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Linkages of Enzymatic Activity and Stoichiometry with Soil Physical-Chemical Properties under Long-Term Manure Application to Saline-Sodic Soil on the Songnen Plain

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Abstract: Excess Na^+ and high pH result in poor structures in Saline-Sodic soils, which reduces extracellular enzyme activity (EEA) and causes nutrient limitations. The application of manure improved the Physical-Chemical properties of soil and balanced the soil nutrient supply, which was reflected in the soil EEAs and stoichiometry. Five experimental treatments were designed according to the manure application duration as follows: manure application for 11 years (11a), 16 years (16a), 22 years (22a), and 27 years (27a) and a control treatment with no manure application (CK). The results of the redundancy analysis (RDA) showed that physical properties (mean weight diameter (MWD)) and EEA (β–glucosidase (BG)) significantly increased and bulk density (ρ_b) significantly decreased when the nutrient content increased. Additionally, soil pH, electrical conductivity (EC), exchangeable sodium percentage (ESP) and sodium adsorption ratio (SAR) significantly decreased after manure application. Based on stepwise multiple linear regression models (SMLR), total nitrogen (TN) was the dominant variable that significantly increased EEA, and the Mantel test showed that soil C:N significantly influenced enzyme stoichiometry. Furthermore, RDA showed that pH, soil C:N and TN were the main factors influencing EEAs and enzyme stoichiometry. Soil EEAs significantly increased with TN and decreased with pH and soil C:N, which affected enzyme stoichiometry. The enzyme stoichiometry increased from 1:2.1:1.2 and 1:2.7:1.5 to 1:1.7:1.2, and the vector angle (vector A) increased, which showed that the N limitation was relieved after the application of manure. The vector length (vector L) showed no significant difference in the C limitation at depths of 0–20 cm and significantly increased at depths of 20–40 cm. In conclusion, soil EEAs and stoichiometry improved with changes in TN and soil C:N, and pH decreased with changes in the soil structure after the application of manure, which accelerated the soil nutrient cycle and balanced the soil nutrient supply.

Keywords: solonetz; cattle manure; ecoenzymatic stoichiometry; pH

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1. Introduction

Salinization is a process of soil degradation that causes desertification and arable land loss in arid and semiarid regions [1,2]. In addition, osmotic stress and toxic ions are important factors in the suppression of plant growth [3], and salinization threatens agricultural production and soil ecosystems [2]. The saline-sodic soil on the Songnen Plain has excess exchangeable Na^+ and soluble Na^+ [4]. A review indicated that excess Na^+ dispersed clay particles, resulting in aggregate breakdown [5], which led to poor soil structure and soil organic carbon (SOC) loss [6]. Additionally, EEAs were inhibited by low soil organic matter (SOM) content and poor structure and decomposed under high salt concentrations [7–9]. Yan reported that soil BG and alkaline phosphatase (ALP) were inhibited by salinity, while the microbial community structure changed with salinity [3]. This resulted in enzyme production and aggregate formation affected by microorganisms being further suppressed [10,11]. Sinsabaugh considered soil EEAs to be an indicator of soil nutrient acquisition effectiveness and SOM decomposition [12]. Therefore, saline-sodic soil is generally characterized by low nutrient cycling and availability [13].

Ecoenzymatic stoichiometry reflects the equilibria between the elemental composition of microbial biomass and detrital organic matter and the efficiencies of microbial nutrient assimilation and growth [14]. Hill considered nutrient availability and ecosystem metabolism to be measured by the relative activities of the functional classes of extracellular enzymes, which may be used to assess large-scale phenomena [15]. Previous studies reported that enzyme stoichiometry was influenced by climate along north-south transects in forest ecosystems [16], and dissolved nutrients, microbial biomass and depth affected enzyme stoichiometry [17,18]. A study showed that vegetation type and not climate explained the variation in enzyme stoichiometry in subalpine forests on the eastern Tibetan Plateau [19], which showed that previous studies were inconsistent regarding enzyme stoichiometry. Zhu proposed that soil microorganisms influence C:N stoichiometry by regulating extracellular enzyme production [20]. Mori thought that enzyme stoichiometry reflected microbial nutrient limitations when cellulose was the predominant C source [21]. Thus, cattle manure contained high amounts of cellulose that were suitable to investigate nutrient limitations.

Researchers considered the effects of the amount of SOM in manure, which stimulates aggregate formation and decreases saline-sodic content [4,22,23]. Soil structure, nutrient content and proportions were altered [24–26], and microbial enzymes were included in the manure [27], the application of which was beneficial to soil nutrient release [28–30]. In addition, EEAs and enzyme stoichiometry change with aggregate size fractions [8,31]. Most studies have focused on the effects of different amendments on the physical-chemical properties in saline-sodic soils, and few studies have focused on the relationship between the nutrient limitations and enzyme stoichiometry of soil when the properties of saline-sodic soil are improved after the long-term application of manure. Thus, the ratio of C-, N- and P-acquiring enzyme activity was measured to investigate the soil nutrient limitations after long-term manure application, which may provide insights into the influence of manure application on saline-sodic soils.

The objectives of our study were to examine (1) how the physical-chemical properties of soil influence EEAs and (2) the response of soil enzyme stoichiometry to long-term manure application in saline-sodic farmland soil. We hypothesized that soil EEAs and stoichiometry vary with increasing nutrients and that mainly saline-sodic properties are improved by long-term manure application.

2. Materials and Methods

2.1. Study Site

The experimental field is located in Zhaozhou County, Heilongjiang Province, China (125.06° E, 45.48° N 136 m altitude), and it was used to examine the response of saline-sodic soils to long-term manure application. The area is located west of the Songnen Plain, with an annual average temperature and precipitation of 3.6 °C and 448.9 mm, respectively, and average evaporation of 1657.79 mm. This area has a mean summer temperature, precipitation and evapotranspiration of 22.02 °C, 158 mm and 142 mm, respectively, based on information from the local Agricultural Extension Department. This area has a temperate, semiarid continental monsoon climate. The soil with a dense and strong structure, a clay illuviation horizon and a high proportion of Na_2CO_3 was strongly alkaline, and it was classified as solonetz according to the FAO World Reference Base [32]. The soil consisted of 26% sand, 22% silt and 52% clay, and the native type of vegetation was *leymus chinensis* in this area. The physicochemical properties of the soil at the 0–20 cm depth prior to the experiment (the data were obtained in 1995 when the experimental field was set up) are shown in Table 1.

Table 1. The physicochemical properties of soil prior to experiment at the 0–20 cm depth. EC is electrical conductivity; ESP is exchange sodium percentage; SOM is soil organic matter; TK is total potassium; CEC is cation exchange capacity.

	Soil		**Cattle Manure**
pH	9.50	pH	8.42
EC (dS m^{-1})	6.23	SOM (g kg^{-1})	590.69
ESP (%)	32.03	TN (g kg^{-1})	13.28
SOM (g kg^{-1})	10.95	TP (g kg^{-1})	12.02
TN (g kg^{-1})	0.37	TK (g kg^{-1})	15.35
TP (g kg^{-1})	0.25		
Available N (mg kg^{-1})	39.11		
Available P (mg kg^{-1})	12.06		
Available K (mg kg^{-1})	125.18		
$CaCO_3$ (g kg^{-1})	103.20		
CEC (cmol kg^{-1})	33.78		

2.2. Experimental Design

The experiment consisted of a randomized complete block design. According to the history of manure application, five treatments were set up as follows: manure applied for 27 years (27a), 22 years (22a), 16 years (16a), 11 years (11a) and a control treatment with no manure application (CK). Each plot was 65 m^2 (6.5 m $\times$ 10 m) in size, rain-fed agriculture and no field operations were carried out except continuous corn cultivation, corn residue return and manure application. The corn yield was measured in October 2022 and was 8153 kg hm^{-1} in the experimental plot with no manure application. Before soil ploughing, cattle manure was applied at 10,000 kg/hm^2 (oven-dry weight) in late April each year, and chemical fertilizer in the form of urea was applied at 400 kg/hm^2 in the elongation stage of corn.

2.3. Soil Sampling

In June 2022, four samples of undisturbed soil were randomly collected from each plot at the 0–20 (topsoil) and 20–40 cm (subsoil) depths to assess the effect of long-term manure application on soil EEAs and stoichiometry during the corn growing season while avoiding interference in the soil samples from chemical fertilizer (urea) during the corn elongation stage. The soil samples were transported to the laboratory and divided into two parts. One part of the soil samples was stored at 4 °C and was used to measure EEAs, and the other part of the soil was stored for measuring physicochemical properties after air-drying.

2.4. Measurement of the Physical-Chemical Properties of Soil

Soil water-stable aggregates were measured by the wet sieving method [33]. One hundred grams of air-dried soil was separated into five fractions: >2 mm, 1–2 mm, 0.5–1 mm, 0.25–0.5 mm and <0.25 mm [4]. The MWD and geometric mean diameter (GMD) were calculated as follows:

$$\text{MWD} = \frac{\sum_{i=1}^{n}(W_i X_i)}{\sum_{i=1}^{n} W_i} \quad (1)$$

$$\text{GMD} = \exp\left(\frac{\sum_{i=1}^{n}(W_i LnX_i)}{\sum_{i=1}^{n} W_i}\right) \quad (2)$$

where MWD is the mean weight diameter, mm; GMD is the geometric mean diameter, mm; X_i is the average diameter of the ith size fraction of the aggregates, mm; W_i is the weight of the aggregates in that size as a fraction of the weight.

Soil ρ_b was measured by the core method using the ratio of the oven-dried undisturbed soil core mass to the total volume [34]. Field capacity (FC) was measured by the gravimetric method when the soil and water reached equilibrium via capillary action after the soil cores were soaked for 24 h [34]. Soil particle density (ρ_d) was measured by a pycnometer [34]. The soil total porosity (f_t) was calculated as follows:

$$f_t = \left(1 - \frac{\rho_b}{\rho_d}\right) \times 100 \quad (3)$$

where ft is the soil total porosity (%). ρ_b is the bulk density (g cm^{-3}). ρ_d is the soil particle density (g cm^{-3}).

SOC was determined by dichromate oxidation with heating (K_2CrO_7-H_2SO_4) [35]. Soil total phosphorus (TP) was measured by spectrophotometry after digestion with $HClO_4$-H_2SO_4 [35]. Soil TN was measured by an elemental analyser and the Kjeldahl digestion procedure. Soil C:N was calculated as the ratio of soil organic C to total N; soil C:P was calculated as the ratio of soil organic C to total P; and soil N:P was calculated as the ratio of total N to total P [24].

Soil pH and EC were measured by a pH meter and conductivity meter, respectively, at a soil-to-water ratio of 1:5 [35]. The soil cation exchange capacity (CEC) was measured by a spectrophotometer after extraction with 1 mol/L NaOAc [35]. Soil exchangeable Na^+ was measured by a spectrophotometer after extraction with 1 mol/L NH_4Oac [35]. Soil soluble Na^+ was measured by a spectrophotometer, and Ca^{2+} and Mg^{2+} were measured by atomic absorbance with a 1:5 soil-to-water extract [35]. The soil ESP was calculated as follows:

$$\text{ESP} = \frac{\text{Na}^{+}{}_{\text{Exc}}}{\text{CEC}} \times 100 \quad (4)$$

where ESP is exchangeable sodium percentage (%), $Na^+{}_{Exc}$ is soil exchange Na^+ (cmol kg^{-1}), CEC is cation exchange capacity (cmol kg^{-1}).

Soil SAR was calculated as follows:

$$\text{SAR}_{1:5} = \frac{\text{Na}^{+}}{\left(\frac{\text{Ca}^{2+} + \text{Mg}^{2+}}{2}\right)^{\frac{1}{2}}} \quad (5)$$

where $SAR_{1:5}$ is sodium adsorption ratio ((mmol L^{-1})$^{1/2}$), Ca^{2+}, Mg^{2+} and Na^+ are soluble cations (mmol L^{-1}).

2.5. Enzyme Activity

The activities of four enzymes were measured by the methods of Tabatabai [36], Parham [37] and Rachel [38], i.e., C-acquiring enzyme (β-glucosidase BG), N-acquiring enzyme (β-N-acetyl glucosaminidase NAG leucine aminopeptidase LAP), and P-acquiring enzyme (alkaline phosphatase ALP) activity. Soil samples (0.1 g) were incubated at 37 °C for 1 h after the substrate and buffer were added (Table S1). Then, the filtrate was analysed at 405 nm by a microplate reader using a 96-well plate. The enzyme activity unit was nmol p-NP/p-NA g^{-1} h^{-1}. Enzyme activity was expressed as a logarithm.

The soil enzyme C:N, C:P and N:P ratios were calculated using ln(BG):ln(NAG + LAP), ln(BG):ln(ALP) and ln(NAG + LAP):ln(ALP), respectively [16]. The vector length and vector angle were calculated as follows [39]:

$$\text{Vector } L = \text{SQRT}\left(X^2 + Y^2\right) \quad (6)$$

$$\text{Vector } A = D\text{EGREES}(\text{ATAN2}(X, Y)) \quad (7)$$

where Vector L is the vector length, Vector A is the vector angle, X is the relative C:P-acquiring enzymatic activity (ln(BG):ln(ALP)), and Y is the relative C:N-acquiring enzymatic activity (ln(BG):ln(NAG + LAP)). Vector length quantifies relative C versus nutrient limitation, and the angle quantifies relative N versus P limitation [39].

2.6. Statistical Analysis

One-way analysis of variance (ANOVA) followed by Duncan's multiple comparisons ($p < 0.05$) was used to analyse the differences in the soil physicochemical properties and enzyme stoichiometry for different manure application treatments using SPSS software (version 25). RDA was conducted using CANOCO 5 to identify relationships among the vector model, EEAs, stoichiometry, and the physical-chemical properties of soil. SMLR was used to identify the relationships between EEAs and the physical-chemical properties of soil using SPSS 25. The Mantel test was used to analyse the relationship between soil C:N:P and enzyme stoichiometry, and figures were constructed in the R 4.3.1 software using the "ggcor" package.

3. Results

3.1. Soil Physical Properties

Water-stable aggregate (WSA) size was greatly influenced by the application of manure (Table S2). Macroaggregates (0.25–0.5 mm, 0.50–1.00 mm, 1.00–2.00 mm, >2.00 mm) increased after the application of manure, and that of microaggregates (<0.25 mm) significantly decreased ($p < 0.05$) at depths of 0–20 and 20–40 cm. The highest values of macroaggregates (0.25–0.5 mm, 0.50–1.00 mm and 1.00–2.00 mm) were found in the topsoil and subsoil of the 11a treatment.

The MWD and GMD of WSAs were significantly higher ($p < 0.05$) than those in CK at depths of 0–20 and 20–40 cm (Figure 1a,b). The trend in the values was similar to that of the MWD and GMD of WSAs with the history of years of manure application. The mean values of MWD and GMD in the 11a treatment were the maximum in the topsoil and subsoil, and they were significantly higher than those of 16a, 22a and 27a ($p < 0.05$) in the subsoil, implying that WSA stability was best in the 11a treatment with manure application. However, there was no significant difference in MWD among all manure application treatments at a depth of 0–20 cm and no significant difference in MWD and GMD among the 16a, 22a and 27a treatments at depths of 0–20 and 20–40 cm.

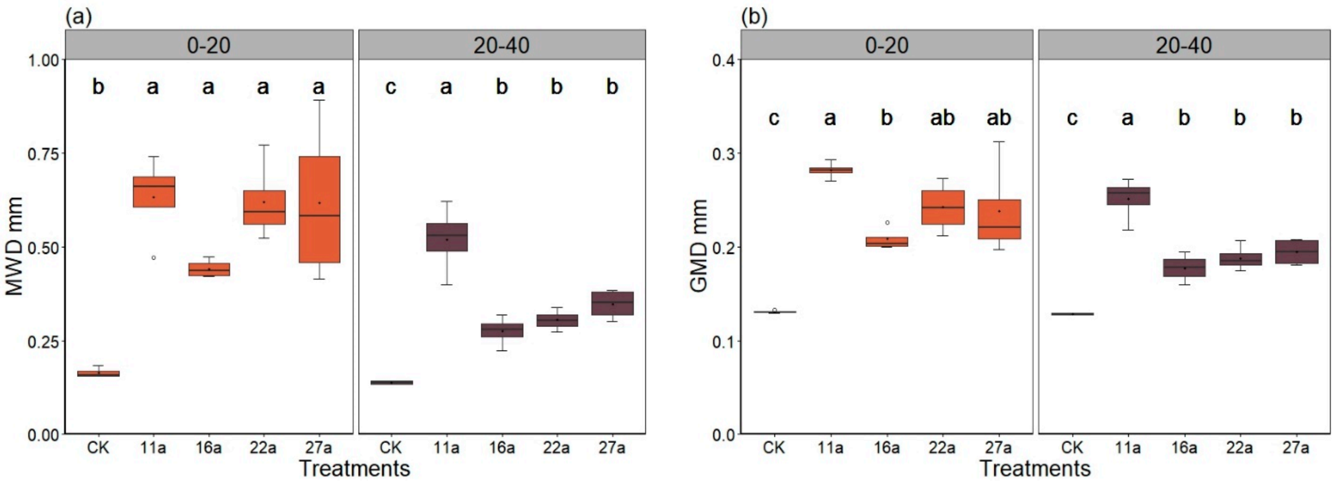

Figure 1. MWD (**a**) and GMD (**b**) under different treatments of application manure. The different letters in the same depth indicate a significant difference at the 0.05 level. Solid and empty circles indicate mean and outlier values, respectively.

Soil ρ_b, FC, ρ_d and f_t at depths of 0–20 and 20–40 cm was greatly affected by the application of manure (Table S3). The value of ρ_b significantly decreased and FC significantly increased ($p < 0.05$) compared with CK at depths of 0–20 and 20–40 cm. The ρ_d significantly decreased and f_t significantly increased ($p < 0.05$) in the topsoil compared with CK. However, there was no significant difference between ρ_d and f_t in the 16a treatment and the CK treatment, and the values in the subsoil of the 11a, 22a and 27a treatments were significantly different from those in CK ($p < 0.05$). The maximum FC and f_t were found in the topsoil in the 11a treatment, and the minimum ρ_b and ρ_d were found in the 27a treatment, whereas there was no significant difference in ρ_b, ρ_d and f_t among all manure application treatments at depths of 0–20 cm.

3.2. Soil pH, Electrical Conductivity, Sodium Adsorption Ratio and Exchangeable Sodium Percentage

The saline-sodic properties, pH, EC, ESP and SAR significantly decreased after manure application compared with CK at depths of 0–20 and 20–40 cm ($p < 0.05$) (Table S4). pH tended to decrease with the history of years of application; i.e., that in 22a and 27a treatments were significantly lower than that in 11a and 16a treatments, while that in 16a treatment was significantly lower than that in 11a treatment ($p < 0.05$). The ESP values at a depth of 20–40 cm in the 16a, 22a and 27a treatments were significantly lower than those in the 11a treatment ($p < 0.05$). However, there was no significant difference in EC and SAR among all manure application treatments at depths of 0–20 and 20–40 cm, and there was no significant difference in ESP amo"g al' manure application treatments in the topsoil.

3.3. Soil SOC, TN and TP Contents and Stoichiometry

SOC, TN and TP were significantly influenced by the application of manure ($p < 0.05$) (Figure 2a–c). SOC, TN and TP significantly increased at depths of 0–20 and 20–40 cm ($p < 0.05$), which indicates that the soil nutrient concentration significantly increased after manure application. The values of SOC and TN in the 11a treatment were the highest and were not significantly different from those in the 22a and 27a treatments at a depth of 20–40 cm. However, there was no significant difference in SOC and TN in the topsoil among all manure application treatments. The value of TP showed no obvious trend, and the 22a treatment had the highest value at depths of 0–20 and 20–40 cm.

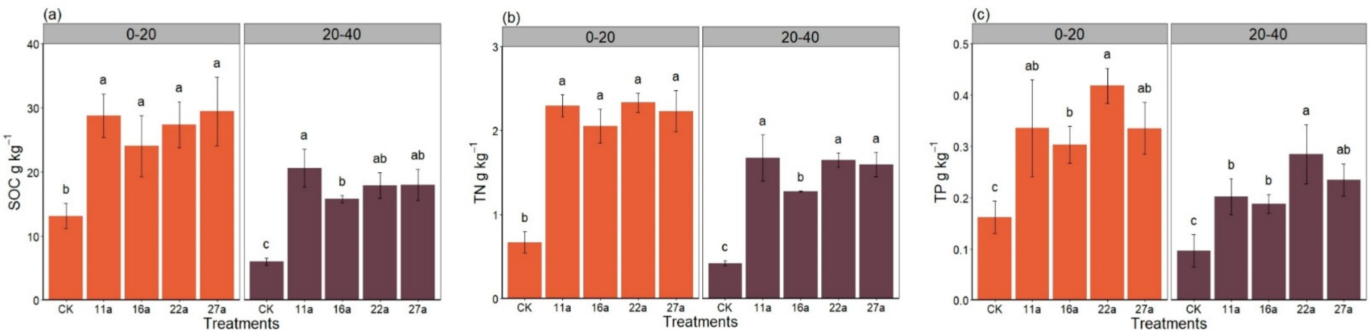

Figure 2. Soil SOC (**a**), TN (**b**) and TP (**c**) concentration under different treatments of application manure. The different letters in the same depth indicate a significant difference at the 0.05 level.

Soil C:N and soil N:P were influenced by the application of manure (Figure 3a,c). However, soil C:P showed no observed trend in response to manure application. At depths of 0–20 and 20–40 cm, soil C:N significantly decreased and soil N:P significantly increased after the application of manure ($p < 0.05$) (Figure 3a,c), which indicated that SOM tended to decompose and that the supply of N and P to plants increased. The value of subsoil N:P was higher than that in the topsoil for all treatments, which was similar to the results for soil C:N and N:P in the manure application treatments, but the opposite trend was observed in the CK treatment.

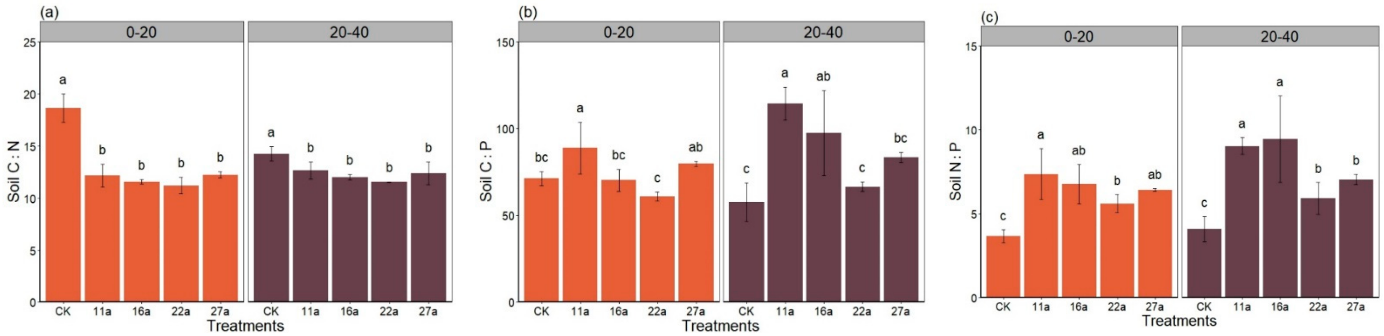

Figure 3. The change in soil C:N (**a**), C:P (**b**) and N:P (**c**) under different treatments of application manure. The different letters in the same depth indicate a significant difference at the 0.05 level.

3.4. Soil Enzyme Activity and Stoichiometry

Soil EEAs increased at depths of 0–20 and 20–40 cm after the application of manure (Figure 4a–c), and the EEAs in the topsoil were higher than that in the subsoil. BG, NAG + LAP, and ALP activities increased compared with CK, while BG and ALP activities significantly increased in all manure application treatments ($p < 0.05$). The maximum NAG + LAP was observed in the 11a treatment, and that in the 11a treatment it was significantly higher than that in the 27a treatment at depths of 0–20 and 20–40 cm ($p < 0.05$). EEAs in the 16a treatment were observed to be minimal, with significant differences in BG, NAG + LAP and ALP in the subsoil in all manure application treatments ($p < 0.05$).

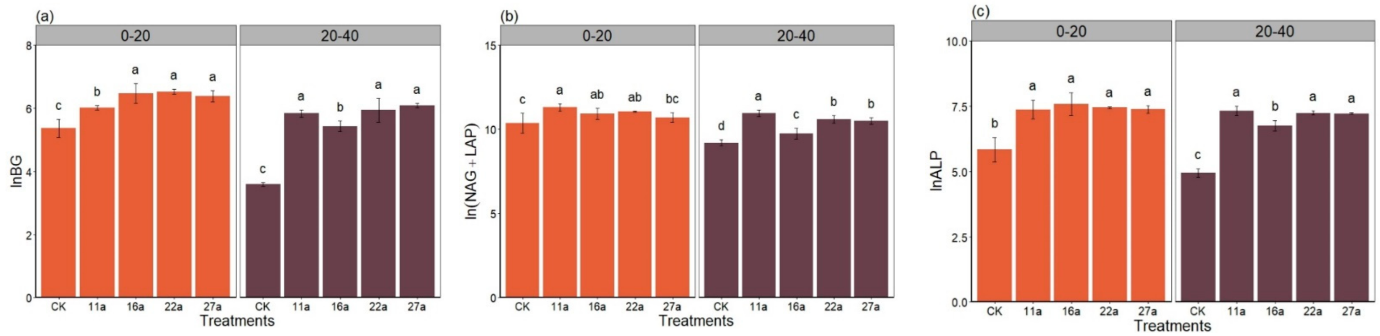

Figure 4. EEAs under different treatments of application manure (lnBG (**a**), lnNAG+LAP (**b**), lnALP (**c**)). The different letters in the same depth indicate a significant difference at the 0.05 level. BG, β-glucosidase; NAG, β-N-acetyl glucosaminidase; LAP, leucine aminopeptidase; ALP, alkaline phosphatase. The value of enzyme activity was expressed with logarithm.

The enzyme C:N and N:P were greatly affected by the application of manure (Figure 5a,c). The enzyme C:N at depths of 0–20 and 20–40 cm increased after manure application (Figure 5a). The enzyme C:N in the topsoil in the 16a, 22a and 27a treatments and in the subsoil in all manure treatments significantly differed from that in CK. The enzyme C:P was not significantly different among all treatments at a depth of 0–20 cm, but the value significantly increased compared with that in CK at a depth of 20–40 cm ($p < 0.05$) (Figure 5b). The enzyme N:P significantly decreased at depths of 0–20 and 20–40 cm after the application of manure ($p < 0.05$) (Figure 5c). However, there was no significant difference among all manure application treatments for the soil C:N, C:P and N:P at 20–40 cm.

3.5. Vector Model

Vector length was not significantly different among all treatments at a depth of 0–20 cm. However, the vector length significantly increased compared with that of CK, and the maximum was in the 27a treatment at a depth of 20–40 cm ($p < 0.05$) (Figure 6a). The vector angle significantly increased at depths of 0–20 and 20–40 cm after the application of manure

($p < 0.05$) (Figure 6b). The maximum vector angle was observed in the 16a treatment at depths of 0–20 and 20–40 cm. This result implied that N limitation was relieved due to the application of manure, and no C limitation occurred in the topsoil. However, SOC supplementation was not sufficient in the subsoil.

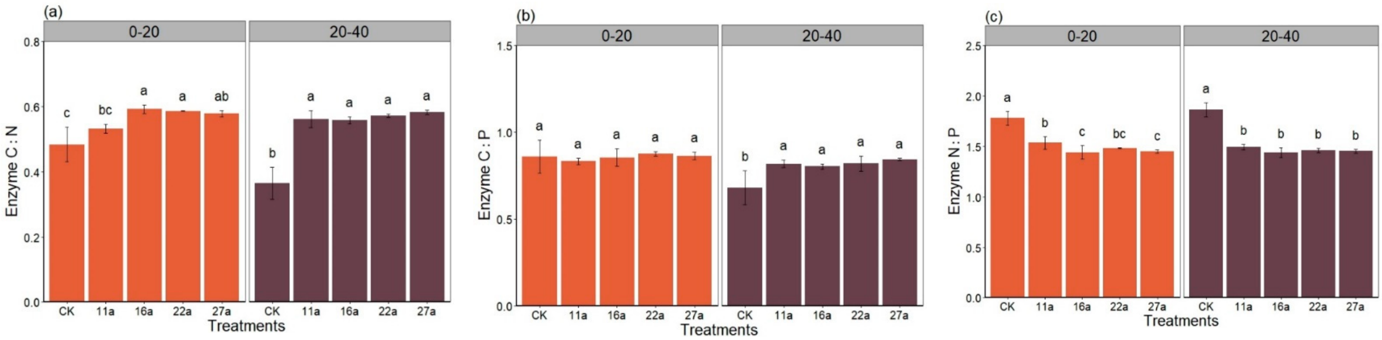

Figure 5. EEAs stoichiometry under different treatments of application manure (Enzyme C:N (**a**), Enzyme C:P (**b**), Enzyme N:P (**c**)). The different letters in the same depth indicate significant difference at the 0.05 level. Enzyme C:N, lnBG:ln(NAG + LAP); Enzyme C:P, lnBG:lnALP; Enzyme N:P, ln(NAG + LAP):lnALP.

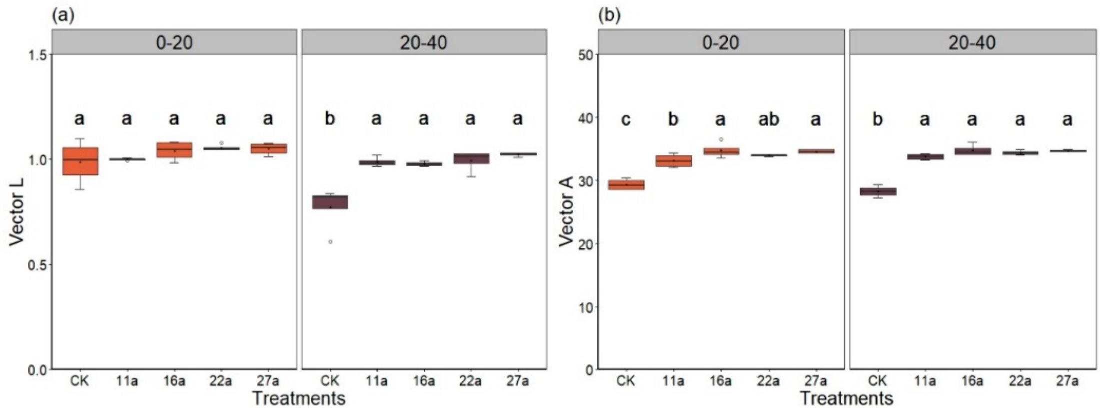

Figure 6. Vector model under different treatments of application manure (Vector L (**a**) and Vector A (**b**)). The different letters in the same depth indicate a significant difference at the 0.05 level. Solid and empty circles indicate mean and outlier values, respectively. Vector L is vector length. Vector A is vector angle.

3.6. The Correlations among the Physical-Chemical Properties and Enzyme Activity and Stoichiometry of Soil

The RDA showed that the physical properties (MWD and ρ_b) and EEA (BG) of soil were significantly influenced by TN, SOC and TP ($p < 0.05$), while TN was the main factor that affected the physical-chemical properties of soil (Figure 7a). The variations in the physical-chemical properties (EC, ρ_b, GMD and SAR) and EEA (BG) of soil significantly affected soil C:N, C:P and N:P ($p < 0.05$), and EC was the main driving factor, explaining 42.80% of the variation (Figure 7b).

RDA showed the correlation of the physical-chemical properties and EEAs of soil, which indicated that TN was the main factor that influenced EEAs ($p < 0.05$). In contrast, pH and soil C:N were negatively correlated with EEAs (Figure 7c). Enzyme stoichiometry was significantly influenced by sodic-saline properties (pH, SAR, ESP and EC), soil C:N and TN ($p < 0.05$) (Figure 7d). The pH explained 59.00% of the variation, which implied that enzyme stoichiometry was mainly affected by pH (Figure 7d).

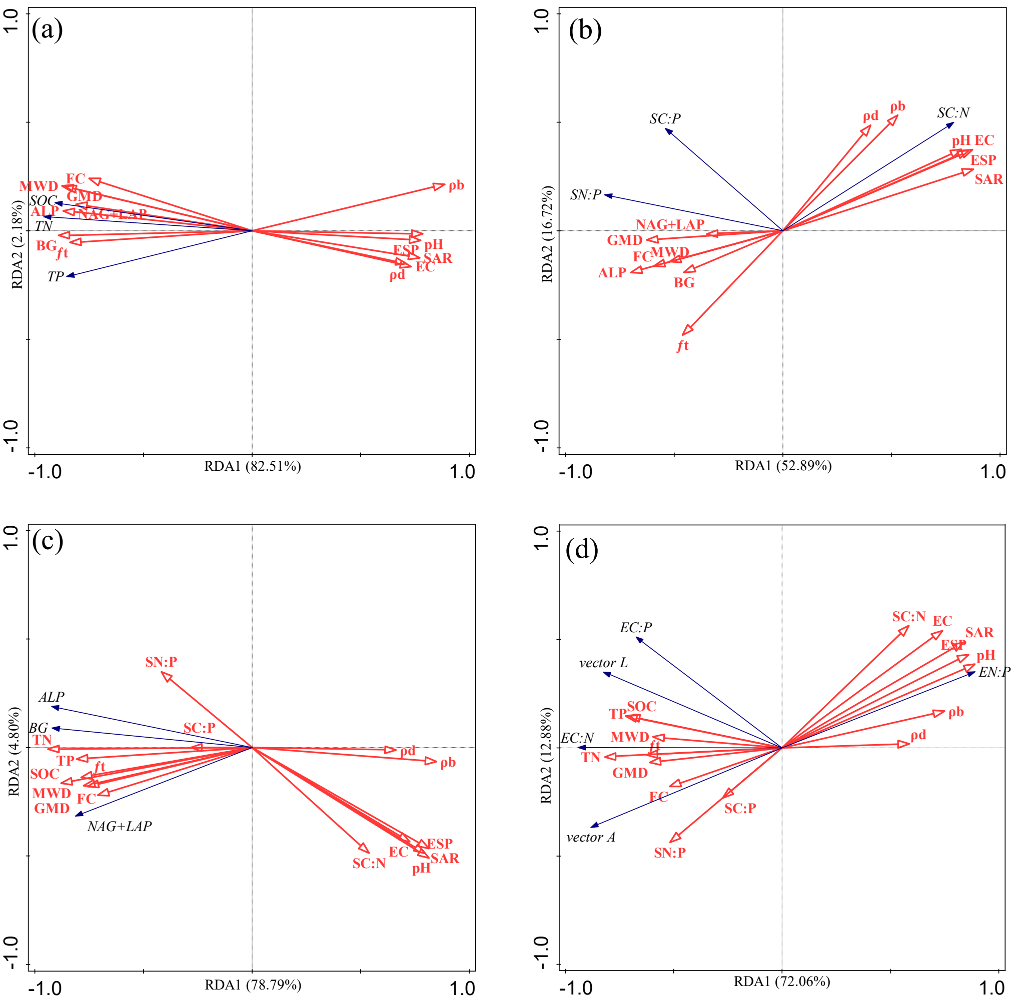

Figure 7. The redundancy analysis (RDA) used to identify the relationship among EEAs, stoichiometry and physical-chemical properties of soil. (**a**) The relationship between nutrient (blue arrows) and physical-chemical properties and EEAs of soil (red arrows). (**b**) The radio of soil nutrient (blue arrows) and physical-chemical properties and EEAs of soil (red arrows). (**c**) EEAs (blue arrows) and physical-chemical properties of soil (red arrows). (**d**) EEAs stoichiometry (blue arrows) and physical-chemical properties of soil (red arrows). The ρ_b, bulk density; FC, field capacity; ρ_d, soil particle density; f_t, soil total porosity; MWD, mean weight diameter; GMD, geometric mean diameter; SOC, soil organic carbon; TN, total nitrogen; TP, total phosphorus; SC:N, soil C:N radio; SC:P, C:P radio; SN:P, N:P radio; EC, electrical conductivity; ESP, exchangeable sodium percentage; SAR, sodium adsorption ratio; BG, β-glucosidase; NAG, β-N-acetyl glucosaminidase; LAP, leucine aminopeptidase; ALP, alkaline phosphatase. The value of enzyme activity was expressed with logarithm. EC:N, lnBG:ln(NAG + LAP); EC:P, lnBG:lnALP; EN:P, ln(NAG + LAP):lnALP; vector L, vector length; vector A, vector angle.

SMLR showed that soil BG, NAG + LAP and ALP had significantly positive effects on TN ($p < 0.01$) (Table S5). BG was mainly influenced by FC. Soil C:N greatly affected the activities of NAG + LAP and ALP. Meanwhile, ALP was suppressed by SAR.

The Mantel test showed that soil C:N:P greatly influenced enzyme C:N:P and affected soil microbial nutrient limitations (Figure S1). Enzyme C:N was significantly negatively influenced by soil C:N and positively influenced by soil N:P. Enzyme N:P was significantly positively influenced by soil C:N and negatively influenced by soil C:P and N:P. Enzyme C:P and the vector L had no significant effect on soil C:N:P. Vector A was significantly influenced by soil C:N and N:P.

4. Discussion

4.1. The Effect of Manure Application on the Physical-Chemical Properties of Soil

The results showed that BG, MWD and ρ_b were mainly affected by the application of manure, which improved the soil nutrient content (TN and SOC) (Figure 7a). Compared with the value prior to the experiment, SOM and TN increased, while TP decreased in the CK treatment at the 0–20 cm depth. These results implied that plant roots may influence the physical structure of soil, saline-sodic properties and SOM accumulation [40,41]; however, this influence of the physical-chemical and biological properties of soil was not sufficient to affect P release in this study. Starke found that the N cycle was dominated by bacteria and that the C cycle was controlled by fungi [42]. Thus, fungi and bacteria may be stimulated by TN, and SOC increases after the application of manure, which facilitates macroaggregate formation due to physical entanglement by fungal mycelium and binding by bacteria amino sugars [10,11]. Rabbi reported that WSAs formed from decomposed SOM, and microbial carbon was the cementing agent [43]. Thus, BG activity increased the decomposition of cellulose from manure, which increased microbial carbon acquisition and accumulation, increasing the formation of microbial carbon-cemented aggregates [43]. The results are consistent with those reported by Wang and Das [44,45], who found that macroaggregates and stability increased with the application of manure in rice-barley rotation and maize cropping systems. However, this study showed that macroaggregates and stability were reduced when the duration of manure application was longer than 11 years, with limited comparison to other studies, and aggregate fraction had no significant difference when the duration of manure application was longer than 16 years. Researchers stated that SOM was occluded within macroaggregates during the initial period and then ultimately sequestered within microaggregates [46,47]. Thus, microaggregates might increase due to SOM changes. Tian considered macroaggregate formation to be a dynamic process in which the aggregates formed, degraded and formed anew [48].

Regelink considered soil f_t to be determined by WSAs [49], and Schjønning reported that ρ_d was predicted by clay and SOM that influenced WSAs [50]. RDA showed that MWD and GMD increased f_t and decreased ρ_b (Figure 7a), which caused the rate of soil infiltration and saline leaching to increase, consistent with the findings reported by Regelink, Kim and Zhang [49,51,52]. Ions (Na^+) were leached, which caused the pH, EC [23] and ESP to significantly decrease. The Na^+ loss, and Ca^{2+} increased, which caused the SAR to significantly decrease [23]. Meanwhile, RDA indicated that the saline-sodic content (pH, EC, ESP and SAR) negatively affected WSA stability (MWD and GMD) (Figure 7a). Researchers have reported that pH, EC and SAR affect clay dispersion [53,54], which influences aggregate formation.

4.2. Factors Driving Enzyme Activity and Stoichiometry

This study demonstrated that EEAs increased with the application of manure (Figure 4a–c). The results are consistent with those reported by Wang [55] and Das [44], who stated that EEAs increased with the application of manure in tobacco and maize cropping systems. The results showed that BG, NAG + LAP and ALP were significantly correlated with TN ($p < 0.01$) (Table S5 and Figure 7c), which indicated that EEAs were mainly influenced by N and implied that microbial activities increased with the N content [56,57]. Chen thought that the C cycle was primed by exogenous organic C and controlled by N [58]. Studies showed that the addition of N increased BG activity [58,59]. However, the result was inconsistent with that reported by Wang [8]; N supply supressed BG activity, which may be related to the form of N [60]. The results indicated that soil C:N was significantly negatively correlated with enzyme C:N and positively correlated with enzyme N:P (Figure 7d and Figure S1). The results implied the indirectly effect on EEAs when soil C:N was regulated by soil ρ_b and GMD (Figure 7b,c). Thus, NAG + LAP activity decreased when the duration of manure application was longer than 11 years, which was likely resulted by GMD decreased. This result was similar with Huang [31], NAG activity in the macroaggregates was higher than microaggregates.

The resource allocation model considers that extracellular enzymes was regulated microbial communities to acquire maximum resources [61]. Thus, EEAs is highest when soil C:N matches microbial demands [58].

SMLR showed that soil C:N was significantly positively correlated with ALP and NAG + LAP ($p < 0.01$) (Table S5), and RDA showed that soil C:N was significantly correlated with EEAs ($p < 0.01$) (Figure 7c). Liu reported that high exogenous organic C:N addition led to low microbial biomass N, which increased N acquisition enzymes [62]. Das indicated that P-acquiring enzymes increased and reported that the N content dominated ALP activity [44].

Li found that the BG, NAG + LAP, and ALP activities increased shortly after manure application but decreased over time, which is inconsistent with our results [25]. It may be that EEAs are also influenced by pH and EC [9,12]. RDA showed that pH was significantly correlated with EEAs ($p < 0.01$) (Figure 7c), similar to the results of Chen and Xu [63,64]. Soil pH is an important factor influencing EEAs. Studies have reported that EEAs decrease with increasing pH, which may be due to changes in enzyme solubility and structure in response to EC and pH [7,65]. SMLR showed that ALP was significantly negatively affected by SAR ($p < 0.01$), and BG and NAG + LAP were not significantly different. Researchers have evaluated the sensitivity of enzymes to pH [12,66]. The saline-sodic content decreased, which changed the enzyme C:N:P.

SMLR showed that the BG was significantly influenced by FC, which indicated that the BG was more sensitive to water content. Steinweg found that BG was influenced by moisture because of diffusion limitations [67].

4.3. Soil C, N or P Limitation after Manure Application

The RDA results showed that enzyme stoichiometry and the parameters of the vector model were significantly correlated with pH, soil C:N, TN, SAR, ESP and EC (Figure 7d). The results indicated that the dominant factors driving enzyme stoichiometry were soil nutrients and saline-sodic properties because pH and salinity directly suppressed EEAs to different degrees. Meanwhile, the soil C:N:P greatly affected enzyme stoichiometry (Figure S1).

Sinsabaugh found that the mean ratio for lnBG:ln(NAG + LAP):lnALP was near 1:1:1 in all habitats [14]. This study showed that enzymes with C:N and C:P < 1 responded to microbial N and P limitations relative to C, and N:P > 1 indicated microbial N limitation relative to P [14]. Thus, the results showed that enzyme C:N increased from 1:2.1 and 1:2.7 to 1:1.7, and the vector A < 45° also indicated that microbial N limitation was relieved, although a shortage of N remained after the application of manure. The vector L was not significantly different among all treatments at depths of 0–20 cm, which indicated that C limitation did not occur after the application of manure. BG activity may have increased with sufficient SOC after the application of manure, but there was a lack of SOC at a depth of 20–40 cm. This result was similar to that of Chen [59]; BG activity and C limitation increased with N availability when the C content was not sufficient. Microbial nutrient demand increased with soil microbial activities, which was reflected in the BG activity, and this implied that SOC continued to leach downwards, but the content was not sufficient to match microbial C demand. The results also showed C limitation at the 20–40 cm depth. Thus, the topsoil was not saturated with respect to SOC due to the high C demand that SOC continued to leach downwards. Meyer considered C saturation to result in soil SOC loss, and the SOC loss increased with the degree of saturation [68]. However, the value of SOC had no significant difference among all application manure treatments, indicating that SOC was not saturated and lost from the topsoil. Compared with the 11a and 16a treatments, this result indicated that macroaggregate turnover or low structural protection capacity resulted in C loss (SOC saturation), which may have taken place at 20–40 cm depth. Fontaine revealed that soil C loss increased when microbial C limitation [69] was affected by spatial patterns [69]. EEAs (BG, NAG + LAP and ALP) and aggregate stability (MWD and GMD) decreased, indicating that SOC may indirectly influence EEAs but not at a significant level. Meanwhile, enzyme stoichiometry and nutrient limitations had no significant effect on the change in SOC and aggregate stability.

The enzyme C:P was 1:1.2 in all treatments at a depth of 0–20 cm but significantly increased from 1:1.5 to 1:1.2 at a depth of 20–40 cm, which implies that available P increased in the subsoil and had no obvious influence on the topsoil after the application of manure. Enzyme N:P decreased from 1:0.6 and 1:0.5 to 1:0.7, which implies that N limitation relative to P was relieved after the application of manure. Yan explained that Ca-P increased after the application of manure and hardly transformed into available P at high pH [70]. Thus, N availability or activation was greater than that of P after the application of manure.

5. Conclusions

The results from our study demonstrated that the physical-chemical properties of saline-sodic soil were improved by the application of manure, and the physical-chemical properties of soil plateaued when the history of the application of manure was longer than 16 years. Soil MWD, ρ_b and BG were crucial variables that influenced soil nutrient content. Macroaggregates and stability as well as BG activity increased with nutrient content, which improved the saline-sodic properties. Meanwhile, BG activity increased showed that microbial saline-sodic stress was reduced. Based on RDA, pH, TN and soil C:N were dominant factors that affected soil EEAs and enzyme stoichiometry. EEAs and enzyme stoichiometry increased with increasing TN, and the pH decreased after the application of manure. In addition, the results of this study showed that the application of manure relieved N limitation and that the nutrient supply tended to be balanced. Additionally, the enzyme stoichiometry results indicated that soil nutrients were not balanced and that C and N limitations existed. Further studies are needed to focus on the effects of manure amendments on the soil N content and C:N in saline-sodic soils.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy13122972/s1, Figure S1. The relationship between soil C:N:P and enzymatic stoichiometry. SC:N, soil C:N radio; SC:P, C:P radio; SN:P, N:P radio; EC:N, lnBG:ln(NAG + LAP); EC:P, lnBG:lnALP; EN:P, ln(NAG + LAP):lnALP; vector L, vector length; vector A, vector angle. Table S1. The substrate and butter pH of enzyme assay. Table S2. Soil water-stable aggregates size under different treatments of application manure. Mean value ± standard deviations in the same column and depth followed by the different letters indicate significant difference at the 0.05 level (n = 4). Table S3. The soil physical properties under different treatments of application manure. Mean value ± standard deviations in the same column and depth followed by the different letters indicate significant difference at the 0.05 level (n = 4). Table S4. Soil saline-sodic properties under different treatments of application manure. Mean value ± standard deviations in the same column and depth followed by the different letters indicate significant difference at the 0.05 level (n = 4). Table S5. The relationship between soil physical-chemical properties and EEAs. Stepwise multiple linear regression models (SMLR) used to identify the relationship between EEAs and soil physical-chemical properties. TN, total nitrogen; FC, field capacity; SC:N, soil C:N radio; SAR, sodium adsorption ratio. BG, β-glucosidase; NAG, β-N-acetyl glucosaminidase; LAP, leucine aminopeptidase; ALP, alkaline phosphatase. The value of enzyme activity was expressed with logarithm.

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Abbreviations

ρb, bulk density; FC, field capacity; ρd, soil particle density; *f*t, soil total porosity; MWD, mean weight diameter; GMD, geometric mean diameter; SOC, soil organic carbon; TN, total nitrogen; TP, total phosphorus; EC, electrical conductivity; ESP, exchangeable sodium percentage; SAR, sodium adsorption ratio; BG, β-glucosidase; NAG, β-N-acetyl glucosaminidase; LAP, leucine aminopeptidase; ALP, alkaline phosphatase; vector L, vector length; vector A, vector angle; RDA, redundancy analysis; SMLR, stepwise multiple linear regression.

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