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Response of Soil Absolute and Specific Enzyme Activities to Long-Term Application of Organic Fertilizer to Solonetz in Northeast China

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Abstract: Soil organic carbon (SOC) and microbial biomass carbon (MBC) are highly correlated with enzyme activities. Specific enzyme activities can exclude the autocorrelation between enzyme activity and SOC and MBC. However, the responses of absolute and specific enzyme activities to saline–alkali properties remains unclear. In this study, the absolute and specific enzyme activities of cellobiose hydrolase, β-glucosidase, arylsulfatase, alkaline phosphatase, and urease were measured in soils with 10, 15, 18, 21, and 26 years of organic fertilizer application in contrast to soils without organic fertilizer application. The results showed that long-term organic fertilizer application led to significantly increased in the absolute and specific enzyme activity and decrease in pH, electrical conductivity (EC), exchangeable sodium percentage (ESP), as well as sodium adsorption ratio ($SAR_{5:1}$). In the structural equation model (SEM), the EC extremely limited the geometric mean of specific enzyme activity per unit of MBC (MBC-GMSEA) (path coefficient, −0.84, $p < 0.001$). Pearson's correlation analysis showed that the correlations between EA/MBC and pH, EC, ESP, and $SAR_{5:1}$ were higher than between the absolute soil enzyme activity and pH, EC, ESP, and $SAR_{5:1}$. Of the parameters tested, EA/MBC was a more sensitive index to reflect the improvement effect of organic fertilizer on soils and evaluate the saline–alkali barrier.

Keywords: specific enzyme activities; geometric mean of enzyme activity; organic fertilizer; Solonetz

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1. Introduction

Soil salinization has become an important problem that limits land use efficiency and the quality of agricultural output [1,2]. Songnen Plain is one of the three major alkalization regions in the world [3]. In the Songnen Plain (northeastern) and northern and northwestern China, Solonetz is mostly scattered in the middle of the saline soil in the form of patches. Solonetz occurs in areas with a semi-arid temperate continental climate, in particular in flat lands with impeded vertical and lateral drainage, and Na_2CO_3 is present (field pH > 8.5) [4], and has an exchangeable Na percentage (ESP) of ≥15% and a sodium adsorption ratio (SAR) ≥ 13 $(\text{mmol L}^{-1})^{0.5}$ throughout the entire natric horizon or within its upper 40 cm, whichever is thinner [5]. In this study, we used pH, electrical conductivity (EC), ESP, and $SAR_{5:1}$ to represent the saline–alkali barrier. The reduction of pH, EC, ESP, and $SAR_{5:1}$ indicates a reduction of the saline–alkali barrier.

Soil salinity and alkalinity significantly affects the availability of soil nutrients in Solonetz, leading to complex relationships between soil enzymes, microorganisms and nutrients in different vegetation types [6,7]. Poor soil structure caused by soil excessive exchangeable sodium is one of the key limiting factors for corn production in Northeast China [8]. Therefore, it is very important to improve Solonetz in Northeast China. Organic matter can form soil aggregates with clay particles and can improve the capacity for organic matter sequestration, nutrient cycling, and soil multifunctionality [9,10]. Applying organic fertilizer is an effective means to improve soil properties and enzyme activity [11–13].

Previous studies have shown that changes in soil properties may be slow or may occur dramatically, so such properties are not suitable for estimating soil quality [14]. Soil enzyme activity is sensitive to biogeochemical cycling and soil organic matter dynamics and is a useful index reflecting soil quality changes [15–17].

However, there is a positive correlation between microbial biomass, organic matter, and soil enzyme activity [15,18,19]. Soil organic matter is the main factor determining enzyme activity [20]. Soil microbial community composition and biomass, directly or indirectly, are the main factors of enzyme synthesis and production potential. Because living microorganisms have a strong influence on soil enzyme activities, which may mask the individual effects of microbial biomass or organic matter on enzyme activity [21], most previous studies could not reflect the relationship between soil enzyme activity and microbial carbon and organic matter. The observed effects based on absolute enzyme activity cannot be determined as being caused by organic matter and soil microbial biomass or as real differences in enzyme activity itself [16,22,23]. Soil nutrients and enzymes can be used as indicators of soil quality. However, in some cases, they may interact with each other and so make the use of both somewhat misleading [24]. The main way to eliminate this effect is to use a specific enzyme activity index. Moreover, based on previous studies, absolute enzyme activity is not as representative as soil specific enzyme activity [21,25–27]. However, the specific enzyme activity is rarely used to evaluate the properties of Solonetz after the long-term application of organic fertilizer.

In this study, the aim was to determine whether the changes in absolute and specific enzyme activity can reflect soil salinity disturbance and to select the most suitable sensitive index for the saline–alkali barrier. We hypothesized that with the application of organic fertilizer, the saline–alkali barrier was reduced, and the absolute and specific soil enzyme activity was increased. The specific enzyme activity could better reflect the reduction of the saline–alkali barrier than absolute enzyme activity.

2. Materials and Methods

2.1. Site Description

The study area is located in a typical Solonetz area (45°44′48′′ N; 125°6′28′′ E; altitude 150 m) in the town of Yongle, Zhaozhou County, in the central and western parts of the Songnen Plain of China. The site is in a temperate zone with a continental monsoon climate, with an annual cumulative temperature of 2800 °C, a mean annual temperature of 3.7 °C, a mean annual temperature range of −24 °C to 30 °C, an average annual precipitation of 436 mm, a mean annual evaporation of 1800 mm, and a frost-free period of 143 days. It is a semi-arid region with a hot and rainy summer climate and a cold and dry winter climate. Evaporation exceeds precipitation. Salt accumulates on the surface soil. The quaternary fluvio-lacustrine deposits are the main soil parent materials. Soil horizons are weakly developed and the natric horizon is within 2 cm to 54 cm of the soil profile in the area. Before soil reclamation, the acric horizon (0–2 cm) pH, ESP, cation exchange capacity (CEC), calcium carbonate ($CaCO_3$), clay, bulk density, and soil organic carbon (SOC) content were 10.3, 54.60%, 38.59 cmol kg^{-1}, 85.7 g kg^{-1}, 46.63%, 1.3 g cm^{-3}, and 5.60 g kg^{-1}, respectively. The natric horizon (2–54 cm) pH, ESP, CEC, $CaCO_3$, clay, bulk density, and SOC were 10.25, 59.7%, 39.55 cmol kg^{-1}, 93.7 g kg^{-1}, 56.36%, 1.4 g cm^{-3}, and 5.85 g kg^{-1}, respectively. The experimental soils are classified as Solonetz [5]. Poor soil properties and structure seriously affect corn production. In 1995, a long-term positioning experiment was set up with organic fertilizer application as the treatment for soil improvement. In a completely randomized block design, ridge tillage was conducted, and cattle manure treatment was applied at a rate of 10,000 kg hm^{-2} (oven-dry weight basis) in late April each year. The area of each plot was 65 m^2, the ridge length was 10 m, and the ridge width was 0.65 m. There were 10 rows in total. The treatment was repeated three times.

The cattle manure pH was 8.61, the EC was 9.89 dS m^{-1}, and the organic carbon content was 346.18 g kg^{-1}. Continuous corn cultivation was practised in the experimental field, and no other field operations were carried out during the corn growth season except for top dressing. Urea (46% N) was applied as a top dressing to corn in the elongation stage at a rate of 400 kg hm^{-2}. There was no irrigation during the growing stages of corn. The unharvested corn was returned to the field. Six treatments were defined in the experiment, including 26 years, 21 years, 18 years, 15 years, and 10 years of manure application continuously and no manure application, which were represented by 26a, 21a, 18a, 15a, 10a, and CK, respectively. Before applying organic fertilizer in different years, no organic fertilizer was applied in the plots, like CK treatment.

2.2. Measurements and Methods

Soil samples (0–20 cm) were collected in April 2021. It was in the spring with arid climate and no rain. Field humidity ranges from 17% to 24%. Each sample was collected at three points and mixed together to form a composite sample. After sampling, a portion of the soil was passed through a 2-mm sieve and stored at 4 °C in the dark for the determination of soil enzyme activity and microbial biomass carbon (MBC), and the other portion was air-dried and used to determine soil chemistry. The exchangeable sodium percentage (ESP) refers to the degree of sodium saturation of the exchangeable complex and is often used in the characterization of Solonetz. The sodium adsorption ratio ($SAR_{5:1}$) is a property that is easier to measure and is used more widely than ESP. $SAR_{5:1}$ represents the relative concentrations of Na^+, Ca^{2+}, and Mg^{2+} in the soil solution. The pH, EC, and $SAR_{5:1}$ were measured at a 5:1 water-to-soil ratio. The pH and EC measurements used a pH-meter electrode and conductivity meter, respectively. Na^+ was determined by flame photometry, and Ca^{2+} and Mg^{2+} by atomic absorption spectroscopy. The CEC was determined by the sodium acetate flame photometry method. The exchangeable Na^+ was extracted using ammonium acetate, and its concentration was measured using flame photometry. ESP and $SAR_{5:1}$ were calculated as follows [28,29]:

$$\mathrm{ESP} = (\mathrm{ExchNa}/\mathrm{CEC}) \times 100\% \tag{1}$$

$$\mathrm{SAR}_{5:1} = \mathrm{Na}^+/\sqrt{\left(\mathrm{Ca}^{2+} + \mathrm{Mg}^{2+}\right)/2} \tag{2}$$

where ExchNa is the exchangeable sodium and CEC is the cation exchange capacity.

SOC was determined using H_2SO_4–$K_2Cr_2O_7$ oxidation [30]. MBC was fumigated with chloroform and extracted with 0.5 mol L^{-1} K_2SO_4 [31], and tested on the total organic carbon analyzer (TOC analyzer, Germany) to determine the MBC content. The activities of cellobiose hydrolase (CBH, 3.2.1.91), β-glucosidase (βG, 3.2.1.21), arylsulfatase (ARY, 3.1.6.1), and alkaline phosphatase (ALP, 3.1.3.1) were determined via microplate p-nitrophenol colorimetry [32]. Briefly, 0.1 g soil sample with 0.4 mL of 0.1 M modified universal buffer (MUB) (added by pipette gun) to stabilize the pH of the solution (buffer pH = 5.0 [33], 6.0 [32], 5.8 [34], 9.5 [33], respectively). After oscillating with a vortex oscillator for several seconds, substrate was added; the substrates were *p*-Nitrophenyl-beta-D-cellobioside [33], *p*-Nitrophenyl-β-D-glucoside, potassium *p*-Nitrophenyl sulfate, and *p*-Nitrophenyl phosphate [34]. The solutions were shaken for several seconds, then incubated at 37 °C for 1h. Then, 0.4 mL of a tris hydroxymethyl aminomethane (THAM) solution (pH = 12) was added to stop the enzymatic reaction, followed by the addition of 0.1 mL of 0.5 mol L^{-1} $CaCl_2$, and the sample was shaken and centrifuged at 12,000 rpm for 10 min. Then, 250 μL of supernatant was pipetted into the microplate. The enzyme activity was measured by a microplate reader (Bio-Rad Benchmark, Hercules, CA, USA) at 405 nm and expressed as the amount of *p*–nitrophenol produced per g of soil after 1 h. There was a substrate-free control for each sample, and soil-free control was set up in the experiment. The urease (URE, 3.5.1.5) activity was determined by sodium phenol–sodium hypochlorite colorimetry [35]: a 5 g soil sample with 10 mL of 10% urea solution, and 20 mL of pH 6.7 citrate buffer solution, shaken for 15 min and incubated at 37 °C for 24 h, and the concentration of $NH_3{}^-$-N released was determined colorimetrically at 578 nm. The soil specific enzyme activity, the geometric mean of enzyme activity (GMEA), the geometric mean of specific enzyme activity per unit of SOC (SOC-GMSEA), and the geometric mean of specific enzyme activity per unit of MBC (MBC-GMSEA) were calculated as follows [36,37]:

$$\text{Microbial carbon specific enzyme activity} = \text{EA}/\text{MBC} \tag{3}$$

$$\text{Organic carbon specific enzyme activity} = \text{EA}/\text{SOC} \tag{4}$$

$$\text{GMEA} = \sqrt[5]{\text{CBH} + \beta\text{G} + \text{ARY} + \text{ALP} + \text{URE}} \tag{5}$$

$$\begin{aligned}&\text{SOC} - \text{GMSEA}\\&= \sqrt[5]{\text{CBH}(\text{Ea}/\text{SOC}) + \beta\text{G}(\text{Ea}/\text{SOC}) + \text{ARY}(\text{Ea}/\text{SOC}) + \text{ALP}(\text{Ea}/\text{SOC}) + \text{URE}(\text{Ea}/\text{SOC})}\end{aligned} \tag{6}$$

$$\begin{aligned}&\text{MBC} - \text{GMSEA}\\&= \sqrt[5]{\text{CBH}(\text{Ea}/\text{MBC}) + \beta\text{G}(\text{Ea}/\text{MBC}) + \text{ARY}(\text{Ea}/\text{MBC}) + \text{ALP}(\text{Ea}/\text{MBC}) + \text{URE}(\text{Ea}/\text{MBC})}\end{aligned} \tag{7}$$

where EA is enzyme activity, MBC is microbial biomass carbon, SOC is soil organic carbon, CBH is cellobiose hydrolase activity, βG is β-glucosidase activity, ARY is arylsulfatase activity, ALP is alkaline phosphatase activity, and URE is urease activity.

2.3. Statistical Analysis

SPSS 25 software was used to conduct one-way ANOVA to compare the differences in soil parameters among the treatments, and Duncan's method was used to test the significance of the differences ($p < 0.05$). Origin 2021 was used to analyze the correlation between absolute and specific enzyme activities and soil properties. An Amos 27 structural equation model (SEM) was used to investigate the influence path and degree of the main driving factors of soil absolute and specific soil enzyme activities.

3. Results

3.1. Soil Properties

The pH and EC of CK were 10.67 and 2.32 dS m^{-1}, respectively, which were significantly higher than those of the five organic fertilizer treatments. The SOC of the organic fertilizer treatments increased by 53.6% to 184.2% compared with that of CK (Table 1). The MBC of the organic manure treatments was significantly higher than that of CK except for 21a and 18a. The application of organic fertilizer significantly reduced ESP and $SAR_{5:1}$ by 80.0% to 94.1% and 74.5% to 96.1%, respectively. Soil saline–alkali stress can be significantly reduced by applying organic fertilizer.

Table 1. Soil pH, electrical conductivity (EC), exchange sodium percentage (ESP), sodium adsorption ratio ($SAR_{5:1}$), soil organic carbon (SOC) and microbial biomass carbon (MBC) contents in different long-term organic fertilizer application years. 26a, 21a, 18a, 15a, 10a, and CK are 26 years, 21 years, 18 years, 15 years, and 10 years of manure application and no manure application treatments, respectively. Values with the same lowercase letters are not significantly different at $p < 0.05$.

Treatment	pH	EC (dS m^{-1})	ESP (%)	$SAR_{5:1}$ [(mmol L^{-1}) $^{0.5}$]	SOC (g kg^{-1})	MBC (mg kg^{-1})
26a	8.21 ± 0.23 c	0.31 ± 0.15 b	2.38 ± 0.23 c	3.34 ± 0.29 c	21.47 ± 2.35 ab	697.48 ± 27.11 a
21a	8.17 ± 0.20 c	0.15 ± 0.02 b	1.73 ± 0.46 c	1.37 ± 0.57 c	19.00 ± 2.91 bc	310.52 ± 12.24 b
18a	8.69 ± 0.05 b	0.30 ± 0.07 b	5.46 ± 0.97 b	7.65 ± 1.80 b	14.56 ± 2.33 cd	405.48 ± 7.57 b
15a	8.73 ± 0.07 b	0.28 ± 0.00 b	5.88 ± 0.37 b	9.03 ± 0.73 b	26.94 ± 2.19 a	593.48 ± 91.15 a
10a	8.34 ± 0.27 c	0.30 ± 0.09 b	5.66 ± 0.99 b	7.53 ± 0.90 b	20.57 ± 6.04 b	644.30 ± 91.83 a
CK	10.67 ± 0.05 a	2.32 ± 0.35 a	29.38 ± 1.60 a	35.41 ± 2.19 a	9.48 ± 1.05 d	416.15 ± 158.00 b

3.2. Absolute Enzyme Activities

The absolute enzyme activities of CBH, βG, ARY, ALP, and URE in the five organic fertilizer treatments increased by 2.8 to 7.9 times, 3.1 to 10.3 times, 2.9 to 8.4 times, 3.2 to 7.3 times, and 1.9 to 9.5 times, respectively, compared with that of CK (Figure 1). The application of organic fertilizer had a significant positive effect on soil enzyme activity.

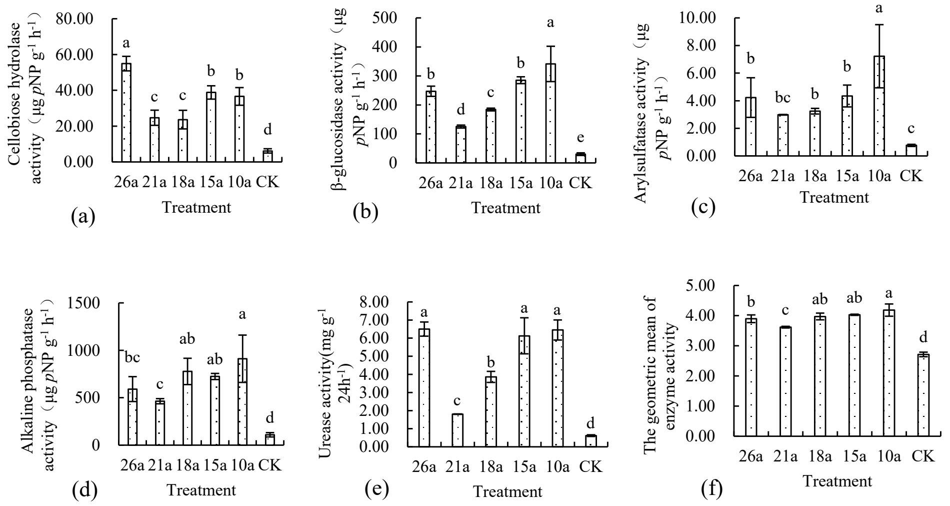

Figure 1. The cellobiose hydrolase (**a**), β-glucosidase (**b**), arylsulfatase (**c**), alkaline phosphatase (**d**), and urease (**e**) activities and the geometric mean of enzyme activity (**f**) in different years of long-term application of organic fertilizer. Values with the same lowercase letters are not significantly different at $p < 0.05$.

3.3. Specific Enzyme Activities per Unit of SOC (EA/SOC) and MBC (EA/MBC)

The EA/SOC of the organic fertilizer treatment was significantly higher than that of CK, except for ALP (Figure 2). The CBH (EA/SOC) and βG (EA/SOC) of the organic fertilizer treatments were obviously higher than those of CK, with increases of 1.0 to 2.9 times and 1.1 to 4.3 times, respectively. The ARY (EA/SOC), ALP (EA/SOC), and URE (EA/SOC) of the organic fertilizer treatments were significantly higher than those of CK, with increases of 1.0 to 3.4 times, 1.1 to 3.6 times, and 0.4 to 3.7 times, respectively.

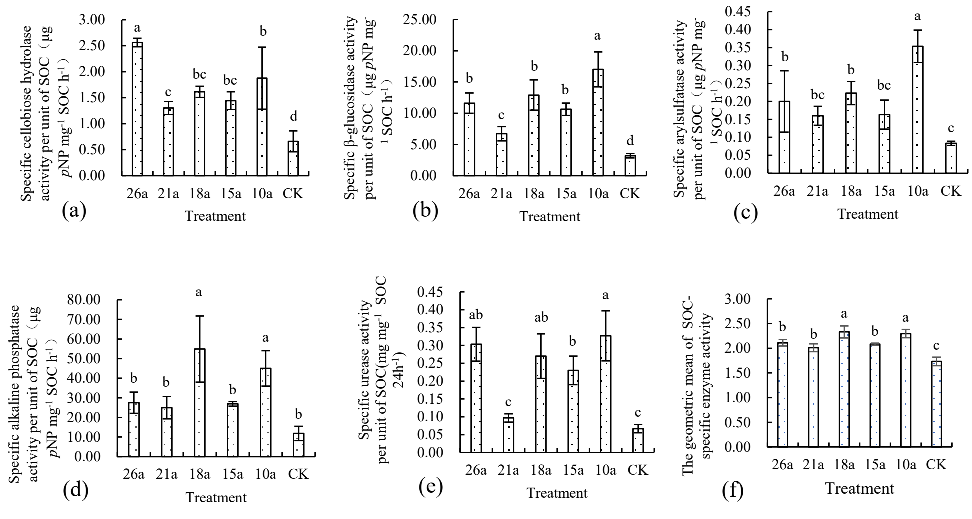

Figure 2. Specific cellobiose hydrolase (**a**), β-glucosidase (**b**), arylsulfatase (**c**), alkaline phosphatase (**d**), and urease (**e**) activity per unit of SOC and the geometric mean of SOC-specific enzyme activity (**f**) in different years of long-term application of organic fertilizer. Values with the same lowercase letters are not significantly different at $p < 0.05$.

The trends of EA/MBC and EA/SOC were similar for different years of organic fertilizer application (Figure 3). The CBH (EA/MBC), βG (EA/MBC), ARY (EA/MBC), ALP (EA/MBC), and URE (EA/MBC) of the organic fertilizer treatments were significantly higher than those of the CK, with increases of 5.0 to 7.0 times, 3.4 to 5.6 times, 5.0 to 12.0 times, 3.7 to 9.7 times, and 5.0 to 10.5 times, respectively.

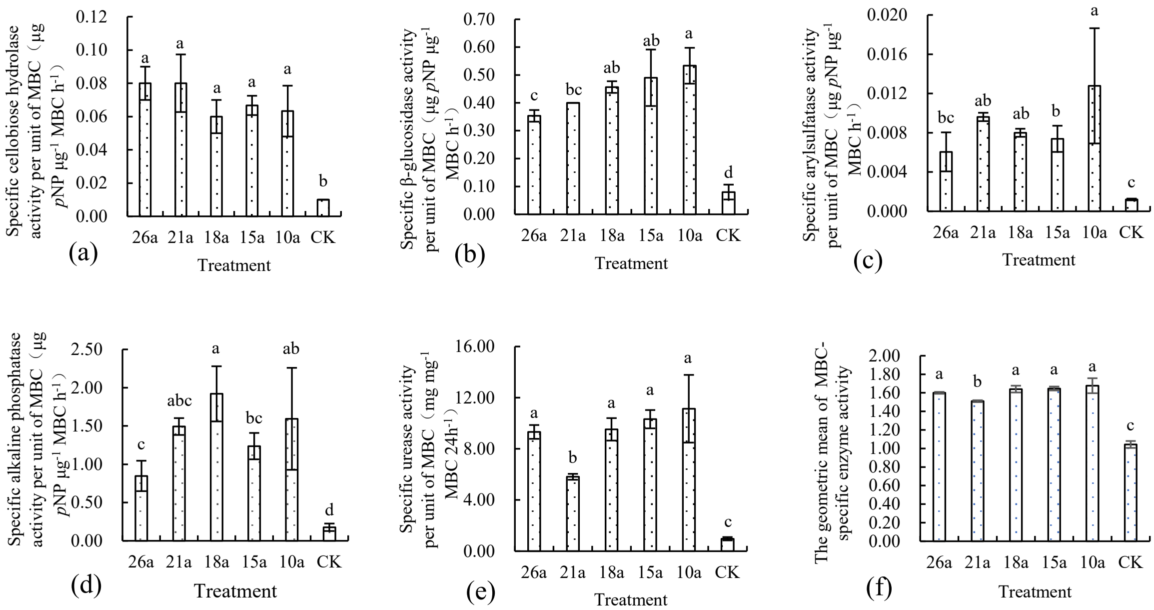

Figure 3. Specific cellobiose hydrolase (**a**), β-glucosidase (**b**), arylsulfatase (**c**), alkaline phosphatase (**d**), and urease (**e**) activity per unit of MBC and the geometric mean of MBC-specific enzyme activity (**f**) in different years of long-term application of organic fertilizer. Values with the same lowercase letters are not significantly different at $p < 0.05$.

In conclusion, long-term application of organic fertilizer can significantly improve soil specific enzyme activities, and the effect of EA/MBC is more obvious than that of EA/SOC.

3.4. Geometric Mean of Absolute and Specific Enzyme Activity

GMEA reflects the overall enzyme activity of the soil of each treatment. Compared with CK, the GMEA of the organic fertilizer treatment was significantly increased by 33.6% to 54.2% (Figure 1). That of the 10a treatment was significantly higher than that of the 26a and 21a treatments. The same trend was observed for SOC-GMSEA and MBC-GMSEA. The SOC-GMSEA and MBC-GMSEA of the organic fertilizer treatments were obviously higher than those of CK, with increases of 16.2% to 34.7% and 45.2% to 61.6%, respectively. The SOC-GMSEA of the 18a and 10a treatments were significantly higher than those of the other organic fertilizer treatments (Figure 2).

3.5. Relationships between the Soil Properties and Enzymes

In Figure 4, blue and red represent negative and positive correlations, respectively. The absolute and specific enzyme activities were negatively correlated with pH, EC, ESP, and $SAR_{5:1}$ but positively correlated with SOC and MBC. Except for alkaline phosphatase, Ea/MBC was more strongly correlated with pH, EC, ESP, and $SAR_{5:1}$ than absolute enzyme activity, Ea/SOC with pH, EC, ESP, and $SAR_{5:1}$ (darker and thinner). There was no correlation between EA/MBC and MBC or EA/SOC and SOC. For ALP, the correlation between absolute enzyme activity and pH, EC, ESP, and $SAR_{5:1}$ was not significantly different from that between specific enzyme activity and pH, EC, ESP, and $SAR_{5:1}$. The correlation between MBC-GMSEA and pH, EC, ESP, and $SAR_{5:1}$ was significantly higher than that between GMEA and pH, EC, ESP, and $SAR_{5:1}$.

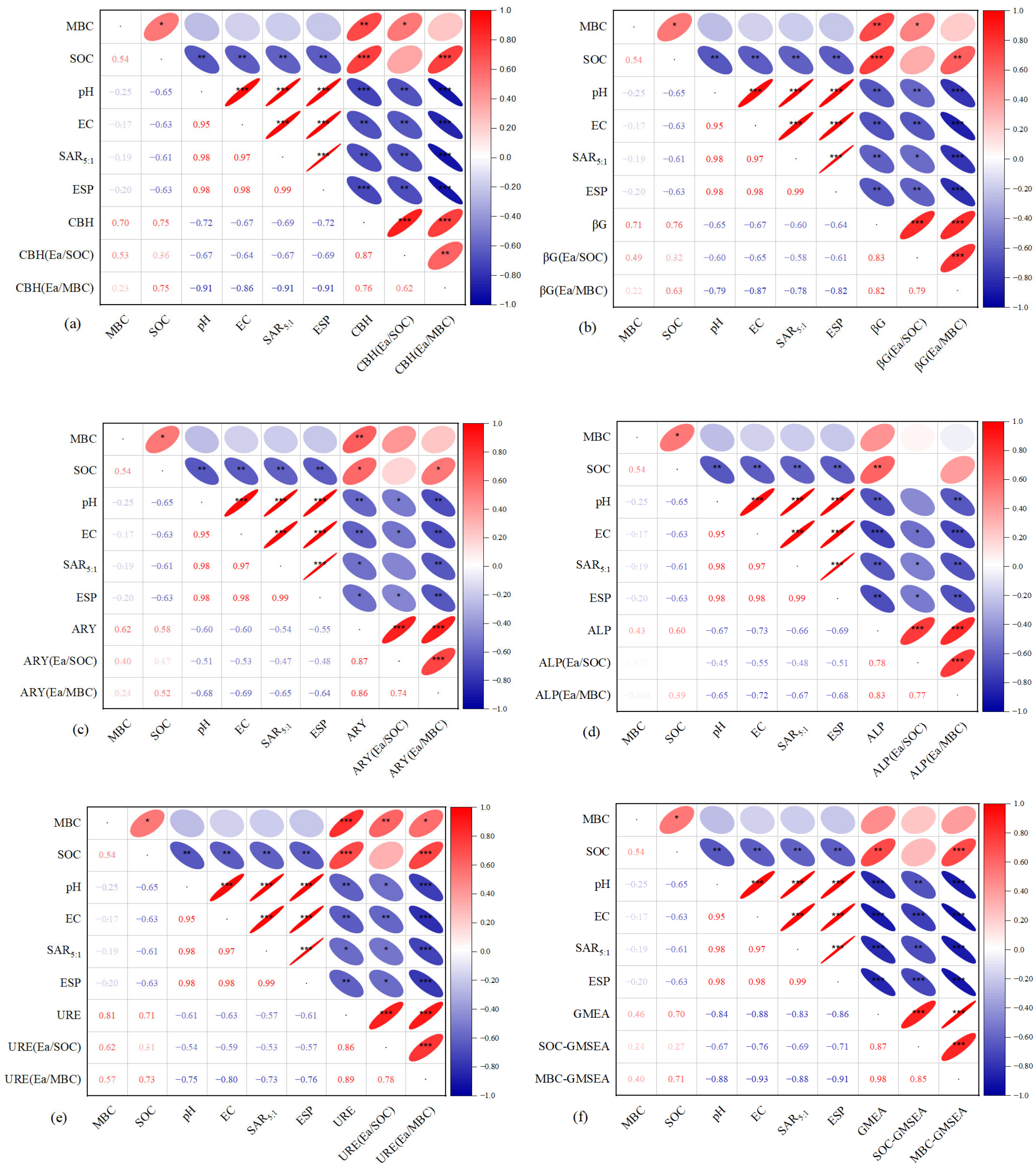

Figure 4. Pearson's correlation between absolute and specific cellobiose hydrolase (**a**), β-glucosidase (**b**), arylsulfatase (**c**), alkaline phosphatase (**d**), urease (**e**), geometric mean of enzyme activity activity (**f**) and soil property index (MBC, SOC, pH, EC, ESP, and $SAR_{5:1}$). Notes: CBH is cellobiose hydrolase activity, βG is β-glucosidase activity, ARY is arylsulfatase activity, ALP is alkaline phosphatase activity, URE is urease activity, EA/SOC is soil activity per unit of SOC, EA/MBC is soil activity per unit of MBC, GMEA is the geometric mean of enzyme activity, SOC-GMSEA is the geometric mean of specific enzyme activity per unit of SOC, MBC-GMSEA is the geometric mean of specific enzyme activity per unit of MBC. *, $p < 0.05$, **, $p < 0.01$, ***, $p < 0.001$.

In addition, the structural equation model (SEM) results can well explain the way to affect absolute and specific enzyme activities by saline–alkali properties (Figure 5). We input SOC, MBC, pH, EC, ESP, GMEA, SOC-SGMEA, and MBC-SGMEA into the SEM model and achieved a great fitting level. The causal relationship in different soil variables can be reflected by SEM. The strong collinearity between ESP and $SAR_{5:1}$ could not meet the precondition of model analysis, and the $SAR_{5:1}$ variable was removed. SOC had large positive effects on MBC, indicated by the effect coefficient of 0.58. SOC provides energy substances and nutrients required by microbial life activities, and it can also promote the improvement of soil structure and other physical properties, making it conducive to microbial activities. The path coefficients from ESP and pH to GMEA were 0.03 and −0.04; although the effect of ESP on GMEA was positive, it showed a lower effect on enzyme activity, and the effect was not significant. The path coefficient from EC to MBC-SGMEA was −0.84. The contribution of soil saline–alkali properties to MBC-SGMEA was greater. The path coefficient from MBC to MBC-SGMEA was 0.42. Soil microorganisms are important sources of soil enzymes, and in the process of decomposing organic materials, microorganisms produce enzymes that catalyse the mineralization of SOC [38]. Soil microorganisms release extracellular enzymes to perform essential metabolic functions [39]. SOC has a different effect on GMEA (0.45) and SOC-SGMEA (−0.46). In our study, SOC had a positive effect on enzyme activity but had a negative effect on unit organic carbon enzyme activity. To maintain soil metabolism, the utilization rate of enzymes for organic matter increased in the soil with low organic matter content, which showed a negative correlation [24]. SOC decomposition rates are strongly associated with soil enzyme activity [40]. SOC is the most critical physicochemical property affecting GMEA and SOC-SGMEA. Soil enzyme activity is often used as a sensitive index to characterize changes in soil organic carbon content [41]. The most critical property affecting MBC-SGMEA is EC (−0.84). In addition, GMEA, MBC-SGMEA, and SOC-SGMEA are also affected by each other.

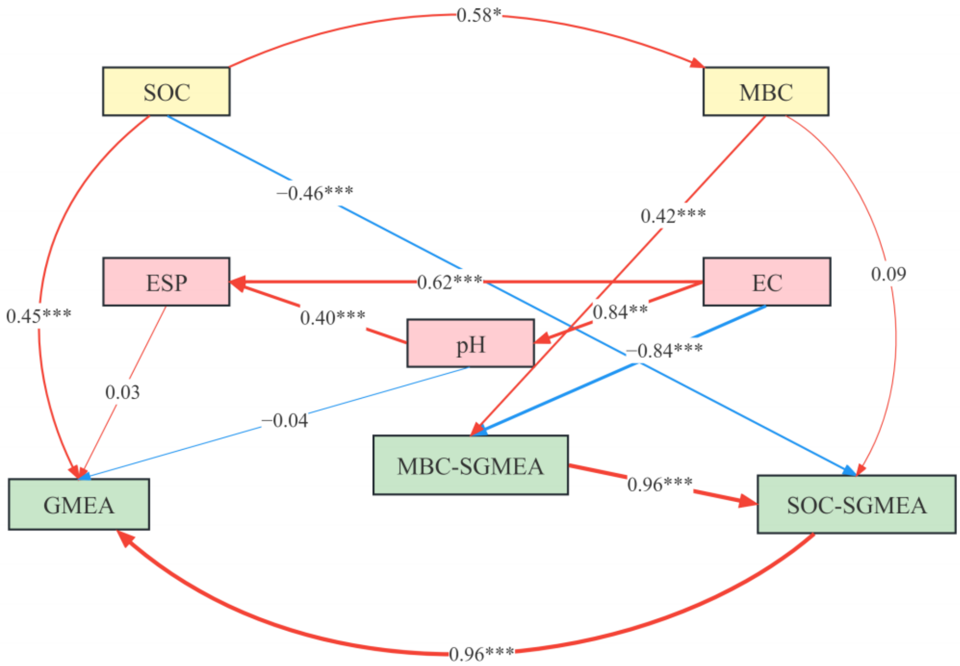

Figure 5. Structural equation modelling (SEM) demonstrates the effects of soil saline–alkali properties on absolute and specific enzyme activities. χ^2/df = 0.691, p = 0.797, RMSEA = 0.000. Notes: Blue and red lines represent negative and positive relationships, respectively. The thickness of each arrow and the value on the arrow represent the path coefficient and its size, respectively. *, $p < 0.05$, **, $p < 0.01$, ***, $p < 0.001$.

In summary, the saline–alkali properties of soil had the greatest correlation with EA/MBC, and the contribution rate to EA/MBC was the highest.

4. Discussion

The organic carbon content of soil treated by long-term application of organic fertilizer was significantly higher than that of CK. There is a certain conversion relationship between soil organic carbon and organic matter (van Bemmelen factor) [42]. The application of organic fertilizer increased soil organic carbon, and the corresponding organic matter content was also increased. The increase in soil organic matter content promoted the formation and stability of microaggregates and macroaggregates and increased the protection of soil enzyme activity [43]. By providing a sufficient nutrient matrix, soil properties, biological communities, and native soil morphology can be changed, thus improving microbial activity and affecting soil enzyme activity [44–46]. The SOC of 18a was not significantly different from CK, and soil properties and enzyme activity fluctuate with different years of manure application. We think this may be due to sampling errors. Solonetz has large variability and uneven distribution, but the trend of the relationship between absolute enzyme activity, specific enzyme activity, and saline–alkali degree is not affected. This fact should be taken very much in account for further studies.

In addition, GMEA can more comprehensively represent the enzyme activity in soil. It can show the overall enzyme activity of soil and is also a commonly used indicator in many studies [16,24,47]. The GMEA, SOC-GMSEA, and MBC-GMSEA of organic fertilizer-treated soil was significantly higher than that of CK, which was consistent with the change in absolute and specific enzyme activity, indicating that organic fertilizer application played a positive role in the improvement of overall enzyme activity in Solonetz.

Microbial activity and organic carbon are two major factors affecting enzyme activity, and absolute enzyme activity cannot eliminate the strong correlation between microbial and enzyme activity, so specific enzyme activities were more appropriate [48,49]. EA/MBC and EA/SOC were studied in this experiment. Although the specific enzyme activity has a higher similarity with absolute enzyme activity in the range of change, it is not exactly the same. The absolute activity of a particular enzyme may change due to induction/suppression of enzyme synthesis in the same microbial species or changes in microbial diversity, and EA/MBC may reflect microbial functional capabilities associated with ecological mechanisms that maintain soil metabolic activity [50]. The increase in EA/MBC in the organic fertilizer treatment indicates that the application of organic fertilizer can increase soil microbial metabolism, thus improving the production and release efficiency of soil microbial enzymes [51]. The interactions among microbial taxa were promoted by organic fertilizer [52]. The application of organic fertilizer reduced pH and increased SOM, which were all factors affecting EA/MBC [53]. Organic fertilizer can promote the release of enzymes that are immobilized in soil aggregates [51]. After applying organic fertilizer, SOC renewal was promoted, and unit organic carbon enzyme activity was stimulated.

The strong positive correlation between the absolute enzyme activity and SOC and MBC provided further support for the utilization of specific enzyme activities. There was no correlation between EA/MBC and MBC or EA/SOC and SOC, which may have been because specific enzyme activity was autocorrelated with MBC and SOC. pH, EC, ESP, and $SAR_{5:1}$ are important saline–alkali characteristics of soil [54]. The correlation between ALP and saline–alkali properties was different from that of other enzymes. Zhang et al. showed that absolute and specific phosphatase activities were affected by pH and phosphorus limitations [51]. In this experiment, the pH of different treatments significantly differed (Table 1), and the related indices may have been affected by pH. SOC-GMSEA and MBC-GMSEA can comprehensively reflect specific soil enzyme activities. In Figure 4f, MBC-GMSEA was more sensitive to the saline–alkali properties of soil and it could be concluded that enzyme activities were more strongly influenced by microorganisms. The organic fertilizer applied to the soil can decompose only after the action of soil microorganisms, releasing nutrient elements, forming humus, improving the structure of the soil, and reducing the saline–alkali properties. Enzymes were negatively correlated with salinity and alkalinity [55]. Salinization was the process of accumulation of free salts such as Na^+ [3]. Na toxicity has an inhibitory effect on enzyme activities, negatively affecting metabolism [56]. Poor physical properties of the soil lead to negative effects on enzymes. Long-term application of organic fertilizer increases the stability of soil aggregates [57] and has a positive effect on enzyme activity.

In the SEM (Figure 5), it is notable that the influence relationship between absolute and specific enzyme activities is positive. We speculate that this might be due to the degree of salinization of CK being too high, affecting the normal metabolism of microorganisms, and that the improved soil can restore normal metabolic function, so that the two are positively correlated. EC is the main limiting factor of MBC-SGMEA, which restricts the production of specific enzyme activities per unit of MBC. The application of organic fertilizers alleviates this limitation and improves the specific enzyme activities in Solonetz.

5. Conclusions

Our data indicated that long-term organic fertilization improved the soil properties, increased the absolute and specific enzyme activities, and decreased the pH, EC, ESP, and $SAR_{5:1}$ in Solonetz. The EA/MBC was more sensitive to pH, EC, ESP, and $SAR_{5:1}$ than absolute enzyme activity and EA/SOC to pH, EC, ESP, and $SAR_{5:1}$. The EC had an extreme limiting effect on MBC-GMSEA (path coefficient, −0.84, $p < 0.001$) according to the SEM. After the strong effects of SOC and MBC on enzyme activity were eliminated by the specific enzyme activity indexes, the study concluded that microorganisms were the main source of enzyme activity change after long-term organic fertilizer improvement of Solonetz.

Nevertheless, more studies are needed analyze absolute and specific enzyme activity from the perspective of C, N, P, and S cycles to fully reveal the pathways by absolute and specific enzyme activity responses in Solonetz. Meanwhile, in order to obtain more stable experimental results, we will continue to monitor changes in soil properties.

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