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Dynamic Change of Soil Enzyme Activities and Soil Microbe During Rice Main Growth Stages in Different Long-term Fertilizer Regimes

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Soil microbe and soil enzyme activities are closely related to the fertilizer inputs. Our objective was to explore the dynamic change of soil microbe and soil enzyme activities in paddy field during early and late rice (*Oryza sativa* L.) main growth stages with different long-term fertilizer managements in the double cropping rice system. we analyzed the soil microbe and soil enzyme activities with mineral fertilizer alone (MF), rice residues and mineral fertilizer (RF), 30% organic matter and 70% mineral fertilizer (LOM), 60% organic matter and 40% mineral fertilizer (HOM), and without fertilizer (CK) basis on long-term fertilizer experiment. The results showed that the soil enzyme activities were increased by application of mineral fertilizer along with manure or rice residues at the main growth stages of early and late rice. The soil urease activities for different fertilizer managements was RF>HOM>LOM>MF>CK at the main growth stages of early and late rice. The soil catalase activities with HOM, LOM treatments was highest, and was significantly higher than that of RF, MF, CK treatments at the main growth stages of rice. The soil invertase and dehydrogenase activities with HOM treatment were highest, and were significantly higher than that of CK at the main growth stages of rice. And the soil cellobiohyrolase activities with RF treatment were highest, and were significantly higher than that of MF, CK treatments at the main growth stages of rice. Meanwhile, the results indicated that different fertilization managements were significantly affected some physiological function soil microbial quantity. The amount of nitrifying and denitrification bacteria in soil for different fertilizer managements was HOM>LOM>RF>MF>CK at the main growth stages of early and late rice. Meanwhile, the amount of ammonifiers and cellulose"decomposing bacteria in soil for different fertilizer managements was MF>RF>LOM>HOM>CK and LOM>RF>MF>HOM>CK at the main growth stages of rice, respectively. The amount of azotobacteria in soil with RF treatment was highest, and was significantly higher than that of MF, CK treatments at the main growth stages of rice. As a result, the soil microbe and soil enzyme activities were increased by applied of fertilizer practices, the effect of mineral fertilizer combined with manure or rice residues is better than that of only mineral fertilizer.

Keywords: Rice; Paddy field; Long-term fertilizer management; Soil microbe; Soil enzyme activity.

Enzymes is an index of soil microbial activity and fertility for that it was respond to changes in soil management more quickly than other soil variables, and they play an important role in the cycling of nutrients in nature. Therefore, it was useful as early indicators of biological changes [1]. It was involved in energy transfer, environmental quality and crop productivity [2,3]. The number and activities of soil enzymes were influenced by soil microorganisms, plants and animals, and is closely related to environmental and ecological factors. Among the different enzymes in soils, the soil urease, catalase, invertase, dehydrogenase and

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cellobiohyrolase activities were important for the soil nutrients, for that they are play a key role in C, N, P cycling in ecological systems.

Soil microbial communities also play a critical role in the cycling of carbon and nutrients in terrestrial ecosystems, as well as regulating plant productivity and diversity [4]. Therefore, there has a closely relationship between soil functions and its community structure, activity [5,6]. Meanwhile, the communities and activities of soil bacterial were affected by the physicochemical properties of the soil, such as the pH [7], water content [8], mineral composition, fertilizer managements [9] and planting systems [10].

In recent years, many studies have indicated that the enzyme activities and microbial communities of soil are known to be affected by the field managements, including the vegetation types and crop rotations [11], soil tillage [12], fertilization regime [13,14] and so on. Meanwhile, fertilization is an important factor that influences the soil enzyme activities and diversity and growth of microorganisms in agricultural soils.

However, relatively few studies have investigated how different fertilizer managements affect the soil enzyme activities and soil microbes in double cropping rice system paddy field in southern China. It is a traditional practice that application with organic manure used as the main nutrient source for rice production in China. In recently yeas, it was commonly accepted that application of mineral fertilizer along with rice residues practices in rice production systems enhance the soil quality [15]. Therefore, the rice residues returning to the field practices is another important source of nutrients during rice production [16]. However, in recent years, there has been a large increase in the use of mineral fertilizer and decrease in the use of organic fertilizer in rice production. Therefore, the soil quality was decreased in this fertilizer regime, such as decrease in soil organic matter (SOM) content and soil microbial community [17]. Rather than applying with mineral fertilizer alone, the fertilizer regime of application mineral fertilizer with manure or rice straw is benefit for maintain both soil quality and high grain yields in double-cropping production systems.

The double-cropping production systems is the main crop rotation in southern China, and the fertilizer regime are very important for rice production and the paddy agroecosystems, however, limited information about the changes of soil enzyme activities and soil microbe under different long-term fertilization schemes in the double-cropping rice systems in southern China. Therefore, the objective of the present research was to study the soil enzyme activities and soil microbe at the early and late rice main growth stages as affected by long-term fertilization managements.

MATERIALS AND METHODS

Sites and cropping system

The experiment was established in 1986. It was located in Ningxiang County (28°07 N, 112° 18 E) of Hunan Province, China. Under a continental monsoon climate, the annual mean precipitation is 1553 mm and potential evapotranspiration of 1354 mm. The monthly mean temperature is 17.2°C. Soil texture in the plough layer (0–20 cm) was silt clay loam with 13.71% sand and 57.73% silt. At the beginning of the study, the surface soil characteristics (0–20 cm) were as follows: soil organic carbon (SOC) 29.4 g/kg, total N 2.0 g/kg, available N 144.1 mg/kg, total phosphorous (P) 0.59 g/kg, available P 12.87 mg/kg, total potassium (K) 20.6 g/kg, and available K 33.0 mg/kg. There were three crops in a year, barley (*Hordeum vulgare* L.), early rice and late rice (*Oryza sativa* L.). Barley was sown in the middle of November and harvested in early May of the following year. Early rice was then transplanted and harvested in the middle of July. The growing season of late rice from late July to the end of October.

Experimental design

The experiment had five treatments: control (without fertilizer input, CK), mineral fertilizer (MF), rice residue and mineral fertilizer (RF), low manure rate and mineral fertilizer (LOM), and high manure rate and mineral fertilizer (HOM). The design ensured all fertilized treatments received the same amount of N, phosphorus pentoxide (P_2O_5), potassium oxide (K_2O) (the amount of N, P_2O_5, K_2O in mineral fertilizer plus that from rice residue or manure) during the early and late rice growing season, respectively. The mineral fertilizers included urea, ordinary superphosphate and potassium chloride. Details about the fertilizer managements are listed in Table 1. Before transplanting rice seedlings, air-dried rice residue was manually spread onto the soil surface and incorporated into the soil at a cultivation depth of 20 cm. For early and late cropped rice, 70% and 60%, respectively, of mineral N fertilizer was applied at seedling and the remaining N fertilizer was applied by top dressing (7–10 days after transplanting) during crop growth. All the P and K fertilizers were applied at seedling. There were three replications and each plot size was 66.7 m^2 (10 × 6.67 m).

Soil sampling

Soil samples were collected from the plow layer (0–20 cm) at seedling stage, tillering stage, jointing stage, heading stage, and mature stage of the early and late rice growing season in 2016, respectively. Three soil samples were taken from each plot at the main growth stages of early and late rice. The soil samples were passed through a 2-mm sieve and kept moist in a refrigerator at 4°C until use.

Laboratory analyses

Enzyme activities

The urease and invertase activity was measured according to Kandeler et al.(2006) [18], and the invertase activity results were expressed as mg/(g soil•h) glucose released. Urease activity is calculated by the conversion of NH_4-N after 24 h and colorimetric assessment at 690 nm.

Catalase activity was measured using the method of Roberge(1978) [19]. Based on the H_2O_2 transformation efficiency, the residual H_2O_2 was determined by titration with potassium permanganate, and catalase activity was expressed as mL $KMnO_4$ consumed per gram per hour. Dehydrogenase activity was measured using triphenyl tetrazolium chloride (TTC) colorimetric analysis [3]. The enzyme substrate TTC is degraded into triphenyl formazan (TPF), which is quantified colorimetrically at 485 nm.

Soil cellobiohyrolase was measured by 3, 5-dinitrosalicylic acid regent. Briefly, 5 g of air-dried soil (<1 mm), 15 mL of 8% sucrose, and 5-mL phosphate buffer at pH 5.5 were added to a 50-mL conical flask with 0.2-mL toluene. After shocking, the suspensions were incubated for 24 h at 37°C. Following filtration, 1-mL filtrate and 3-mL 3,5-dinitrosalicylic acid were added to a 50-mL flask and heated for 5 min at 100°C. The solution was immediately cooled and diluted to 50 mL with distilled water. Absorbance was then measured at 508 nm using a spectrophotometer [20].

Soil microbial populations

The number of culturable nitrifying and denitrification bacteria, ammonifiers bacteria and azotobacteria in a sample was determined by counting colony forming units (CFU). The number of culturable nitrifying and denitrification bacteria, ammonifiers bacteria and azotobacteria were measured using the method of Xu and Zheng (2006) [21]. The density of culturable cellulose-decomposing bacteria was determined as colony forming units (CFU) using a soil dilution plate-count technique [22].

Statistical analysis

All data were expressed as mean ± standard error. The data were analyzed as a randomized complete block, using the PROC ANOVA procedure of SAS [23]. Mean values were compared using the least significant difference (LSD) test, and a probability value of 0.05 was considered to indicate statistical significance.

RESULTS

Dynamics of soil urease activities

At the early rice main growth stages, the activities of soil urease were affected by the different long-term fertilizer managements. The RF treatment has the highest soil urease activities and the CK had the lowest activities (Fig. 1), and the activity increased in the following order: CK<MF<LOM<HOM<RF. At the late rice main growth stages, the highest activity was the treatment that the rice straw was used as residues, and the activity trend was as follows: CK<MF<LOM<HOM<RF.

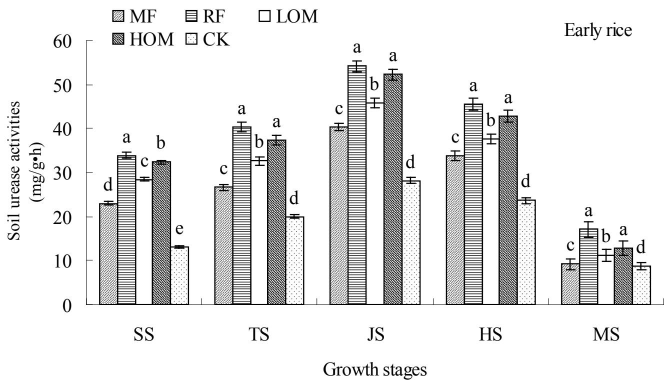
Dynamics of soil catalase activities

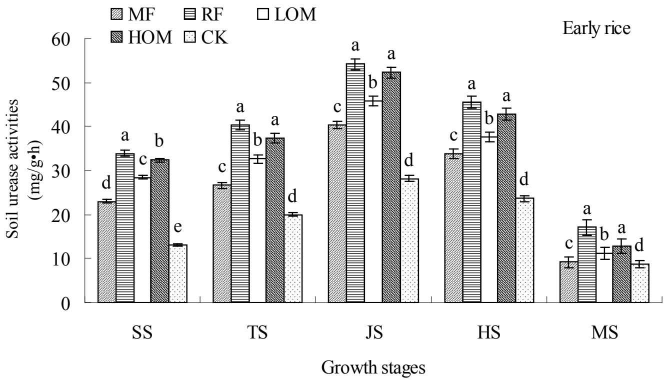
With the addition of the rice residue and manure, soil catalase activities in the HOM, LOM and RF treatments were higher than that of in CK (Fig.2). In other words, the soil catalase activities were enhanced by application of mineral fertilizer along with manure or rice residues during the rice growth season. In the early and late rice seasons, soil catalase activities decreased as follows: HOM>LOM>RF>MF>CK. And there were no significant differences (P>0.05) in soil catalase activities under the HOM and LOM treatments at the early and late rice main growth stages. In the early and late rice seasons, the soil catalase

Table 1. Nutrient supply from rice straw, manure and mineral fertilizer under different fertilizer treatments

Treatment	Early rice			Late rice			Total		
	N	P_2O_5	K_2O	N	P_2O_5	K_2O	N	P_2O_5	K_2O
CK	0+0*	0+0	0+0	0+0	0+0	0+0	0	0	0
MF	142.5+0	54.0+0	63.0+0	157.5+0	43.2+0	81.0+0	300.0	97.2	144.0
RF	124.4+18.1	50.4+3.6	38.3+24.7	133.0+24.5	37.8+5.4	48.2+32.8	300.0	97.2	144.0
LOM	96.0+46.5	33.0+21.0	33.6+29.4	110.2+47.3	21.8+21.4	51.1+29.9	300.0	97.2	144.0
HOM	49.6+92.9	12.0+42.0	4.2+58.8	63.0+94.5	0.5+42.7	21.2+59.8	300.0	97.2	144.0

MF: mineral fertilizer alone; RF: rice residues and mineral fertilizer; LOM: 30% organic matter and 70% mineral fertilizer; HOM: 60% organic matter and 40% mineral fertilizer; CK: without fertilizer.
* Input from mineral fertilizer + input from rice residue or manure. The numbers are in kg/ha.
For the RF treatment, rice straw return rate (air dry) was 2780, 3600 kg/ha for early and late rice.
For the LOM treatment, manure application rate (decomposed) was 2625.0, 2670.0 kg/ha for early and late rice.
For the HOM treatment, manure application rate (decomposed) was 5250.0, 5340.0 kg/ha for early and late rice.
The N, P, and K content of air-dry early rice straw was 6.5 g/kg, 1.3 g/kg, and 8.9 g/kg, N, P, and K content of air-dry late rice straw was 6.8 g/kg, 1.5 g/kg, and 9.1 g/kg, respectively, and N, P, and K content of decomposed chicken manure was 17.7 g/kg, 8.0 g/kg, and 11.2 g/kg, respectively.





MF: mineral fertilizer alone; RF: rice residues and mineral fertilizer; LOM: 30% organic matter and 70% mineral fertilizer; HOM: 60% organic matter and 40% mineral fertilizer; CK: without fertilizer.
SS: seedling stage; TS: tillering stage; JS: jointing stage; HS: heading stage; MS: mature stage.
Bars represent standard deviation (S.D.) of three replicates. Different letters indicate significance at $P < 0.05$ among the fertilization treatments at the same rice growth stage, according to the least significant difference test.

Fig. 1. Effects of different long-term fertilizer treatments on soil urease activities during rice growth stages in paddy field

activities of different treatments at different growth stages was in the range of 15.23–41.07 and 16.48–42.12 0.1 mol/L $KMnO_4$ ML/(g•h), respectively. The highest activities were detected at the tillering stage.

Dynamics of soil invertase activities

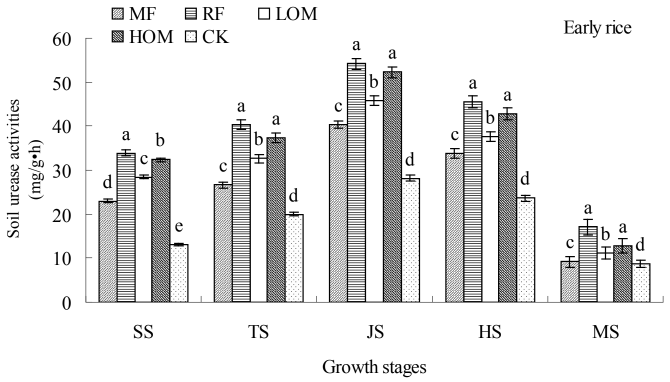
At the early and late rice main growth stages, soil invertase activities with different treatments was in the range of 4.34–5.39 mg/(g•h) and 4.56–5.52 mg/(g•h), respectively (Fig. 3). During the rice growth period, there was no significant difference in soil invertase activities among the HOM, LOM, RF and MF treatments, but the activity of this enzyme in HOM, LOM, RF and MF treatments was significantly (P<0.05) higher than that of in CK treatment at the different main growth stages of early and late rice.

Dynamics of soil dehydrogenase activities

In the early and late rice growing seasons, soil dehydrogenase activities decreased as follows: HOM>LOM>RF>MF>CK. And there were no significant differences (P>0.05) in soil dehydrogenase activities under the HOM, LOM and RF treatments at the early and late rice main growth stages, but there were significant differences (P<0.05) among other treatments. And there were no significant differences (P>0.05) in soil dehydrogenase activities under the RF and MF treatments at the early and late rice main growth stages. In the early and late rice growing seasons, the soil dehydrogenase activities of different treatments at different growth stages was in the range of 25.46–72.64 μg/(g•h) and 26.21–73.66 μg/(g•h), respectively. The highest activities were detected at the jointing stage.

Dynamics of soil cellobiohyrolase activities

At the early rice main growth stages, the activities of soil cellobiohyrolase were affected by the different fertilizer managements. The RF treatment has the highest soil cellobiohyrolase activities and the CK had the lowest activities (Fig. 5), and the activity decreased in the following order: RF>HOM>LOM>MF>CK. At the late rice main growth stages, the highest activity was the RF treatment, and the activity trend was as follows: RF>HOM>LOM>MF>CK.



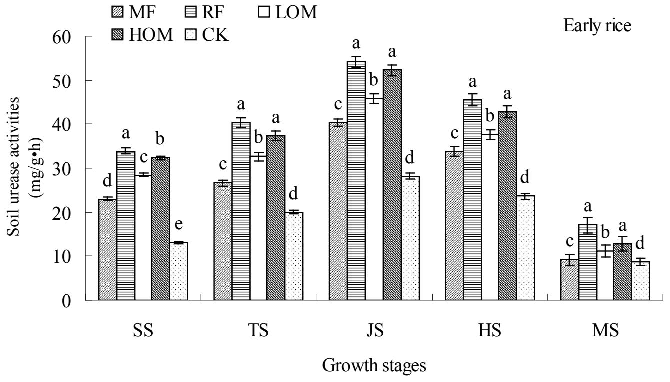


Fig. 2. Effects of different long-term fertilizer treatments on soil catalase activities during rice growth stages in paddy field

Dynamics of soil nitrifying and denitrification bacteria

At the early and late rice main growth stages, the numbers of soil nitrifying and denitrification bacteria decreased as follows: HOM>LOM>RF>MF>CK. There were significant differences (*P*<0.05) in numbers of soil nitrifying and denitrification bacteria under the HOM, LOM and RF, MF, CK treatments at the early and late rice main growth stages. But there were no significant differences (*P*>0.05) in numbers of soil denitrification bacteria under the HOM and LOM treatments at the early and late rice main growth stages (Table 2).

Dynamics of soil ammonifiers bacteria

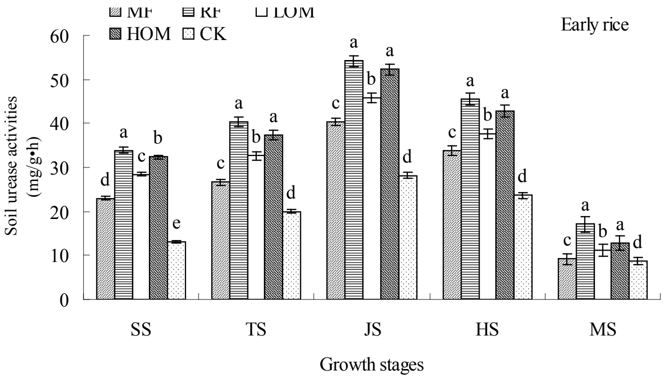
At the early and late rice main growth stages, the numbers of soil ammonifiers bacteria decreased as follows: MF>RF>LOM>HOM>CK. The MF treatment has the highest numbers of soil ammonifiers bacteria and the CK had the lowest numbers of soil ammonifiers bacteria (Table 3). There were significant differences (*P*<0.05) in numbers of soil ammonifiers bacteria under the RF, MF and HOM, LOM, CK treatments at the early and late rice main growth stages.

Dynamics of soil cellulose-decomposing bacteria

At the early and late rice main growth stages, the numbers of soil cellulose-decomposing bacteria were affected by the different fertilizer managements. The LOM treatment has the highest numbers of soil cellulose-decomposing bacteria and the CK had the lowest numbers of soil cellulose-decomposing bacteria (Table 3), and the numbers of soil cellulose-decomposing bacteria decreased in the following order: LOM>RF>MF>HOM>CK. The numbers of soil cellulose-decomposing bacteria in LOM treatment was significantly (*P*<0.05) higher than that of in MF, HOM, and CK treatments at the different main growth stages of early and late rice.

Dynamics of soil azotobacteria

In the early and late rice growing seasons, the numbers of soil azotobacteria decreased as follows: RF>LOM>HOM>MF>CK. There were significant differences (*P*<0.05) in the numbers of soil azotobacteria under the RF, LOM and HOM,



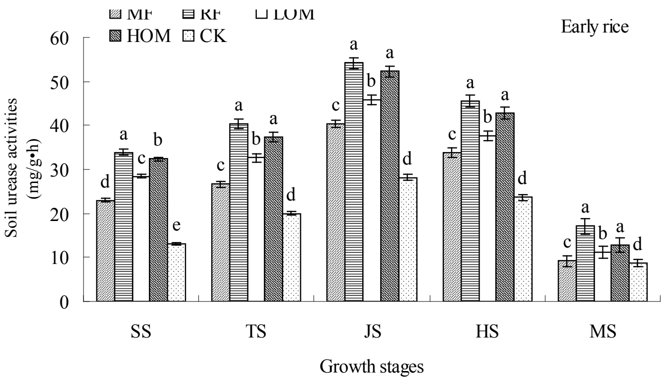


Fig. 3. Effects of different long-term fertilizer treatments on soil invertase activities during rice growth stages in paddy field

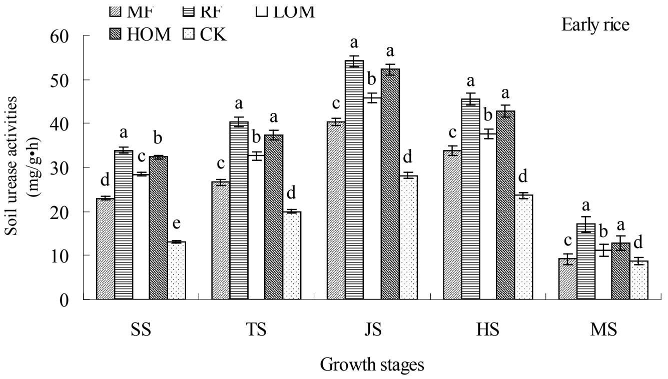
MF, CK treatments at the early and late rice main growth stages. In the early and late rice growing seasons, the numbers of soil azotobacteria of different treatments at different growth stages was in the range of 8.05–85.17 $\times 10^6$ cfu/g and 8.67–86.02 $\times 10^6$ cfu/g, respectively.

DISCUSSION

Soil enzyme activities and fertilizer managements

Soil enzymes are an index of soil microbial activity and fertility, and they are playing an important role in C, N, P cycling in ecological systems. Zhao et al. (2009) [24] results showed that the soil invertase activity were increased by combined application of straw and manure. In our study, compared with MF and CK treatments, the soil urease and catalase activities were increased by application of mineral fertilizer along with manure or rice residues at the early and late rice main growth stages. The reason maybe application of mineral fertilizer and organic (manure or rice residues) provides energy material for soil microbial activity, conducive to the straw mineralization and nutrient release, soil microbial metabolic activity and soil enzyme activity were increased [25]. Secondly, with the increase of temperature and rice growth, the plant root exudates were promoted, which also stimulated the microbial activity and enhanced the metabolic activity of microorganisms [26]. In the present study, the RF treatment has the highest soil urease activities at the early and late rice main growth stages. This may be application of rice straw provides energy and nutrition source for microorganisms, so that the soil urease activity were promoted. In the low and high rate of manure combine with mineral fertilizer treatments, the soil urease activities increased with the increase of organic application, that is, the soil urease activities in HOM treatment were higher than that of the LOM treatment, this may be application of high rate of manure and mineral fertilizer is benefit to soil C/N ratio and organic decomposition [27].

In our study, compared with the RF, MF



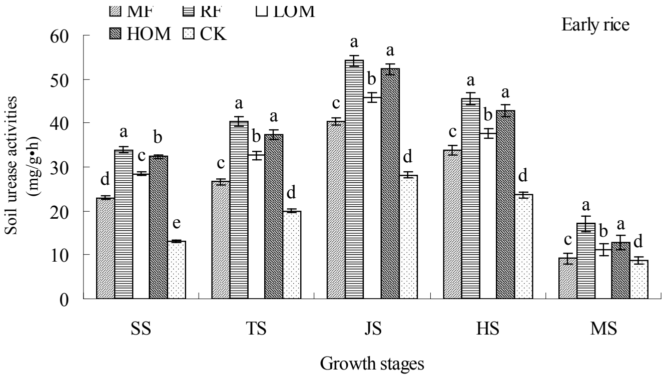
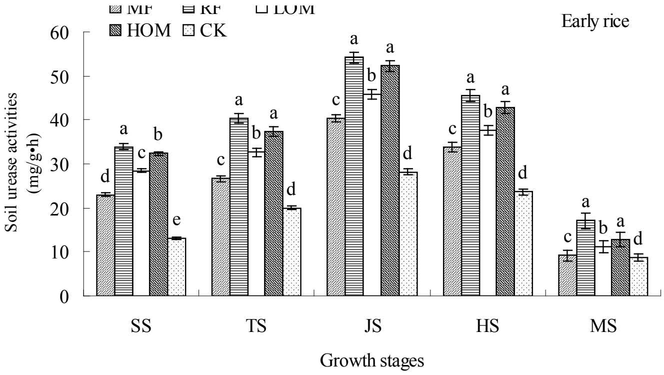


Fig. 4. Effects of different long-term fertilizer treatments on soil dehydrogenase activities during rice growth stages in paddy field

and CK treatments, the soil catalase, invertase and dehydrogenase activities were increased by application of manure along with mineral fertilizer (LOM and HOM treatments) at the early and late rice main growth stages. The reason maybe application of manure were promote the growth and physiological metabolism of the underground of rice plant, increased the root exudates, and promoted the activity of soil enzyme [28]. Meanwhile, application of the manure provides carbon and nitrogen source for soil enzyme activity. Secondly, soil physical and chemical properties were improved under long-term application of organic practices, which also promoted the improvement of enzyme activity [29].

Soil invertase activities play a critical role in the cycling of carbon in terrestrial ecosystems, it is an indictor of the accumulation and decomposition characteristic of soil organic carbon (SOC). And it was closely related to mineralization intensity of soil organic matter [28]. In the present study, soil invertase activities with application of mineral fertilizer along with manure or rice residues were higher than that of the merely mineral fertilizer inputs and without fertilizer input treatments, which suggested that application with organic manure (chicken manure or rice residues) practices had increased of soil organic matter content in a long-term experiment, and provide substrate for the soil invertase activities. Thus, the soil invertase activities were greatly increased by application of mineral fertilizer along with manure or rice residues practices.

Soil catalase is an important oxidation–reduction enzyme, which is involved in the synthesis of humic compounds and protects the cells from damage caused by hydrogen peroxide [30]. SOC content was the most important factor influencing soil catalase activities. During the rice growth period, the soil catalase activities were increased by application of mineral fertilizer along with manure or rice residues compared with application of mineral fertilizer practices. That is, the soil catalase activities were inhibited



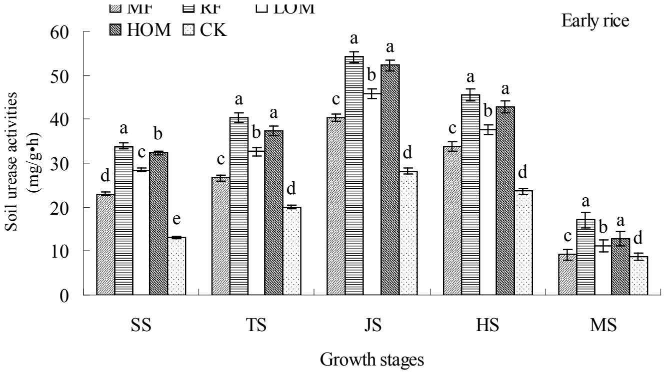


Fig. 5. Effects of different long-term fertilizer treatments on soil cellobiohyrolase activities during rice growth stages in paddy field

Table 2. Effects of long-term fertilization treatments on the amount of nitrifying bacteria and denitrification bacteria in soil at early and late rice main growth stages

Items	Treatment	Early rice					Late rice				
		SS	TS	JS	HS	MS	SS	TS	JS	HS	MS
Nitrifying	MF	10.24±0.69d	159.05±1.88d	69.81±1.97d	31.59±1.15d	11.51±0.12d	12.38±0.82d	153.48±5.61d	68.45±3.91d	34.66±2.21d	13.37±1.46d
bacteria	RF	12.18±0.40c	240.39±3.31c	120.15±1.97c	42.93±1.62c	25.37±1.12c	14.55±0.57c	231.62±4.88c	117.73±4.03c	43.82±1.65c	26.42±1.15c
(×10^5 cfu/g)	LOM	17.84±0.33b	282.54±4.22b	177.30±2.17b	56.19±1.21b	38.74±0.67b	19.92±0.42b	273.28±4.68b	174.38±3.39b	57.37±1.26b	39.89±1.76b
	HOM	24.59±0.27a	307.06±4.88a	206.82±1.98a	75.34±0.85a	53.15±0.22a	28.75±0.35a	298.47±2.43a	204.74±2.97a	76.62±1.01a	50.58±1.38a
	CK	8.17±0.26d	38.41±1.11e	28.17±0.81e	20.52±0.59e	8.13±0.11e	10.31±0.29e	29.78±1.85e	25.81±0.74e	17.54±0.51e	8.92±0.25e
Denitrification	MF	22.93±2.38c	39.63±3.79c	30.99±3.05c	27.83±2.16c	22.22±1.74d	25.47±2.45c	42.76±3.59c	33.46±3.12c	29.63±2.20c	24.37±1.80d
bacteria	RF	41.73±2.28b	76.31±2.93b	64.99±2.48b	37.09±2.03b	30.35±1.53c	44.24±2.35b	79.48±3.05b	67.43±2.55b	38.39±2.07b	32.26±1.58c
(×10^5 cfu/g)	LOM	79.14±1.20a	101.62±2.20a	85.94±1.87a	70.51±1.07a	53.12±0.87b	81.73±1.27a	105.73±2.29a	98.45±1.94a	71.73±1.11a	54.94±0.93b
	HOM	82.57±0.66a	111.53±1.14a	95.85±0.89a	74.93±0.81a	60.46±0.64a	85.15±0.73a	114.66±1.23a	108.35±0.96a	76.36±1.85a	62.36±1.71a
	CK	12.31±0.35d	38.42±1.11d	22.78±0.65d	17.25±0.49d	10.63±0.31e	14.87±0.42d	41.58±1.21d	25.28±0.72d	18.58±0.53d	12.47±0.36e

MF; mineral fertilizer alone; RF; rice residues and mineral fertilizer; LOM; 30% organic matter and 70% mineral fertilizer; HOM; 60% organic matter and 40% mineral fertilizer; CKÿwithout fertilizer.
SS; seedling stage; TS; tillering stage; JS; jointing stage; HS; heading stage; MS; mature stage.
Different letters indicate significance at $P < 0.05$ among the fertilization treatments at the same rice growth stage, according to the least significant difference test.

Table 3. Effects of long-term fertilization treatments on the amount of ammonifiers bacteria, azotobacteria and cellulose-decomposing bacteria in soil at early and late rice main growth stages

Items	Treatment	Early rice					Late rice				
		SS	TS	JS	HS	MS	SS	TS	JS	HS	MS
Ammonifiers	MF	65.53±1.89a	71.26±2.05a	56.93±1.64a	45.83±1.32a	47.96±1.38a	66.78±1.92a	72.17±2.08a	57.26±1.65a	46.62±1.34a	48.12±1.38a
bacteria	RF	40.36±1.16b	42.26±1.21b	40.26±1.16b	39.17±1.13b	29.83±0.86b	41.58±1.21b	43.54±1.25b	41.05±1.18b	35.04±1.01b	37.95±1.09b
(×10^6 cfu/g)	LOM	35.68±1.03c	37.84±1.09c	29.96±0.86c	28.73±0.82c	20.04±0.57c	36.74±1.06c	38.63±1.11c	32.33±0.93c	27.23±0.78c	28.53±0.82c
	HOM	12.99±0.37d	28.86±0.83d	15.56±0.44d	10.88±0.31d	13.32±0.38d	14.31±0.41d	29.75±0.85d	15.94±0.46d	12.69±0.33d	13.25±0.38d
	CK	11.25±0.32d	19.48±0.73e	11.53±0.33e	8.85±0.25d	9.93±0.28e	12.29±0.35d	13.36±0.38e	11.82±0.34e	9.67±0.27e	10.14±0.29e
Cellulose-	MF	34.25±1.44c	39.09±1.70c	33.06±1.39c	30.88±1.17b	32.07±1.23b	36.56±1.59c	41.24±1.76c	36.49±1.51c	31.88±1.08b	32.06±1.12b
decomposing	RF	46.11±1.33b	51.70±1.49b	41.58±1.20b	39.11±1.12a	40.55±1.17a	48.63±1.40b	54.41±1.57b	45.97±1.32b	35.11±1.01a	36.25±1.04a
bacteria	LOM	50.08±0.98a	58.97±1.12a	48.27±0.95a	40.63±0.89a	42.95±0.92a	55.12±1.05a	61.28±1.19a	52.37±1.05a	37.63±0.92a	38.67±0.92a
(×10^3 cfu/g)	HOM	29.37±0.84d	33.28±0.96d	28.06±0.81d	23.14±0.66c	25.58±0.73c	28.68±0.82d	32.78±0.94d	31.06±0.89d	28.14±0.81c	30.74±0.89b
	CK	17.42±0.51e	21.11±0.61e	12.74±0.36e	10.17±0.29d	11.06±0.31d	16.95±0.48e	19.46±0.56e	15.48±0.44e	12.12±0.34d	13.35±0.39c
Azotobacteria	MF	18.54±0.72d	25.78±1.25d	20.06±1.13d	17.38±0.78c	10.93±0.33b	19.97±0.76d	27.62±1.31d	22.54±1.17d	18.42±0.82c	12.45±0.41b
(×10^6 cfu/g)	RF	50.26±0.53a	85.17±0.74a	66.36±1.98a	34.15±0.50a	23.52±0.67a	51.62±0.57a	86.02±0.79a	67.86±0.65a	34.95±0.53a	24.68±0.35a
	LOM	31.75±1.45b	55.48±2.45b	43.71±1.91b	32.42±0.98a	20.85±0.31a	33.15±1.49b	57.35±2.48b	46.25±1.95b	33.26±1.01a	21.76±0.71a
	HOM	25.23±0.91c	43.63±1.60c	39.35±1.26c	27.31±0.93b	11.65±0.60b	26.56±0.95c	45.47±1.65c	40.85±1.33c	28.55±0.65b	14.26±0.62b
	CK	14.36±0.41e	20.13±0.58e	16.26±0.46e	10.22±0.29d	8.05±0.23c	15.72±0.45e	20.95±0.60e	17.86±0.51e	10.56±0.30d	8.67±0.33c

under application of mineral fertilizer condition, compared with the combined application of organic and inorganic fertilizers treatments.

Soil dehydrogenase activity can be used as the index of microbial redox system, and its activity is closely related to soil fertility. Soil dehydrogenase is typically present in all intact and viable microbial cells [31]. We found that the soil dehydrogenase activities were higher in RF, LOM and HOM treatments than that of in MF and CK treatments. This might because the soil physical and chemical properties and soil micro environment were improvement by combined application of manure or rice straw with miner fertilizer, which provides a good environment and carbon sources for growth and reproduction of soil dehydrogenase [32]. Meanwhile, the application of organic fertilizer were contained some microorganisms, which provide source for activities of the soil dehydrogenase.

Soil microbial community and fertilizer managements

Fertilizer managements are important practices for soil microbial community, soil quality and sustainable development for agriculture production [33]. Soil microbial community was control by different fertilizer managements, and it was closely related to the amount of application with manure. In the present study, the results indicated that different fertilization managements were significantly affected some physiological function soil microbial quantity, including the soil nitrifying and denitrification, ammonifiers, cellulose-decomposing and azotobacteria bacteria. Compared with the RF and CK treatments, the number of some physiological function soil microbial in the paddy field were increased by application of mineral fertilizer along with manure or rice residues at the early and late rice main growth stages. The reason may be application of mineral fertilizer and organic (manure or rice residues) increase crop growth and root biomass [26]. Therefore the number of some physiological function soil microbial is raised by inputs of organic and straw residues.

Meanwhile, the communities and activities of soil physiological function bacterial (nitrifying and denitrification, ammonifiers, cellulose-decomposing and azotobacteria bacteria) were affected by the soil physicochemical properties [7,8], fertilizer regimes [9, 34], type of crops and planting systems [10], and the fertilizer managements is an important factor for affect the communities of soil bacterial. In the present study, the numbers of soil bacterial were increased by application of mineral fertilizer along with manure or rice residues at the early and late rice main growth stages. The reason may be animal manure contains large amounts of soluble, readily available, organic substrates for microorganisms growth by application of mineral fertilizer and organic (manure or rice residues). Secondly, both manure and rice residues act as a substrate for microbes, and this will result in an increase in the numbers of soil bacterial. Meanwhile, with the increase of temperature during the rice growth period, the plant physiological activity and root exudates were increased, which also contribute to increase of the numbers of soil bacterial. And there have straw cellulose and hemicellulose contained in the application of straw, which provide the carbon and energy source for growth and reproduction of the microbial, therefore, the microorganisms were promoted during the rice growth period. Similarly, in a long-term experiment, some studies results showed that the soil microbial were greater in application of manure (farmyard manure, straw) along with NPK fertilizer than that of the merely NPK fertilizer inputs in field conditions [35].

The numbers of soil nitrifying and denitrification bacteria are related with soil redox potential (Eh), soil organic matter and pH. The numbers of soil nitrifying bacteria were higher in application of manure (manure or rice residues) along with mineral fertilizer than that of the without fertilizer input treatment. The reason maybe it did not inhibit nitrification by application of manure. Meanwhile, the numbers of soil denitrification bacteria were increased by application of mineral fertilizer along with manure or rice residues at the early and late rice main growth stages, which suggested that application with organic manure (chicken manure) practices had decreased of soil Eh and pH in a long-term experiment. This results was coincides with application of organic manure boosted bacterial growth and increased the number of bacterial in agroecosystems [36].

CONCLUSIONS

The results showed that the activities of soil enzyme were affected by the different long-term fertilizer managements at the rice main growth stages. Compared with the MF and CK treatments, the soil enzyme activities were increased by application of mineral fertilizer along with manure or rice residues at the early and late rice main growth stages. That is, application of manure or rice residues can improve the soil urease, catalase, invertase, dehydrogenase and cellobiohyrolase activities at the rice main growth stages. Compared with CK treatment, the soil urease activities with RF treatment were increased by 20.92, 20.45, 25.95, 21.86, 8.44 mg/(g•h) and 20.90, 19.63, 25.75, 21.76, 8.36 mg/(g•h) at the early and late rice main growth stages, respectively. And the soil catalase activities with HOM treatment were increased by 6.65, 12.31, 12.34, 10.97, 7.66 mol/L $KMnO_4$ ML/(g•h) and 6.94, 12.11, 12.12, 10.67, 7.76 mol/L $KMnO_4$ ML/(g•h) at the early and late rice main growth stages, respectively. But there was no significant difference in soil invertase activities among the HOM, LOM, RF and MF treatments. The soil dehydrogenase activities with HOM treatment were increased by 7.26, 7.95, 37.19, 20.49, 9.75 µg/(g•h) and 7.40, 8.92, 37.27, 20.53, 9.80 µg/(g•h) at the early and late rice main growth stages, respectively. And the soil cellobiohyrolase activities with RF treatment were increased by 1.23, 2.45, 1.96, 1.58, 1.03 mg/(g•h) and 1.24, 2.44, 2.06, 1.60, 1.02 mg/(g•h) at the early and late rice main growth stages, respectively.

The number of some physiological function soil microbial in the paddy field also increased by application of mineral fertilizer along with manure or rice residues at the rice main growth stages. At the early and late rice main growth stages, the numbers of soil nitrifying and denitrification bacteria higher in HOM treatment, and the numbers of soil ammonifiers bacteria higher in MF treatment, the numbers of soil cellulose-decomposing bacteria higher in LOM treatment, the numbers of soil azotobacteria bacteria higher in RF treatment, respectively. In conclusion, application of mineral fertilizer along with manure or rice residues is an effective way of enhancing soil enzyme activities and soil microbial community.

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