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Soil enzymes as indicators of saline soil fertility under various soil amendments

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ABSTRACT

Soil salinity, caused by natural of anthropogenic factors, has been recognized as a challenge to cultivation. Coastal saline soil is widely distributed in China. The relationships between soil properties and enzyme activities under different amendment types were investigated in Yellow River Town, Kenli County, Shandong Province. The aim of our study was to determine the appropriate treatments for alleviating salinity. Hekang (a saline soil modifier), chemical fertilizers, microbial inoculant, and organic fertilizer were applied to coastal saline soil in this study. The results showed that urease and catalase activities were improved under conditions of Hekang, organic fertilizer and microbial inoculant, but not under single chemical fertilizer applications. All the amendment applications improved alkaline phosphatase activity. Urease activity, alkaline phosphatase activity and catalase activity were all significantly positively correlated with soil organic matter (SOM) or soil nitrogen (N), and were negatively correlated with soil salinity or pH. In addition, Catalase activity was significantly negatively correlated with available phosphorus (P); urease activity showed a significantly positive correlation with soil available nitrogen (N) and a negative correlation with available P or available potassium (K).

1. Introduction

Soil salinization, either caused by natural factors or anthropogenic activities, has been recognized as a serious problem worldwide, where there is notably in arid and semi-arid regions (Wichelns and Qadir, 2014; Singh et al., 2013; Wang et al., 2008).

Salt toxicity has negative effects on soil properties, including high pH, high level of sodium adsorption ratio (SAR) and exchangeable sodium percentage (ESP), poor soil structure and also low water permeability (Wong et al., 2008; Shukla et al., 2011; Singh et al., 2012a, 2012b). Salt accumulation also has detrimental effects on enzyme activities, microbial and biochemical activities (Rietz and Haynes, 2003; Karlen et al., 2008), limiting agriculture productivity (Rady, 2011). Yuan et al. (2007) conducted a survey in a salt-affected area located in Gansu Province, China to investigate the effects of soil salinity on biomass activity and community structure of soil microorganisms, showing that salinity is a stressful factor which reduces microbial communities and has harmful effects on soil organic matter decomposition and available nutrients uptake. This is similar to the results obtained by Rietz and Haynes (2003).

Soil enzyme activities are closely related to soil properties, soil types and environmental conditions, and are now widely used as important indicators of soil quality and soil biological activities (Rietz and Haynes, 2003; Yuan et al., 2007). Therefore, the study of factors influencing soil enzyme activities is important (Dick and Burns, 2011). Among all the enzymes, urease, alkaline phosphatase and catalase activity are more sensitive to environmental changes. Urease promotes the hydrolyze of nitrogen-containing organic matter specially and is closely related to the formation and availability of N in the soil (Liang et al., 2003). Activity of urease is sensitive to high salinity and sodicity. This suggests that urease activity can be used as an indicator of soil quality (H.S. Zhang et al., 2014). Phosphorus (P) in soil is mainly in the organic form. Alkaline phosphatase is the main enzyme involved in the cycling of P

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because it can transform organic P into inorganic P which is the available nutrient for plants (Dick and Burns, 2011). Alkaline phosphatase reacts to external environments sensitively and is an indicator of the organic P mineralization and biological activity of soils (Krämer and Green 2000; T.B. Zhang et al., 2014; T. Zhang et al., 2014). Catalase can enable the peroxide produced during metabolism to decompose, thus preventing its toxic effects on organisms. These enzyme activities play an important role in the cycling of soil carbon (C), N and P. Also, they participate in a great number of soil biochemical processes and they are directly involved in various biochemical reaction in the soil. However, few studies have compared enzyme activity in coastal saline soils.

Various soil amendments have been documented to improve soil properties. Chemical amendments are a common approach to reclaim saline soils which function to provide a source of Calcium (Ca) to replace the exchangeable sodium (Na) on the exchangeable sites. The replaced Na is then leached from the root zone through irrigation (Qadir and Oster, 2004; Qadir et al., 2006; Sahin et al., 2011). However, chemical amendments have become expensive for farmers with the increasing use by industry.

Microbial application for amelioration of sodic and saline–sodic soils is gaining popularity due to its better amelioration and reduction in economic and environmental costs. A study by Sahin et al. (2011) on irrigated land in the Igdir plain, Northeast Turkey was designed to determine the effects of microbial inoculation (fungi and bacteria) on saturated hydraulic conductivities of four saline-sodic soils ameliorated with gypsum. The results suggested that microbial mixtures played a key role in increasing the saturated hydraulic conductivities under saline soils.

Recently, applications of organic amendments (manure, compost and mulch) have been widely used as a strategy to improve soil quality. Liu et al. (2010) found that the use of organic manure with chemical fertilizers could increase microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN) as well as the increase of enzyme activities (dehydrogenase, alkaline phosphatases, β-glucosidase and urease activity). Furthermore, organic amendments may improve nutrient availability and therefore enhance plant growth (Alburquerque et al., 2007). Also, the application of organic matter to saline soils increased soil structural stability and soil bulk density (Tejada and Gonzalez, 2005).

Hekang, saline soil amendment, is a kind of organic high polymer amendment. The principle of Hekang is to improve the solubility of $CaSO_4$ and $CaCO_3$ and to activate the solidified Ca in the soil based on the function of solubilization of anion organic acid complexes. The activated Ca replaces Na adsorbed by soil colloids. The displaced sodium ions and negative valency form water-soluble complexes which are leached from the soil along with chloride and sulphate ions allowing the H+, carbonate and bicarbonate ions to interact producing water and CO2. In China, the area of coastal saline land is $14.0 \times 10^4\,km^2$ along 1.8×10^4 km of coastline adjacent next to the Pacific Ocean. Coastal areas play an important role in the global system. However, these areas are so vulnerable to climatic changes because of poor drainage system and the intrusion of seawater (Datta et al., 2013; Lv et al., 2013). Due to periodic tidal activity, the salinity changes seasonally (Shi et al., 2005; Sun et al., 2012) which makes the soil complicated to manage and unsuitable for sustainable use. High salinity and pH in the coastal saline soils might be the two main limiting factors to enzyme activities. So, it is of great importance to study the effects of amendments on enzyme activities.

In our research, it was hypothesized that the soil amendment treatments Hekang, organic fertilizer and microbial inoculant can promote transformation of organic matter, thus increasing enzyme activity resulting in improved quality of severely salinized soil. The objectives of this study are to 1) find appropriate amendments from Hekang, chemical fertilizer, organic fertilizer and microbial inoculant to ameliorate coastal severe salinized soils based on the indicator—enzyme activity; 2) to reveal the relationship between soil enzyme activities and soil salinity, organic matter or some other soil properties.

2. Materials and methods

The soil for the test was collected from the topsoil (0–20 cm) of the typical zone located in Yellow River Town, Kenli County, Shandong Province (37°45′58. 7″ N, 118°58′40.1″E). This coastal soil is a loamy sand with sever salinity developed in the alluvial fan of Yellow River delta. The soil is with 8.71 of pH and 8.52 g kg^{-1} of salt content, and contained 12.69 g kg^{-1} of soil organic carbon, 0.78 g kg^{-1} of total nitrogen (TN), 42.73 mg kg^{-1} of available nitrogen (N), 16.14 mg kg^{-1} of available P, 178.67 mg kg^{-1} of available K.

2.1. Amendments for the experiment

a Hekang (HK): an organic polymer saline soil amendment (Registration No. 2000 [0277]);
b Chemical fertilizer (CF): urea as N, superphosphate as P, potassium (K) chloride as potash;
c Organic fertilizer (OF): name: Shi Danli; the content of organic matter is 30%, the amount of NPK is 8% and the ratio of N: P: K = 3: 2: 3;
d Microbial inoculant (MI): name: Jinbaobei microbial inoculant; registration No. 2005 [0176] of China; the number of effective viable cells $\geqq 10^{10}\,g^{-1}$.

2.2. Experimental design

There are one control (CK, original soil) and four kind of amendments—HK, CF, OF and MI, with amendment doses: 1, 2, 3 levels, a total of 13 treatments. Each treatment was repeated three times and arranged randomly. Details of the treatments are shown in Table 1.

All treatments received the same chemical fertilizer rate of 75 kg N (urea), 24.6 kg P (P_2O_5) and 46.7 kg K (K_2O) per ha. All fertilizers (half of the Nitrogen) were applied once to the soil before planting, individually. The other half of the Nitrogen was applied as a top dressing during the growing season of *S. salsa*. These doses were established based on the recommendation in the local area.

At June 20, 2014, soil (6 kg) and soil amendments were mixed to bulk density 1.4 g cm^{-3} (the solution of HK was poured into the pot after mixing the soil). The pots with 20 cm in depth, have drainage holes at the bottom and a piece of filter paper was placed on the hole. There is a tray at the bottom of the pot, and if there was some

Table 1
Treatments of the experimental study.

Factor	Fertilizer/kg ha^{-1}		
	Level 1	Level 2	Level 3
CK	–	–	–
HK	15	20	30
CF	N: 75	N: 150	N: 225
	P: 24.6	P: 49.2	P: 73.8
	K: 46.7	K: 93.4	K: 124.5
OF	37.5	75	112.5
MI	7.5	15	30

Note: application of HK, MI and OF with basal fertilizer— N-75 kg ha^{-1}, P-24.6 kg ha^{-1}, K-46.7 kg ha^{-1}. CK: control; HK: Hekang; CF: chemical fertilizer; OF: organic fertilizer; MI: Microbial inoculant.

seepage in the tray, the seepage solution would be poured back to the pot.

300 ml distilled water was irrigated per time according to the wet and dry conditions. 12 weeks later (September 26, 2014), soil samples were collected from each pot to determine enzyme activities. Soil samples were air dried, crashed and passed through 2 mm mesh sieve in the laboratory for chemical analysis.

2.3. Measurement methods

Soil pH and EC were measured in 1:5 soil-water solution. SOC was analyzed by the dichromate oxidation titration method. Total N (TN) was determined by Kjeldahl Method. Extractable P and K were measured using Olsen's method and NH_4AC extraction-flame photometry method, respectively. Urease activity, expressed as mg NH_4^+-N released g^{-1} dry soil 24 h^{-1} at 37 °C, was measured using the indophenol blue colorimetric method. Alkaline phosphatase activity, expressed as mg g^{-1} dry soil 24 h^{-1} at 37 °C, was measured using the disodium phenyl phosphate colorimetric method and catalase activity was measured using the permanganimetric method, which was expressed as ml (0.1 mol/L $KMnO_4$ h^{-1} g^{-1}). All these methods were according to Lu (1999).

2.4. Data analysis and processing

Microsoft Excel 2010 was used for data processing and graphics making. Data significant test (Duncan Test), correlation and path analysis were performed using SPSS statistics software. Path analysis not only shows the relationship between two variables, but also expresses the importance of factors on the results. The correlation coefficient can be divided into a direct effect and an indirect effect, revealing the weights of various factors on the results.

3. Results

3.1. Enzyme activities

Fig. 1 showed that the urease activity was higher than CK and reached a significant difference under the application of HK, OF or MI, except for CF treatments. The level 2 and level 3 of MI were the best treatments with an increase of 32.8% and 31.4% compared with CK, respectively, and reached a significant difference compared with the rest of the treatments. The urease activity under OF treatments was significantly higher than that under CK, CF or HK. We can conclude that MI and OF can significantly improve urease activity. However, urease activity was lower in CF compared with CK.

Fig. 2 showed that CF, OF, MI and level 1 and level 2 of HK could significantly improve alkaline phosphatase activity compared to CK. For HK or CF treatments, alkaline phosphatase activity was highest in level 2 and decreased in level 3. For OF or MI treatments, alkaline phosphatase activity increased with the increasing application amount. Alkaline phosphatase activity of the level 3 of OF relatively increased by 52.58% compared to CK, significantly higher than other treatments and level 1 and level 2 of OF relatively increased by 16.11% and 38.53%, respectively; Three levels of MI increased by 26.42%, 41.16 and 43.27%, respectively.

Fig. 3 showed that HK, OF and MI could improve catalase activity and the activity increased with the increase of fertilizer amount. CF treatments reduced catalase activity, indicating that CF has a negative impact on catalase activity under severe salinization.

3.2. Correlation between soil enzyme activities and other soil properties of the soil

3.2.1. Simple correlation analysis

The correlation analysis between soil enzyme activities and other soil properties (EC, pH, available N, P, K, TN and SOM) was shown in Table 2. The data of correlation analysis is based on all the treatments and all the levels. Urease activity, alkaline phosphatase activity, and catalase activity, were significantly or very significantly correlated with N, OC, soil salinity and pH, suggesting that these enzyme activities of coastal saline soil were significantly correlated with soil nutrient properties, thus reflecting the fertility of coastal saline soils to a certain extent. Furthermore, the research showed that urease, alkaline phosphatase and catalase activities were all positively correlated significantly with each other, indicating that any soil enzyme activity can reflect another two kinds of soil enzyme activities to a large degree.

3.2.2. Path analysis

The path analysis was used to analyze the effects of soil salinity, pH, available N, P, K, TN and OM on soil enzyme activity. Taking the effect of soil properties on soil urease activity as an example, with urease activity as the dependent variable, and soil salinity, pH, N, P, K, TN and OM as the independent variables, the analysis got the

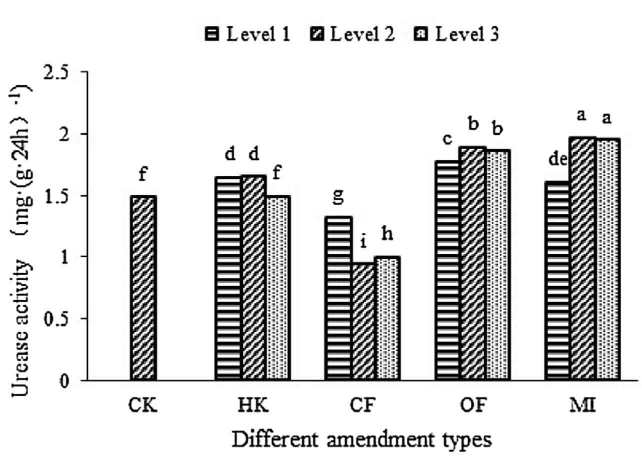

Fig. 1. Soil urease activity under different amendments, values marked with different letters is significantly different at P ≤ 0.05 according to Duncan test. CK: control; HK: Hekang; CF: chemical fertilizer; OF: organic fertilizer; MI: Microbial inoculant.

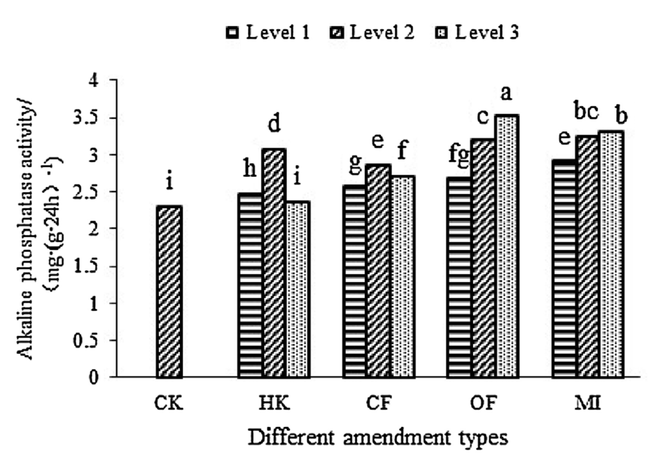

Fig. 2. Soil alkaline phosphatase activity under different amendments, values marked with different letters is significantly different at P ≤ 0.05 according to Duncan test. CK: control; HK: Hekang; CF: chemical fertilizer; OF: organic fertilizer; MI: Microbial inoculant.

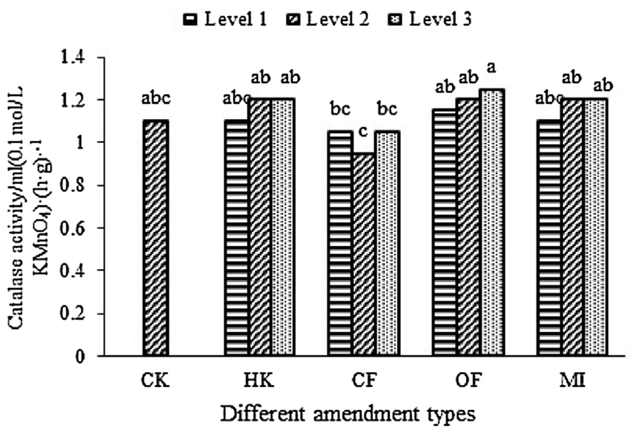

Fig. 3. Soil catalase activity under different amendments, values marked with different letters is significantly different at $P \le 0.05$ according to Duncan test. CK: control; HK: Hekang; CF: chemical fertilizer; OF: organic fertilizer; MI: Microbial inoculant.

correlation coefficients (Table 2) and path coefficients (Table 3). The path analysis of the effect of each factor on alkaline phosphatase activity and catalase activity is similar to this.

The standardized multiple regression equation of urease activity (U) on the influencing factors is:

$$U = 0.023\ \text{Salinity} + 0.344\ \text{pH} + 0.643\ \text{N} - 0.446\ \text{P} + 0.314\ \text{K} + 0.588\ \text{TN} + 0.074\ \text{SOM} \quad (1)$$

Where: equation coefficient is the direct path coefficient, which was multiplied by the correlation coefficient between the various soil properties to derive indirect path coefficients (Table 3).

Table 3 showed that the direct path coefficients of TN and available N on soil urease activity were 0.643 and 0.588, suggesting that the direct effects of them on soil urease activity are the most significant. Soil salinity has little direct impact on urease, but its indirect path coefficients via available N and TN were −0.315 and −0.417, which were 13.70 and 18.13 times of its direct path coefficient. The direct path coefficient of SOM was 0.074, but its indirect path coefficients via available N and TN were 0.206 and 0.406, respectively. The indirect effect of soil pH on urease activity was also affected via available N and TN to some extent. Urease activity is mainly affected via available N and TN. OM had a high indirect path coefficient, indicating that it had no significant direct effect but do have important indirect effects via other soil properties on urease activity.

The correlation analysis of the effects of each factor on alkaline phosphatase activity or catalase activity showed that different soil properties also have effects on these two enzyme activities. The direct path coefficient of TN on alkaline phosphatase activity was 0.676, indicating a highest direct impact of N on alkaline phosphatase activity. pH had little direct impact on alkaline phosphatase activity (path coefficient was −0.019), but its indirect path coefficient via TN was −0.277, which were 14.58 times of the direct path coefficient. The effects of soil salinity, available N and OM on alkaline phosphatase activity were also indirectly affected via TN. Alkaline phosphatase activity was directly affected via TN, and indirectly affected via other soil properties through indirect effects of TN.

SOM had the highest direct path coefficient on catalase activity with the coefficient 0.440, indicating the highest direct effect of OM on catalase activity. The direct path coefficients of soil salinity, pH, TN and available N on catalase activity were obviously lower than indirect path coefficients via OM, indicating that the factors described above had indirect effects on catalase activity via OM.

4. Discussion

Soil enzyme activities play an important role in decomposing soil organic matter and recycling nutrients in the soil (Masto et al., 2006; Demisie et al., 2014). Furthermore, enzyme activities have been regarded as potential indicators of soil fertility (Schloter et al.,

Table 2
Correlation coefficients between enzyme activities and soil properties.

	Soil salinity	pH	N	P	K	TN	SOM	Catalase activity	Urease activity	Alkaline phosphatase activity
Soil salinity	1	0.69**	−0.49**	0.65**	0.36	−0.71**	−0.75**	−0.86**	−0.71**	−0.58**
pH		1	−0.57**	0.60**	0.19	−0.41*	−0.54**	−0.53**	−0.49**	−0.45*
N			1	−0.59**	0.69**	0.14	0.32	−0.32	0.54**	−0.2
P				1	0.67**	−0.27	−0.44*	−0.53**	−0.58**	−0.29
K					1	−0.18	−0.40*	−0.36	−0.49**	−0.14
TN						1	0.69**	0.77**	0.63**	0.71**
SOM							1	0.85**	0.55**	0.59**
Catalase activity								1	0.72**	0.63**
Urease activity									1	0.66**
Alkaline phosphatase activity										1

Note: Pearson was used in the correlation analysis.
* $p < 0.05$.
** $p < 0.01$.

Table 3
Path coefficients between soil properties and urease activity.

	(Soil salinity)	(pH)	(N)	(P)	(K)	(TN)	(SOM)	Sum
Soil salinity	0.023	0.237	−0.315	−0.290	0.113	−0.417	−0.056	−0.705
pH	0.016	0.344	−0.367	−0.268	0.060	−0.241	−0.040	−0.496
N	−0.011	−0.196	0.643	0.263	−0.217	0.082	−0.024	0.540
P	0.015	0.206	−0.379	−0.446	0.210	−0.159	−0.033	−0.585
K	0.008	0.065	−0.444	−0.299	0.314	−0.106	−0.030	−0.490
TN	−0.016	−0.141	0.090	0.120	−0.057	0.588	0.051	0.636
SOM	−0.017	−0.186	0.206	0.196	−0.126	0.406	0.074	0.553

Notes: The data with underline are direct path coefficients and the rest are indirect path coefficients, and the sum is the sum of direct and indirect path coefficients for each property.

2003) because they are more sensitive, rapid, inexpensive and representative of the potential metabolic capacity of the soil than SOM which are hard to measure directly in a short time (Bosatta and Ågren, 1994; Liang et al., 2014). However, there are few studies about the effects of salinity and sodicity on enzyme activities, especially in coastal saline areas. Pandey et al. (2011) reported that researchers need focus on enzyme activities of saline soils.

Our data suggested that organic fertilizer could improve alkaline phosphatase activity. Organic amendments provide SOM, thereby increasing SOC and increasing the water hold capacity, as well as improving soil physical properties and soil structure (Tejada and Gonzalez, 2005; Aparna et al., 2014). According to Saha et al. (2008), alkaline phosphatase activity increased by long-term application of farmyard manure while was inhibited with inorganic fertilizer. Liu et al. (2010) showed an increase in alkaline phosphatase activity with manure and optimum NP application, while the activity decreased with the application of N fertilizer. This is in agreement with several other reports (Mandal et al., 2007; Garg and Bahl, 2008), who suggested that alkaline phosphatase activity increased by the application combined organic with inorganic fertilizer. Organic amendments could increase alkaline phosphatase activity might be due to more substrate availability and enhanced microbial activity and diversity (Tejada et al., 2006). Overall, organic amendments used for reclaiming saline soils are crucial to improve alkaline phosphatase activity. In our study, chemical fertilizer could increase alkaline phosphatase activity to a small degree which was not consistent with the results exhibited in the above articles.

Urease activity showed a significant increase by using organic amendments whereas decreased in chemical fertilizer in our study, which were similar with several results (Zhu et al., 2008; Dick and Burns, 2011) and Bandick and Dick (1999) who showed that urease activity decreased with increasing application of inorganic N, hypothesized that the addition of the end product of the enzymatic reaction (NH_4^+) suppressed urease synthesis (Dick and Burns, 2011; Chang et al., 2007). Also, chemical fertilizer may increase electrolyte concentration under condition of severe salinization. However, this observation is contrast to the results obtained by Liang et al. (2014) who investigated the effects of mineral fertilizer on enzyme activities and found that urease activity increased with the application of mineral fertilizer which might be because the mineral fertilizer included both ammonia-based N fertilizer and urea as N source. Urease activity was positively correlated with TN (Ralte et al., 2005) and SOM (Askin and Kizilkaya, 2006; Makoi and Ndakidemi, 2008). In specific, the direct and indirect effect of soil properties on urease activity was identified by path analysis. TN and available N had positive and highest direct effects on urease activity, indicating that TN and N affected urease activity significantly. This might be because the urease hydrolyzes urea to ammonia, and urease activity is related to soil N availability. However, Bai et al. (2014) found that NO_3–N had direct positive effects on soil urease activities while TN and SOM were not significantly related with urease activities.

Catalase is an enzyme that acts as an indicator of aerobic microorganisms, and reflects the ability of redox in the soil. The catalase activity was closely related to the number of aerobic microorganisms and soil fertility. The reason why single chemical fertilizer inhibited the activity may be that single chemical fertilizer could not increase the number of the aerobic microorganisms since it might increase electrolyte concentration under condition of severe salinization.

5. Conclusions

(1) Hekang, organic fertilizer and microbial inoculant can improve soil urease and catalase activity. Urease, alkaline phosphatase and catalase activity were significantly positively correlated with soil nitrogen or organic matter, and were significantly negatively correlated with soil salinity and pH. In addition, organic matter had no significant direct effect but had important indirect effects via other soil properties on urease and alkaline phosphatase activity.
(2) Four kinds of amendment applications in this study could increase alkaline phosphatase activity, among which organic fertilizer of 112.5 kg ha^{-1} is the best.
(3) Soil enzymes are better indicators of saline coastal soil quality.

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