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Effects of returning organic waste on soil enzymes and microbial quantity in dryland farming**

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A b s t r a c t. The application of animal manure combined with straw is an important strategy for sustainable agricultural production. The objective of this study was to investigate the effects of animal manure combined with straw on soil enzymes and microbial quantity. The two-year experiment involved four treatments: maize straw only, maize straw plus ox manure, maize straw plus chicken manure, and maize straw plus pig manure. In 2018 and 2019, treatments with animal manure combined with straw led to increased levels of soil microbial quantity, soil enzyme activity, and yields. Compared to other treatments, higher catalase activities were achieved in both years for the maize straw plus ox manure and maize straw plus pig manure treatments whereas the cellulase activities were higher for the maize straw plus ox manure and maize straw plus chicken manure treatments. The maize straw plus ox manure treatment had the highest number of soil bacteria, and the quantity of actinomycetes was higher after the applications of maize straw plus ox manure and maize straw plus chicken manure as compared to the other treatments. Moreover, compared to the application of maize straw only, treatment maize straw plus pig manure had the most significant effect on the soil urease activity, invertase activity, fungal quantity, and maize yield with 43.9, 35.9, 52.0, and 31.7% increases, respectively. In conclusion, our findings suggested that animal manure combined with straw, especially the application of maize straw plus pig manure was the most effective treatment for enhancing soil enzymes and microbial quantity and also promoting maize yield.

K e y w o r d s: animal manure, maize straw, enzyme activities, microbial quantity

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INTRODUCTION

Corn straw and animal manure are high-quality organic materials which contain a large amount of organic matter as well as nitrogen (N), phosphorus (P), and other trace elements necessary for plant growth and agricultural sustainability (Kumar *et al*., 2018). Statistical data have shown that every year, about 700 million tonnes of crop straw and 3.8 billion tonnes of animal manure are produced as agricultural waste in China (Wang *et al*., 2017; Wang *et al*., 2020). The burning of straw and the inappropriate disposal of animal manure leads to a waste of resources and environmental pollution (Ji, 2015). Returning straw to the field is an effective practice that facilitates the management of agricultural residues (Blumfield *et al*., 2003). Hou *et al*. (2012) reported that the application of straw mulch is a traditional agricultural practice used to reduce evaporation and improve crop yields, however, the traditional ways of applying mulch do have some shortcomings, such as the slow decomposition rate of the straw which leads to a low emergence rate for seedlings (Hu *et al*., 2016). Lafond *et al*. (2009) found that straw mulch had no significant effects on crop production and soil quality. Therefore, this research seeks to test a new method of returning straw, which may serve to alleviate the shortcomings of the traditional straws techniques in order to improve sustainable agricultural production.

Soil enzymes are involved in the biogeochemical cycle of most nutrient elements, the decomposition and synthesis of humus, and the transformation of organic compounds (Benavente *et al*., 2018). They play an important catalytic role in ecosystem metabolism, which is not only an important index of soil fertility but also one of the most important indicators of soil metabolism (Dominik *et al*., 2018; Gajda *et al*., 2013). Previous studies have illustrated that the application of N fertilizer, as well as returning straw to the soil can be used to promote enzyme activities (Zhao *et al*., 2016; Jian *et al*., 2016). Some studies have also shown that organic fertilizer is more efficient at stimulating soil enzymes, while inorganic fertilizers had a weaker effect on soil enzyme activities (Yu *et al*., 2012; Zhang *et al*., 2015). In terrestrial ecosystems, soil microorganisms play important roles in the biogeochemical cycling of soil nutrients and the decomposition of organic matter (Cusack *et al*., 2011; Mierzwa-Hersztek *et al*., 2019). The number of soil bacteria, fungi, and actinomycetes can be used to determine the total distribution of soil microorganisms and the decomposition and transformation of organic matter (Zimmerman, 2010). Soil urease activity is an important factor in the evaluation of the soil N status (Albiach *et al*., 2000) while soil invertase is an important hydrolytic enzyme which can increase the content of soluble nutrients in the soil and can also be used to assess the soil carbon (C) transformation process (Plaza *et al*., 2004). Soil urease and invertase play an important role in the release of simple N and C sources for the multiplication and growth of soil microorganisms (Antonious *et al*., 2020). Zhang *et al*. (2016) demonstrated that the application of straw greatly enhanced the activities of soil urease and invertase. Soil cellulase is an enzyme that can accelerate straw decomposition, thereby releasing C compounds, it contributes to the formation of humus to improve soil fertility (Han and He, 2010). Karami *et al*. (2012) showed that the application of crop straw increased soil nutrient levels and soil enzyme activity, it also provided a favourable chemical, physical and biological soil environment, which had a positive effect on crop yield and soil productivity. The application of animal manure, as well as straw, contributes to the renewal of soil organic matter which in turn changes the characteristics and amount of soil C and N components (Gao *et al*., 2018). Jiao *et al*. (2011) observed that returning straw to the soil promoted the microbial population and microbial biomass C or N, thereby providing a favourable environment and energy for the accumulation of soil enzymes. Cima *et al*. (2015) demonstrated that the application of manure contributed to an increase in soil organic C. Zhu *et al*. (2010) reported that crop yields were significantly increased by straw incorporation based on an eight-year field experiment in China. Several studies have reported the effects of straw or manure incorporation on the properties of soil, but there are few studies to date concerning the biological results of simultaneously returning straw and different animal manures to the soil in dryland farming.

This study aims to determine the combined effect of animal manure and straw on soil enzyme activity levels, microbial quantity, and maize yield in the field, to serve as a reference for agricultural residue management.

MATERIALS AND METHODS

The experimental site was located in Liaoyuan County, Jilin Province, northeastern China (42°50′55″N, 125°20′31″E). The region is very cold during winter and hot during summer, having a temperate continental monsoon climate. The average annual temperature is 5.4°C, and the mean annual precipitation value is 666.5 mm. The frost-free period is approximately 140 d, and the average yearly sun exposure is 2 507 h. The soil is classified as dark-brown soil, the term used is Cryumbreps in the American soil classification system, and Humic Cambisols in WRB with a pH of 6.3. The total organic C, total N, alkali-hydrolysable N, available P, and available K in the 0-20 cm soil layer are 12.3 g kg^{-1}, 1.3 g kg^{-1}, 100.4 mg kg^{-1}, 20.3 mg kg^{-1}, and 125.1 mg kg^{-1} respectively. Artificial irrigation was not applied during the experiment although the location is classified as a dryland area.

The straw was returned to the field by applying the straws in strips. In this method, 20 cm deep furrows were first created using a plow. Afterwards different animal manures were added to the furrows (according to the respective treatment plot) together with the straw, and finally, the incorporated organic materials were covered by the surrounding soil. Each year (2018 and 2019) the straw and/or animal manure were incorporated in the field before maize was sown in spring. Maize was planted in the ridges during the planting season.

The field was arranged in a randomized block design consisting of four treatments in three replicates. Each plot had a dimension of 10 m × 5 m and the treatments were maize straw only (S), maize straw plus ox manure (SO), maize straw plus chicken manure (SC), maize straw plus pig manure (SP). The chicken manure, ox manure, and pig manure were collected from chicken farms, ox farms, and pig farms in Liaoyuan County and they were composted for a few months before application. The basic properties of the organic materials used in this study are shown in Table 1. The basic properties of the soil after the application of animal manure combined with straw (AM-S) are shown in Table 2.

In this experiment, the same amount of maize straw (3 088 kg C ha^{-1}) was applied to each plot for each year. The application of animal manure was adjusted so that equal amounts of carbon (7 738 kg C ha^{-1}) may be applied to each plot in 2018 and 2019. The S treatment received 10 826 kg C ha^{-1} (from only straw) while the other treatments received 3 088 kg C ha^{-1} from straw and 7 738 kg C ha^{-1} from manure each. The application rate for animal manure was 32 500 kg ha^{-1} for chicken manure, 25 123 kg ha^{-1} for ox manure, and 24 333 kg ha^{-1} for pig manure.

Table 1. Basic properties of organic materials

Material	Maize straw	Ox manure	Chicken manure	Pig manure
Organic C (g kg^{-1})	423.05±1.93a	308.15±2.10c	238.61±3.09d	313.54±2.19b
Total N (g kg^{-1})	6.52±0.46d	13.25±0.64c	15.77±0.58b	17.20±1.01a
C/N	65.11±4.47a	23.29±0.97b	15.14±0.37c	18.27±0.95c
Lignin (%)	6.32±0.20b	7.23±0.11a	3.21±0.24d	5.09±0.31c
Cellulose (%)	32.28±0.64a	23.53±1.40b	7.04±0.18d	14.41±0.24c
Hemicellulose (%)	22.37±1.10a	15.38±0.46b	4.26±0.12d	13.24±0.30c
Polyphenol (%)	0.87±0.02a	0.73±0.10b	0.68±0.06b	0.69±0.07b
Lignin/N	9.71±0.38a	5.47±0.35b	2.04±0.21d	2.97±0.35c
Soluble substance (%)	32.00±1.15d	42.24±0.51b	40.24±0.29c	47.56±0.50a

Data with the same letter within the same row do not differ significantly at the 5% level. Mean ± standard error. C – carbon, N – nitrogen.

Table 2. Basic properties of soil after the application of animal manure combined with straw

Treatments	pH	SOC (g kg^{-1})	DOC (mg kg^{-1})	EOC (g kg^{-1})	MBC (mg kg^{-1})	TN (g kg^{-1})	AN (mg kg^{-1})
S	6.42±0.43d	14.66±0.30c	138.70±6.54c	1.35±0.07d	89.54±3.37d	1.36±0.07b	118.31±4.74c
SO	6.58±0.52c	16.07±0.32b	150.57±8.13b	3.46±0.09b	124.55±5.13a	1.44±0.12b	129.60±8.09b
SC	6.71±0.91b	17.32±0.51a	154.32±7.78ab	3.19±0.09c	98.13±2.03c	1.66±0.07a	153.94±3.84a
SP	6.84±0.64a	16.61±0.40b	167.01±7.64a	3.80±0.09a	115.73±3.96b	1.58±0.07a	139.63±3.10b

SOC – soil organic carbon, EOC – easily oxidizable carbon, DOC – dissolved organic carbon, MBC – microbial biomass carbon, TN – total nitrogen, AN – available nitrogen, S – maize straw only, SO – maize straw plus ox manure, SC – maize straw plus chicken manure, SP – maize straw plus pig manure. Other explanation as in Table 2.

Soil samples were collected in October 2018 and 2019. Five soil cores were randomly collected from each plot following the “S” method at a depth of 0-20 cm. All samples were packed into aseptic PET resin bags, placed in iceboxes and taken to the laboratory for further processing. The soil samples were immediately stored in a refrigerator at 4°C. One part was used for soil enzyme analysis and the other part was used for soil microbial quantity determination.

The moisture content was determined using the oven-drying method, that is, samples were dried in an oven at a temperature of 105°C until a constant mass was reached. The activities of soil urease and catalase were determined following the methods described by Zhang *et al.* (2011). The activity of soil invertase was determined according to methods described by Parthasarathi and Ranganathan (2000). The activity of soil cellulase was measured using 3,5-dinitrosalicylic acid colorimetry (Breuil and Saddler, 1985). The blank (reagents) and reference samples (only soil without reagents) were used to measure soil enzyme activity (as mentioned in the sample test). The activity of soil cellulase was measured by weighing 5 g of a soil sample into a shaking bottle and adding 10 mL of carboxymethyl cellulose solution, 5 mL acetate buffer (pH 4.8) and 0.1 mL toluene. After the sample was fully mixed, it was placed in an incubator at 37°C for 72 h. The glucose released by cellulase was measured by 3,5-dinitrosalicylic acid colorimetry in a spectrophotometer at 540 nm. In order to analyse the urease activity in the soil, urea solution was used as a substrate. Fresh soil (5 g) was incubated at 37°C with 5 mL of substrate and 5 mL of citrate buffer (pH of 6.7) for 24 h. The reaction mixture was diluted to 50 mL with distilled water. The urea was hydrolysed to ammonium nitrogen through the catalysis of urease. The level of ammonium was measured in a spectrophotometer at 578 nm. The activity of soil urease was determined from the filtered mixture using the phenol-sodium hypochlorite colorimetric method (Zhang *et al*., 2011). The activity of soil catalase was measured by weighing 2 g of fresh soil into a shaking bottle and adding 40 mL of distilled water and 5 mL of 0.3% H_2O_2. After the sample was fully mixed, it was placed in an oscillator and shaken for 20 minutes. After filtration, 5 mL of 3 mol L^{-1} sulfuric acid was added to the filtrate, and the filtrate was titrated with 0.1 mol L^{-1} $KMnO_4$ (Zhang *et al*., 2011). In order to analyse the activity of the soil invertase, a glucose solution was used as the substrate wherein a 5 g soil sample was incubated at 37°C with 15 mL of the substrate, 0.1 mL toluene, and 5 mL phosphate buffer (pH 5.5). The glucose released by invertase was determined using 3,5-dinitrosalicylic acid colorimetry (Parthasarathi and Ranganathan, 2000). The glucose was analysed colorimetrically at 508 nm.

The activity of the soil enzyme was expressed in terms of the dry soil weight. The activities of soil urease, catalase, invertase, and cellulase were expressed in terms of mg NH_4^+-N g^{-1} soil 24 h^{-1}, 0.1 mol L^{-1} $KMnO_4$ g^{-1} soil 20 min^{-1}, mg glucose g^{-1} soil 24 h^{-1}, mg glucose kg^{-1} soil 72 h^{-1}, respectively. The activities of soil urease, invertase, and cellulase were calculated using the following Eq. (1),

$$E = (a - b - c)\frac{n}{m}, \tag{1}$$

where: E is the activity of the soil enzyme; a, b, c are the contents of the measured substance (mg) corresponding to the optical density values of the test sample, reagent, and reference sample on the standard curve respectively; n is the split multiple; m is the mass of the soil sample (g).

The activity of soil catalase was calculated using the following Eq. (2),

$$The\ activity\ of\ soil\ catalase = (A - B)\frac{T}{M}, \quad (2)$$

where: A is the amount of $KMnO_4$ (mL) consumed by the titrating reagent, and B is the amount of $KMnO_4$ (mL) consumed by the titrating soil sample, T is the correction value of the $KMnO_4$ titration, M is the mass of the soil sample (g).

The microbial quantity was determined using the plate count method. The bacteria were cultivated in a beef-protein medium at 37°C for 7 d, the actinomycetes were cultivated at 28°C for 7 days using GAUZE´s medium and the fungi were cultivated in Martin medium at 28°C for 7 days (Zhou, 1993). The number of microbial colonies was counted and recorded in order to calculate the number of microorganisms per gram of dry soil (cfu g^{-1} dry soil).

The three central rows of maize with a length of 5 m for each plot were manually harvested, then the grain yield was determined at 12% grain moisture content.

Statistical analyses were carried out using the SPSS 17.0 statistical software (SPSS). The means and standard errors for three replicates were calculated. A one-way analysis of variance (ANOVA) was used to evaluate the differences in the selected parameters (e.g. the properties of organic materials and soil, soil enzymes, microbial quantity and yield). A two-way ANOVA was carried out to assess the effect of the experimental year and the various treatments on microbial quantity, soil enzyme activities and yield. Pearson's correlation analysis was used to investigate the relationship between microbial quantity, soil enzyme activities, and yield. Multiple comparisons were carried out using Duncan's new multiple ranges test. Significant differences between treatments were set at confidence levels of $p<0.05$ and $p<0.01$.

RESULTS

Compared with the S treatment, higher yields ($p<0.01$) were achieved in the treatments with AM-S (Fig. 1). The yields for 2018 and 2019 followed the order SP>SO>SC>S, and were 5.9-12.0% higher in 2019 than in 2018. Compared with the S treatment, the AM-S-treated plots significantly increased the yield by 17.8-31.0% in 2018 and 24.0-32.4% in 2019 ($p<0.05$). In 2018, compared with SO and SC, the yield from SP was 5.4 and 11.2% higher respectively, whereas in 2019, the yield from SP was 0.7 and 6.7% higher than from SO and SC, respectively (Fig. 1).

The enzyme activity for the different treatments is shown in Fig. 2. Compared with S, higher urease activities were observed for the AM-S treatments ($p<0.01$). The urease activity levels followed the order SP>SC>SO>S in both 2018 and 2019 and were 17.1-41.8% higher in 2019 than in 2018. Urease activity with the SP, SO, and SC treatments were 56.1, 51.6, and 37.0% higher than those with the S treatment in 2019, respectively ($p<0.01$).

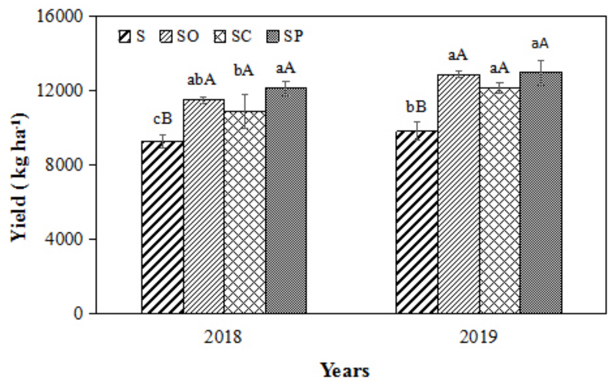

Fig. 1. The maize yields after the application of maize straw and manure in 2018 and 2019. Note: Bars represent the standard errors of three replications. Different lowercase and capital letters above the bars in the same year indicated significance among the treatments at $p<0.05$ and $p<0.01$, respectively. S – maize straw only, SO – maize straw plus ox manure, SC – maize straw plus chicken manure, SP – maize straw plus pig manure.

Compared to 2018, the catalase content was greater in 2019. The soil catalase contents were ranked as follows: SO>SP>SC>S in both 2018 and 2019. Compared to the S treatment, the catalase content in the SO treated soils significantly increased by 37.7 and 51.6% in 2018 and 2019, respectively ($p<0.01$).

The invertase activity of soil significantly increased in the AM-S treatments compared to the S ($p<0.01$), and the activity of invertase followed the order SP>SC>SO>S in both years and were 18.9-30.6% higher in 2019 than in 2018. In 2018 and 2019, the invertase activity in SP was 38.9 and 32.8% higher than that of S, respectively ($p<0.01$).

The soil cellulase activity followed the order SO>SC>SP>S in 2018 and 2019 and was 6.9-13.0% higher in 2019 than in 2018. The cellulase activity of SO, SC, and SP were 45.0, 26.9, and 8.6% higher respectively compared with S in 2019.

The effects of AM-S on the soil microbial quantity are shown in Table 3. Compared to S, the AM-S treated soils significantly increased their microbial quantity in both years. In 2018 and 2019, the highest quantity of soil bacteria was recorded for the SO treatment. Moreover, the fungal quantity in the SP, SC and SO treated soils were 52.0, 18.4, and 40.1% higher than for the S treatment in 2019, respectively. Compared with S, the quantity of actinomycetes after the applications of SO and SC increased by 57.8 and 87.9% respectively, while SP had little impact on their quantity in 2019.

The two-way ANOVA results showed that the experimental year and the treatments were the main factors influencing the microbial quantity, soil enzyme activities, and yield parameters (Table 4). The AM-S treatments had a significant effect on microbial quantity, soil enzyme activity, and yield ($p<0.01$). Similarly, the effect of the experimental years was significant for yield, soil enzyme activities, and fungal quantity ($p<0.01$). The interactions

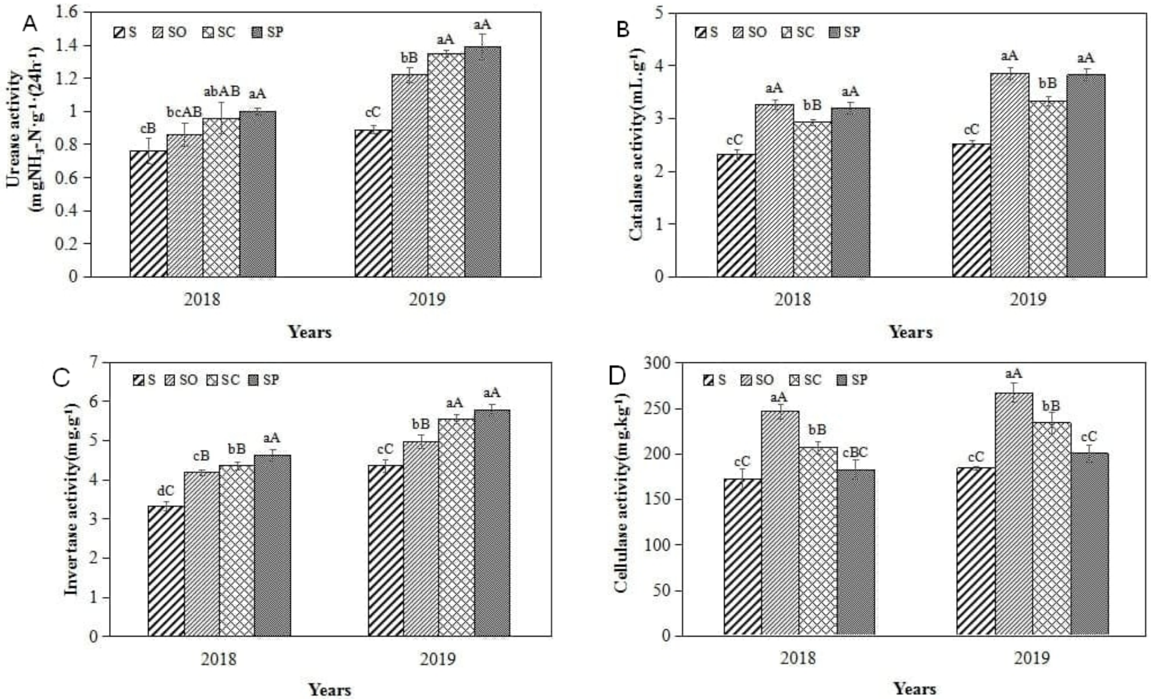

Fig. 2. Soil enzymatic activities after the application of maize straw and manure in 2018 and 2019. Note: A, B, C, and D represent soil urease activity, catalase activity, invertase activity, and cellulase activity respectively. For significant difference among treatments, seen in Fig. 1. S – maize straw only, SO – maize straw plus ox manure, SC – maize straw plus chicken manure, SP – maize straw plus pig manure.

Table 3. Effect of animal manure combined with straw on the number of microorganisms in the soil

Treatments	Bacterial quantity ($\times10^6$ cfu g^{-1} soil)		Fungal quantity ($\times10^3$ cfu g^{-1} soil)		Actinomyces quantity ($\times10^4$ cfu g^{-1} soil)	
	2018	2019	2018	2019	2018	2019
S	2.4±0.1d	2.5±0.2c	3.6±0.2c	4.5±0.4d	4.3±0.3d	4.2±0.2d
SO	8.4±0.1a	8.0±0.5a	5.6±0.3b	6.3±0.1b	6.3±0.1b	6.6±0.2b
SC	4.8±0.5c	5.0±0.4b	5.2±0.2b	5.3±0.2c	7.3±0.1a	7.9±0.2a
SP	7.4±0.2b	7.8±0.1a	6.5±0.2a	6.8±0.1a	5.3±0.1c	5.2±0.1c

Other explanation as in Table 1 and 2.

Table 4. Effects of treatments, year of experiment, and their interactions (two-way ANOVA) on bacterial quantity, fungal quantity, actinomyces quantity, soil urease activity, catalase activity, invertase activity, cellulase activity and yield

Main effects	Yield		Urease activity		Catalase activity		Invertase activity		Cellulase activity		Bacterial quantity		Fungal quantity		Actinomyces quantity	
	F value	Sig.	F value	Sig.	F value	Sig.	F value	Sig.	F value	Sig.	F value	Sig.	F value	Sig.	F value	Sig.
Treatments (T)	44.59	**	45.90	**	283.90	**	153.72	**	85.89	**	515.48	**	154.49	**	237.33	**
Years (Y)	24.79	**	168.71	**	219.85	**	473.14	**	26.63	**	1.02	ns	28.75	**	4.54	*
T × Y	0.89	ns	6.48	**	10.05	**	3.73	*	0.71	ns	1.93	ns	3.88	*	2.89	ns

* significant at $p<0.05$, ** significant at $p<0.01$, ns – not significant, n = 48.

Table 5. Correlations among microbial quantity, soil urease activity, catalase activity, invertase activity, cellulase activity and yield

	Yield	Urease	Catalase	Invertase	Cellulase	Bacterial quantity	Fungal quantity	Actinomycetes quantity
Yield	1.000							
Urease	0.813**	1.000						
Catalase	0.949**	0.803**	1.000					
Invertase	0.751**	0.928**	0.699*	1.000				
Cellulase	0.594*	0.369	0.597*	0.207	1.000			
Bacterial quantity	0.900**	0.708**	0.973*	0.617*	0.586*	1.000		
Fungal quantity	0.858**	0.714**	0.921**	0.701*	0.385	0.953**	1.000	
Actinomycic quantity	0.517	0.618*	0.437	0.493	0.745**	0.316	0.162	1.000

Date were collected in 2019. * significant at $p<0.05$, ** significant at $p<0.01$, n= 24.

between the treatments and experimental years were significant at $p<0.01$ for soil urease activity and catalase activity, and at $p<0.05$, the interactions had a significant effect on invertase activity and fungal quantity.

The correlation results showed that yield was positively correlated with urease activity ($r = 0.813$, $p<0.01$), catalase activity ($r = 0.949$, $p<0.01$), invertase activity ($r = 0.751$, $p<0.01$), cellulase activity ($r = 0.594$, $p<0.05$), bacterial quantity ($r = 0.900$, $p<0.01$), and fungal quantity ($r = 0.858$, $p<0.01$) (Table 5). Moreover, the urease activity exhibited a positive correlation with the catalase activity, invertase activity, bacterial quantity, and fungal quantity ($p<0.01$). Similarly, catalase activity also showed a positive correlation with bacterial quantity, invertase activity, cellulase activity ($p<0.05$), and fungal quantity ($p<0.01$). In addition, a positive relationship was found between the invertase activity, bacterial quantity, and fungal quantity ($p<0.05$).

DISCUSSION

Some studies have shown that the incorporation of organic wastes and maize straw applications into the soil increases soil fertility and crop yields (Maltas *et al*., 2018, Mandal *et al*., 2013). In our study, the AM-S treatments increased maize yield, this was possibly due to the use of animal manure as an additional C source. In addition, other properties of the soil such as pH and available C and N content in the soil (Table 2), structure, and microbial activity were enhanced to create favourable soil conditions supporting maize yield (Li *et al*., 2020). The higher yield in SP treatment may be due to the fact that pig manure contains more organic carbon and soluble components (Table 1), which are more accessible to microorganisms to improve soil conditions for crop growth (Wu *et al*., 2017).

Various soil enzymes are important indicators for soil biochemistry and fertility and are involved in the conversion processes and biological cycling of soil nutrients (Maxwell *et al*., 2020). In our study, when compared with S, the AM-S treatments greatly increased the activities of soil enzymes under straw and manure. The activity of urease in the soil can be used to represent the N supply capacity of the soil, and the improvement of urease is conducive to the conversion of organic N in the soil to available N which may be absorbed and utilized by plants (Cao *et al*., 2016). In our study, urease activity under S treatment was relatively low, while the AM-S treatments had a higher urease activity. Previous studies showed that the application of organic manure increased the content of urease in the soil, which was similar to the results achieved by our study (Ghollarata and Raiesi, 2007). The enhancement of soil urease activity in SC and SP was mainly due to chicken and pig manure which is rich in nitrogen and provides an adequate substrate for urease-producing microorganisms (Wei *et al*., 2010). Moreover, chicken and pig manure have a low C/N ratio (Table 1), hence the incorporation of straw regulated the C/N ratio thereby creating favourable conditions for the improvement of microbial activities and enzyme activity (Zhang *et al*., 2020).

Catalase is an important redox enzyme in the soil which can decompose hydrogen peroxide in the soil and prevent crops from being poisoned (Huang *et al*., 2014). In 2019, catalase activity was higher than it was in 2018, which may be due to the accumulation of nutrients after the return of animal manure and straw to the field in each year (Samuel *et al*., 2008). The ox manure accelerated soil C and N cycling and provided a significant amount of nutrients and C for catalase-producing microorganisms thereby improving the soil quality and catalase activities (Bowles *et al*., 2014). Soil catalase activity is associated with soil organic matter content, respiration, and the activity of the soil microbial community (Brzezińska *et al*., 2005). The activity of catalase in the SC treatment was lower, which may be due to the fact that chicken manure contains relatively less organic matter and fewer catalase-producing microorganisms compared to pig and ox manure. Zhang *et al*. (2016, 2020) similarly attributed the increases in soil enzymatic activities to increases in soil carbon content due to the addition of organic fertilizer (Table 2).

Soil invertase is directly involved in the metabolism of organic matter and can be used to represent the soil fertility index and soil biological activity (Plaza *et al*., 2004). In this study, compared with other treatments, the highest activity of soil invertase was recorded under SP. This may be because pig manure contains more organic C, which increases the amount of soil organic matter and substrate for enzymes after its application to the field, thus enhancing enzyme activity (Bocar *et al*., 2009). Similarly, Wei *et al*. (2015) and Sharma *et al*. (2020) respectively reported increases in soil invertase and enzymatic activity due to an increase in soil organic matter and organic C content.

Cellulase is particularly important in the C cycle (Gander *et al*., 1994). In our study, it was found that compared with other treatments, the activity of soil cellulase under the SO treatment was higher. This may be due to the release of cellulose after the application of SO thereby promoting the enhancement of cellulase activity. This improved the cellulose decomposition rate which in turn lead to the production of more glucose and other metabolites to provide more and better nutrients for maize growth (Shi *et al*., 2019). The organic waste treatments were applied annually, the materials that failed to decompose from 2018 would later decompose in 2019 to improve the soil environment, enhance microbial activity, and thus increase enzyme activity and yield. This phenomenon also applies to the control group though only straw was applied.

The activity of soil enzymes is closely related to the activities of microbial communities and has become an important indicator of microbial ecological status (Yan *et al*., 2018). In the present study, the AM-S-treated soils significantly increased the microbial quantity. This corresponds to the small size of the microbial community which is responsible for microbiological changes in the environment. The utilization of chemical nutrients and rich organic matter produced by the farm manure may improve soil conditions, further increase the number of soil microorganisms and the activity of enzymes, and thus accelerate the straw nutrient release rate (Singh *et al*., 2016).

The findings of this study confirmed the relationship between the soil microbial quantity and enzymatic activities and crop yield as it had been ascertained by previous studies (Karami *et al*., 2012; Tripathy and Singh, 2004). In our study, soil enzymes (invertase, urease, and catalase) had a close relationship with soil bacterial quantity and fungal quantity. This indicated that the changes to soil microorganisms were closely related to soil enzyme activity (Evgenia and Yakov, 2013). In this paper, the yield was positively correlated with soil microbial quantity and enzyme activity and also the application of AM-S increased microbial quantity and soil enzyme activity, thus increasing the yield. This shows that improving soil microbial and enzymatic activities through the application of organic wastes enhances the soil conditions for crops thereby improving the growth and yield of crops. The results of this study may help to alleviate the shortcomings of the traditional modes of straw application and also provide a reference for straw and animal manure management. Further research should characterize soil microbial communities after the application of animal manure and straw using next-generation sequencing and help to identify their relationships with labile organic C fractions in the soil.

CONCLUSIONS

1. In our two-year study, the combination of animal manure with straw improved soil enzyme activity, microbial quantity, and the yield of maize, however, the effects varied in each treatment.

2. The catalase activities were higher for the maize straw plus ox manure and maize straw plus pig manure treatments, while the highest cellulase activities were recorded in the maize straw plus ox manure and maize straw plus chicken manure treatments. The invertase and urease activities in maize straw plus pig manure were higher compared to other treatments.

3. A higher quantity of actinomycetes was achieved for the treatments amended with maize straw plus chicken manure and maize straw plus ox manure as compared to other treatments. The highest number of soil bacteria was recorded in the maize straw plus ox manure treatment.

4. Maize straw plus pig manure had the highest maize yield and significantly improved the fungal quantity, urease activities, and invertase activities. We recommend the application of pig manure combined with straw as the most effective agronomic practice for improving the activity of soil enzymes, microbial quantity, and crop yields.

5. Only the microbial quantity of soil was tested in the present study, therefore, future research will be required to identify the microbial community using next-generation sequencing. This will provide more information concerning changes to the microbial community occurring as a result of the application of various treatments.

Conflict of interest: The authors declare no conflict of interest.

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