure 5c)**.

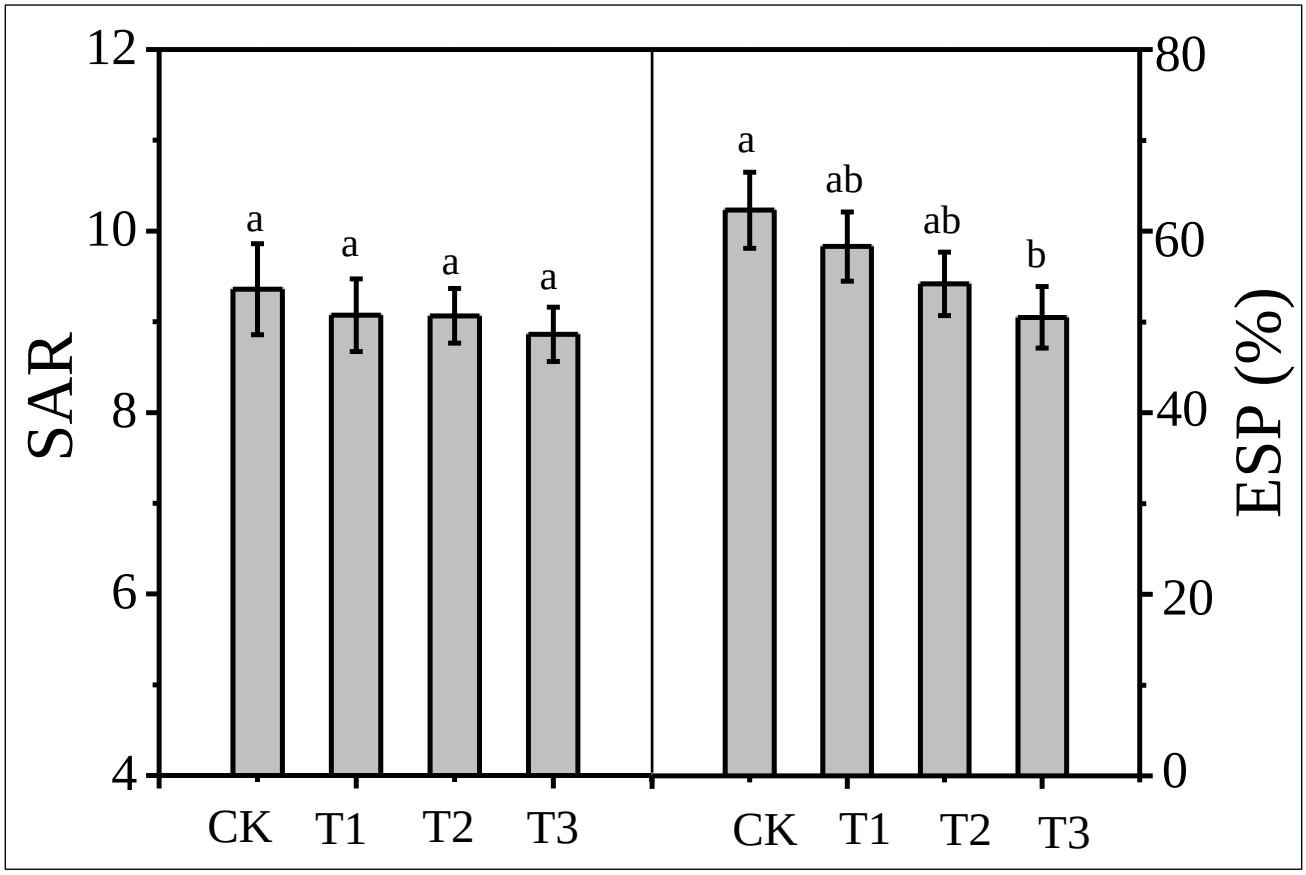


Figure 2: Effect of fermenting straw application on SAR and ESP in saline–sodic soil.

Treatment	MBC(mg kg–1)	MBN(mg kg–1)	C/N	Bacteria OTU count
CK	93.5±10.2 d	8.7±0.9 c	10.7	1619±310 c
T1	185.6±20.5 c	15.2±1.9 b	12.2	2117±320 b
T2	265.3±38.4 b	19.4±2.3 ab	13.7	2554±442 ab
T3	342.2±45.2 a	22.3±1.8 a	15.3	2606±150 a

Note：Values are expressed as means ± standard error. Within each column, different letters indicate statistically significant differences ($P < 0.05$).

MBC: microbial biomass carbon; MBN: microbial biomass nitrogen.

Table 3: Effect of fermenting straw application on MBC, MBN and OUT count of saline–sodic soil.

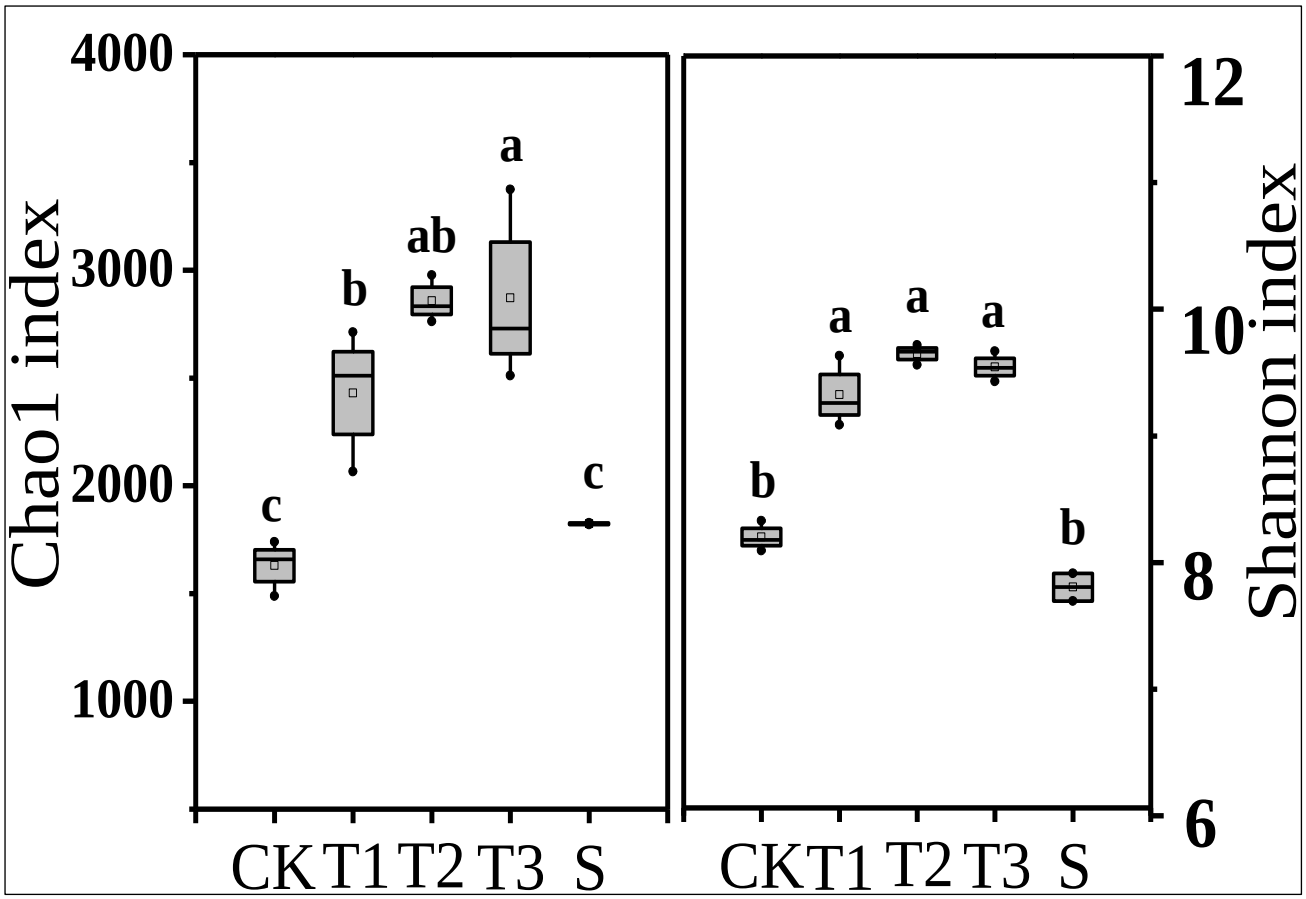


Figure 3: Chao1 and Shannon of soil bacterial community. Note: S–fermenting straw.

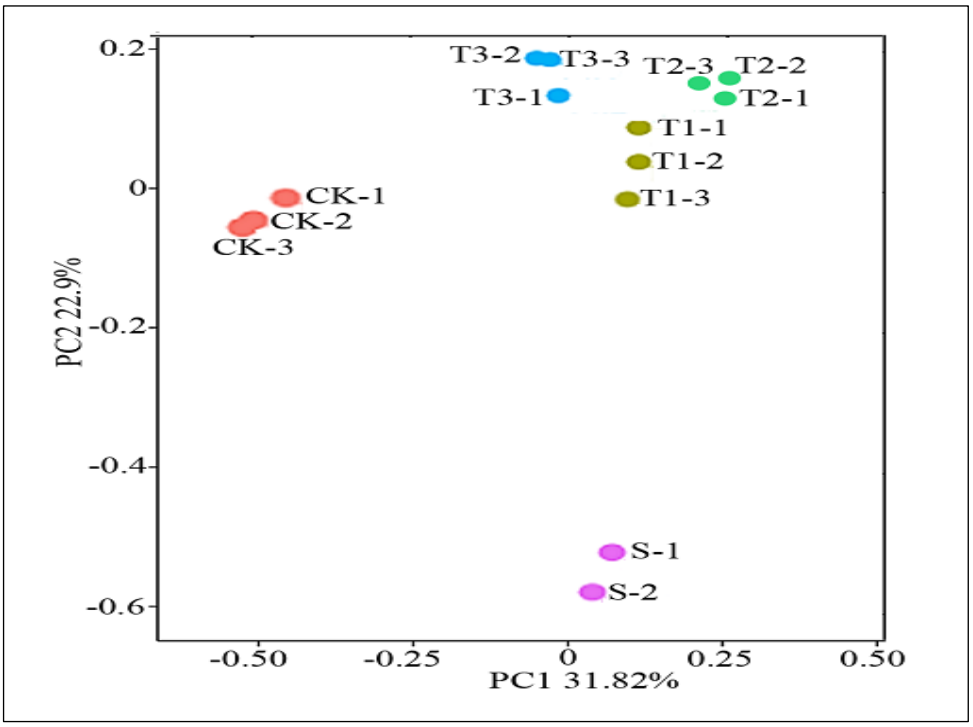
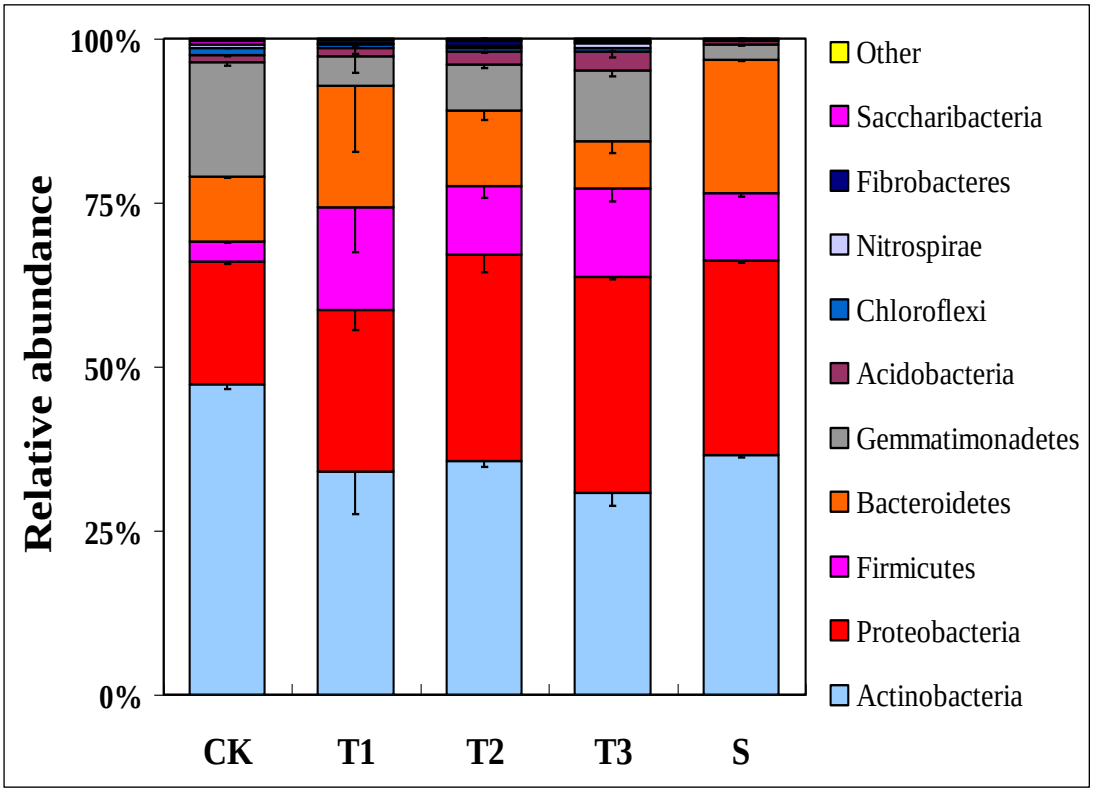
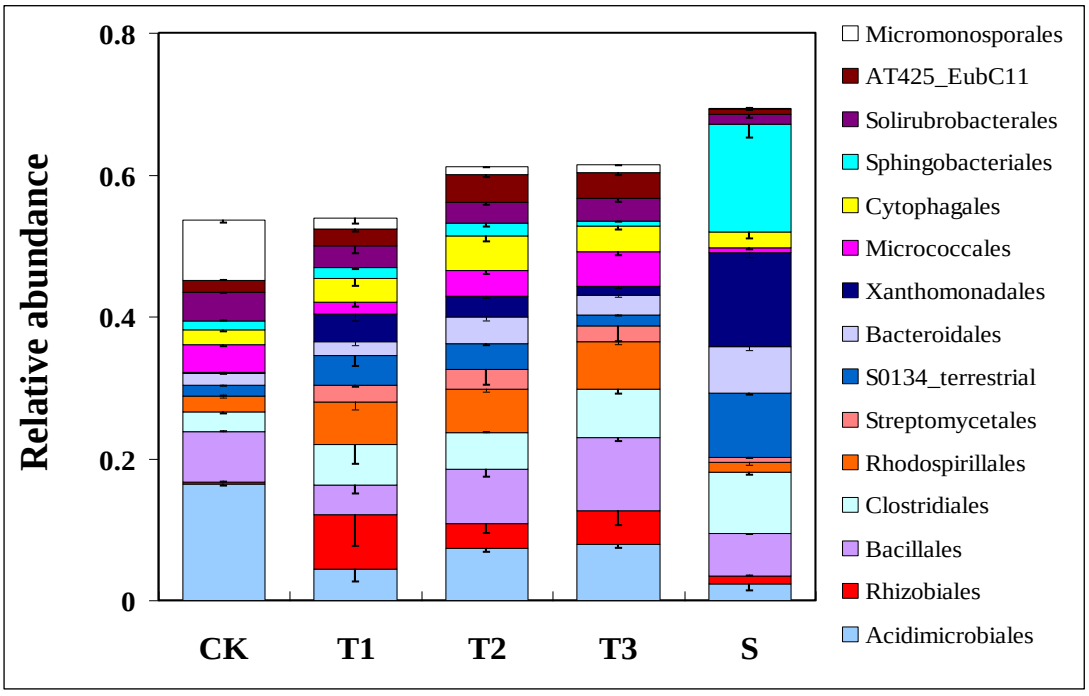


Figure 4: PCoA Analysis of fermenting straw application of soda alkali soil. Note: S–fermenting straw.


100%
75%
50%
25%
0%
Relative abundance
CK
T1
T2
T3
S
Other
Saccharibacteria
Fibrobacteres
Nitrospirae
Chloroflexi
Acidobacteria
Gemmatimonadetes
Bacteroidetes
Firmicutes
Proteobacteria
Actinobacteria

(a)


0.8
0.6
0.4
0.2
0
Relative abundance
CK
T1
T2
T3
S
Micromonosporales
AT425_EubC11
Solirubrobacterales
Sphingobacteriales
Cytophagales
Micrococcales
Xanthomonadales
Bacteroidales
S0134_terrestrial
Streptomycetales
Rhodospirillales
Clostridiales
Bacillales
Rhizobiales
Acidimicrobiales

(b)

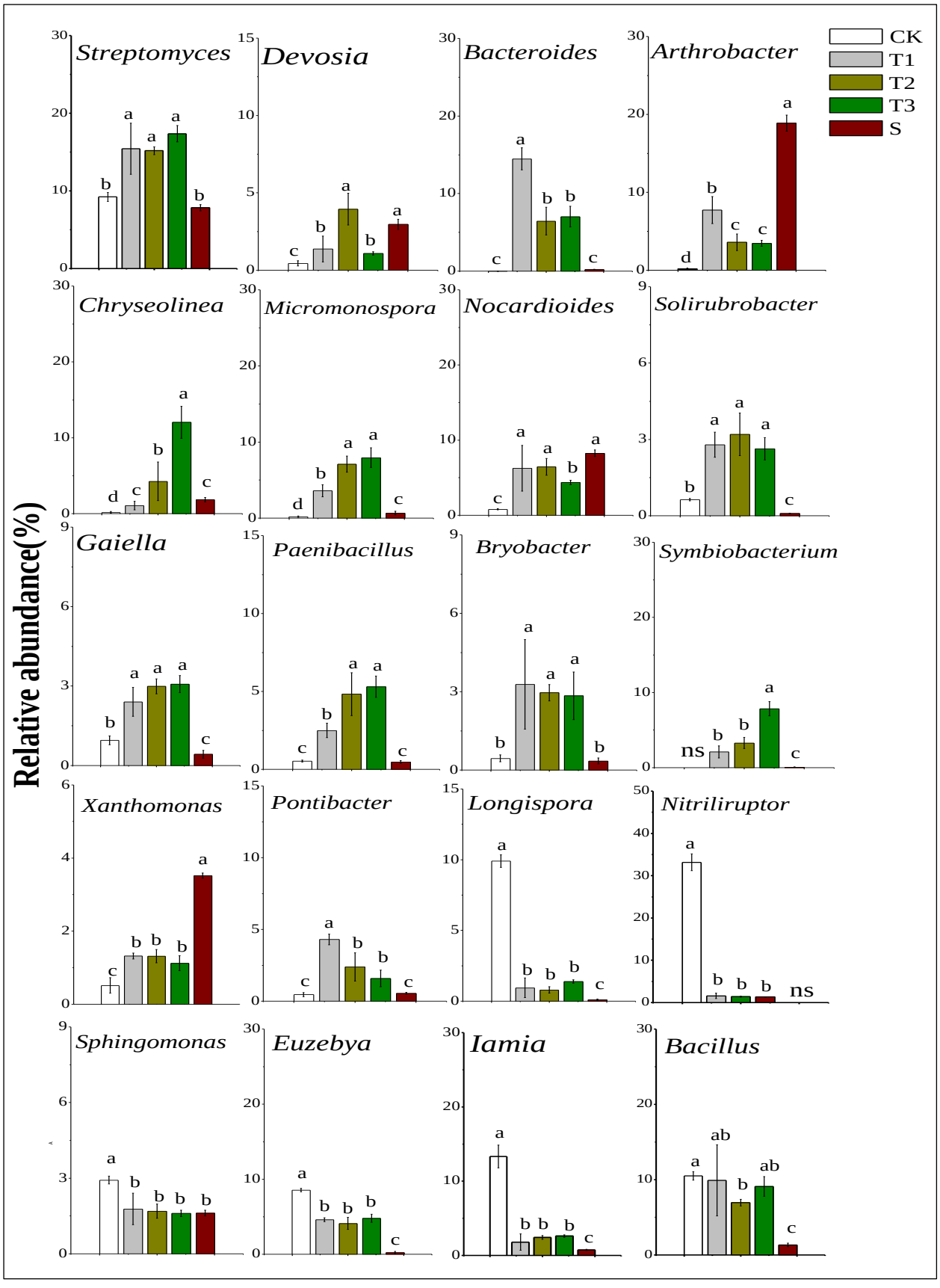


Figure 5: Effect of fermenting straw application on characteristics of bacterial community structure (a, phylum; b, order; c, genus) in saline–sodic soil. Note: S–fermenting straw. ns–the value is too small to be ignored.

3.4. Ryegrass Growth

After straw return to the soda alkali–soil, allelopathy effects were verified by the growth performance of ryegrass. As shown in Table 4, root length and plant height of ryegrass increased with increasing straw content, and then the biomass increased significantly. We found that the shoot dry biomass of ryegrass relative to CK increased by 71.2%, 220.3%, and 341.5% in T1, T2 and T3, respectively ($P < 0.05$); simultaneously, the root dry biomass increased by 89.5%, 198.8%, and 347.6%, respectively ($P < 0.05$). The allelopathy index (*RI*) of total biomass increased from 0.44 to 0.77 after fermenting straw return treatment, which showed that the positive effect was greater than the negative effect upon fermenting straw addition to the experimental saline–sodic soil. The growth of ryegrass was promoted by the increase in nutrient content in the experimental soda alkali–soil, and by the reduction in pH.

3.5. Correlation between microbial taxa, ryegrass growth and environmental factors

In RDA analysis (Fig. 6), the explanation of the first axis was 63.7%, the explanation of the second axis was 19.0%, and the total explanation was 82.7%, which fully reflected the correlation between microbial taxa, ryegrass growth and environmental factors. The arrow line of SOM, AP, TN, SAR and pH were longest, indicating that SOM, AP, TN, SAR and pH played a better role in explaining soil salinity. The relative abundances of *Bacteroidales*, *Clostridiales*, *Streptomycetales*, *Micromonosporales*, *Rhodospirillales*, *Bacteroidales*, *Micrococcales* and *Cytophagales* had a significant positive correlation with soil SOM, TN, AP, and porosity. While ryegrass root–biomass had a significant positive correlation with SOM, TN, AP, and porosity, but a significant negative correlation with soil pH, ESP and SAR.

4. Discussion

4.1. Fermenting straw return proved beneficial for reducing the extent to which a saline–sodic soil impedes plant growth

The area of saline–sodic soils in the Songnen Plain reaches approximately three million ha; pH of strongly salinized soils in the region may be as high as 10.5 [25]. Extremely high pH, limits normal crop growth so severely that any attempt of reclamation of such a large area for cropping purposes is inevitably bound to fail if not scientifically based. Our analysis of ion composition of the tested soils showed that the sum of CO_3^{2-} and HCO_3^- accounted for approximately 70%–80% of the total anions. The hydrolysis of CO_3^{2-} in soil solution is one of the main reasons for the characteristic high pH in these soils. The following chemical reactions will occur in the soil after adding fermenting straw：

$RCOOH+Na_2CO_3 \rightarrow RCOONa + NaHCO_3$ ①,

$NaHCO_3 + RCOOH \rightarrow RCOONa+CO_2+H_2O$ ②.

The results obtained using an isotope technique of this experiment can be confirmed from the point of view. The results in Table 1 showed that $^{13}C_i$ accumulation as calcium carbonate precipitate increased from 0.88 mg (CK) to 11.68 mg (T3); further quantitative analysis of the proportion of CO_3^{2-} reduction to total CO_3^{2-} found that T3 treatment accounted for the largest proportion, 4.90%. Thus, clearly the addition of fermenting straw to saline–sodic soils can indeed produce a small amount of CO_2 by reacting with CO_3^{2-} in the soil to reduce the CO_3^{2-} concentration, and further significant reduction in pH. Therefore, our hypothesis that fermenting straw reduces the potential of soil anion salts to damage crop growth was verified.

Treatment	Root length (cm)	Height (cm)	Shoot dry (g m–2) biomass	Root dry (g m–2) biomass	Total dry biomass	
					(g m^{-2})	*RI*
CK	3.8±0.5 c	10.2±1.2 b	73.5±10.3 d	25.6±3.6 d	99.1±9.8 d	– –
T1	4.2±0.3 b	13.4±2.1 b	125.8±15.3 c	48.5±5.8 c	174.3±12.5 c	0.43
T2	4.8±0.4 b	15.6±2.3 a	235.4±25.4 b	76.5±9.4 b	311.9±35.4 b	0.68
T3	5.9±0.5 a	18.3±2.0 a	324.5±35.4 a	114.6±12.5 a	439.1±22.4 a	0.77

Note：Values are expressed as means ± standard error. Within each column, different letters indicate statistically significant differences ($P < 0.05$).

Table 4: Effect of fermenting straw addition on growth of ryegrass.

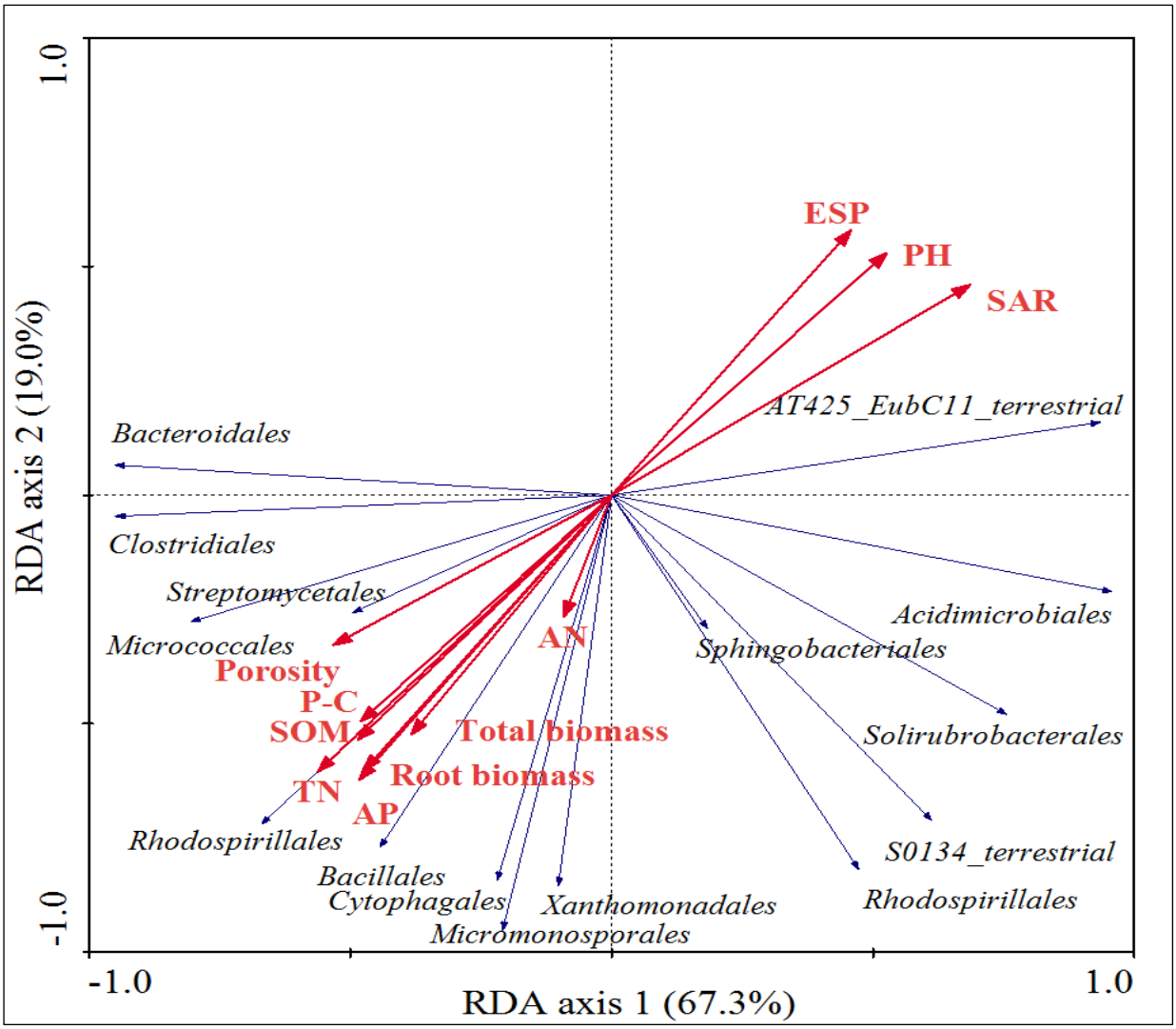


Figure 6: Effect of fermenting straw application on characteristics of bacterial community structure (a, phylum; b, order; c, genus) in saline–sodic soil.

The groundwater level in the area of highly saline–sodic soils in the Songnen Plain is relatively high, additionally, evaporative demand can be very high, particularly in the higher latitudes of the plain region. During the resting period of rice production, the upper soil first freezes into ice, and the salt migrates to the surface with soil capillary water under a freeze–thaw action, thus forming a severe re–salinization phenomenon [26]. We observed that under T1, T2 and T3 treatments, soil porosity increased by 3.6%, 8.0% and 9.9%, respectively, compared with the CK treatment. Therefore, we inferred that adding fermenting straw can significantly improve the porosity of saline–sodic soils [27], and effectively break the continuity of soil capillaries to inhibit the re–salinization phenomenon [28].

Due to the long–term abandonment of farmland, the nutrient content of heavily saline–sodic soils is poor, which significantly restricts land reclamation for agricultural utilization [29]. For example, saline–sodic soils are rich in exchangeable calcium ions and calcium carbonate [30], thus, when calcium ions and phosphate ions form insoluble calcium phosphate precipitates, the soil has a strong fixation effect on phosphorus. Although the total phosphorus content in the soil is very high, there is a severe lack of available phosphorus [31]. The application of fermenting straw, as a carrier of nutrient elements, significantly improved the soil fertility level [32]. Thus, SOM increased by 47.5%–90.4%, and TN increased by 26.0%–68.0%, and soil alkali–hydrolyzed nitrogen increased by 47.5%–90.4% ($P< 0.05$) after straw return. Further, the increase in SOM, which competes with soil particles, reduced the number of soil adsorption sites of phosphorus [33]; this was effectively complemented by the organic acids produced by organic decomposition, which can form chelates that further weaken the fixation effect of phosphorus in the soil and increase the bioavailability of phosphorus in saline–sodic soils [34]; additionally, straw contains large amounts of phosphorus, therefore, the concentration of available phosphorus after straw return increased by 107.7%–421.1% ($P<0.05$). Furthermore, the application of straw carried a large number of K^+, Ca^+ and Mg^+ ions, which not only increased the nutrient content of the soil, but also reduced SAR and ESP of the experimental saline–sodic soil.

4.2. The Application of Fermented Straw Benefits Soil

The activity of soil diversity is one of the key indexes to characterize soil quality [35]. In these experiments, the total amount of microorganisms was negatively correlated with soil salt content and alkalinity in a saline–sodic soil; further, the greater the degree of salinity, the lower the diversity of markers; a finding that fully reflected the inhibitory effect of soil saline environment on microorganisms [36]. The results of this study showed that the dominant phylum in primitive saline–sodic soils were *Actinobacteria* (47.4%), *Proteobacteria* (18.6%), *Gemmatimonadetes* (17.6%) and *Bacteroidetes* (9.8%), with a low Alpha diversity. Fermenting rice straw added to the soil, bred a large number of *Bacteroidetes* and *Firmicutes* (Fig. 5a; S treatment). Therefore, the addition of fermenting straw significantly increased MBC and the bacterial Chao1 and Shannon indexes. Meanwhile, fermenting straw supply increased the relative abundance of functional bacteria of decaying organic residue（i.e., *Micromonospora*, *Xanthomonas*）, and of functional bacteria of hydrolysis of organic matter（i.e., *Bacillus*, *Chryseolinea*, *Nocardioides*, *Bacteroides*, *Paenibacillus*, *Arthrobacter*）, and of functional bacteria of biological carbon sequestration（i.e., *Streptomyces*, *Gaiella*）. In turn, RDA analysis showed that the relative abundances of *Bacteroidales*, *Clostridiales*, *Streptomycetales*, *Micromonosporales*, *Rhodospirillales*, *Bacteroidales*, *Micrococcales* and *Cytophagales*, correlated significantly and positively with soil SOM, TN, AP, and porosity, whereas they correlated significantly, but negatively, with soil pH, ESP and SAR. These findings indicate that the addition of saprophytic materials significantly decreased soil alkalinity,

increased soil nutrient content, and was beneficial to the breeding of microorganisms [37]. In turn, the increase of soil microbial biomass and diversity is beneficial to the activation of soil nutrients [38-39] and to the improvement of soil structure [40] in saline–sodic soils.

4.3. Fermenting Rice Straw can be Used in Reclamation of Saline–Sodic Soils

Many studies have found that the return of straw to normal soil will limit the growth of crops because of allelopathy. However, this study found that the main obstacle factors limiting the normal growth of crops in a severely saline–sodic soil were high pH and nutrient deficiency, rather than any allelopathic effects; under such conditions, straw addition to the saline–sodic soil used here, increased shoot and root biomass of ryegrass. The results showed that the positive effect of straw application to the saline sodic soil used experimentally, on crop growth, was greater than the negative effect. We recommend that straw be collected and artificially decomposed after rice harvest, and once it has fully matured (i.e., ripe) then it should be returned to the soils, as it is clearly beneficial to the reclamation of saline–sodic soils.

5. Conclusions

Upon addition of fermenting straw, a small amount of CO_2 was produced by reaction with CO_3^{2-} present in a soda–alkali soil, thereby reducing soil CO_3^{2-} concentration. Concomitantly, the applied straw reduced soil pH, SAR and ESP and increased soil porosity and K^+, Ca^{2+}, Mg^{2+}, TN, SOM, and MBC concentrations, as well as bacterial alpha diversity in the soil. The positive effect of fermenting straw application on ryegrass growth was greater than the negative effect in the saline–sodic soil used. We conclude that rice straw should be collected and artificially decomposed after harvest to benefit process of reclamation of saline–sodic soils for agricultural production.

6. Author's contribution statement

Xuejun Du put forward the research idea and designed the experimental scheme. Shunyi Wang is in charge of conducting the experiment. Haiqing Huang is responsible for collecting and analyzing data. Yiying Zhang is responsible for analyzing the data. Xuejun Du and Xueqin Ren are in charge of drafting the paper. Shuwen Hu is responsible for the revision of the final version.

7. Acknowledgements

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8. Conflict of Interest

The authors declare they have no conflict of interest.

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