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Soil carbon and nitrogen regulation using organic and inorganic fertilizers for leaf physiological activity, grain yield and nutritional quality improvements in rice

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Research Article

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Abstract

Organic fertilizers are widely used in agriculture production and change the soil carbon (C) and nitrogen (N) contents, thus improving crop production. The increased amount of soil C and N exhibit a greater potential to improve the leaf physiological activity, yields and grain guality of rice by improving soil fertility indices. To understand the relationship between soil C and N contents with leaf physiological activity and grain quality; organic fertilizers (i.e., cattle manure (CM) and poultry manure (PM)) coupled with chemical fertilizer (CF) was applied at the different proportion. The recommended rate of N 150 (kg ha⁻¹) was provided from manure and CF using six treatments, 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 soil organic C (SOC), total N (TN), leaf net photosynthetic rate (Pn), SPAD values, grain yield and grain quality attributes were significantly increased with combined organic and inorganic fertilizer application. Averaged over the years, T₆ treatment significantly improved SOC, TN, Pn, starch content (SC), amylose content (AC), and grain yield by 16%, 12%, 9%, 7%, 12%, and 24%, respectively, compared to CF-only. However, no significant differences among T₄ and T₆ were observed for studied parameters. In addition, the linear regression exhibited that SOC (R^2 = 0.70** & R^2 =0.50*) and TN (R^2 = 0.62* & R^2 =0.58*) were positively correlated with grain SC and AC, respectively. Likewise, Pn (R²= 0.51* & R²=0.62*) were also positively associated with SC and AC, respectively. The correlation analysis showed that improving SOC and TN played a key role in enhancing leaf physiological activity and grain nutritional guality. Thus, the combination of organic and inorganic fertilizers at a 30:70 ratio is a promising option for the improvement of soil fertility and grain yield of rice as well as grain nutritional traits.

1. Introduction

Improving crop production and quality to feed the global rising population and sustain high living standards has become a great challenge, recently (Fischer et al., 2014). After green revaluation, the modern farming system had played a crucial role in feeding the world's population (Jensen et al., 2011) but relied on the usage of heavy chemical fertilizers (CF) (Chen et al., 2014; Zhang et al., 2016). However, the usage of CF had led to a significant decrease in soil quality and is a major concern for sustainable agricultural productivity and crop quality (Sarma et al., 2017; Agegnehu et al., 2014). In addition, the extreme N application causes significant environmental problems, such as increased greenhouse gas emission, soil acidification, and groundwater pollution (Mahajan et al., 2008; Cai et al., 2018). Application of N fertilizer for a long time has also decreased soil microbial diversity, and make compaction of arable soils, thereby suppressed plant growth and crop production (Guo et al., 2017; Yue et al., 2015). In China, it has been documented that the CF application rates are usually far higher than the crop requirements (Ju et al., 2009). It is perceived by the farmers treat CFs are the most reliable and effective way to improve crop production and their revenue (Yan et al., 2013; Qiao et al., 2012), but have totally ignored the ill effects on soil and environment by using the N fertilizers continuously. For example, in winter wheat and summer maize rotation, the annual fertilization of mineral N (550-600 kg N ha⁻¹) was almost three times greater than the minimum fertilization rate i.e., about 180 kg N ha⁻¹ (Zhao et al., 2006; Cui et al., 2008).

Consequently, a huge amount of the applied nutrients, particularly N lost to the environment and have negative effects.

Organic fertilizers, such as farmyard manure (FYM), green manure, straw and compost are an alternative source of fertilizers that can provide various benefits over CF, such as improved soil health (Thangarajan et al., 2013), increased soil fertility (lqbal et al., 2019a; Ali et al., 2020a), maintain soil quality and health (Xie et al., 2014; Ali et al., 2020b), and in particular, similar or even greater crop grain yields in some cases (lqbal et al., 2020b; Seufert et al., 2012). Moreover, organic manure fertilization could provide all essential nutrients required for plant growth and is also considered eco-friendly (Ullah 2020; Gu et al., 2015). But organic manure is quite low in nutrients content and its releasing capability is also low to meet plant demands in a short time, therefore the sole fertilization of manure cannot meet the usual demands of plants nutrients. Therefore, the organic fertilizers if coupled with mineral fertilizer, can be considered a better approach to enhance soil quality on a sustainable base and crop productivity than sole CF application (Kumar et al., 2017; Bandyopadhyay et al., 2010). However, gaps still exist in the knowledge presently available about the responses of soil chemical properties and leaf physiological traits with grain quality attributes and grain yield of rice using the various proportion of organic and inorganic N fertilization.

Rice is one of the major stable crops in China with a per capita consumption of 100 to 120 kg year⁻¹, which is almost 2.2 times the world average (FAO, 2016; Ding and Zhai, 2013). Rice grain quality determination can judge by four components, i.e., appearance, cooking, milling and nutritional quality (Li et al., 2003; Stockdale et al., 2001). Environmental factors, such as temperature, precipitation, ecological zone, and amount and type of fertilization significantly affecting rice grain quality traits (lgbal et al., 2020; Patindol et al., 2015). Most importantly, the accumulation of starch content in the grain is closely related to the rate and timing of N fertilization which can be improved by using the optimum rate of N (Yang et al., 2020; Zhou et al., 2020). In a conventional agricultural system, the excessive use of N is not suitable for the accumulation of grain starch and therefore does not result in high-quality rice grains (Guo et al., 2017). This may be due to the starch mobilizing enzyme activities, which is severally affected by N fertilization (Tang et al., 2019; Iqbal et al., 2020). Moreover, earlier findings have also shown that rice grain filling rate (starch accumulation) is strongly associated with the rice grain processing and appearance qualities (Shen et al., 1997; Jin et al., 2000; Sun and Yang, 2008). However, we do not yet have full knowledge of the mechanism for linking soil properties to grain quality characteristics under the joint application of manure and mineral N fertilizers. Therefore, in conventional farming systems, improving the quality of grain is very important by adjusting the proportion of organic and inorganic fertilizer.

It is well documented that fertilization improves the grain yield, however, most of the studies were conducted on a weight basis of organic fertilizers rather than the specific rate and N concentration along with mineral N fertilizer (Zhang et al., 2015; Arif et al., 2015). Therefore, the primary mechanism of N on a concentration basis remains unknown. Moreover, there is a lack of information on the role and relationship of soil chemical and leaf physiological characteristics to the quality and yield of rice. So, it

was expected from the present study that organic N along with synthetic N fertilization could improve soil health, which in turn will have a significant role in higher leaf physiological activity, rice yield and grain qualitative traits. The aims of this experiment are: (1) to explore the combined effect of organic and inorganic N on soil quality; (2) to evaluate the integrated effect of organic and mineral fertilizers on leaf physiological activity, rice yields and grain qualitative traits; (3) To examine the relationship among soil quality, leaf physiological activity and grain nutritional quality.

2. Materials And Methods

2.1 Site description

Field experiment at the research farm of Guangxi University, China was conducted in 2019 and 2020 during early season runs (from March-July) and the late season (from July-November). This site experiences a subtropical monsoon climate, with a total annual rainfall of 1398 mm, and an average annual temperature of 24.8°C (Figure 1). The soil is classified as Ultisols (USDA soil classification), which is slightly acidic with a pH of 5.96. The soil physico-chemical properties indicated that the soil contained 18.75 g kg⁻¹ SOC and 1.64 g kg⁻¹ total nitrogen (TN), with other details shown in Table 1.

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	-	-
C:N ratio	7.16	14.92	12.98

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

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 season experiment was arranged in a randomized complete block design having three replications. The plot size was 3.9 m × 6 m (23.4 m²). Organic fertilizers at the recommended rate of N (150 kg ha⁻¹) i.e., poultry manure (PM), cattle manure (CM), and chemical fertilizer (CM) as urea was used in this study in six different proportions: $T_1 - CF_0$; $T_2 - 100\%$ 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 nutrient contents of organic fertilizers and the quantity of all treatments are shown in Table 2. The N and K fertilizers were applied in three splits: 50% initially before transplanting, 30% at tillering, and 20% at the heading stage. All phosphate fertilizer was applied before transplanting as a basal dose. Flooding was maintained from transplantation to physiological maturity.

During the entire rice growth period, standard agronomic practices, including irrigation and pesticide application was performed the same for all amendments.

Table 2

Nutrient contents and amount of nutrients provided to each plot and application time.							
Treatment	Ν	Urea	CM,	Basal fertilization	Tillering	Panicle initiation	
	(g	(gplot ⁻¹)	PM,	//	(g		
	plot⁻')		(kgplot ⁻¹)	(kg plot ')	plot ')	(g plot ')	
T ₁ : CF ₀	0	0	0	P ₂ O ₂ : 0.93, KCI: 0.30	KCI: 0.30	Urea: 0	
T ₂ : 100% CF	351	753	0	Urea: 0.45,	Urea: 150	Urea: 150	
				P ₂ O ₂ : 0.93, KCI:	100,		
				0.30	KCI: 0.30		
T ₃ : 60% CM +40% CF	351	301	21.5	Urea: 0, CM: 21.5,	Urea: 150,	Urea: 150	
				P ₂ O ₂ : 0.93, KCI: 0.30	KCI: 0.30		
T ₄ : 30% CM +70% CF	351	527	10.7	Urea:1.17, CM: 10.7,	Urea: 150,	Urea: 150	
				P ₂ O ₂ : 0.93, KCI: 0.30	KCI: 0.30		
T ₅ : 60% PM + 40% CF	351	301	15.5	Urea: 0, PM: 15.5,	Urea: 150,	Urea: 150	
				P ₂ O ₂ : 0.93, KCI: 0.30	KCI: 0.30		
T ₆ : 30% PM +70% CF	351	527	7.7	Urea: 1.17, PM: 7.7,	Urea: 150,	Urea: 150	
				P ₂ O ₂ : 0.93, KCI: 0.30	KCI: 0.30		

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 measurement

2.3.1 Soil properties

Samples were obtained by a core sampler at a depth (0-20 cm) from each treatment immediately after the late-season rice harvest in 2019–2020. Soil sampling was done at five different points and then mix

to make a composite sample, and divide into two parts, one part was used for the measurements of soil nutrients, and the other was stored at 4°C for molecular analysis.

Soil organic C was determined by the $K_2Cr_2O_7$ - H_2SO_4 oxidation method (Wang et al., 2003). For soil TN analysis, 200 mg of the samples were processed using the salicylic acid–sulfuric acid–hydrogen peroxide method (Ohyama et al. 1991), and total N was measured using the micro-Kjeldahl technique according to Jackson (1956).

2.3.2 Leaf physiological traits

The *Pn* and SPAD value was found at tillering, heading and ripening stage during both years. From every plot, fully expanded flag leaves were targeted for *Pn* and SPAD value assessment. Portable photosynthesis (Li-6400, Li-COR Inc., Lincoln, NE, USA) system was used for *Pn* measurement on a shiny day: air humidity (70%), light intensity (1200 µmol m⁻²s⁻¹), CO₂ (375 µmol mol⁻¹), and temperature (28°C). A SPAD meter (SPAD-502, Minta Camera Co., Osaka, Japan) was used to take SPAD values of the 4 uppermost leaves on each plant at tillering, heading and ripening stage.

2.3.3 Rice grain yield, grain nutritional and noodles appearance traits

Rice plants from the whole plot area were harvested at maturity to measure the grain yield of rice and accommodated to 14% moisture content. For AC and SC concentration, rice grains were ground using a mortar, and the powder was then degreased two times with anhydrous ether. The total grain SC and AC were determined via the dual-wavelength iodine binding method as suggested by He (1985) and Zhu et al. (2008). Grain crude protein content was measured as the product of grain N contents and 5.75 (protein conversion coefficient) according to Fujihara et al. (2008). The value of dissolved starch was measured according to the method of Weiguo et al. (2005). The noodles expansion rate and evenness were determined according to the procedure as suggested by Shaomao and Mao, (2004) and Guo et al. (2016), respectively.

2.3.4 Statistical analysis

The collected data on soil chemical traits, leaf physiology, yield, and grain qualitative attributes were analyzed according to the ANOVA techniques relevant to RCB design using Statistics 8.1 software. The data were first checked for a normal test. Furthermore, data in percentage were arcsine transformed to normalize the variables before analysis. The analysis was performed combined over the years, to detect changes between years as well as in fertilizer treatments. Fertilizer treatments were considered as a fixed factor, years were considered as a repetitive measured factor and also a fixed effect. Likewise, the interaction among fertilizer treatments and the years was taken as a fixed effect. But, the interaction of years and treatment with replications was taken as a random effect. The means were separated using Tukey's test at p < 0.05. Linear regression analysis was performed to evaluate the relationship of SOC, TN, *Pn*, and SPAD value with grain SC and AC.

3. Results

3.1. Soil Chemical properties

The effects of combined organic and inorganic N fertilization treatments on soil chemical attributes are shown in Figure 2. The co-applied fertilizers significantly increased SOC and soil organic matter (SOM) during both years. In both years, the SOC and SOM content were maximum in T_3 and T_5 treatments than in the rest of the treatments. Furthermore, the SOC and SOM contents among the different treatments showed the same pattern across years, and average increases in SOC and SOM content were 29.3% and 29.3% in the T_3 treatment, respectively, compared with control (T_2). But, T_5 was non-significant (P < 0.05) with T_3 . Compared with control, the combined treatments, such as T_4 and T_6 showed significantly higher SOC and SOM content. Moreover, substantial enhancements were detected in SOC and SOM 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 TN and available N (AN) content at the 0–20 cm soil depth is shown in Figure 3. Soil TN and AN contents were considerably improved in the combined treatments compared with CT in both years. The average increases in TN and AN were 24.2%, and 17.02%, respectively, in the T₃ treatment compared with control. But, T₅ was similar (P < 0.05) non-significant with T₃. Likewise, the integrated treatments like T₄ and T₆ had considerably higher soil TN and AN contents compared to control. Furthermore, significant improvements were observed in TN and AN contents during the seceding year. The average increment in TN and AN contents in the year 2020 was 9.55% and 12.28%, respectively, which was higher than the year 2019.

3.2. Soil Leaf physiological traits

Leaf photosynthetic rate (*Pn*) and SPAD values at the tillering, heading, and ripening stage was significantly affected by N amendments during the year 2019 and 2020 (Fig. 4 & 5). Both traits exhibited a quadratic trend across growth, with higher values at heading and lower values at the ripening stage in both years. Further, the *Pn* and SPAD values showed a similar trend across the years. Average across the years, at the tillering stage, the *Pn* and SPAD were 20% and 23%, respectively, higher in T_2 compared to T_1 , while at the heading and ripening stage, *Pn* and SPAD were significantly higher in T_6 and T_4 compared with other treatments. Average increases across the growth stages and years in *Pn* and SPAD were 16% and 18%, respectively, in T_6 treatment compared to T_1 . Furthermore, the joint N treatments, such as T_3 and T_5 also improved leaf *Pn* and SPAD values.

3.3. Rice grain nutritional and appearance qualities

Grain nutritive quality is a primary feature of rice, such as starch, amylose and protein content. The collective use of organic and mineral fertilizers had significantly improved rice grain SC, AC and PC in

both years 2019 and 2020 (Table 3). The integrated treatment i.e., T_6 significantly enhanced the SC, AC and PC by 6.4%, 12.7% and 12.2%, respectively, compared to T_2 . But, the treatment T_4 and T_6 were statistically (P<0.05) non-significantly different from each other. Similarly, the co-applied organic and synthetic N treatments i.e., T_3 and T_5 also significantly increased the nutritive traits of grain compared to sole urea. The lowest content of SC, AC and PC were noted in control plots during both years.

The influence of joint manure and synthetic N fertilizer application on rice noodle appearance traits are presented in Table 3. The combined fertilization had no significant effects on starch dissolving value, noodles expansion rate and evenness.

3.4. Rice grain yield

The combined organic-inorganic fertilizer application significantly affected the grain yield of rice in both 2019 and 2020 (Table 3). Compared with control (T_2), the co-applied N fertilization considerably improved the grain yield of rice 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, such as T_5 and T_3 also significantly increased grain yield, compared to control. Significant improvements in grain yield were observed among the different years, and the average grain yield was increased by 14% in 2020 compared to 2019.

3.6. Relationship of the soil chemical traits with grain starch and amylose contents

Rice grain starch and amylose content were strongly reliant on soil parameters. This was confirmed by linear regression analysis, which showed that the grain SC was positively correlated with SOC content (R^2 =0.70** Fig. 6 [A]) and TN (R^2 =0.62* Fig. 6 [C]). Similarly, the AC showed a positive relationship with SOC (R^2 =0.52* Fig. 6 [B]) and TN (R^2 =0.58* Fig. 6 [D]). The leaf physiological traits, such as *Pn* and SPAD values were correlated with SC and AC. The positive correlation was observed of SC with *Pn* (R^2 =0.51* Fig. 7 [A]) and SPAD (R^2 =0.60* Fig. 7 [C]); as well as of AC and SC with *Pn* (R^2 =0.62* Fig. 7 [B]) and SPAD (R^2 =0.63* Fig. 7 [D]). Such results showed that the improvements in soil SOC, TN and leaf physiological activity directly affect the starch and amylose accumulation in rice grain.

4. Discussion

The present agricultural system is massively dependent on CFs, which have adversely affected soil quality, environment and cereal yields as well as grains quality. The improvement of soil fertility and cereal yields and its quality on a sustainable basis is novel research these days (Adekiya et al., 2019; Iqbal et al., 2019). Therefore, the influence of a cumulative application of manure and mineral fertilizers on soil chemical properties. Leaf physiological traits, grain yield and its qualitative traits of rice were

undertaken in field studies to devise a sound strategy for improvement in these indicators on a sustainable basis.

4.1. Soil quality

The collective use of organic and inorganic N significantly increased the SOC, SOM, TN and AN concentration in the soil as compared with sole urea application (Fig. 2 & 3). The decomposition of organic fertilizer slowly released nutrients to the soil and exhibited that rising the organic fertilizer ratio from 30–60% had enhanced the soil health. Soil organic C is a vital indicator of soil health and fertility (Stockmann et al., 2013; Johanne's et al., 2017), soil having greater SOC would have better health. A significant improvement in SOC content was noted under combined treatments compared with control (Fig. 2). The enhancement in SOC and SOM could be explained by two possible mechanisms, i.e., the direct addition of C by adding organic fertilizer and improvement in rhizosphere quality by increased root exudate as a result of improved plant root activities (Bitew and Alemayehu, 2017). In fact, the SOC 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). In accordance with our finding, Purakayastha et al., (2008), documented that manure significantly improves soil C in the top layer.

The co-applied fertilization of manure and mineral fertilizers significantly improved the content of total and available N (Fig. 3). This is mainly due to the addition of organic fertilizer such as CM or PM, which had a positive effect on the soil N and other nutrients. This might be related to the direct addition of N when the organic fertilizer was added to the soil had increased the TN, but its decomposition had increased the AN (Wei et al., 2013). Another possible explanation for enhancements in soil nutrients might be related to the ability of organic fertilizers in reducing the leaching of nutrients and thus had a greater capability of absorbing these leachates. The greater absorbing capability is due to improvement in water holding capacity, a decline in nutrients leaching, and consequently greater availability of NPK (Adekiya et al., 2019; Murmu et al., 2013). Our results are also in line with Purakayastha et al. (2008), who concluded that organic manure coupled with mineral fertilizer improves soil organic C by 11-80% and soil total N by 56-90% in the upper layer.

4.2. Leaf physiological activity

The chlorophyll content directly affects the plant photosynthetic activities (Wittmann et al., 2001), and hence crop production. Photosynthesis is the key driver of crop production by increasing plant growth and dry matter accumulation as well as displayed a strong response to N-uptake and water (Wittmann et al., 2001). Leaf *Pn* and SPAD values were noted higher under co-applied manure and mineral N fertilizers treatments compared with the sole synthetic N fertilization (Fig. 3). This increment might be due to improvement in soil health and fertility, declines in nutrients leaching downside (Geng et al., 2019) due to increased organic and inorganic fertilization. We confirmed that the combined manure and mineral N improved soil fertility (Fig. 2 & 3), which eventually increased the root's ability to absorb more water and nutrients, lead to higher stomatal conductance and CO_2 fixing (Iqbal et al. 2019). The higher *Pn* and SPAD values of the plant at heading and ripening stages under combined fertilization treatment could be due to

faster release of nutrient from mineral fertilizers and slow and steady releases of the plant needed nutrient from manure upon decomposition (Iqbal et al., 2020; Ali et al., 2020).

4.3. Rice grain yield

The integrated use of manure and synthetic N significantly increased the grain yield of rice compared with that of sole synthetic fertilizer (Table 3). The improvements in grain yield could be owing to the enhanced soil fertility and functionality under mutual treatments, which ultimately increased crop growth, biomass and yield. Our findings are also in agreement with the results of Yang et al. (2008) and Akhtar et al. (2018) who reported that cereals grain yield is closely associated to soil properties and fertilization application. Similarly, Mangalassery et al. (2018) concluded that the continued application of organic manure coupled with mineral fertilizers improves the biomass and rice yield.

4.4. Rice grain nutritional and appearance traits

The concentration of SC and AC strongly affects the eating and cooking quality of rice noodles (Adu-Kwarteng et al., 2003). The combined (organic fertilizer and CF) fertilization considerably improved the AC and PC of grain compared with sole CF fertilization (Table 3). The observed improvement in PC and AC under integrated amendments explores that both fertilizers deliver enough amount of nutrients (macro + micro), mainly N, which is essential for growth and development for rice. Kumar et al. (2007), finding are in agreements with our finding, and had reported that co-application of inorganic and organic fertilizers enhance grain quality and AC by 7% than sole inorganic fertilizer. Similarly, Sravan and Sing (2019) concluded that the higher grain protein content of rice was associated with the improvement of soil fertility. Moreover, the manure coupled mineral fertilizers did not significantly influence rice noodle physical traits, such as starch dissolving value, expansion rate, and evenness (Table 3). In general, rice grain and noodle physical quality do not indicate variability in the short-time intake under manure fertilization. However, the combined organic and inorganic amendment improves the physical quality of rice grain, which is also supported by earlier researchers (Kesarwani et al., 2016).

Soil quality and leaf physiological traits had affected the grain nutritional quality. The correlation analysis showed a strong relationship between soil properties and rice grain starch and amylose (Fig. 5 & 6). Changes in soil supply C and N and the improvement in the potential and regulation of source-sink-translocation might be the possible explanation for this shift, which had a positive impact on the grain filling process and grain quality (Wu et al., 2020). The leaf *Pn* and chlorophyll content showed a significant and positive relation with grain quality (Fig. 6). The higher leaf *Pn* and SPAD values were primarily due to the enhanced soil health under integrated organic and inorganic armaments (Fig. 2 & 3), which eventually increased root growth, nutrients and water uptake. Such improvement had increased the leaf physiological activity. Moreover, the combined treatments prolonged N supply and leaf physiological activity during the ripening period; and thus, might have enhanced the accumulation capacity of the "source", and ultimately improved the grain filling process.

5. Conclusion

In this study, the combined application of manure and synthetic fertilizers significantly improved the soil fertility, leaf physiological activity, rice yield and grain quality attributes. Higher SOC, TN, *Pn*, AC and PC, as well as grain yield, were observed in the treatments as compared to sole urea application during both years. Improvements in leaf *Pn*, SPAD value, grain yield and grain nutritional characteristics were primarily due to enhanced soil quality under manure application. In addition, according to the linear regression analysis, the nutritional characteristics of rice grain are strongly dependent on soil fertility and leaf physiological activity. It is concluded that the combined application of manure and chemical fertilizers at 30:70 ratio is the best strategy for producing higher rice yields both quantitatively and qualitatively along with improvement in soil fertility.

Abbreviations

С carbon CM cattle manure PM poultry manure CF chemical fertilizer SOC soil organic carbon SOM soil organic matter ΤN total nitrogen Pn net photosynthetic rate SC starch content AC amylose content FYM farm yard content RCBD randomized complete design.

Declarations

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Conflicts of Interest: The authors declare no conflicts of interest.

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Table 3

Year	Treatment	SDV	ER (%)	Eve (%)	PC (%)	SC (%)	AC (%)	GY (kg ha⁻¹)
	T ₁	6.27 a	278 a	92 a	5.85 c	71 d	23.00 c	2899 d
	T ₂	5.92 a	273 a	92 a	6.55 b	73 c	24.00 b	4798 b
2019	T ₃	5.52 a	285 a	92 a	6.41b	74 b	24.00 b	4236 c
	T ₄	5.44 a	279 a	93 a	7.20 a	76 a	25.64 a	5412 a
	Τ ₅	5.53 a	290a	91 a	6.45 b	74 b	24.66 b	4109 c
	T ₆	5.80 a	280 a	93 a	7.35 a	76 a	26.68 a	5428 a
Average		5.75	280	92	6.64	74	24.66	4480
	T ₁	6.89 a	265 a	93 ab	5.72 c	70 c	21.00 d	3278 d
	T ₂	6.81 a	258 ab	94 a	6.54 b	71 c	23.00 c	5216 c
2020	T ₃	6.11 a	260 ab	93 ab	6.58 b	75 b	24.07 b	6159 b
	T ₄	6.34 a	252 ab	94 a	7.46 a	76 a	26.11 a	6444 a
	Τ ₅	6.84 a	255 ab	93 ab	6.57 b	75 b	24.11 b	6294 b
	Τ ₆	6.66 a	258 ab	94 a	7.40 a	77 a	26.18 a	6553 a
Average		6.61	258	94	6.71	74	24.08	5657
ANOVA								
Treatment	t (T)	ns	ns	ns	*	**	**	**
Year (Y)		*	*	*	ns	ns	ns	**
Τ×Υ		ns	ns	ns	ns	ns	ns	Ns

Table 3. Changes in grain yield, grain nutritive and appearance traits under the combined organic and inorganic fertilization.

Note: T_1 -CFo, 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, SDV-starch dissolving value, ER-expansion rate, Eve-evenness, PC-protein content, SC-starch content, AC-amylose content, and GY-grain yield. Means sharing same letter within a category are non-significantly different from each other using Tukey HSD test at P < 5%. **, *= significant at 5% and 1%, respectively and ns= non-significant.

Figures



Figure 1



Mean monthly rainfall and air temperature during the experimental years.

Figure 2

Changes in soil organic carbon (g kg-1) and soil organic matter (g kg-1) as influenced by combined organic and inorganic N fertilization. Means sharing the same letter within a category are non-significantly different from each other using the Tukey HSD test at P < 5%. **= significant at 1%, and ns= non-significant. For treatments combination please see Table 3.



Figure 3

Changes in soil total nitrogen (g kg-1) and soil available nitrogen (mg kg-1) as influenced by combined organic and inorganic N fertilization. The mean comparison was made using Tukey tests for treatments and years and the lettering was on basis of Tukey HSD test at 5% using simple effect. **= significant at 1%, respectively and ns= non-significant. For treatments combination please see Table 3.



Figure 4

Changes in leaf net photosynthetic rate as influenced by combined organic and inorganic N fertilization. The mean comparison was made using Tukey tests for treatments and years and the lettering was on basis of Tukey HSD test at 5% using simple effect. **, *= significant at 5% and 1%, respectively and ns= non-significant. For treatments combination please see Table 3.



Figure 5

Changes in leaf SPAD values as influenced by combined organic and inorganic N fertilization. The mean comparison was made using Tukey tests for treatments and years and the lettering was on basis of Tukey HSD test at 5% using simple effect. **, *= significant at 5% and 1%, respectively and ns= non-significant. For treatments combination see please Table 3.



Figure 6

Linear regression of starch and amylose content with soil organic carbon and total nitrogen content. **, *= significant at 5% and 1%, respectively. n= 6.



Figure 7

Linear regression of starch and amylose content with net photosynthetic rate (Pn) and SPAD values *= significant at 1%. n= 6.