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Soil Enzyme Activities and Soil Microbe Population as Influenced by Long-term Fertilizer Management under An Intensive Cropping System

Haiming Tang[1]*, Yilan Xu[2], Jimin Sun[1], Xiaoping Xiao[1], Ke Wang[1], Weiyan Li[1], Wenguang Tang[1] and Guangli Yang[1]

[1]Hunan Soil and Fertilizer Institute, Changsha, 410125, PR China.
[2]College of Biological and Electromechanical Polytechnic, Changsha 410127, China.

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The objective of this study was to characterize the changes of soil enzyme activities and soil microbe population as related to mineral fertilizer and crop residues based on a long-term field experiment. The experiment, initiated in 1986, has three treatments: unfertilized (CK), mineral fertilizer alone (MF) and rice residues plus mineral fertilizer (RF). The cropping system consists of barley (*Hordaum vulgare* L.), early rice, and late rice, three crops in a year. In May 2013, after early and late rice transplanting, soil samples were collected from the 0-20 cm layers to determine soil enzyme activities and soil microbe population. The results indicated that during the rice growing seasons, the enzyme activities were significantly affected by application of crop residue and mineral fertilizer practices: they were greater in the MF and RF than in the CK and were similar during the early and late rice season. The β-glucosidase and alkaline phosphatase activities reached peak values at the tillering stage and booting stage after crop residue and mineral fertilizer application, respectively, and gradually decreased up to the maturity stage. Arylsulfatase and arylamidase activities reached peak values at the maturity stage. Combined application of crop residue and mineral fertilizer also improved the numbers of aerobic bacterial, actinomycete and fungus, but the improvement by mineral fertilizer alone was limited. Under the intensive rice-rice-barley cropping system, joint application of crop residue and chemical fertilizer improved soil enzyme activities and soil microbe population, but the change of soil enzyme activities and soil microbe population from chemical fertilizer alone was not significant.

Key words: Soil enzyme; Soil microbe; Mineral fertilizer; Crop residue; Fertilizer management.

Enzymes play an important role in the cycling of nutrients in nature, and soil enzyme activity can be used as an index of soil microbial activity and fertility[1]. Soil enzymes are involved in energy transfer, and consequently affect environmental quality and crop productivity[2-3]. The rate of enzyme production, and the activity and the stability of free and adsorbed enzymes are controlled by environmental conditions and ecological interactions. Enzymes may respond to changes in soil management more quickly than other soil variables and therefore might be useful as early indicators of biological changes. Enzyme activity profiles reflect soil functional diversity, which is influenced by the genetic diversity of soil microorganisms, plants and animals and is closely related to environmental factors and ecological interactions[4]. Among the different enzymes in soils, arylamidase, alkaline phosphatase, β-glucosidase and arylsulfatase are important for the transformation of plant nutrients. β-glucosidase catalyzes glucose formation and is an important enzyme in the terrestrial carbon cycle, glucose being an important energy source for microbial

* To whom all correspondence should be addressed.
Tel.: +86 731 84691345; Fax: +86 731 84691581;
E-mail: tanghaiming66@163.com

biomass[3]. Phosphatases play an important role in transforming organic phosphorus into inorganic forms that are suitable for plants. Arylamidase catalyzes one of the most important reactions in N mineralization: it releases amino acids that are used as substrates for amidohydrolases from soil organic matter. Arylsulfatase is an extracellular enzyme that catalyses the hydrolysis of organic sulphate esters, releasing SO_2 that can be used by plants.

Soil microbes play an important role in ecosystem function and may act as filters or valves that regulate the intra-system cycling of soil nutrients[5]. Soil microbes play an important role in the maintenance of soil fertility (i.e. nutrient cycling and organic matter decomposition). The ability of microbes to maintain soil fertility and regulate nutrient cycling may be largely dependent on the composition of soil microbial communities[6-7], and changes in microbial diversity or community structure could have dramatic impacts on ecosystem processes[8].

In recent years, many studies have shown that the enzyme and microbial activities of soil are affected by soil tillage and crop residue management[9], application of fertilizer and organic matter[10-12], crop rotations[13], and field management[14, 15]. However, limited information exists on the influences of long-term fertilizer management on soil enzyme activities and soil microbe population under the intensive rice production system in southern China.

The middle and lower Yangtze River Plain is one of the most important rice (*Oryza sativa* L.) production bases in China. Since the 1980s, traditional fertilizer management practices have been altered considerably. With the continuous increase of mineral fertilizer application rates, manure inputs have been declining dramatically. Meanwhile, returning crop residue to field is being accepted gradually. There is a growing concern that the new fertilization systems may not be sustainable due to their detrimental effects on soil properties. Does mineral fertilizer alone reduce soil enzyme and soil microbial activities? Is crop residue application a viable option to maintain soil organic matter content, physical quality and soil enzyme and soil microbial activities?

The objectives of this research were to determine the effect of long-term application of crop residue, and mineral fertilizer in a double-cropping rice system on soil enzyme activities and soil microbial community in a paddy field.

MATERIALS AND METHODS

Sites and cropping system

The experiment was established in 1986. It locates in Ning Xiang County (28°07'N, 112°18'E, and altitude 36 m) of Hunan Province, China. Under a continent monsoon climate, the annual mean precipitation is 1553 mm and potential evapotranspiration is 1354 mm. The monthly mean temperature is 17.2°C. Soil texture of the plough layer (0–20 cm) is silt clay loam with 13.71% sand and 57.73% silt. At the beginning of the study, the characteristics of the surface soil (0–20 cm) are as follows: soil organic carbon (SOC) 29.4 g kg^{-1}, total nitrogen 2.0 g kg^{-1}, available N 144.1 mg kg^{-1}, total phosphorous 0.59 g kg^{-1}, available P 12.87 mg kg^{-1}, total potassium 20.6 g kg^{-1}, and available potassium 33.0 mg kg^{-1}. There are three crops in a year, barley (*Hordeum vulgare* L.), early rice, and late rice. Barley is sown in the middle of November and is harvested in early May of the following year. Early rice is then transplanted, and harvested in the middle of July. The growing season of late rice lasts from late July to the end of October.

Experiment design

The experiment had three treatments: control (without fertilizer input, CK), mineral fertilizer only (MF), and rice residue plus mineral fertilizer (RF). The design made all the fertilize treatments receiving the same N rate (the amount of N in mineral fertilizer plus that from rice residue). The mineral fertilizers included urea, ordinary superphosphate, and potassium chloride. Details about the fertilizer management are listed in Table 1. Before crop seeding or transplanting seedling, air–dried rice residue was spread onto soil surface manually and was incorporated into soil. The cultivation depth was about 20 cm. For early rice, late rice, and barley, 40%, 30%, and 30% of mineral nitrogen fertilizer was applied at seeding, and the remaining nitrogen fertilizer was applied by top dressing in the growth periods. All the phosphorus and potassium fertilizers were applied at seeding. There were three replications and each plot size was 66.7 m^2.

Soil sampling and measurements

Data were collected in 2013. In each plot,

the soil samples in the ploughed layer (0–20 cm) were collected from the centre of four hills of rice plants at different rice growth periods, using a drill. The soil samples were passed through a 2-mm sieve and kept moist in a refrigerator at 4°C until use. Arylamidase (EC 3.4.11.2) activity was assayed by incubating 1.0 g moist soil with 3.0 mL of 0.1 M THAM buffer (pH 8.0) and 1.0 mL of an 8.0 mM solution of L-leucine β-naphthylamide hydrochloride [16]. Alkaline phosphatase (EC 3.1.3.1), β-glucosidase (EC 3.2.1.21) and arylsulfatase (EC 3.1.6.1) activities were determined as described by Tabatabai (1994)[3], and the activity was reported as µg *p*-nitrophenol g^{-1} h^{-1}. All the measurements were repeated for three times.

Colony forming units (CFUs) of soil aerobic bacteria, actinomycetes, and fungi were enumerated by a 10-fold dilution plate technique. And the number of aerobic bacteria were identified by spreading 100 ¼l of diluted sample on LB agar medium. The medium for actinomycetes contained 1% (w/v) soluble starch, 0.2% (w/v) $(NH_4)_2SO_4$, 0.1% (w/v) K_2HPO_4, 0.1% (w/v) $MgSO_4 \cdot 7H_2O$, 0.7% (w/v) NaCl, 0.3% (w/v) $CaCO_3$, and 2% (w/v) agar. The number of CFUs of actinomycetes formed on this medium was determined by probing colonies that developed with a dissecting needle: if the colony remained as a discrete, small mass, it was considered to be an actinomycete. The number of CFUs of fungi was estimated on Martin's agar medium containing 1.25 g streptomycin l^{-1} and 33 mg rose bengal l^{-l}. Three replicates of the inoculated agar plates were incubated at 28°C for 3 d for bacteria, 5 d for fungi, and 7 d for actinomycetes, after which colonies were counted[14].

Statistical analysis

All data were expressed as mean α standard error. The data were analyzed as a randomized complete block, using the PROC ANOVA procedure of SAS[17]. 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 alkaline phosphatase activity during the rice growth period

Under different fertilization treatments in double–cropping rice system, alkaline phosphatase activity in the MF and RF soils was higher than that in without fertilizer input soil (Fig. 1). In other words, the alkaline phosphatase activity was enhanced by the application of residue and mineral fertilizer in the rice growth season. The alkaline phosphatase activity of soils in the early rice season was higher than that in the late rice season. In the early and late rice seasons, alkaline phosphatase activity decreased as follows: RF>MF>CK, and there were significant differences ($P > 0.05$) between RF, MF and CK. And there were significant differences ($P > 0.05$) in alkaline phosphatase activity under the RF and MF treatments at the main stages. In the early and late rice seasons, the alkaline phosphatase activity of different soils collected at different growth stages was in the range of 76.92–266.89 and 74.16–252.57 µg *p*-nitrophenol g^{-1} soil h^{-1}, respectively. The highest activities were detected at the booting stage.

Dynamics of β-glucosidase activity during the rice growth period

In the early rice season, the activity of β-glucosidase in soils was significantly affected by

Table 1. Nutrient supply from rice straw, and mineral fertilizer under different fertilizer treatments. The treatments are without fertilizer (CK), mineral fertilizer alone (MF), and crop residue plus mineral fertilizer (RF). The numbers are in kg hm^{-2}

Treatment	Early rice			Late rice			Barley			Total		
	N	P	K	N	P	K	N	P	K	N	P	K
CK	0+0*	0+0	0+0	0+0	0+0	0+0	0+0	0+0	0+0	0	0	0
MF	143+0	54+0	63+0	157.5+0	27+0	81+0	157.5+0	27+0	81+0	458	108	225
RF	117+26	34+20	9+54	130.5+27	23+4	24+57	130.5+27	23+4	24+57	458	108	225

* Input from mineral fertilizer + input from crop residue.

Note: 1) For the RF treatment, rice straw return rate (air dry) was 2.85, 3.0, and 3.0 t/(hm^2· a) for early rice, late rice, and barley, respectively. 2) The N, P, and K content of air-dry rice straw was 9.1%, 1.3%, and 18.9 %, respectively.

Table 2. Variations in the number aerobic bacteria, actinomycetes and fungi in a paddy soil under different fertilization systems.

Items	Treatment	Early rice					Late rice				
		SS	TS	BS	FS	MS	SS	TS	BS	FS	MS
Aerobic bacterial ($\times10^4$cfu g^{-1} dry soil)	MF	98.74±3.44a	264.54±7.75a	109.36±3.71b	86.26±2.79a	96.59±2.95a	68.66±2.33a	97.60±3.36b	92.70±3.11a	87.34±2.7ab	95.40±3.01a
	RF	119.33±2.76a	268.56±7.64a	128.38±3.12a	96.65±2.49a	102.05±2.79a	80.86±1.84a	116.56±2.82a	107.66±2.62a	93.52±2.52a	104.18±2.75a
	CK	91.33±2.64b	124.97±3.61b	53.53±1.55c	79.81±2.3b	94.98±2.74b	57.62±1.66b	87.48±2.53c	81.58±2.36b	80.52±2.32b	73.63±2.13b
Fungi ($\times10^2$cfu g^{-1}) dry soil)	MF	26.04±0.93b	37.02±1.07b	34.92±1.01a	10.98±0.99b	23.111.02b	38.87±1.73b	51.12±2.34b	45.44±1.75b	14.37±0.57b	35.78±1.38b
	RF	32.36±0.89a	73.54±2.12a	42.62±1.23a	34.56±0.56a	35.38±0.76a	59.76±1.13a	81.15±1.94a	60.47±1.35a	19.73±0.45a	47.91±1.24a
	CK	12.31±0.36c	28.3±0.82c	20.58±0.59b	7.61±0.22c	17.19±0.5c	30.03±0.92c	40.04±1.44c	29.36±0.85c	9.97±0.29c	31.55±0.91c
Actinomycetes ($\times10^3$ cfu g^{-1} dry soil)	MF	120.49±3.99b	160.59±5.78b	78.93±3.71b	139.35±5.08b	91.15±3.66b	126.81±4.56b	170.07±6.95b	128.8±5.18b	108.23±4.46b	125.14±4.86b
	RF	138.38±3.48a	200.28±5.53a	128.38±2.77a	175.82±4.39a	126.66±3.29a	158.10±4.30a	240.68±6.44a	179.41±4.68a	154.41±4.3a	168.39±4.18a
	CK	56.63±1.63c	113.29±3.27c	57.01±1.65c	76.34±2.20c	56.08±1.62c	82.87±2.39c	116.16±3.35c	64.95±1.88c	88.32±2.55c	96.24±2.78c

MF: mineral fertilizer; RF: rice residues plus chemical fertilizer; CK: without fertilizer.
SS: seedling stage; TS: tillering stage; BS: booting stage; FS: full heading stage; MS: maturity stage.

the residue and mineral fertilizer management practice. The highest β-glucosidase activity values were observed with RF and the lowest activity with CK (Fig. 2), and the activity decreased in the following order: RF>MF>CK. In the late rice season, the highest activity was noted in soils where crop straw was used for residue treatment, and the activity trend was as follows: RF>MF>CK (Fig. 2).

Dynamics of arylsulfatase activity during the rice growth period

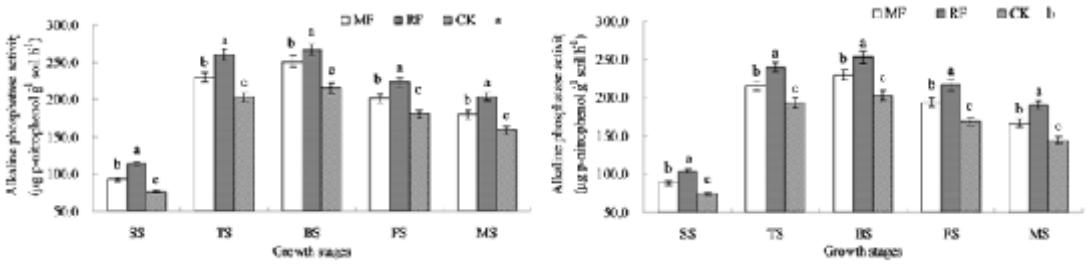
In the early and late rice seasons, arylsulfatase activity in the soils was in the range of 25.10–36.30 and 28.02–36.68 µg *p*–nitrophenol g^{-1} soil h^{-1}, respectively (Fig. 3). At the seedling stage, there was no significant difference in arylsulfatase activity among the MF, RF and CK, but the activity of this enzyme in RF soils was significantly ($P < 0.05$) higher than that in CK at the tillering and booting stage, and the activity of this enzyme in MF and RF soils was significantly ($P < 0.05$) higher than that in CK at the full heading and maturity stage.

Dynamics of arylamidase activity during the rice growth period

Application of the rice straw and mineral fertilizer management practices has significantly affect arylamidase activity in the soil (Fig. 4). At 0–20 cm soil depth, there was no significant difference ($P > 0.05$) in arylamidase activity under the MF and RF. But the activity of this enzyme in RF soils was significantly ($P < 0.05$) higher than that in CK soil at the main growth stages. In the early and late rice seasons, arylamidase activity changed in the range of 21.42–40.67 and 21.75–40.29 ¼g *p*-nitrophenol g^{-1} soil h^{-1}, respectively.

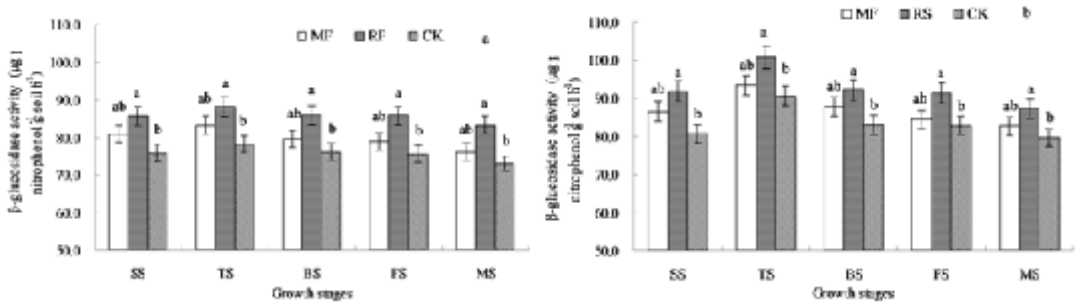
Enumeration of aerobic bacteria, actinomycetes and fungi in the paddy soil

Significant differences ($P < 0.05$) were observed in the numbers of aerobic bacteria, actinomycetes and fungi between samplings throughout the rice growth season. The number of aerobic bacteria decreased in the order RF>MF>CK in the early and late rice season (Table 2). The number of aerobic bacteria in the paddy soil under the RF was significantly higher than that in the CK. However, the numbers of fungi and actinomycetes in the soil with rice straw and mineral fertilizer were significantly higher ($P < 0.05$) than that in the without fertilizer soil throughout the



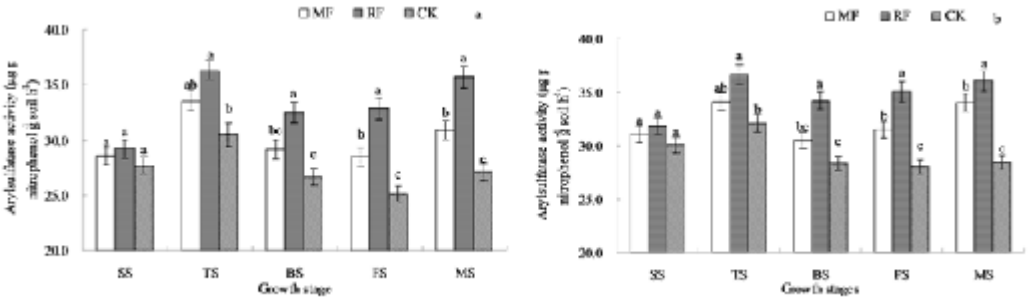
MF: mineral fertilizer; RF: rice residues plus chemical fertilizer; CK: without fertilizer.
SS: seedling stage; TS: tillering stage; BS: booting stage; FS: full heading stage; MS: maturity stage.
Error bars represent the standard error of mean. Different letters indicate significance at $P < 0.05$, according to the least significant difference test.

Fig. 1. Dynamics of alkaline phosphatase activity in paddy fields during the rice growth period (a for the early rice season and b for the late rice season)



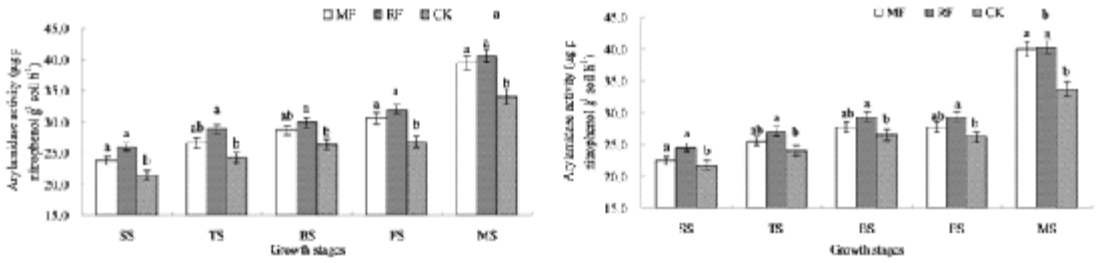
MF: mineral fertilizer; RF: rice residues plus chemical fertilizer; CK: without fertilizer.
SS: seedling stage; TS: tillering stage; BS: booting stage; FS: full heading stage; MS: maturity stage.
Error bars represent the standard error of mean. Different letters indicate significance at $P < 0.05$, according to the least significant difference test.

Fig. 2. Dynamics of β-glucosidase activity in paddy fields during the rice growth period (a for the early rice season and b for the late rice season)



MF: mineral fertilizer; RF: rice residues plus chemical fertilizer; CK: without fertilizer.
SS: seedling stage; TS: tillering stage; BS: booting stage; FS: full heading stage; MS: maturity stage.
Error bars represent the standard error of mean. Different letters indicate significance at $P < 0.05$, according to the least significant difference test.

Fig. 3. Dynamics of arylsulfatase activity during the rice growth period (a for the early rice season and b for the late rice season)



MF: mineral fertilizer; RF: rice residues plus chemical fertilizer; CK: without fertilizer.
SS: seedling stage; TS: tillering stage; BS: booting stage; FS: full heading stage; MS: maturity stage.
Error bars represent the standard error of mean. Different letters indicate significance at $P < 0.05$, according to the least significant difference test.

Fig. 4. Dynamics of arylamidase activity during the rice growth period (a for the early rice season and b for the late rice season)

main growth stages. And the number of fungi and actinomycete decreased in the order RF>MF>CK in the early and late rice season.

DISCUSSION

Soil enzyme activities and fertilizer management

Enzymes may respond to changes in soil management more quickly than other soil variables and there fore might be useful as early indicators of biological changes. In the early and late rice seasons, alkaline phosphatase activity decreased as follows: RF> MF>CK, and there were significant differences ($P > 0.05$) between RF, MF and CK. Our results suggest that residue management practices have effects on the activities of this enzyme. Temporal variations in activity, induced by crop residue decomposition, may modulate the enzymes by the microbial biomass. Alkaline phosphatase activity increase in RF and MF suggested that there were fundamental differences in C-supplying mechanisms to the microbial community in both treatments in addition to fertilizer or crop-derived inputs[18]. And there were significant differences ($P > 0.05$) in alkaline phosphatase activity under the RF and MF treatments at the main stages. Temporal variations in the enzyme activities can be differentiated from that representing long-term change in the enzyme activity in response to mineral fertilizer, straw incorporation or crop rotation with the statistical approaches used in this study.

β-glucosidase is the rate-limiting enzyme in the microbial degradation of cellulose to glucose and plays a crucial role in the C cycle in soils. β-glucosidase releases important energy sources for microorganisms. Ekenler and Tabatabai (2003) [9] showed that the activity of β-glucosidase in soils was significantly affected by the different residue management practices. We found a significant difference in β-glucosidase activity among the mineral fertilizer, rice residue plus mineral fertilizer and without fertilizer input treatments at the rice growth stages, that was, at the rice growth stages, β-glucosidase activity in the without fertilizer input treatment is always lower than in the mineral fertilizer and rice residue plus mineral fertilizer treatments because MF and RF with the higher C inputs should have stimulated β-glucosidase activity[15]. Furthermore, there also was not the expected significant difference in β-glucosidase activity between the mineral fertilizer and rice residue plus mineral fertilizer treatments at the rice growth stages which is probably related to the different C inputs by the different fertilizer managements[9].

Arylsulfatase is produced by both plants and microorganisms[11]. It is an extracellular enzyme that catalyses the hydrolysis of organic sulphate esters, releasing SO_2 that can be used by plants. In our study, the activity decreased in the following order: RF>MF>CK in the early and late rice season. These observations indicate that rice residue plus mineral fertilizer made a higher contribution to the active pool of C in soil organic matter than without fertilizer, which may explain the higher immobilization in rhizosphere soil containing rice residue plus mineral fertilizer and fertilizer than in

rhizosphere soil containing without fertilizer. We found arylsulfatase activity in soil from the RF treatment at the main stages of rice to be significantly higher than in soil of those treatments that did not receive any additional organic amendments (MF and CK). Arylsulfatase catalyzes the hydrolysis of ester sulfate bonds. Castellano and Dick (1988)[19] estimated biennial inputs of ester sulfate (straw plus other organic amendments if present) to the four agricultural treatments at the residue utilization plots. In our study, differences in enzyme activity were directly related to the quantity of substrate contained in the organic amendments and rice straw incorporation.

It has been suggested that arylamidase are one of the major enzymes involved in N mineralization in soils[16]. Previous studies demonstrated that the activity of arylamidase is significantly affected by crop residue managements[20]. In our study, statistical analyses suggested that different fertilizer managements affected significantly ($P < 0.05$) arylamidase activity (Fig. 4). There was no significant difference ($P > 0.05$) in arylamidase activity under the MF and RF in the early and late rice seasons. But the activity of this enzyme in RF soils was significantly ($P < 0.05$) higher than that in CK at the main growth stages. Dick *et al.* (1988)[21] reported a decrease in the arylamidase activity with long-term addition of inorganic N whereas crop residues additions increased the activity in a wheat–fallow system. The low activity at the main growth stages could be related to lower microbial biomass. The different ranking of treatments in soil enzyme activities might be related to the fertilizer management practices. This might have been because there was significant difference decomposable organic material in the crops straw-returned soil which favored soil enzyme activities[22-23].

Soil microbial community and fertilizer management

Yoshinari *et al.* (2001)[24] observed that rice straw affected soil microbial community structure. In the laboratory, we verified the increased numbers of aerobic bacteria, actinomycetes, and fungi in the soil with rice residue. In our study, the number of aerobic bacteria decreased in the order RF>MF>CK in the early and late rice season. The reason for this difference is being investigated. On the one hand, both of the soils in which rice straws had been incorporated showed considerable increases in the numbers of aerobic anaerobic microorganisms compared to the without straws-added soil (mineral fertilizer and without fertilizer input treatments). The number of aerobic bacteria in the paddy soil under the RF was significantly higher than that in the CK (Table 2). This might have been because there was significant difference of decay rates in the crops straws-returned soil which favored aerobic bacteria. And the numbers of aerobic bacteria were increased under rice residue plus mineral fertilizer in the early and late rice season, respectively.

Numerous studies indicate that fungi play an important role in both the formation and stabilization of soil aggregates but they are sensitive to disturbance, pollution and environmental change[25,26]. In our study, the number of fungi decreased in the order RF>MF>CK in the early and late rice season. This may be that the changes of soil environmental on fungal diversity could influence ecosystem function via decomposition of crops straws, fungi populations increased by returning rice straws in the early and late rice season, respectively.

And the number of actinomycete decreased in the order RF>MF>CK in the early and late rice season. Different ranking of treatments in the number of actinomycetes might be related to the application of crop residue and mineral fertilizer practices[14, 27-29]. We believe that the use of rice residue as organic manure resulted in an increase in the number of actinomycete. In general, the differences in the fertilization treatments may have caused differences in the microbial and which resulted in the significant differences in the numbers of aerobic bacteria, actinomycetes, and fungi between the paddy soils added with rice straws and without rice straws. In our study, the approaches for enumeration of microbial groups are severely limited by the fact that only a fraction of microorganisms is amenable to cultivation using the traditional methods applied here. For example, it doesn't allow all aerobic microorganisms to grow by using the LB agar medium. Therefore, some advanced methods to study the aerobic microorganisms should be investigated. Further studies could be helpful to better understand on how these changes in the number of microbial might actually impact microbial functions and nutrient availability in paddy soils.

CONCLUSION

Soil microbial and enzyme activities are the potent driving factors for organic matter decomposition and nutrient transformation in soil. This study revealed that the soil enzyme activities and soil microbes were affected by application of crop residue and mineral fertilizer practices during the early and late rice growth stages, and that these two factors could be used as potential soil quality indicators. Combined application of crop residue and mineral fertilizer stimulate ²-glucosidase, alkaline phosphatase, arylsulfatase and arylamidase activities in soils. The population of soil microbes also increased when crop residue and mineral fertilizer was applied. Application of mineral fertilizer was also beneficial to the population of soil microbes, but the enhancement was smaller than that of combined application of crop residue and mineral fertilizer. Thus, our findings indicate that management practices which combined application of crop residue and mineral fertilizer in rotations can improve soil enzyme activities and soil microbe population. Under the experimental condition, a combination of crop residue and mineral fertilizer is encouraged. If conditions are permitted, mineral fertilizer should be accompanied by crop residue.

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