Manure Applications Combined With Chemical Fertilizer Improves Soil Functionality, Microbial Biomass and Rice Production in a Paddy Field

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Research Article

Keywords: Rice, cropping system, soil enzyme, microbial biomass, carbon

Posted Date: April 29th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-461485/v1

Version of Record: A version of this preprint was published at Agronomy Journal on January 21st, 2022. See the published version at https://doi.org/10.1002/agj2.20990.

Abstract

Synthetic fertilizer with organic fertilizer (OF) is an approach for the improvement of soil health and quality without compromising crop yield. Therefore, a two-year field experiment was conducted to explore optimal chemical fertilizer (CF) management strategies in the context of OF, such as cattle manure (CM) and poultry manure (PM) fertilization to Ultisol soil to improve soil microbial biomass production, enzyme activities and nutrient contents, as well as grain yield of rice. A total of six treatments in the following combinations were used: i.e., T_1— CF_0; T_2—100% CF; T_3—60% CM + 40% CF; T_4—30% CM + 70%CF; T_5—60% PM + 40% CF, and T_6—30% PM + 70% CF. Results showed that the combined fertilization significantly increased soil enzymatic activities such as soil invertase, acid phosphatase, urease, catalase, β-glucosidase, and cellulase as compared to sole CF application. Similarly, the integrated manure and inorganic fertilizers led to significant increases in soil microbial biomass carbon (MBC), microbial biomass nitrogen (MBN), soil pH, soil organic carbon (SOC), total nitrogen (TN), available nitrogen (AN), available phosphorous (AP) and grain yield of rice. Average increases in soil MBC, MBN, SOC AN, and AP in the 0–20 cm soil depth were 62.2%, 54.5%, 29.2%, 17.4%, and 19.8%, respectively, across the years in treatment T_3 compared with T_2. Interestingly, the linear regression analysis displayed that soil enzymatic activities were highly positively correlated with MBC and MBN. Furthermore, the PCA exhibited that the improved soil enzyme activities and microbial biomass production played a key role in the higher grain yield of rice. Overall, the results of this study demonstrate that the combined use of CF and OF in paddy soil could be beneficial for the farmers in southern China by improving soil functionality and yield of rice on a sustainable basis.

Highlights

- Insight and basis are provided into N management in double rice cropping systems.
- Reduced N fertilization combined with manure improved rice grain yield and soil fertility.
- 60% organic N replacement chemical N had the greatest effect on soil biochemical properties and enzyme activities.
- Correlation analysis showed that the enhancements in soil enzymatic activities played a key role in higher soil quality and rice yields.

1. Introduction

Appropriate management strategies are important for maintaining soil health and ensuring the long-term sustainability of agricultural production (Ramesh et al., 2009; Li et al., 2015). Intensive agriculture has resulted in a clear decline in soil quality over the last two decades, which is a major issue for long-term agricultural production and food security (Agegnehu et al., 2014; Sarma et al., 2017). Despite playing an important role in feeding the world population (Jensen et al., 2011), extreme use of chemical fertilizers (CFs) is one of the primary causes of soil quality and fertility loss (Akhtar et al., 2019; Zhang et al., 2016; Chen et al., 2014). Farmers treat CFs as the most reliable and efficient way to improve crop production and revenue, without seeing the effects on soil or environmental protection (Yan et al., 2013; Qiao et al., 2012). Furthermore, the excessive use of mineral nitrogen (N) and phosphorous (P) also decline the soil microbial population and increase soil acidity (Zhang et al., 2015; Luo et al., 2020). Hence, full knowledge of soil's response to CFs additions is critical to protect and even improve soil health, particularly in paddy fields.

Recently, there is an increasing demand for agricultural output not only to reach high-quality standards but also to adopt eco-friendly techniques. Several researchers have recommended that OF application meet the requirements of sustainable farming, and thus organic fertilizers have a clear advantage over CFs in many aspects (Iqbal et al., 2020a; Ali et al., 2020a; Gu et al., 2015). Organic fertilizer has greater organic matter and richer nutrients contents; and can improve soil physicochemical and biological properties primarily by enhancing soil structure and reducing bulk density (Iqbal et al., 2019; Zhang et al., 2009). Furthermore, OF could provide additional benefits over CFs, such as enhanced soil structure (Thangarajan et al., 2013; Iqbal., 2019), soil richness (Akhtar et al., 2018), maintaining soil health (Luo et al., 2018), and in particular, comparable or even greater crop grain yields in some cases (Iqbal et al., 2020b; Ali et al., 2020b). These assistances have mainly been affiliated with responses in soil biochemical and biological properties (Huseo et al., 2011; Ling et al., 2016). However, there are gaps in the knowledge presently available about the responses of soil biochemical and microbial activities in the soil to continued fertilizer applications. Particularly, the effect of OF and CF on specific combination ratios on soil enzyme activity and microbial biomass production is still unknown.

The soil enzymatic activity has been used to evaluate soil quality and crop productivity (Akhtar et al., 2019; Ge et al., 2010). They are direct indicators of the biological metabolism of soil biota (Dick and Kandeler, 2015; Burns et al., 2013). In addition, their activity can expose microbial activity, organic matter decomposition rate, and substrate availability for microbial or plant uptake (Lagomarsino et al., 2009). For example, cellulases and glucosidase are a class of hydrolytic enzymes generated by soil microorganisms that decompose polysaccharides (Deng and Tabatabai, 1994). Soil available and total P contents are related to the change in soil phosphatase activity, and more loss in P content was noted in paddy fields (Wang et al., 2012). Furthermore, soil enzymes are the biological catalysts, and facilitate the transformations of various forms of energy, and participate in the processes related to the cycling of bio-elements, such as C, N, P, and S (Xia et al., 2017; Luo et al., 2018). Mooshammer et al. (2014) stated that increment in soil microbial biomass also facilitates C and N use efficiency. Fertilizer-mediated microbial activity can also cause changes in C and N dynamics (Brilli et al., 2017; Mooshammer et al., 2014), which can control the balance of N and C use efficiency (Zhang et al., 2015).

Activities of an enzyme associated with C, N and P nutrients cycling in soils frequently enhance when organic wastes are added (Zhang et al., 2015; Bohme et al., 2005). Organic fertilizers can stimulate microbial activity and improved plant growth (Nayak et al., 2007; Ge et al., 2010); protect and sustain soil enzymes in their active forms; serve as readily available sources of energy and nutrients that increase microbial biomass and crop production (Bi et al., 2009; Akhtar 2018), and provide substrates for living microorganisms in the soil (Saha et al., 2008). In addition, previous research has revealed that various land use management and fertilization amendments have influenced soil enzyme activities and their production (Raiesi and Beheshti, 2014; Medeiris et al., 2015). However, little is known about the effect of combined organic and inorganic N fertilization in specific prorations on soil enzymatic activity, microbial biomass production and grain yield of rice under paddy fields condition.

In an agroecosystem, the relationships between soil enzyme activities, microbial biomass, and soil quality traits are crucial. Therefore, the purpose of this study was to investigate how different forms of manure, such as cattle and chicken manure in various proportions combined with synthetic fertilizer (urea) influence soil enzymatic activities and their relationships with soil quality, microbial biomass production, and rice yield under paddy field. We collected the soil samples from a 4-seasons (2 years) continued fertilizers trial on a rice paddy soil treated by combined organic and inorganic fertilizers. The soil enzyme activities (invertase, urease, catalase, β-glucosidase, acid phosphatase and cellulase), microbial indicators (MBC and MBN), soil environmental factors (pH value, SOC, TN, AN, AP, and AK contents) and grain yield of rice were investigated. The aims of this work are: (1) to evaluate the effects of different types and ratios of OF and CF treatments on the enzyme activities, microbial biomass production and soil quality; (2) to analyze the correlations among the enzyme activities, microbial production, and soil fertility and; (3) to expose how the changes of soil nutrients and enzymes activities influences rice yields.

2. Materials And Methods

2.1 Site description

The ongoing field experiment at the rice experimental station of Guangxi University (22°49'12" N, 108°19'11" E), China was initiated in 2019. This site experiences a subtropical monsoon climate, with a total annual rainfall of 1398 mm, and an average temperature of 24.8°C (Fig. 1). The soil is classified as Ultisols (USDA soil classification), which is slightly acidic with a pH of 5.96. A soil test revealed that the SOC was 18.75 g kg^{-1}, and total nitrogen (TN) was 1.64 g kg^{-1}; details of other nutrients are shown in Table 1.

Table 1
Physical and chemical properties of soil and manure before the experimentation.

Properties		Cattle	Poultry
	Soil	Manure	Manure
Porosity (%)	40.52	-	-
Moisture content (%)	11.93	-	-
Bulk density (g cm^{-3})	1.36	0.81	0.74
pH (water)	5.94	7.75	7.95
SOC (g kg^{-1})	14.56	146.33	164.22
SOM (g kg^{-1})	25.08	254.63	282.42
Total N (g kg^{-1})	1.41	9.76	13.58
Total P (g kg^{-1})	0.75	10.12	7.32
Total K (g kg^{-1})	-	14.22	9.76
Available N (mg kg^{-1})	134.7	-	-
Available P (mg kg^{-1})	23.12	-	-
Available K (mg kg^{-1})	233.3	-	-
Note: SOC—soil organic carbon, SOM—soil organic matter, N—nitrogen, P—phosphorous, K—potassium, C: N—carbon to nitrogen ratio.			

2.2 Experimental design

The double rice-growing season experiment (the early season runs from (March to July) and the late season runs from (July to November)) was arranged in a randomized complete block design having three replications. The plot size was 3.9 m × 6 m (23.4m^2). Organic manure (PM and CM) and CF (urea) was used in this study, and the treatments combination were: T_1— CF_0; T_2—100% CF; T_3—60% CM + 40% CF; T_4—30% CM + 70%CF; T_5—60% PM + 40% CF, and T_6—30% PM + 70% CF used. The rice seeds were germinated in plastic trays, and the uniform-sized 25 days old seedlings were transferred into the rice field. The recommended dose of NPK (150:75:150) was used for each treatment, except T_1. The nutrient contents of OF and the quantity of all treatments are shown in Table 2. The N and K fertilizers were delivered in three splits: 50% was applied initially before transplanting, 30% at tillering, and 20% at the heading stage. All superphosphate fertilizer was applied before transplanting as a basal dose. In addition, even flooding was maintained from transplantation to physiological maturity. During the entire season, standard farming practices, including irrigation and insecticides application was performed the same for all treatments.

Table 2
Nutrient content and amount of nutrient provided of each plot and application time.

Treatment	N (g $plot^{-1}$)	Urea (g$plot^{-1}$)	CM, PM, (kg$plot^{-1}$)	Basal fertilization (kg $plot^{-1}$)	Tillering (g $plot^{-1}$)	Panicle initiation (g $plot^{-1}$)
T_1: CF_0	0	0	0	P_2O_2: 0.93, KCl: 0.30	KCl: 0.30	Urea: 0
T_2: 100% CF	351	753	0	Urea: 0.45, P_2O_2: 0.93, KCl: 0.30	Urea: 150, KCl: 0.30	Urea: 150
T_3: 60% CM + 40% CF	351	301	21.5	Urea: 0, CM: 21.5, P_2O_2: 0.93, KCl: 0.30	Urea: 150, KCl: 0.30	Urea: 150
T_4: 30% CM + 70% CF	351	527	10.7	Urea:1.17, CM: 10.7, P_2O_2: 0.93, KCl: 0.30	Urea: 150, KCl: 0.30	Urea: 150
T_5: 60% PM + 40% CF	351	301	15.5	Urea: 0, PM: 15.5, P_2O_2: 0.93, KCl: 0.30	Urea: 150, KCl: 0.30	Urea: 150
T_6: 30% PM + 70% CF	351	527	7.7	Urea: 1.17, PM: 7.7, P_2O_2: 0.93, KCl: 0.30	Urea: 150, KCl: 0.30	Urea: 150

Note: N—nitrogen, CK—control, CF—chemical fertilizer (urea), CM—cattle manure, PM—poultry manure, P_2O_2—superphosphate, KCl—potassium chloride.

2.3. Soil sampling and analysis

2.3.1 Soil properties

Soil samples were obtained by a core sampler at depth (0–20 cm) from each treatment after the late-season rice harvest in 2019–2020. Soil sampling was done at different points and then mix to make a composite sample, and divide into two parts, one for the measurements of soil nutrients, and the other was stored at 4℃ for the determination of soil enzymes

Soil organic C was measured by the $K_2Cr_2O_7$-H_2SO_4 oxidation method followed by titration (Wang et al., 2003). For soil total nitrogen (TN) analysis, 200 mg of the samples were processed using the salicylic acid–sulfuric acid–hydrogen peroxide method defined by Ohyama et al. (1991), and total N was measured using the micro-Kjeldahl technique according to Jackson (1956). The other chemical traits, including soil pH, available N (AN), available P (AP), and available K (AK), were measured using the methods defined by Lu (2000).

2.3.2 Soil enzyme activities

The determination of soil enzymatic activity was performed by the described procedure of Jin et al. (2009) and Zhang et al. (2011). Soil catalase determination was performed by adding distilled water of 40 ml and 5 ml of 0.3% H_2O_2 in two g of soil, and then properly shake for 20 min at 150 rpm, and then filtered. The filtrate was titrated with 0.1 mol L^{-1} $KMnO_4$ in sulfuric acid. The determination of urease activity was checked in a 5 g fresh soil sample by using 10% of urea solution as substrate, and was incubated for one day at 37℃ with 5 mL of citrate solution at pH 6.7 and 5 mL of the substrate, and then filtered. One ml filtrate was treated with 4 mL of sodium phenol solution and 3 mL of 0.9% sodium hypochlorite solution. The ammonium released from urea hydrolysis was measured using an ultraviolet spectrometer subsystem at 578 nm.

Soil invertase activity was measured using 8% glucose solution as the substrate. A 5-g fresh soil sample was incubated with 15 mL of the substrate, 5 mL of 0.2 M phosphate buffer (pH 5.5), and 5 drops of toluene for one day at 37°C. After incubation, the mixture was filtered (Whatman 2V) immediately, and a 1-mL aliquot was reacted with 3 mL of 3, 5-dinitrylaslicylate in a volumetric flask and heated for 5 min. When cool, the soil solution in the flask was measured in a UVS at 508 nm. The soil acid phosphatase activity was measured in one g sample using p-nitrophenyl phosphate disodium as substrate, followed by incubation in a modified universal buffer (acid phosphatase: pH 6.5) for 1 h at 37°C.

Cellulase was measured following the method of Pancholy and Rice (1973). Reducing sugars were produced when soil samples were incubated at 37°C with carboxymethyl cellulase. Soil β-glucosidase activity was determined by the colorimetric method described by Eivazi and Tabatabai (1994). Briefly, 1 g of soil was incubated with the substrate at pH 6.0 and 37°C. After 1 h, 0.5 M CaCl2 and pH 12.0 modified universal buffer were added to extract p-nitrophenol. The amount of p-nitrophenol released by glycosidases was determined calorimetrically at 410 nm.

Activities of soil catalase, urease, invertase, acid phosphatase, cellulase, and β-glucosidase were expressed as 0.1 mol KMnO4 g^{-1} soil 20 h^{-1}, mg NH3-N g^{-1} day, mg glucose g^{-1} 24 h^{-1}, mg phenol g^{-1} h^{-1}, mg glucose g^{-1} day^{-1}, and μg p-nitrophenol g^{-1} soil, respectively.

2.4. Rice grain yield

Rice plants from the whole plot area were harvested at maturity to measure the rice grain yield adjusted to 14% moisture content.

2.3.3. Statistical analysis

One-way analysis of variance (ANOVA) test was applied to study the effects of organic and inorganic fertilizers on soil enzymatic activities, biochemical traits, and grain yield of rice Tukey's posthoc test was used to compare multiple means for the variables where effects of experimental factors were significant. Principal component analysis (PCA) was used to test the difference among treatments for studied variables (Canoco5). Relationships between soil enzyme activities, chemical properties, and grain yield were studied using redundancy analysis (RDA). Statistical analyses were performed with SPSS for Window Software v. 19.

3. Results

3.1. Soil Chemical properties

The effects of co-application of manure and synthetic fertilizer on soil chemical attributes are shown in Table 3. Co-application of OF and CF significantly enhanced the soil pH compared with soil chemical N fertilization (T_2). In 2019 and 2020, the soil pH of the T_3 and T_5 treatments was considerably ($P < 0.05$) greater compared with the rest of the treatments. Soil pH showed the same pattern across years, and an average increase in soil pH by 5.5% and 5.3% was exhibited in the T_3 and T_5 regimes, respectively, compared with the control (T_2). The other combined treatments also had significantly higher soil pH during both years compared with T_2.

Table 3

Changes in soil properties under the combined organic and inorganic N fertilization.

		pH	SOC	TN	AN	AP	AK
Year	Treatment	(water)	(g kg^{-1})	(g kg^{-1})	(mg kg-1)	(mg kg^{-1})	(mg kg^{-1})
	T_1	5.93c	13.47c	1.41c	138.00c	23.14d	225.81d
	T_2	5.92c	14.73c	1.42c	139.50c	24.15c	228.86d
2019	T_3	6.23a	17.24a	1.72a	154.30a	28.41a	292.37a
	T_4	6.10b	15.81b	1.61b	148.80b	26.29b	276.66c
	T_5	6.21a	17.08a	1.71a	155.40a	27.63ab	288.73b
	T_6	6.09b	15.81c	1.60b	149.60b	27.52b	275.06c
Average		6.06b	15.69b	1.57b	147.43b	26.29b	264.58b
	T_1	5.94c	14.39d	1.42d	142.52c	24.12d	226.57c
	T_2	5.93c	16.25c	1.46c	144.05c	26.52c	230.81c
2020	T_3	6.27a	22.95a	1.95a	177.50a	34.41a	294.38a
	T_4	6.23b	21.08b	1.78b	163.14b	32.29b	280.62b
	T_5	6.28a	23.25a	1.93a	184.51a	33.63a	289.75a
	T_6	6.24b	20.88b	1.77b	181.52ab	33.52a	277.08b
		6.15a	19.97a	1.72a	165.54a	30.75a	266.54a

Note: T_1—control, T_2—100% CF, T_3—60%CM + 40%CF, T_4—30% CM + 70% CF, T_5—60% PM+ 40%CF, T_6—30%PM + 70%CF, SOC—soil organic carbon, TN—total nitrogen, AN—available nitrogen, AP—available phosphorous, AK—available potassium. Values followed by the same letters, within column, are not significantly different at $p \leq 0.05$.

The positive effects of OF and CF treatments on SOC content are shown in Table 3. SOC content increased significantly under integrated treatments during both years. In both years, the SOC content was maximum in the T_3 and T_5 treatments than in the rest of the treatments. Furthermore, the SOC content among the different treatments showed the same pattern across years, and average increases in SOC content were 29.2% and 29.8% in the T_3 and T_5 treatment, respectively, compared with control. Compared with control, the T_4 and T_6 treatments also showed significantly higher SOC content. Moreover, substantial enhancements were detected in SOC content during the second year, the average increase in SOC content in the year 2020 was 27.2% compared with the year 2019.

The difference in soil total N (TN) content at the 0–20 cm soil depth is shown in Table 3. Soil TN content was significantly increased ($P < 0.05$) in the combined treatments compared with CT in both years. The TN content of all treatments showed similar patterns across years. The average increases in TN were 26.3%, and 25.9 %, respectively, in the T_3 and T_5 treatment compared with control. Similarly, the T_4 and T_6 treatments also had considerably superior soil total N content compared with Control. Furthermore, significant improvements were observed in TN content during the seceding year, the average increment in TN content in the year 2020 was 9.55% compared with the year 2019.

The variations in the soil available N, P, and K content in the top layer are shown in Table 3. The co-applied organic and mineral N fertilization considerably ($P < 0.05$) improved soil available N, P, and K content across years compared with control (T_2). The AN, AP, and AK content of all treatments showed similar patterns across years, and the average increases in AN, AP, and AK were 17.02% and 19.84%, respectively, in the T_3 and T_5 treatment compared with control. However, AN, AP, and AK content did not significantly differ ($P < 0.05$) between the treatments T_3 and T_5. The N, P and K content was also considerably greater in the rest of the integrated treatments compared with CT. Moreover, significant developments were noted in AN, AP, and AK content during the following year, and the average increase in AN and AP content in the year 2020 was 12.28% and 16.96%, compared with the year 2019.

3.2. Soil microbial biomass

Soil MBC and MBN were significantly different at the 0–20 cm soil depth among the treatments in both years as shown in Fig. 2. The integrated use of manure with urea (CF) significantly enhanced the soil MBC and MBN, compared to urea-only (T_2). Across the years the treatments showed the same behaved. Averaged across the years, the combined treatment T_3 enhanced soil MBC and MBN by 62% and 54%, respectively, compared with T_2. But, T_3 was statistically ($P < 0.05$) similar to T_5. Likewise, the joint treatments T_4 and T_6 also significantly enhanced soil MBC and MBN compared with sole urea fertilization. In-addition, significant improvements in soil MBC and MBN were observed among the different years, and the average soil MBC and MBN was increased by 9.2% and 21%, respectively, in 2020 compared with 2019.

3.3. Soil enzyme activity

3.3.1. Soil phosphatases and catalase activity

The activities of phosphatases (POH) and catalase (CAT) soil enzymes at the 0–20 cm soil depth are shown in Fig. 3. The co-applied organic and inorganic N fertilization treatments significantly affected soil POH and CAT activity in 2019 and 2020. The POH and CAT activity were considerably ($P < 0.05$) higher in the T_3 and T_5 treatments compared with the other treatments. Patterns of POH and CAT activity among the treatments were similar across years. Average across the years, POH activity 7.45 and 9.21 (mg phenol g^{-1} h^{-1}) were recorded in T_3 and T_5, respectively, and average CAT activity 19.99 and 21.33 (0.1 mol $KMnO4$ g^{-1} soil h^{-1}) was noted in T_3 and T_5, respectively. The POH and CAT activity of the T_3 and T_5 treatment was increased by 43 & 48%, and 35 & 39%, respectively, compared with T_2 averaged across years. The rest of the combined treatments also had significantly higher POH and CAT activity compared with control. Furthermore, significantly enhances were observed in POH and CAT activity during the seceding year in 2020, compared with 2019.

3.3.2. Soil urease and cellulase activity

The combined fertilization significantly enhanced the activity of cellulase and urease in both years (Fig. 4). Soil urease and cellulase enzyme activity was considerably (P< 0.05) superior in the T_5 treatment than in all other treatments. However, treatments T_3 were significantly (P< 0.05) similar to T_5. Patterns of urease and cellulase activity among all the treatments were similar across years. Average across the years, urease activity 1.15 and 1.19 ($NH3$-N g^{-1} day^{-1}) were noted in T_3 and T_5, respectively, and average cellulase activity 2.35 and 2.54 (mg glucose g^{-1} day^{-1}) was noted in T_3 and T_5, respectively. The urease and cellulase activity of the T_5 treatment was 52% and 48% higher, respectively, compared with control (T_2) averaged across years. The other combined treatments, such as T_4 and T_6 had significantly higher urease and cellulase activity compared with CT. Moreover, significantly enhances were observed in POH and CAT activity during the seceding year in 2020, compared with 2019.

3.3.3. Soil -glucosidase and acid invertase activity

The influence of joint use of manure and synthetic fertilizers on soil glucosidase and invertase activity is shown in Fig. 5. During 2019 and 2020, glucosidase and invertase activity were higher in the combined treatment compared with sole urea application. Furthermore, patterns of glucosidase and invertase activity among treatments were similar across years. Average across the years, maximum soil glucosidase activity 15.14 and 14.45 (µg p-nitrophenol g^{-1} soil) were observed in T_3 and T_5, respectively, and invertase activity 44.54 and 43.71 (mg glucose g^{-1} $24h^{-1}$) was observed in T_3 and T_5, respectively. The glucosidase and invertase activity of the T_5 treatment was 62% and 58% higher, respectively, compared with control averaged across. But, T_3 was noted non-significant with T_5. Similarly, the T_4 and T_6 also had notably higher glucosidase and invertase activity compared with control. Moreover, substantially enhances were detected in POH and CAT activity during the year 2020, compared with 2019.

3.4 Grain yield

The combined organic-inorganic fertilizers application significantly affected the grain yield of rice in both 2019 and 2020 (Fig. 7). Compared with control, co-applied N fertilization considerably improved the grain yield of rice across years, and the treatments showed a similar pattern across years. The grain yield of the T_6 treatment was enhanced by 15% and 33% during 2019 and 2020, respectively, relative to control. However, there were no significant (P< 0.05) differences in grain yield among the T_4 and T_6 treatments. Similarly, the combined treatments T3 and T5 also significantly improved grain yield, compared with control. Significant improvements in grain yield were observed among the different years, and the average grain yield was increased by 14% in 2020 compared with 2019.

3.5. Relationships among soil characteristics, soil enzyme activity, and rice grain yield

Significant correlation among soil chemical and biological properties and enzymatic activities are displayed in Figs. 6 and 8. The linear regression analysis exhibited that the soil enzymatic activity was strongly positively associated with soil MBC and MBN. Moreover, RDA showed that soil biochemical traits, such as MBC, MBN, SOC, TN, AN, AP, and AK had a positive relationship with soil enzymes. Moreover, soil MBC, MBN, SOC, and TN, and AN were highly associated with all six enzyme activities when compared with other soil traits.

The PCA revealed a significant difference in soil biochemical properties, enzyme activity, and rice grain yield between the various combined OF and CF treatments (Fig. 9). All the variables for soil biochemical attributes, soil enzyme activities, and rice grain yield were grouped into 4 well-differentiated classes. The first group contained the sample from T1, the second group from T_2, the third form T_4 and T_6, and the fourth from T_3 and T_6. The cumulative variance of contribution reached 99.3% (PCA 1 explained 95.7%, and PCA 2 explained 3.6%) over the years. Furthermore, the analysis showed that rice grain yield, MBC, MBN, SOC, TN, AN, AP, pH, glucoside, cellulase, acid invertase, acid POH, CAT, and urease were highly affected by the co-applied of both OF and CF fertilization treatment.

4. Discussion

The traditional farming system heavily depends on CFs, which adversely affected soil quality, the environment and cereal yields. To enhance soil quality, nutrient contents, and feasible farming, maintaining C and N in soil has recently become the main research subjects (Adekiya et al., 2019; Iqbal et al., 2019). Furthermore, SOC, N, and other soil nutrients are the indicator of soil health and fertility. Soil fertility is strongly related with organic matter level in soil and thus affect soil richness and microorganism (Luo et al., 2020). Additionally, the accretion of SOC and N and their related environmental significance have strongly enhanced the soil ecological activity (Kwiatkowska-Malina, 2018). The aim of this study was therefore to evaluate the effect of a cumulative application of OF and CF on soil biochemical properties and grain yield of rice.

4.1. Soil properties

Soil qualitative traits (i.e., SOC, TN, AN, AK, AP and pH) were significantly enhanced in integrated organic and inorganic N fertilization compared with soil urea fertilization in the present study (Table 3). We found that the decomposition of organic fertilizer slowly released nutrients to the soil and demonstrate that rising the manure level from 30–60% enhanced soil chemical attributes. In this work, sole urea application decreased pH, while integrated organic and inorganic N fertilization treatments significantly increased soil pH. A feasible reason for this is that farmyard manure, such as cattle or chicken manure influences soil acidification because it often contains sufficient basic cations and carbonate ions that neutralize soil acidity (Whalen et al., 2000; Duruigbo et al., 2007). Furthermore, the alkaline nature of organic fertilizer is one of the main reasons for the increase in soil pH (Xu et al., 2006).

Soil organic C is a significant parameter of soil health and fertility in long-term experiments (Stockmann et al., 2013; Johanners et al., 2017). In this study, a significant enhancement in the SOC content was exhibited under combined treatments compared with control (Table 3). In fact, the soil organic C at any certain position strongly relies on the annual organic turnover (i.e., plant root exudates, root and shoot stubbles, and their recycling) (Adekiya et al., 2019; Biratu et al., 2019). Moreover, the significant enhancement of soil organic C could be related to the significant effects of OF application, because the soil organic C change rate is resultant from both direct C inputs from manure and indirect C inputs from an increase in crop biomass return to the soil, such as root and crop residues (Bitew and Alemayehu, 2017). Furthermore, Purakayastha et al., (2008), documented that OF significantly improved soil C in the top layer. Furthermore, the enhances in the soil enzyme activities, microbial biomass production related to C cycling and accumulation also plays role in higher C in this study. The RDA analysis in this study showed that the soil enzyme activities, MBC and SOC are strongly associated with each other.

In addition, soil nutrients, N, Kand P were significantly improved under combined treatments in this study (Table 3). This is mainly due to the addition of organic fertilizer such as CM or PM had a positive effect on the soil N, P and K content. This might be allied with the incorporation of organic fertilizers remains, which directly added nutrients into the soil after decomposition (Wei et al., 2013). Another possible explanation for enhancements in soil nutrients in this work was related to organic fertilizers absorbing more leachate produced during the process, which caused improved water holding capacity, decline nutrients leaching, and consequently more available NPK (Adekiya et al., 2019; Murmu et al., 2013). Moreover, organic fertilizers application provides a great amount of P to the soil, and decreases the fixation of delivered P in the soil, resulting in the enhanced competition of organic molecules with PO_4^{3-} ions for P retention sites (Xie et al., 1991). The higher AK under combined treatments could be associated with the release of organic acids in decomposition, which generate negative electron charges in the soil with a preference for di or tri valent cations, such as Al3+, Ca2+, and Mg2+, leaving K + to be absorbed by negatively charged soil colloids (Timsina et al., 2006). This process could help to decrease K fixation and improve its accessibility in soil. Furthermore, organic manure fertilization could also enhance the population of soil microbes and the activity of enzymes about nutrient transformation, therefore enhancing soil available nutrients (Iqbal., 2019; Lazcano et al., 2013).

4.2. Soil microbial biomass

The rate of microbial biomass C and N production reflects the features of the microbial community composition and structure (Norris and Congreves, 2018). In the present study, organic manure along with CF significantly enhanced the production of MBC and MBN (Fig. 2). The increments in MBC and MBN may be associated with organic fertilizer, along with CF fertilization increased the physicochemical and biological traits of soil, leading to an increase in the absorption and uptake of mineral N by the crop (Ahmad et al., 2008; Mahmood et al., 2017). In addition, manure promotes the conservation of mineral N to microbial N and other forms (Fan et al., 2017). Another possible explanation is that the combined organic amendments fertilization might improve the soil fertility and rice biomass production in the present study (Tables 4–5) ultimately lead to increase crop residues. This so useful for the spread of soil microorganisms, and thereby facilitates the conversion of carbon and nitrogen (Lima et al., 2009). Furthermore, the enhancement in soil enzymatic activities also a reason for higher C and N production under combined treatments.

4.3. Soil enzymes activity

Soil enzymes are a kind of special protein owing to biochemical and catalytic properties and are engaged in many crucial biochemical reactions in soil and have a strong affiliation with soil quality and health (Das & Varma, 2010; Bohme et al., 2005). As a sensitive indicator of soil fertility and health, soil enzyme activity changes with land management practices and fertilization (Lagomarsino et al., 2009; Ge et al., 2010). In the current study, manure coupled with inorganic N fertilization improved soil enzyme activities (i.e., acid phosphatase, catalase, urease, cellulase, and β-glucosidase) in the 0–20 cm soil layer (Figs. 3–5). Similarly, several studies found that organic manure fertilization significantly increases the enzyme activity, such as β-glucosidase, urease, and phosphatase compared with the chemical fertilized soils, and thus improves soil biochemical properties (Liang et al., 2014; Lazcano et al., 2013). Another possible explanation for the increase in soil enzyme activity could be because the microbial activities were enhanced by organic manure fertilization (Fig. 2), which supplied organic matter and was used as a substrate for soil enzymes (Marterns et al., 1992), and enzyme activity is positively related with the content of SOC as shown in RDA analysis in this study (Fig. 7).

More importantly, our result showed that the combined application of manure and mineral N fertilization at the ratio of (30% OM + %70 CF) and (60% OF + 40% CF) significantly enhanced soil enzyme activities during both years, and high ratio of organic manure supplementation caused higher enzyme activity (Figs. 3–5). This was associated with the enhanced inputs of organic substrates that promote microbial population and growth as well as and enzyme synthesis. Alike outcomes were also found from the study of Marcote et al. (2001). In addition, the RDA analysis exhibited a very strong and significant relationship between soil enzyme activity and soil properties (Fig. 7). In relative, chemical fertilizer application has a weaker influence on the activity of soil enzymes (Gu et al., 2010; Yu et al., 2012). Similarly, Fan et al. (2012) reported that CFs application may decline the activities of soil enzymes associated to C, N and P cycling, maybe because enzymes are not required by microorganisms to obtain N and P nutrients.

4.4. Rice grain yield

Combined manure and mineral N fertilization significantly improved the grain yield of rice compared with that of sole inorganic fertilizer (Fig. 6). The enhancements in grain yield could be due to the enhanced soil fertility and functionality under treatment combinations, which ultimately increased crop growth, biomass and yield accumulation by providing sufficient nutrients throughout the crop growing period. Furthermore, the RDA analysis showed that the soil biochemical and enzyme activates in the current study were strongly related to the grain yield of rice (Fig. 7). Hence, changes in cereal grain yield are closely associated with soil properties and enzymatic activities (Yang et al., 2008; Akhtar et al., 2018). The addition of organic manure coupled with mineral fertilizer improved the biomass and yield of rice considerably relative to the application of mineral fertilizer in isolation (Mangalassery et al., 2018).

5. Conclusion

Our findings indicated that the continuous application of OF and CF significantly improved soil qualitative traits, including pH, SOC, TN, AN, AP, MBC and MBN compared with sole urea (CF) fertilization. The combined use of both fertilizers led to higher soil enzymatic activities (i.e., soil acid phosphatase, urease, catalase, invertase, glucosidase, and cellulase). Improvements in these soil enzymatic activities further improved soil microbial biomass production and grain yield of rice. Moreover, redundancy analysis showed that the contents of SOC, TN, AN, AK, AP, MBC and MBN in soil were considerably and positively associated with the potential activities of soil enzymes examined in this experiment. The combined application of organic and inorganic fertilizers could provide an effective strategy for the increase in soil fertility and yield of rice in a sustainable manner.

Abbreviation

CF—chemical fertilizer, OF—organic fertilizer; CM—cattle manure, PM—poultry manure SOC—soil organic carbon; TN—total nitrogen; AN—available nitrogen; TP—total phosphorus; AP—available phosphorus; TK—total potassium; AK—available potassium; MBC—microbial biomass carbon; MBN—microbial biomass nitrogen; C: N—carbon to nitrogen ratio; POH—phosphatases; CAT—catalase; Fig.—figure, PCA—principal component analysis.

Declarations

Acknowledgments:

We thank collaborators at Agriculture Station, Guangxi University for help in conducting this research.

Funding:

This study was fiscally funded by the National Key Research and Development Project of China (2016YFD030050902).

Conflicts of Interest:

The authors declare no conflicts of interest.

Ethics approval:

Not applicable

Consent to participate:

Not applicable

Consent for publication:

The authors declare that consent is given for publication.

Availability of data and material:

Data will be made available upon request from the corresponding author.

Code availability:

Not applicable

Authors' contributions:

Anas Iqbal: Conceptualization, Methodology, Investigation, Data curation, Software, Formal analysis and Wrote the manuscript

Liang He: Resources, Investigation and Data curation

Steven G McBride: Review & editing

Izhar Ali: Software and Formal analysis

Kashif Akhtar: Software, Formal analysis, Review & editing

Rayyan Khan: Review & editing

Maid Zaman: Review & editing

Shangqin Wei: Resources and Data curation

Zixiong Guo: Data curation and Software

Ligeng Jiang: Project administration, Conceptualization, Methodology , Supervision, review & editing

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Tables

Tables 4 and 5 are not available with this version.

Figures

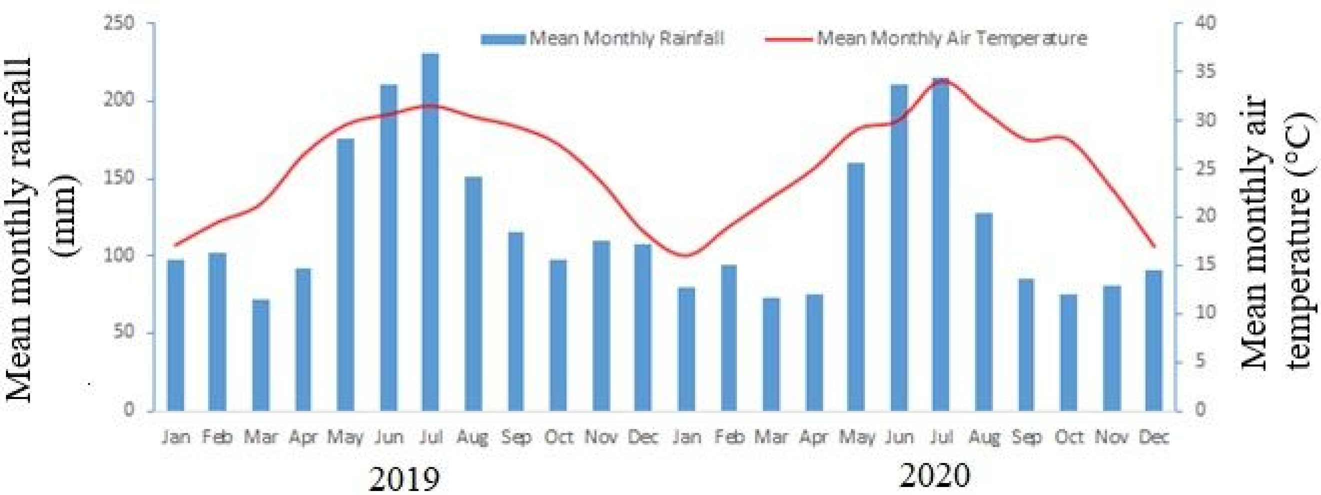

Figure 1

Mean monthly rainfall and air temperature during the experimental years

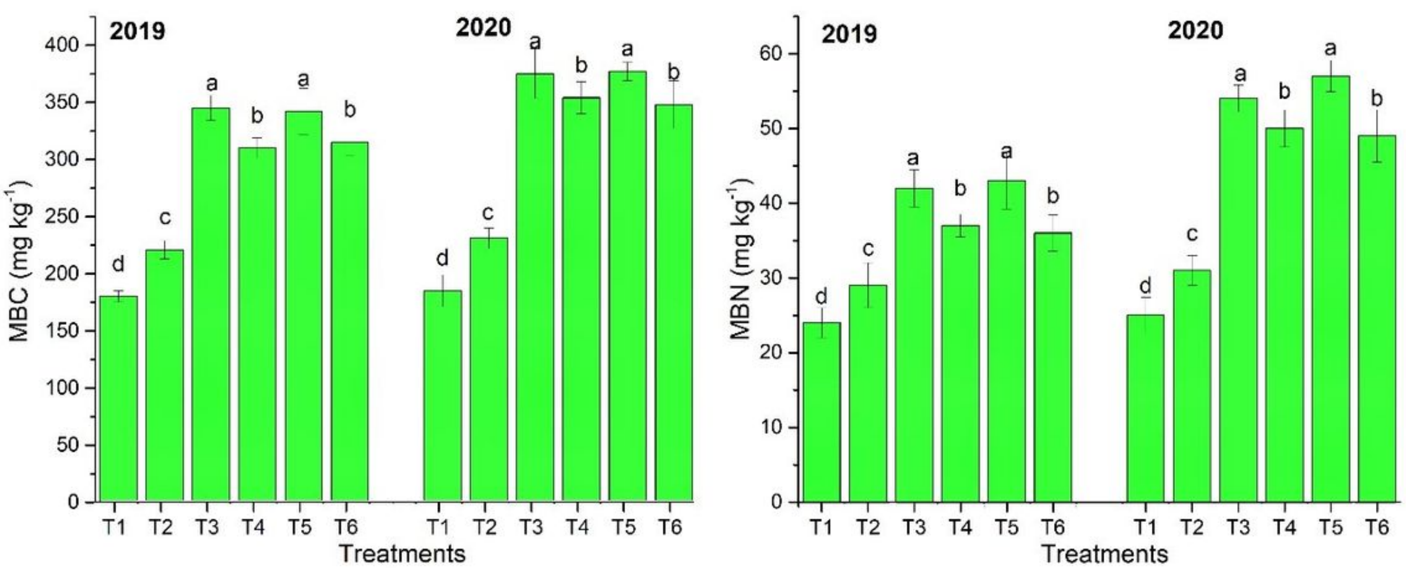

Figure 2

Changes in soil microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN) as influenced by combined organic and inorganic N fertilization. The mean comparison was made using the least significant differences (LSD) test for treatments and seasons means based on the LSD test at 5%. Different letters within the line are not significantly different at $p < 0.05$. Note: T1—control, T2—100% CF, T3—60%CM + 40%CF, T4—30% CM + 70% CF, T5—60% PM+ 40%CF, T6—30%PM + 70%CF.

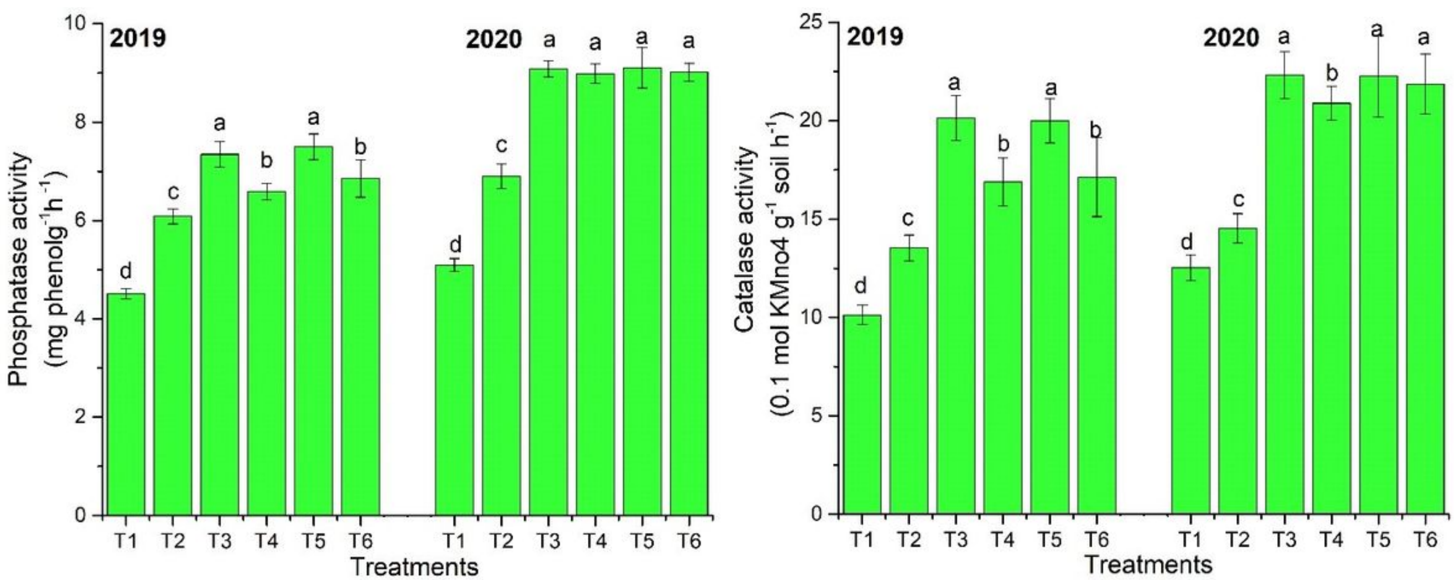

Figure 3

Changes in soil enzyme phosphatase and catalase activity as influenced by different combined organic and inorganic N fertilization. Vertical bars represent the standard error of the mean. The mean comparison was made using the least significant differences (LSD) test for treatments and seasons means based on the LSD test at 5%. Note: For treatments combination please see Fig. 2.

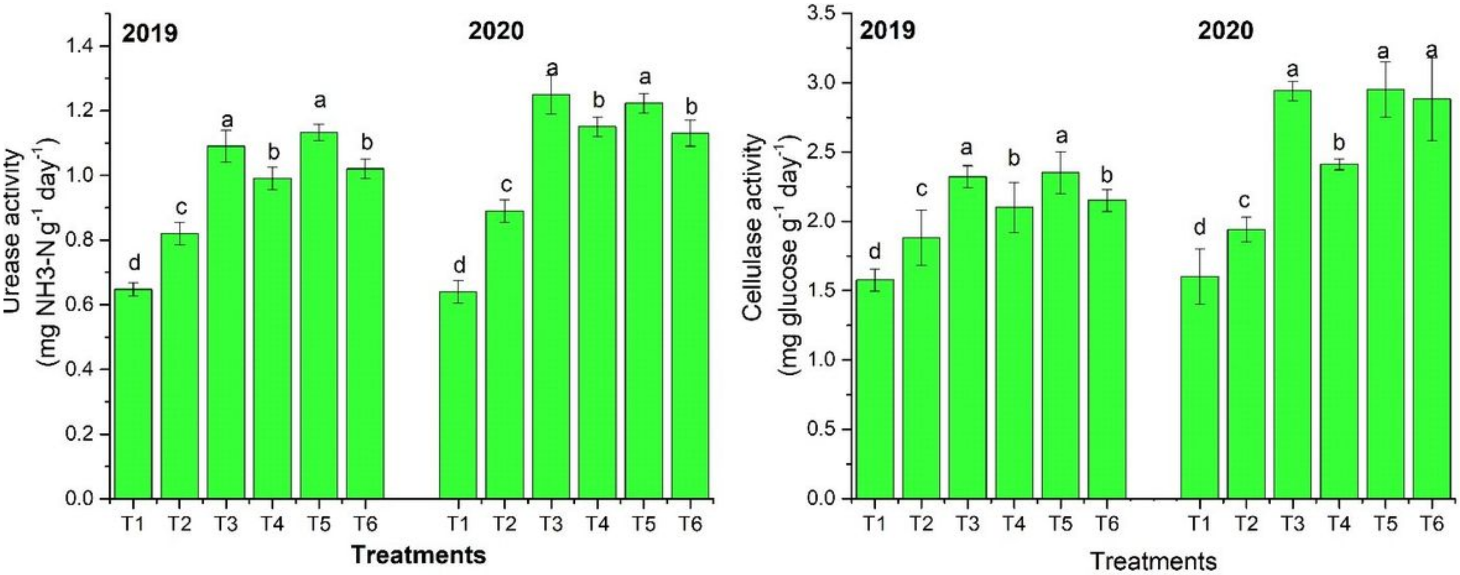

Figure 4

Changes in soil enzyme urease and cellulase activity as influenced by different combined organic and inorganic N fertilization. Vertical bars represent the standard error of the mean. The mean comparison was made using the least significant differences (LSD) test for treatments and seasons means based on the LSD test at 5%. Note: For treatments combination please see Fig. 2.

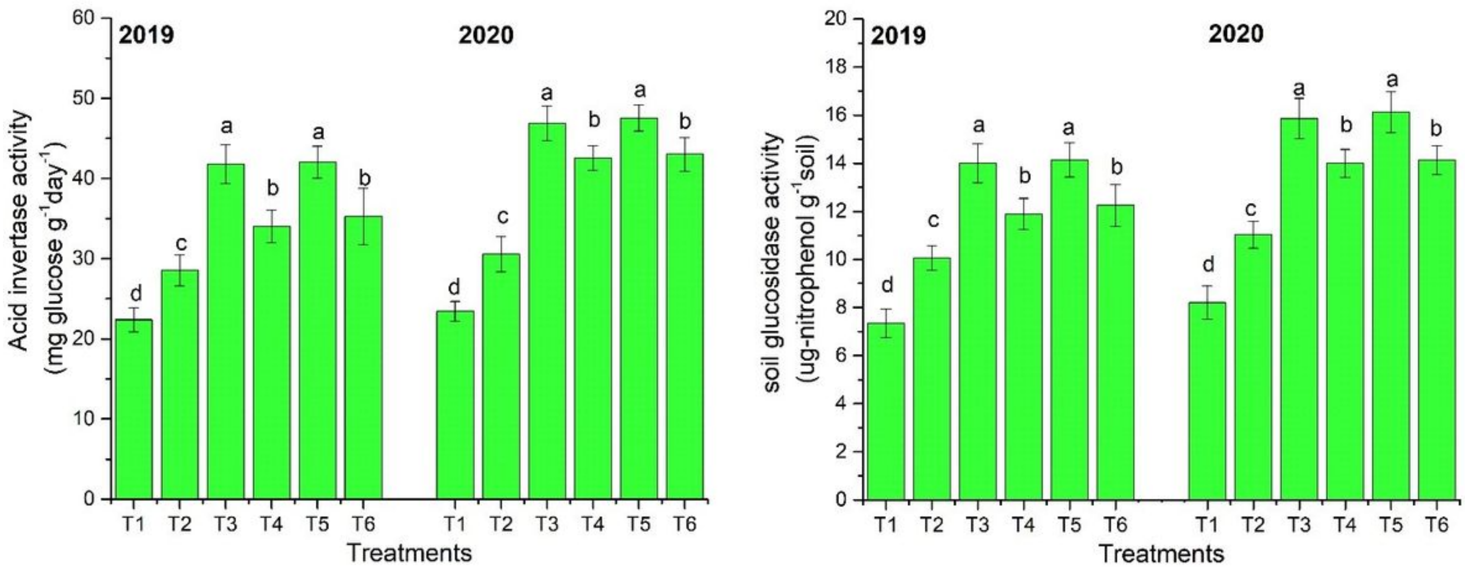

Figure 5

Changes in soil enzyme acid invertase and β-glucosidase activity as influenced by different combined organic and inorganic N fertilization. Vertical bars represent the standard error of the mean. The mean comparison was made using the least significant differences (LSD) test for treatments and seasons means based on the LSD test at 5%. Note: For treatments combination please see Fig. 2.

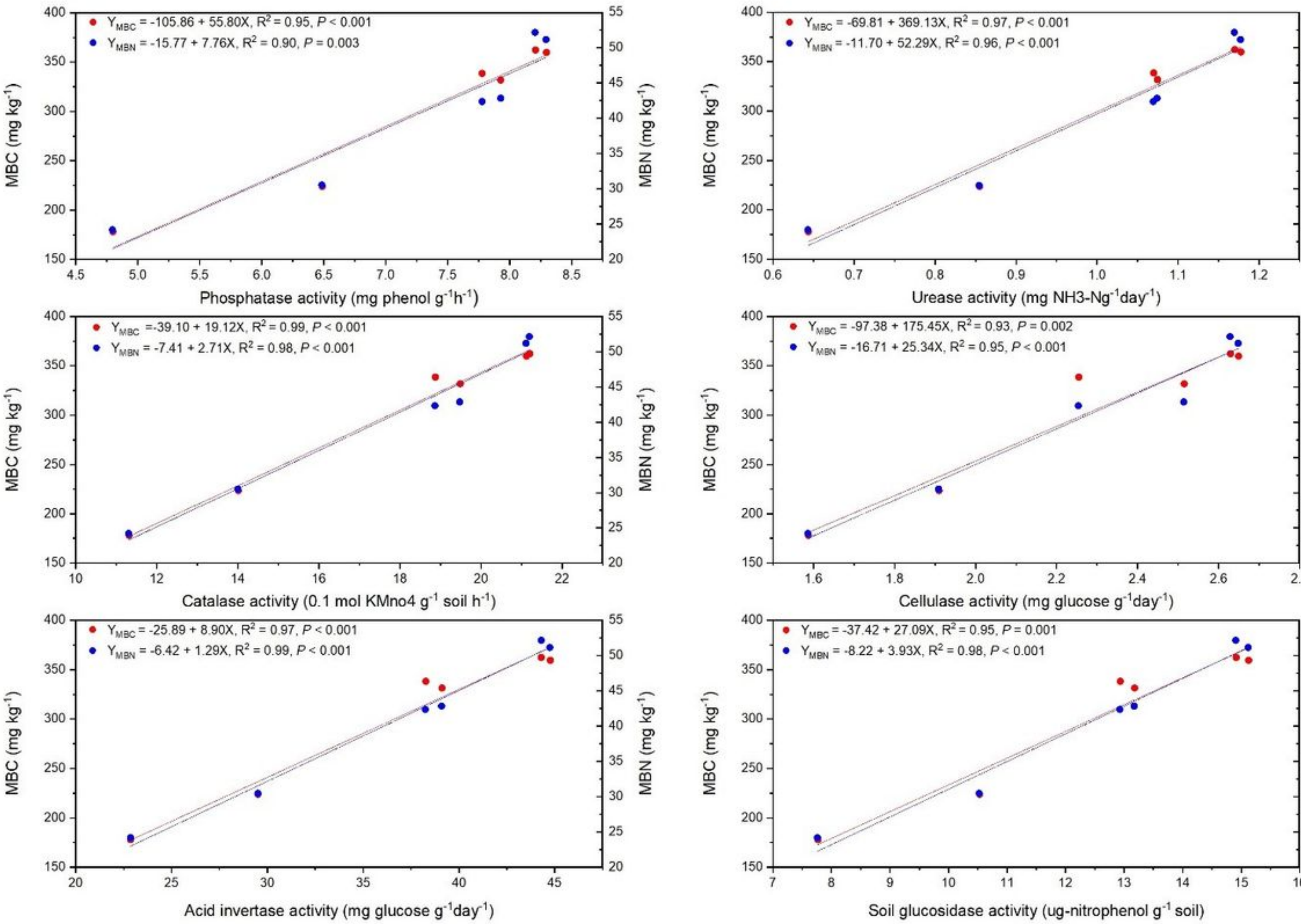

Figure 6

Linear relationship of soil microbial biomass carbon (MBC) and soil microbial biomass nitrogen (MBN) with soil enzymatic activities.

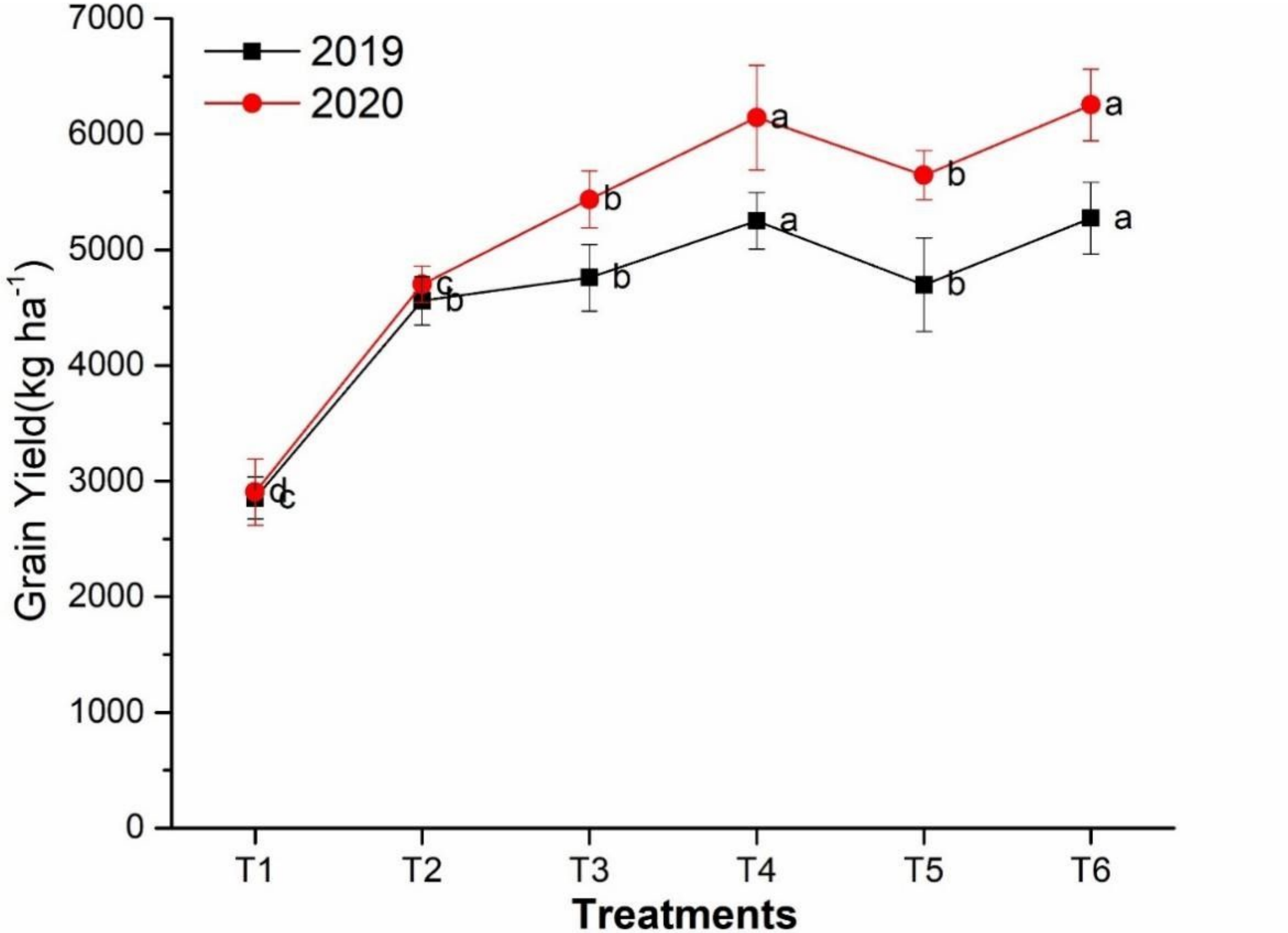

Figure 7

Changes in grain yield of rice as influenced by different combined organic and inorganic N fertilization. The mean comparison was made using the least significant differences (LSD) test for treatments and seasons means based on the LSD test at 5%. Different letters within the line are not significantly different at $p < 0.05$. Note: For treatments combination please see Fig. 2.

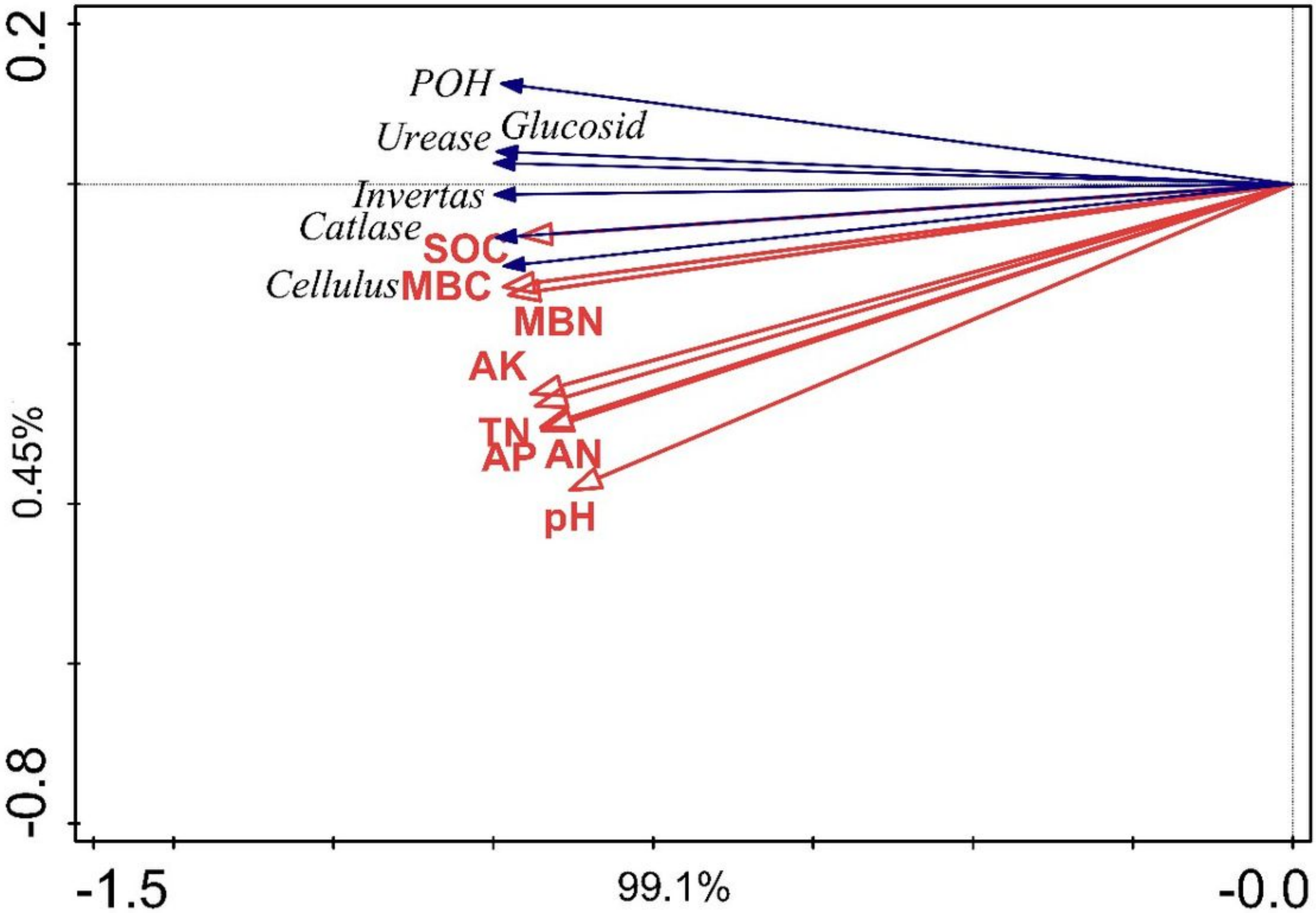

Figure 8

Ordination plot results from redundancy analysis to identify relationships between soil enzymes, such as phosphatase (POH), β-glucosidase (Glucoside), urease, invertase, catalase, and cellulose and soil properties, such as microbial biomass carbon (MBN), microbial biomass nitrogen (MBN), soil organic carbon (SOC), total nitrogen (TN), available nitrogen (AN), available phosphorous (AP), available potassium (AK).

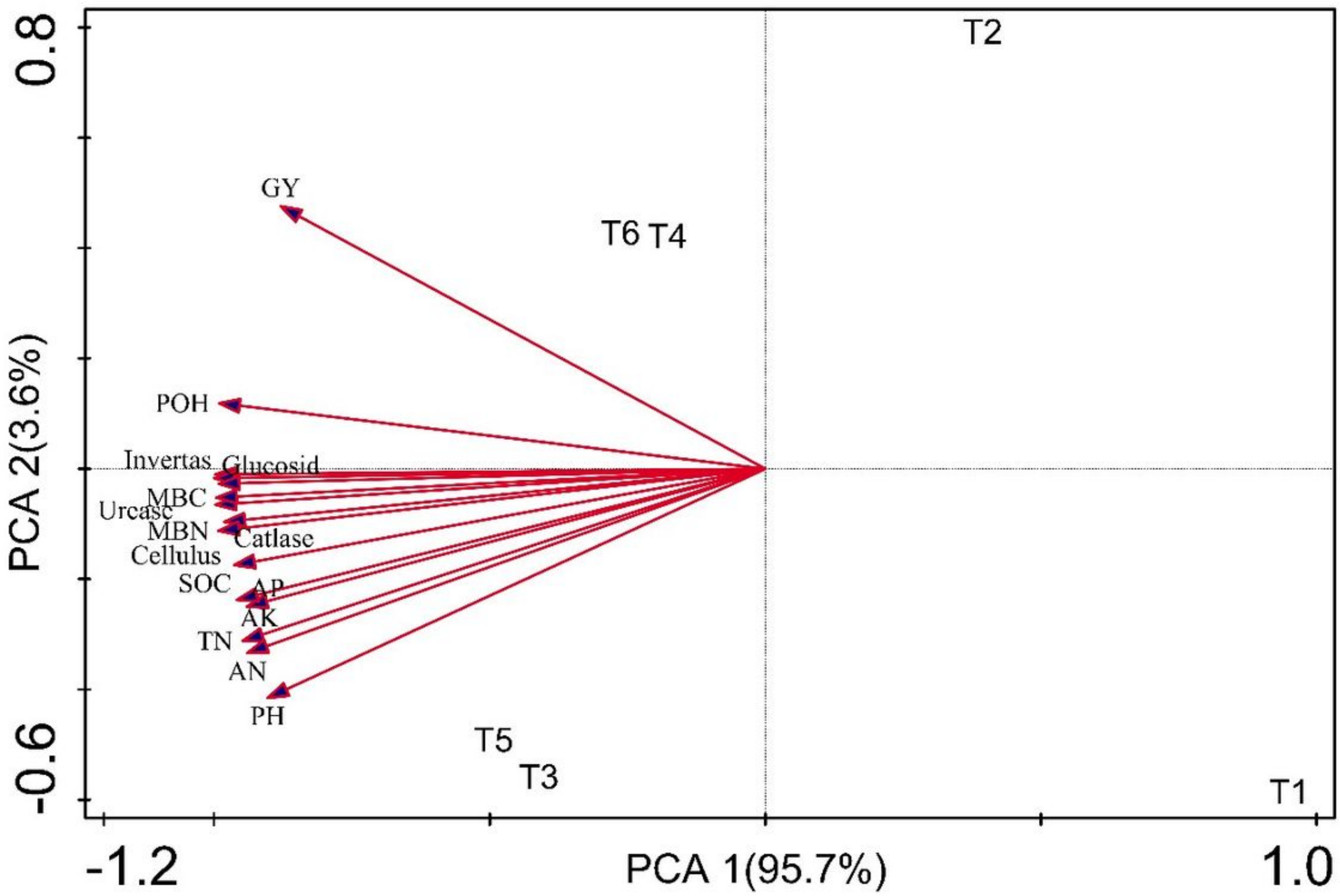

Figure 9

Principal components analysis of all soil enzymes, soil chemical properties, and rice grain yield under combined organic and inorganic N fertilization at 0—20cm soil depth over the two-year study. Note: GY—grain yield, POH—phosphatase, MBN—microbial biomass carbon, MBN— microbial biomass nitrogen, SOC—soil organic carbon, TN—total nitrogen, AN—available nitrogen, AP—available phosphorous, AK—available potassium. For treatments combination please see Fig. 2.