

Article

Effect of Integrated Organic–Inorganic Amendments on Leaf Physiological and Grain Starch Viscosity (Rapid Visco-Analyzer Profile) Characteristics of Rice and Ultisols Soil Quality

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Abstract: Current farming systems are highly reliant on chemical fertilizers (CF), which negatively affect soil health, the environment, and crop productivity. Substituting organic fertilizer for chemical fertilizer (CF) is an important agricultural practice that improves soil health and crop productivity and is eco-friendly. To explore the effects of organic fertilizer in the form of cattle manure (CM) or poultry manure (PM) combined with CF on soil properties, leaf physiological traits, and grain physiochemical characteristics of rice, a 2-year field experiment was conducted in a dual cropping system with six treatments: Neg-CF (no N fertilizer control), Pos-CF (100% CF), High-CM (60% CM + 40% CF), Low-CM (30% CM + 70% CF), High-PM (60% PM + 40% CF), and Low-PM (30% PM + 70% CF). The results exhibited that the adding of organic manure significantly improved soil chemical traits such as soil organic C (SOC), total N (TN), and available N (AN). Similarly, applying the combined fertilizer led to significant increases in the leaf net photosynthetic rate (P_n), SPAD values, and rice grain nutritional and cooking characteristics according to Rapid Visco-Analyzer (RVA) profile. Average increases in SOC, TN, P_n , starch content (SC), and amylose content (AC) were 16%, 12%, 9%, 7%, 9%, and 12%, respectively, across the year in the Low-PM compared to the Pos-CF treatment. Moreover, the manure amendments significantly altered the RVA profile attributes, including peak viscosity, tough viscosity, final viscosity, and the setback and pasting temperatures compared to Pos-CF. Linear regression analysis revealed that SOC and TN were positively associated with leaf P_n and SPAD values. Further, leaf P_n and SPAD were positively correlated with rice grain SC and AC. The correlation heat map analyses revealed a positive correlation in the RVA profile between the soil properties and leaf physiological traits. These correlation analyses showed that the increase in soil chemical traits and leaf physiological activities played a significant role in higher rice grain nutritional and cooking quality. Overall, the findings of this study show that the integrated use of organic fertilizers and CF in rice fields enhances soil functionality and the quality of rice on a sustainable basis.

Keywords: leaf photosynthetic rate; rice; RVA profile; soil; starch viscosity

1. Introduction

Improving the crop production and quality of crops to feed the increasing global population and sustain high living standards has become a significant challenge [1]. After

the green revolution, the modern farming system played a crucial role in feeding the world's population, but this approach depends on the excessive usage of chemical fertilizers (CFs) [2,3]. Overuse of CFs has led to a significant decrease in soil quality and is a primary concern for sustainable agricultural productivity and crop quality [4,5]. The adverse effects of CFs have gradually emerged from their overuse, such as declines in nutrient use efficiency, deterioration in soil quality, and decreased soil microbial activities, as well as loss of biodiversity and lower crop yield and quality [6,7]. The application rate of CFs in China is often far higher than the crop requirements [8]. Farmers perceive CFs as the most reliable and effective way to improve crop production and revenue [9,10] but have ignored the ill effects on the soil and the environment through the continuous use of synthetic N fertilizers. For example, the annual fertilization rate of synthetic N ($550\text{--}600\text{ kg N ha}^{-1}$) is almost three times greater than the recommended fertilization rate for wheat, rice, and maize, i.e., about 180 kg N ha^{-1} [11,12]. Consequently, a large amount of the applied nutrients particularly N are lost to the environment and has adverse effects.

Alternatively, organic fertilizers, such as farmyard manure, green manure, straw, and compost manure are the alternative fertilizer sources that can provide various benefits over CFs, such as improved soil health [7], increased soil fertility [13], maintenance of soil quality and health [14], and in particular, similar or even greater crop grain yields [15]. Applying manure change a soil physical and biochemical properties, improve soil enzyme activities, and decrease or eliminate the harmful effects of the CF-only overuse on soil health [14,16]. However, organic fertilizer is quite low in nutrient content, and its nutrient releasing ability is also low to meet crop requirements in a short time; hence, applying only manure does not meet the needs of agriculture production. Organic manure coupled with synthetic fertilizers is a better approach to improve and sustain soil fertility and crop production than applying chemical fertilizer or organic manure alone [17,18].

Rice is one of the main staple crops in China, with a per capita consumption of 100 to 120 kg year^{-1} , which is almost 2.2 times the world's average [19,20]. Rice grain quality is judged by four components: appearance, cooking, milling, and nutritional quality [21]. Starch is the major component in rice grain and describes its physicochemical properties [22,23]. Starch comprises 75–90% of the dry weight of the rice endosperm and consists of amylopectin and amylose [24]. The physicochemical properties of rice grains and particularly, the nutritional traits are measured using the amylose to amylopectin ratio [25,26]. Most manufacturers prefer rice flour to prepare important snacks, noodles, and other food materials in Asian countries [27]. In addition, the starch viscosity characteristics, such as peak viscosity (PV), trough viscosity (TV), final viscosity (FV), set back (SB), peak time (PT), and pasting temperature (PT) which are described by the Rapid Visco-Analyzer (RVA) profile are useful for evaluating of cooking and eating quality of rice [28,29].

The rice grain starch structure, content, and resulting features are substantially affected by environmental factors, such as geographical location, temperature, precipitation, N fertilization rate, and soil fertility status [30]. Nitrogen fertilization plays a key role in many characteristics of rice, such as crop grain yields and quality [31]. Zhou et al. [32] reported that the accumulation of starch content and improved grain quality attributes are closely correlated to the amount and timing of N fertilization. Moreover, Tang et al. [33] stated that N fertilization strongly affects starch synthesis by altering enzymes activities and the expression of the genes involved in starch accumulation. However, in the current farming systems, synthetic N fertilizer is overused to produce maximum grain yield, and the adverse effects of CFs gradually emerge due to their overuse, such as a decline in nutrient use efficiency, deteriorating soil, poorer grain quality, and lower grain production [5,7]. Furthermore, Zhu et al. [32] reported that the apparent amylose content (AC) and gel consistency decrease continuously with a rise in the N fertilization rate, which in reduces rice cooking and eating quality. Therefore, a change in the source of N fertilizer is urgently needed to reduce the adverse effects of synthetic N fertilization. However, more research is required to identify methods to increase soil fertility, crop yield and quality, achieve sustainable crop production, and reduce the use of synthetic N.

Soil organic carbon (SOC), which is the main constituent of soil organic matter (SOM), forms the basis of soil fertility and is considered an important indicator of soil quality and sustainable agriculture [34,35]. Previous studies have reported that applying organic fertilizers increases SOC content by direct carbon inputs from organic fertilizer and indirect carbon inputs from increased plant biomass returned to the arable soil [7,36]. The physiology of plants is greatly affected by environmental conditions, including induced factors, such as protected farming systems, soil fertility conditions, and nutrient applications [37,38]. Photosynthesis is the main physiological process affected by changes in growth conditions and it can be evaluated by gas exchange measurements based on CO₂ assimilation and chlorophyll content, which are described by the SPAD index [39]. However, few studies have analyzed the effects of organic fertilizers (cattle manure or poultry manure) and synthetic fertilizer on paddy field soil chemical properties, leaf physiological traits, and its relationship with grain physicochemical and nutritional properties, particularly in the Ultisol soil of southern China. We used the Zhenguai, an inbred cultivar which is widely cultivated in southern China for rice noodles. This cultivar has short growth duration and a good morphological structure with a high grain filling percentage [40]. This study is based on continuous research in paddy fields under a dual cropping system, with the following research objectives: (i) illustrate the effect of applying manure and CFs application on soil properties, leaf physiological activities, rice grain yield, and grain qualitative traits; and (ii) investigate the effect of soil chemical properties on leaf physiological activity and grain physicochemical traits and the relationships between them. This study aimed to develop a theoretical framework for scientific fertilization practices and sustainable rice production while minimizing the use of CFs.

2. Materials and Methods

2.1. Site Description

A field experiment was conducted at the Guangxi University Research Farm, China, was conducted in 2019 and 2020 during the late season (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) and is slightly acidic with a pH of 5.95. The soil physicochemical property analysis indicated that the soil contained 15.74 g kg⁻¹ SOC and 1.41 g kg⁻¹ total N (TN). Other details are shown in Table 1.

2.2. Experimental Design

The double rice season experiment was arranged in a randomized complete block design with three replications. The plot was 6 × 3.9 m. (23.4 m²). Organic manure (CM or PM and CF (urea) were used in this experiment, and the treatment combinations were: Neg-CF (no N fertilizer), Pos-CF (100% CF), High-CM (60% CM + 40% CF), Low-CM (30% CM + 70% CF), High-PM (60% PM + 40% CF), and Low-PM (30% PM + 70% CF). Rice seeds were grown in plastic trays, and 25-day-old seedlings of uniform size were transplanted into the field. The suggested N–P–K dose (150–75–150) kg ha⁻¹ was applied for each regime, except the Neg-CF treatment. Table 2 shows the nutrient levels of organic manure as well as the quantities used in each treatment. The N and K were supplied in three stages: 50% before transplantation, 30% during tillering, and 20% during heading. All P was used as a basal fertilizer before transplanting. Standard rice farming practices, including flooding irrigation and applications of insecticides, such as chlorantraniliprole and Omethoate were performed identically for all plots.

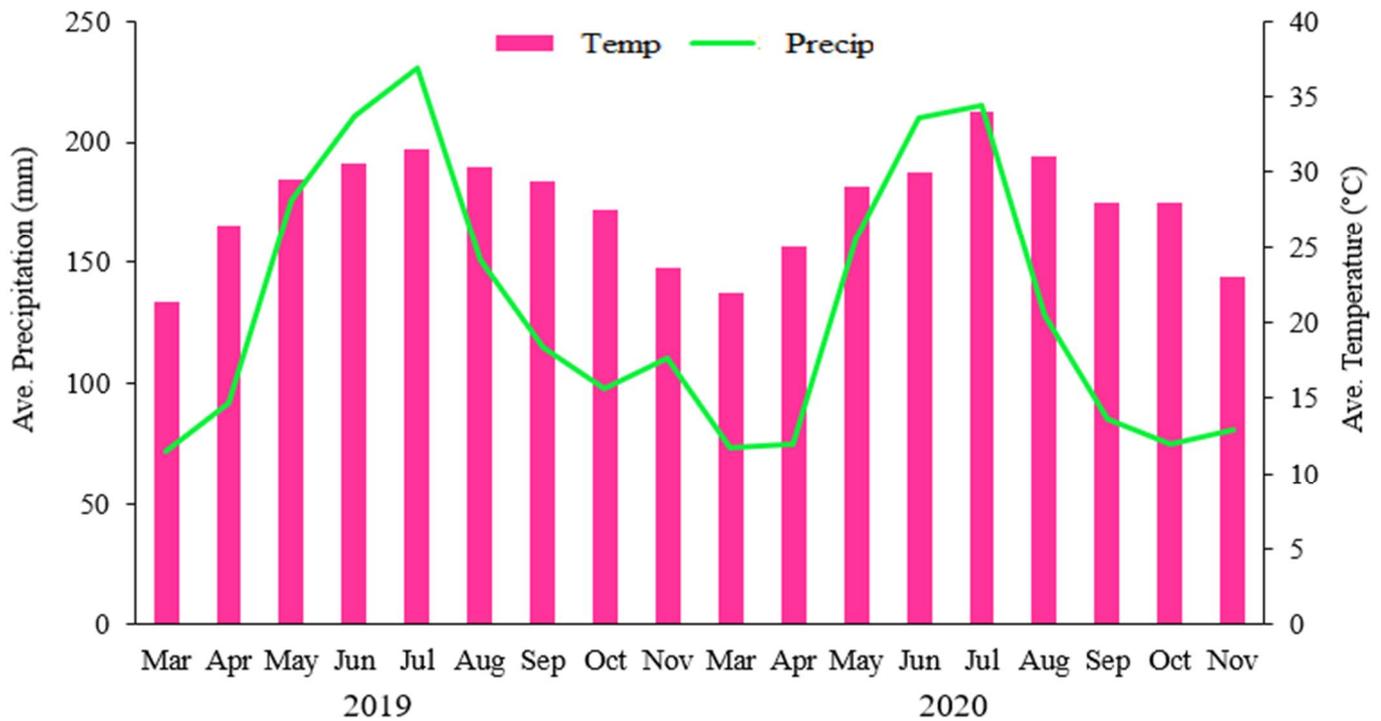


Figure 1. Mean monthly precipitation and air temperature during the experimental years.

Table 1. Soil and manure chemical properties before the experimentation.

Properties	Soil	CM	PM
pH (water)	5.95	7.75	7.95
SOC (g kg ⁻¹)	15.74	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

Note: SOC—soil organic carbon; SOM—soil organic matter; N—nitrogen; P—phosphorous; K—potassium; C:N—carbon to nitrogen ratio.

Table 2. Nutrient content and quantity of nutrients supplied to each plot and fertilization time.

Treatment	N (kg/ha)	Urea (kg/ha)	CM, PM, (kg/ha)	Basal Fertilization (kg/ha)	Tillering (kg/ha)	Panicle Initiation (kg/ha)
Neg-CF:	0	0	0	KCl: 128, P ₂ O ₅ : 397	KCl: 128	Urea: 00
Pos-CF:	150	322	0	Urea: 192, KCl: 128, P ₂ O ₅ : 397	KCl: 128, Urea: 65	Urea: 65
High-CM	150	130	9188	Urea: 0, CM: 9188, KCl: 128, P ₂ O ₅ : 397	KCl: 128, Urea: 65	Urea: 65
Low-CM	150	225	4572	Urea: 94, CM: 4572, KCl: 128, P ₂ O ₅ : 397	KCl: 128, Urea: 65	Urea: 65
High-PM	150	128	6623	Urea: 0, PM: 6623, KCl: 128, P ₂ O ₅ : 397	KCl: 128, Urea: 65	Urea: 65
Low-PM	150	225	3290	Urea: 94, PM: 3290, KCl: 128, P ₂ O ₅ : 397	KCl: 128, Urea: 65	Urea: 65

Note: N, nitrogen; CF, chemical fertilizer; CM, cattle manure; PM, poultry manure; P₂O₅, superphosphate; KCl, potassium chloride.

2.3. Sampling and Analysis

2.3.1. Chemical Properties of Soil

Soil samples were obtained from each treatment with a core sampler at a 0–20 cm depth immediately after the late-season rice harvest in 2019–2020. The soil was collected at five different points and then mixed to prepare a composite sample divided into two parts, one part used for the measurements of soil nutrients, and the other stored at 4 °C for molecular analyses.

Soil organic C (SOC) was measured by the $K_2Cr_2O_7-H_2SO_4$ oxidation method followed by titration [41]. We calculated soil organic matter (SOM) using the measured soil C content and an approximate conversion factor: $SOM = \text{soil C} \times 1.72$, as described by Sullivan et al. [42]. In addition, for soil TN analysis, Ohyama's salicylic acid–sulfuric acid–hydrogen peroxide technique was used to process 200 mg of the sample [43], and TN was calculated using the micro-Kjeldahl method as described by Jackson [44]. The soil pH was measured with a digital pH meter (Thunderbolt PHS-3C) after shaking the soil with distilled water at a 1:2.5 (*w/v*) solid: water ratio for 1 h [45]. Available N (AN) was extracted from the soil samples with hot water. Furthermore, available P (AP) was extracted using Olsen's method with a 0.5 M $NaHCO_3$ solution adjusted to pH 8.5 [46]. Available K (AK) was determined from air-dried soil samples that passed through a 2-mm sieve, transferred to a 100-mL polyethylene bottle, and mixed with 50 mL of an ammonium acetate/acetic acid solution. AK was extracted by the method of Leaf [47].

2.3.2. Rice Leaf Physiological Attributes

The net photosynthetic rate (*P_n*) and the SPAD value was measured during the tillering, heading, and milking stage. Flag leaves from all plots were selected for SPAD value and *P_n* assessments. A portable photosynthesis system (Li-6400, Li-COR Inc., Lincoln, NE, USA) system was used at a sunny day: light intensity ($1200 \mu\text{mol m}^{-2}\text{s}^{-1}$), air humidity (70%), CO_2 ($375 \mu\text{mol mol}^{-1}$), and temperature (28 °C). The SPAD values of the four highest leaves on each plant were determined using a SPAD meter (SPAD-502, Minolta Camera Co., Ltd. Osaka, Japan).

2.3.3. Rice Grain Nutritional and Cooking Attributes

The total grain starch content (SC) and amylose content (AC) were determined via the dual-wavelength iodine binding method as suggested by He [48] and Zhu et al. [49]. Rice grains were ground using a mortar, and the powder was then degreased twice with anhydrous ether. Grain crude protein content was measured as the product of grain N contents and 5.75 (protein conversion coefficient) according to Fujihara et al. [50]. The dissolved starch was measured according to Weiguo et al. [51]. The expansion rate and evenness were determined according to the procedures of Ruan and Mao [52], and Guo et al. [53], respectively.

2.3.4. Rapid Visco-Analyzer (RAV) Profile Characteristics of the Rice Grains

RVA paste viscosity was measured on an RVA (Newport Scientific Pty Ltd., Warriewood NSW 2012, Australia), and analyzed with Thermal Cycle for Windows software according to the American Association of Cereal Chemists Standard Method AACC 61-02 [54]. From each sample, 3 g of flour was weighed, placed in an aluminum canister, and then 25 mL of distilled water was added. The RVA dispersed the samples by rotating the paddle at 960 r/min for the first 10 s of the test and detected viscosity using a constant paddle with a rotation speed of 160 r/min. The idle temperature was set to 50 °C, and the following protocol was run: (1) hold at 50 °C for 1.0 min; (2) ramp up to 95 °C in 3.8 min; (3) hold at 95 °C for 12.5 min; (4) ramp down to 50 °C in 3.8 min; (5) hold at 50 °C for 12.5 min. Heating and cooling were linearly ramped between the setpoints. Standards were used to calibrate the instrument before each assay. The paste viscosity properties of the rice grains such as PV, TV, FV, SB, peak time, and PT were examined as described by Raina et al. [55].

2.3.5. Statistical Analysis

The data on soil chemical traits, leaf physiology, yield, and grain qualitative attributes were analyzed by analysis of variance relevant to a randomized controlled block design using Statistics 8.1 software (Analytical Software, Tallahassee). The data were first checked for a standard test. Percentage data were arcsine transformed to normalize the variables before analysis. The analysis was combined over the years to detect changes between years and fertilizer treatments. The fertilizer treatments were a fixed factor; the year was a repetitive measured factor and a fixed effect. The interaction among the fertilizer treatments and years was a fixed effect. However, the interaction between years and treatments with replications was a random effect. The means were separated using Tukey's post-hoc test, and a p -value < 0.05 was considered significant. Linear regression analysis was performed to evaluate the relationship among soil traits, leaf physiological attributes, and grain nutritional traits. R software (corrplot package: The R Foundation for Statistical Computing, Vienna, Austria) was used to perform the correlation analysis.

3. Results

3.1. Soil Chemical Properties

The effects of the combined organic and inorganic N fertilization treatments on soil chemical attributes are shown in Figure 2. The treatments significantly increased SOC and SOM during both years. SOC and SOM contents were maximum in the High-CM and -PM regimes during both years compared to the other treatments. In addition, the average increases of SOC and SOM were 29.3% and 29.3% in the High-CM treatment, respectively, compared to the control (Pos-CF). However, SOC and SOM contents did not change significantly ($p > 0.05$) among the High PM and CM treatments. Similarly, the low organic fertilizer plots had significantly greater SOC and SOM contents during both years compared to Pos-CF. Moreover, substantial enhancements were detected in SOC and SOM contents during the succeeding year; SOC content increased an average of 27.2% in 2020 compared to 2019.

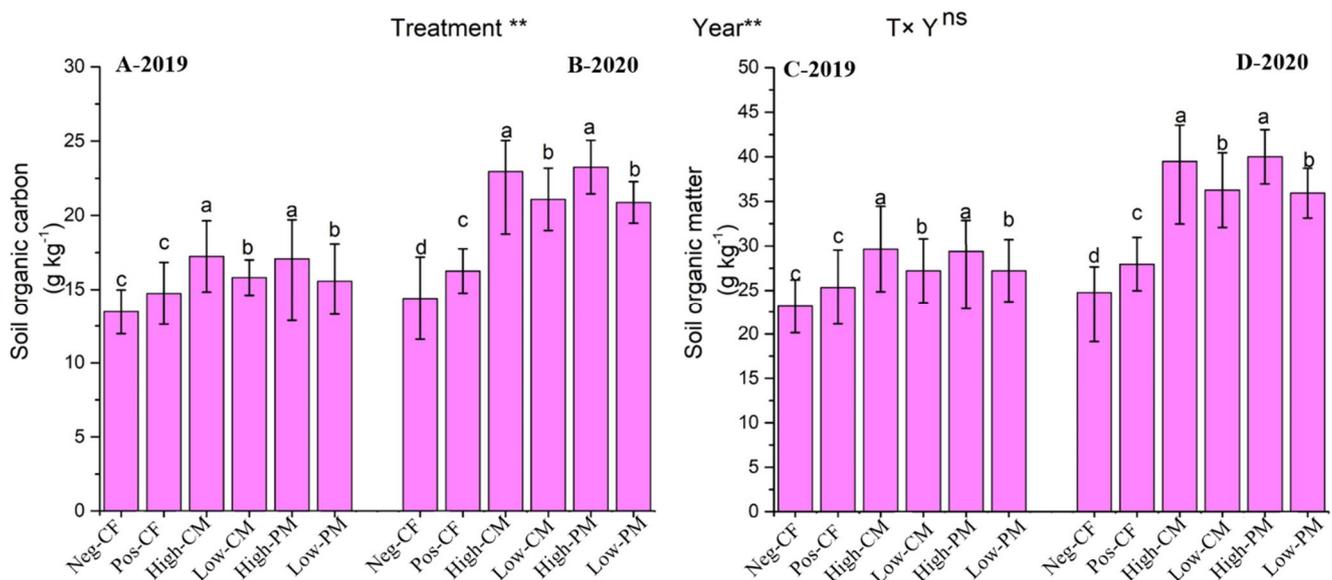


Figure 2. Changes in soil organic carbon in the year 2019 (A) and 2020 (B) and soil organic matter in the year 2019 (C) and 2020 (D) as affected by integrated manure and chemical N fertilizer application. Means sharing the same letter within a category are not significantly different according to Tukey's HSD test at $p < 0.05$. ** = significant at 1%, and ns = non-significant. Note: Neg-CF, no N fertilizer; Pos-CF, 100% chemical fertilizer (CF); High-CM, 60% cattle manure (CM) + 40% (CF); Low-CM, 30% CF + 70% CF; High-PM, 60% poultry manure (PM) + 40% CF; Low-PM, 30% PM + 70% CF.

Soil total N and available N levels of all regimes exhibited similar trends across years, and the average enhances in TN and AN was 24.20% and 17.04%, correspondingly, in the High-CM compared to the Pos-CF (Figure 3). However, the concentrations of TN and AN in High-PM and High-CM treatments did not differ substantially ($p < 0.05$). The TN and AN contents were also significantly higher in the remaining integrated treatments compared to Pos-CF. Moreover, significant increases in TN and AN were observed following year 2020. The average increases in total N and AN in 2020 were 9.55% and 12.28%, respectively, compared to 2019.

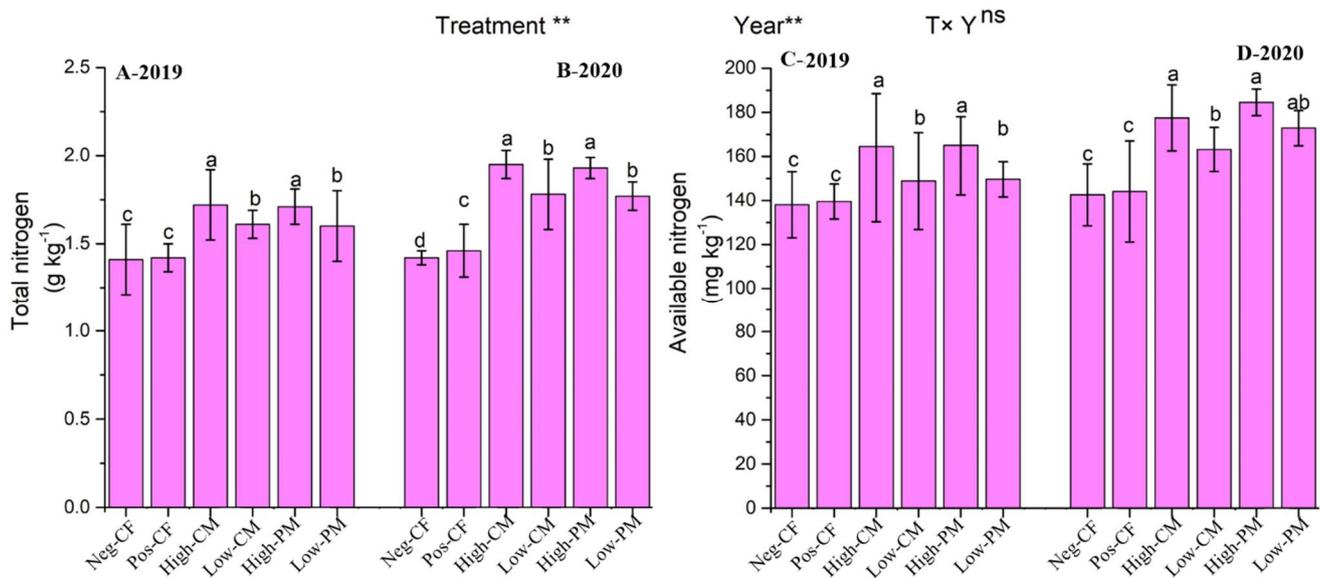


Figure 3. Changes in soil total nitrogen in the year 2019 (A) and 2020 (B) and soil available nitrogen in the year 2019 (C) and 2020 (D) as affected by integrated manure and chemical N fertilizer application. Means sharing the same letter within a category are not significantly different according to Tukey's HSD test at $p < 0.05$. ** = significant at 1%, respectively and ns = non-significant. Please see Figure 2 for the treatment combinations.

3.2. Leaf Physiological Traits

The combined manure and CF applications significantly increased the leaf net photosynthetic rate (P_n) and SPAD values at the tillering, heading, and ripening stages during both years (Figures 4 and 5). Both traits showed a quadratic trend during growth, with higher values at the heading stage and lower values at the ripening stage. The treatments showed the same behavior across years. The average increases in the P_n and SPAD values across the years at the tillering stage in the Pos-CF treatment were 20% and 23%, respectively, compared to Neg-CF. However, P_n and SPAD were significantly higher in the Low-PM and Low-CM treatments during the heading and ripening stages across the years, compared to Neg-CF. The P_n and SPAD values averaged across the growth stages and years increased by 16.32% and 18.45%, respectively, in the Low-PM compared to the Neg-CF treatment. However, the P_n and SPAD values did not differ between the Low-PM and Low-CM regimes. Furthermore, the P_n and SPAD values were significantly higher in the remaining manured plots compared to Neg-CF.

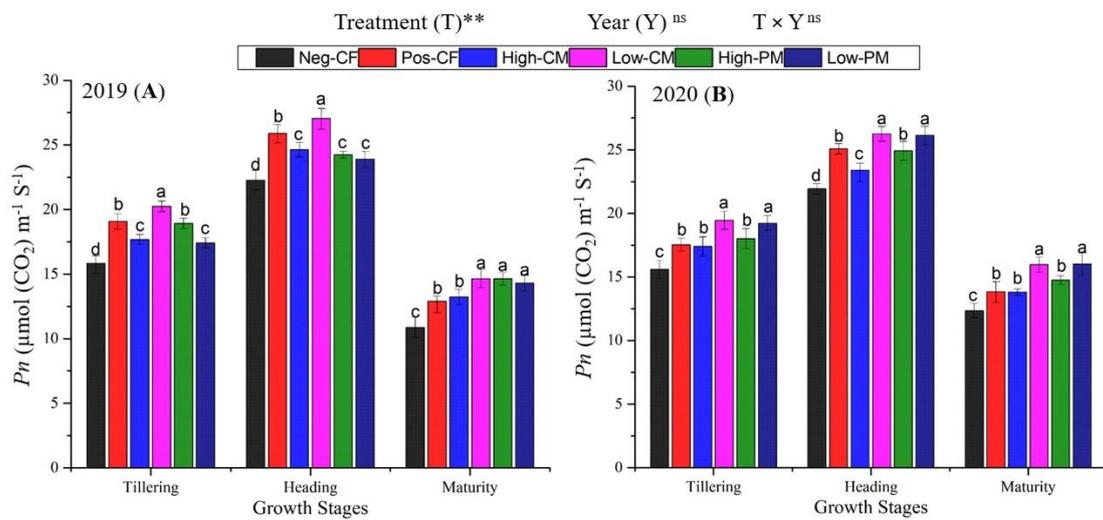


Figure 4. Changes in leaf net photosynthetic rate in the year 2019 (A) and 2020 (B) as affected by integrated manure and chemical N fertilizer application. Means sharing the same letter within a category are not significantly different according to Tukey's HSD test at $p < 0.05$. ** = significant at 1%; ns = non-significant: Please see Figure 2 for the treatment combinations.

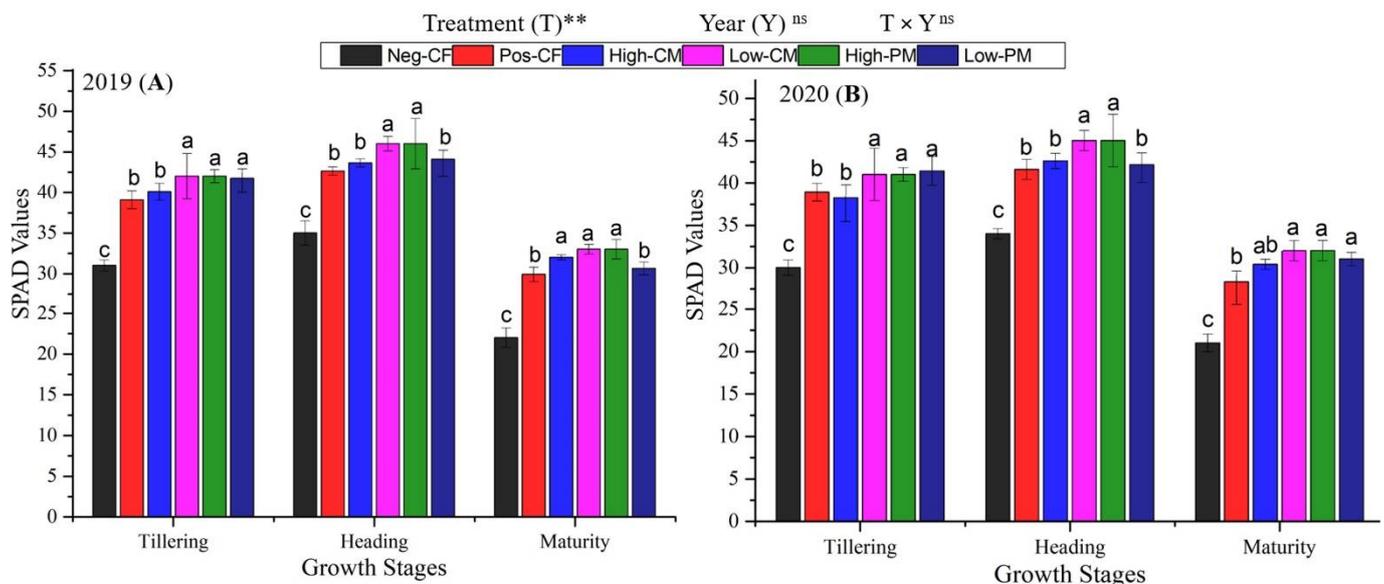


Figure 5. Changes in the leaf SPAD values in the year 2019 (A) and 2020 (B) as affected by integrated manure and chemical N fertilizer application. Means sharing the same letter within a category are not significantly different according to Tukey's HSD test at $p < 0.05$. ** = significant at 5% and 1%, respectively; ns = non-significant. Note: Please see Figure 2 for the treatment combinations.

3.3. Rice Grain Nutritional and Cooking Qualities

Grain nutritional quality is a primary feature of rice, such as the SC, AC, and protein content (PC). The combined organic and chemical N fertilization treatments increased rice grain SC, AC, and PC in 2019 and 2020 (Table 3). The SC, AC, and PC of rice were significantly higher in the Low-PM and Low-CM regimes than in the other regimes. The SC, AC, and PC trends between treatments were consistent over the years. The averaged increased in SC, AC, and PC of the Low-CM treatment by 5.50%, 10.10%, and 12.07% across the years, respectively, relative to Pos-CF. However, the SC, AC, and PC contents non-significant ($p > 0.05$) in between Low-CM and Low-PM. Similarly, the high manure treatments had significantly higher SC, AC, and PC during both years than the Pos-CF. The

lowest SC, AC, and PC levels were noted in the plots that were not treated with N. The effects of combined manure and CF on noodle-cooking qualities are presented in Table 3. Combined fertilization had no significant effects on the starch dissolving value, noodle expansion rate, or evenness.

Table 3. Changes in grain nutritive and cooking traits under combined organic and inorganic fertilization.

Year	Treatment	SDV	ER (%)	Eve (%)	PC (%)	SC (%)	AC (%)
2019	Neg-CF	6.27 a	278 a	92 a	5.85 c	71 d	23.00 c
	Pos-CF	5.92 a	273 a	92 a	6.55 b	73 c	24.00 b
	High-CM	5.52 a	285 a	92 a	6.41 b	74 b	24.00 b
	Low-CM	5.44 a	279 a	93 a	7.20 a	76 a	25.64 a
	High-PM	5.53 a	290 a	91 a	6.45 b	74 b	24.66 b
	Low-PM	5.80 a	280 a	93 a	7.35 a	76 a	26.68 a
Average		5.75 b	280 a	92 b	6.64 a	74 a	24.66 a
2020	Neg-CF	6.89 a	265 a	93 a,b	5.72 c	70 c	21.00 d
	Pos-CF	6.81 a	258 a,b	94 a	6.54 b	71 c	23.00 c
	High-CM	6.11 a	260 a,b	93 a,b	6.58 b	75 b	24.07 b
	Low-CM	6.34 a	252 a,b	94 a	7.46 a	76 a	26.11 a
	High-PM	6.84 a	255 a,b	93 a,b	6.57 b	75 b	24.11 b
	Low-PM	6.66 a	258 a,b	94 a	7.40 a	77 a	26.18 a
Average		6.61 a	258 b	94 a	6.71 a	74 a	24.08 a
ANOVA							
Treatment (T)		ns	ns	ns	*	**	**
Year (Y)		*	*	*	ns	ns	ns
T × Y		ns	ns	ns	ns	ns	ns

Note: SDV, starch dissolving value; ER, expansion rate; Eve, evenness; PC, protein content; SC, starch content; and AC, amylose content. Means sharing the same letter within a category are did not different ($p < 0.05$) from according to Tukey's HSD test. **, * = significant at 5% and 1%, respectively and ns = non-significant. Please see Figure 2 for treatments combination.

3.4. Starch Viscosity Profile Characteristics

The rice starch viscosity characteristics, which are described by the RVA profile, are useful for evaluating the cooking and eating quality of rice. Data regarding the RVA profile as affected by the combined manure and CF applications are presented in Table 4. The integrated manure and CF fertilization significantly affected the characteristics of the RVA profile, i.e., PV, TV, FV, SB, and PT. Similarly, the difference between the years was also significant ($p < 0.05$). The combined manure and CF applications increased the starch viscosity characteristics across the years compared to Pos-CF, and the treatments exhibited a similar trend over both years. The highest PV, TV, FV, DB, and PT values in the 2019 Low-PM treatment were 2347, 2148, 4305, 2221, and 6.78, respectively. Similarly, the highest PV, TV, FV, SB, and PT values in the 2020 Low-PM treatment were 3721, 3236, 5214, and 6.22. PV, TV, FV, DB, and PT increased in the Low-PM treatment by averages of 9.72%, 9.03%, 5.66%, 6.47%, and 5.54%, respectively, compared to Pos-CF. However, PV, TV, FV, DB, and PT considerably differ among the Low-PM and Low-CM treatments. Furthermore, the starch viscosity characteristics were significantly greater in the remaining combined treatments compared to Pos-CF.

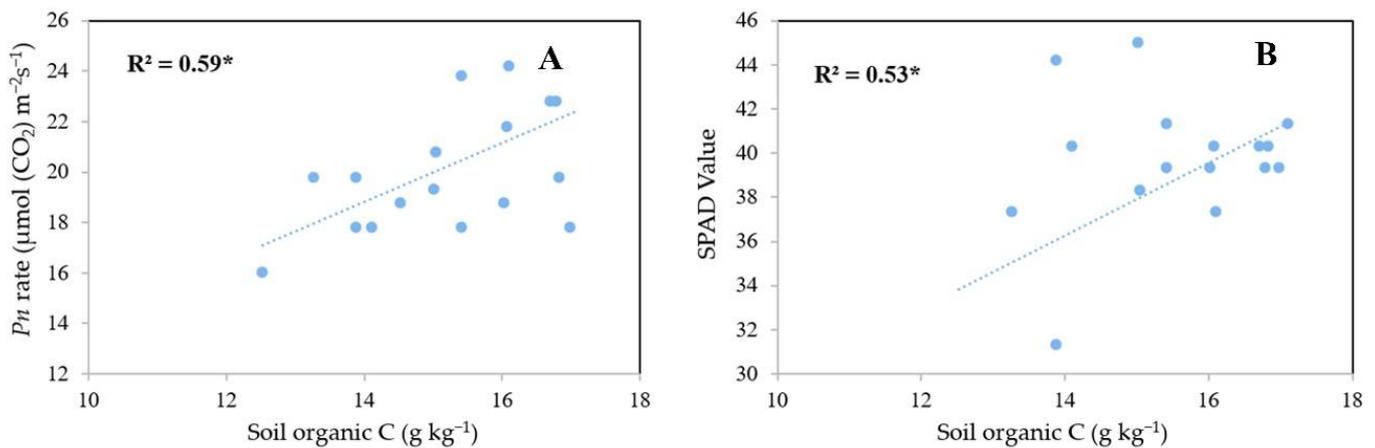
Table 4. Changes in the grain starch viscosity (RVA profile) characteristics under the combined organic and inorganic fertilization.

	Treatment	Peak Viscosity	Tough Viscosity	Final Viscosity	Set Back	Peak Time	Pasting Temp
2019	Neg-CF	2015 b	2032 b	4247 a	2127 b	6.69 b	90.42 a
	Pos-CF	1928 b	1858 c	4080 b	2221 a	6.38 d	90.70 a
	High-CM	2625 a	2333 a	4468 a	2134 b	6.51 c	87.72 b
	Low-CM	1912 b	1863 c	3990 b	2215 a	6.49 c	91.20 a
	High-PM	2038 b	1882 c	4025 b	2143 b	6.60 b	90.67 a
	Low-PM	2347 a,b	2148 b	4305 a	2157 b	6.78 a	85.02 b
	Mean	2144 b	2019 b	4186 b	2166 a	6.57 a	89.29 a
2020	Neg-CF	3829 a,b	3024 a,b	5047 b	1967 c	6.20 a,b	82.10 a,b
	Pos-CF	3602 b	3080 b	4954 b	2005 a	6.09 b	82.62 a,b
	High-CM	3724 a,b	3128 a,b	4923 b	1795 a	6.16 a,b	83.95 a
	Low-CM	3846 a	3168 a,b	5031 b	1864 b	6.18 a,b	82.62 a
	High-PM	3774 a,b	3143 a,b	4949 b	1924 b	6.20 a,b	83.67 a,b
	Low-PM	3721 a	3236 a	5241 a	1812 c	6.22 a	81.25 c
	Mean	3749 a	3130 a	5024 a	1895 b	6.17 b	82.70 b
Treatment (T)	*	*	*	*	*	*	
Year (Y)	*	*	*	*	*	*	
T × Y	ns	ns	ns	ns	ns	ns	

Note: Please see Figure 2 for the treatment combinations. Means sharing the same letter within a category are not significantly different according to Tukey's HSD test at $p < 0.05$. * = significant at 5% and 1%, respectively; and ns = non-significant.

3.5. Relationship between Soil Chemical Traits, Leaf Physiological Activities, Grain Nutritional Attributes, and the RVA Profile

The physiological traits of plants such as photosynthesis and leaf chlorophyll content are strongly affected by environmental conditions, including induced factors, such as protected systems, soil fertility conditions, and nutrient applications. This was further confirmed in this study by linear regression analysis. The significant correlations between soil properties and leaf net photosynthetic rate (P_n) and leaf SPAD values are displayed in Figure 6. The linear regression analysis showed that the soil chemical traits, such as SOC ($R^2 = 0.59^*$, Figure 6A) and TN ($R^2 = 0.67^{**}$, Figure 6C), were strongly correlated with leaf P_n . Similarly, the soil chemical traits, such as SOC ($R^2 = 0.53^*$, Figure 6B) and TN ($R^2 = 0.68^{**}$, Figure 6D), were positively correlated with the leaf SPAD values. These results suggest that the increase in soil chemical traits played a key role in enhancing leaf physiological activities.

**Figure 6.** Cont.

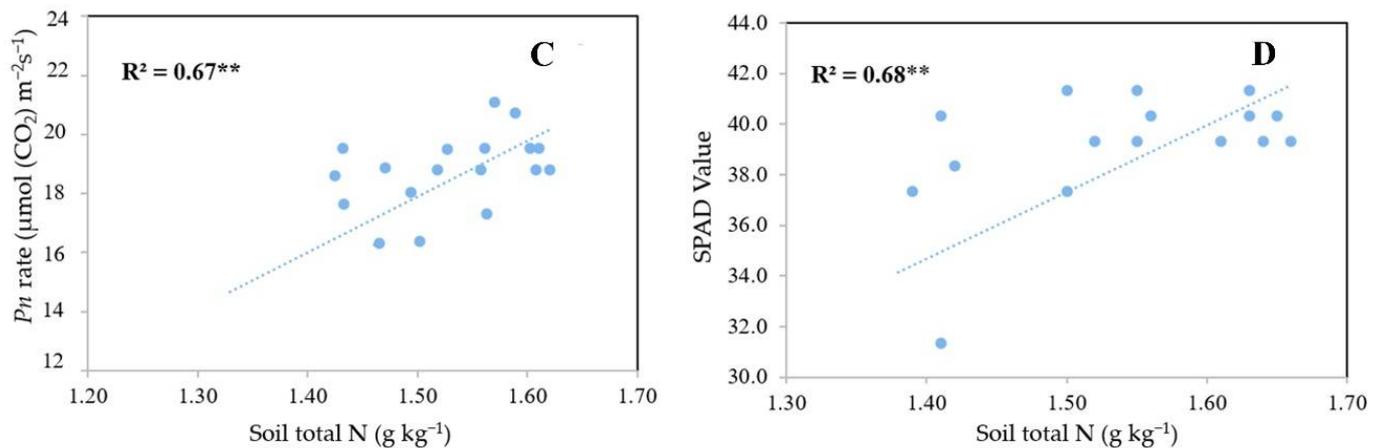


Figure 6. Linear regression analysis of soil organic C (A,B) and total N (C,D) with soil net photosynthetic rate and the SPAD values. **, * = significant at 5% and 1%, respectively. $n = 18$.

In addition, we also performed a correlation analysis among the leaf physiological traits and rice grain nutritional attributes (Figure 7). The linear regression analysis revealed that grain nutritional traits, such as SC ($R^2 = 0.50^*$, Figure 7A) and AC ($R^2 = 0.58^*$, Figure 7B), were positively correlated with leaf P_n . Similarly, the grain nutritional traits, such as SC ($R^2 = 0.60^*$, Figure 7C) and AC ($R^2 = 0.61^*$, Figure 7D), were positively correlated with the leaf SPAD values. Moreover, the heatmap correlation analysis revealed a positive correlation among the soil properties (SOC, TN, and AN), leaf physiological traits (P_n and SPAD values), grain nutritional attributes (SC, AC, and PC), and starch RVA profile features (PV, TV, FV, SB, and PT) (Figure 8). The linear regression analysis results confirmed that the increases in soil nutrient content, particularly in SOC and TN, described the highest proportion of the difference in the leaf physiological activities. Hence, changes in leaf physiological activities are closely associated with rice grain nutritional traits.

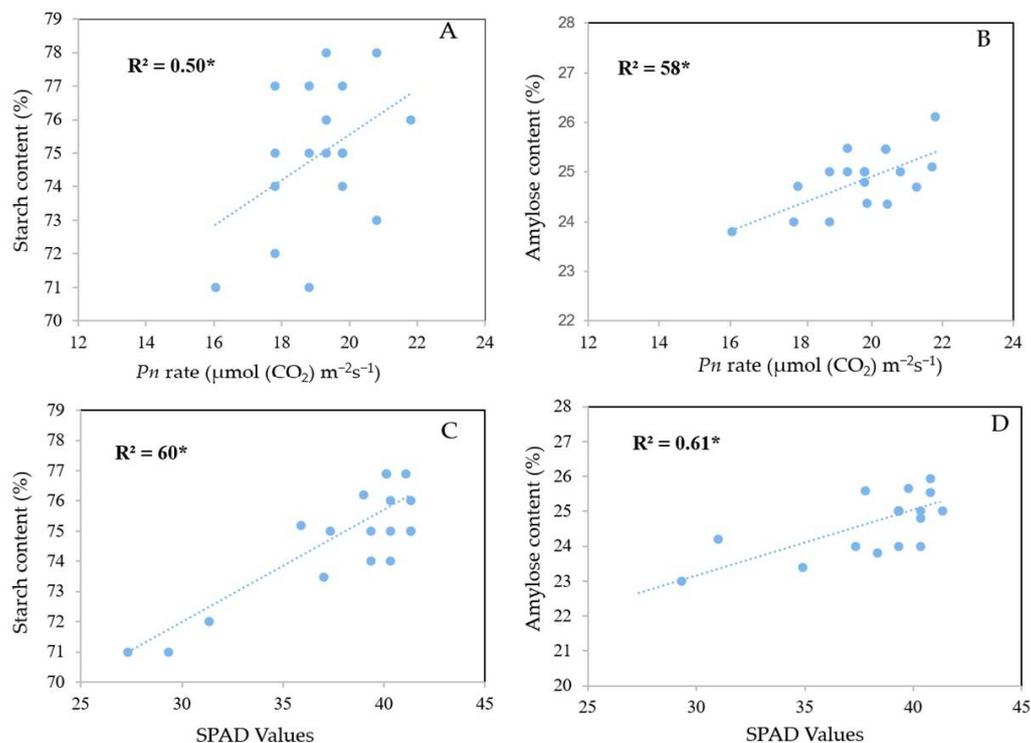


Figure 7. Linear regression analysis of starch content (A,B) and amylose content (C,D) with the net photosynthetic rate and the SPAD values. * = significant at 1%. $n = 18$.

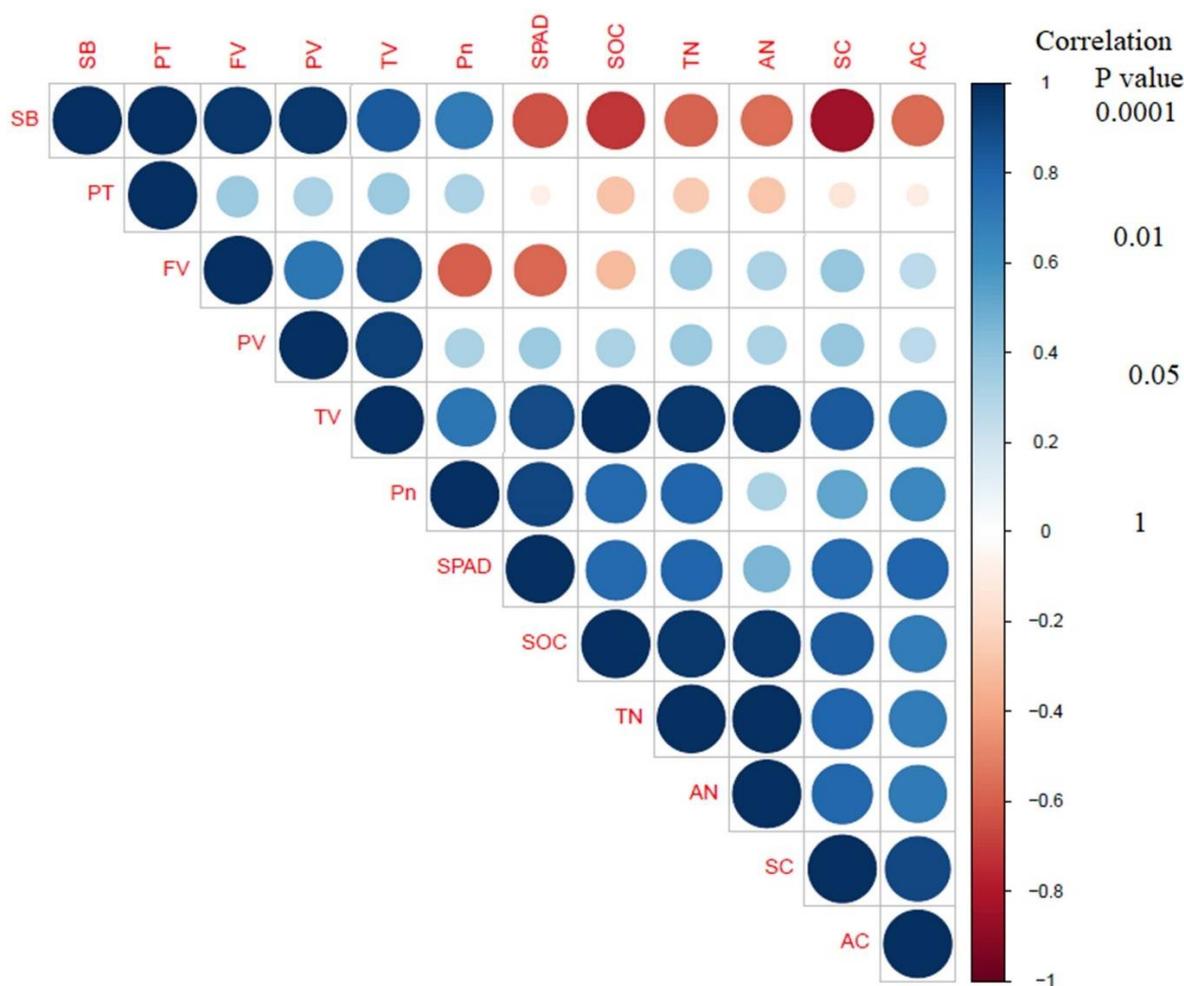


Figure 8. Correlation heat map analysis among the soil chemical properties, leaf physiological traits, and RVA profile characteristics. Note: SOC, soil organic carbon; TN, total nitrogen; AN, available nitrogen; Pn, net photosynthetic rate; PV, peak viscosity; TV, tough viscosity; FV, final viscosity; SB, set back; PT, peak time.

4. Discussion

In current and future agricultural development, the integrated use of manure and CFs is a critical management practice. Several authors have reported that using animal manure improves crop yield and soil quality. However, the influence of continuously substituting different types and amounts of organic fertilizers for CFs on paddy soil chemical properties, leaf physiological activities, and grain physiochemical attributes in a dual cropping system has remained unknown. This research investigated the effects of integrated applications of organic manure (i.e., PM or CM) and CF on soil chemical properties, leaf physiological traits, and rice grain physiochemical characteristics, and how variations in soil chemical properties and leaf physiological activities affected the nutritional and cooking quality of rice grain.

In this study, the combined application of manure and CF improved soil chemical properties, leaf physiological traits, and grain physiochemical characteristics. In contrast, applying only CF decreased the soil properties and grain physiochemical characteristics. During both years, soil chemical properties, such as SOC, SOM, TN, and AN improved in the organic amendment treatments (Figures 2 and 3). Organic fertilizers generally contain more extra organic matter and micronutrients than synthetic fertilizers. Their continued application activates soil nutrients, increases soil nutrient content, maintains available nutrient stability, and improves soil quality and cereals grain yield [5–7].

Soil organic C is a vital indicator of soil health and fertility [34]. Soil organic C content was significantly enhanced in the present experiment under the integrated treatments compared to the soil CF application (Figure 2). In the current study, the combined treatments resulted in a considerable rise in SOC contents as relative to a Pos-CF. The SOC at any given position is heavily reliant on yearly organic turnovers (plant residues) and its recycling [56]. Moreover, the considerable increase in soil organic C could be attributed to the substantial effects of the animal manure because changes in soil organic C result from direct C inputs from manure and indirect C inputs from an increase in crop biomass returned to the soil, such as root and crop residues [57]. Purakaystha et al. [58] stated that organic fertilizers significantly improve soil carbon content.

Soil nutrients, such as TN and AN, improved significantly under the integrated treatments in the present work. This was primarily due to the manure, which positively affected soil N content. This fertilizer was coupled with incorporating organic matter remains, which immediately contributed nutrients to the soil after decomposition [7]. Another possible explanation for the enhancement of the soil chemical traits in this study was associated with organic manure, which absorbs more leachate, improves water holding capacity, and decrease leaching of nutrients; thus providing more available NPK [6,59]. Furthermore, fertilizing with organic manure may increase the populations of soil microorganisms and the activities of soil enzymes involved in nutrient transformation, thus increasing the availability of soil nutrients [17,36].

The leaf chlorophyll content directly affects plant photosynthetic activities [60], hence it affects crop production. Photosynthesis is the crucial driver of crop production by increasing plant growth and dry matter accumulation and shows a good response to N uptake and water [60]. Significant increases in the leaf Pn and SPAD values were noted in the present study under the combined treatments compared to Neg-CF (Figures 3 and 4). The increase in the net photosynthetic rate and SPAD indices under the organic manure coupled with inorganic fertilizer treatment might be related to the faster release of nutrients from mineral fertilizers, which would increase photosynthetic capacity during early growth, while the slow and gradual release of nutrients from the organic manure throughout the growing season would enhance photosynthetic ability, particularly at the grain filling stage [61]. Sufficient water and N decrease water-soluble nutrients, and stress-producing root-sourced signals (abscisic acid) lead to stomatal opening, improved leaf water potential, and enhanced leaf physiological traits [62]. The present results demonstrate that the combined manure and mineral fertilizer treatment improved soil fertility and root growth (Figures 2 and 3), which ultimately boosted the ability of roots to absorb water and nutrients, leading to enhanced stomatal conductance, which enhanced leaf gas exchange and CO_2 fixation before the heading and milking stages. In addition, the linear regression analysis further confirmed that the leaf physiological activities were strongly associated with soil fertility.

Starch and amylose are the major components of rice grains and describe the physiochemical properties, such as gel strength and pasting properties [22,63]. The starch and amylose concentrations in rice grains strongly affect eating and cooking quality [21]. It is well reported that environmental factors, such as air temperature, precipitation, fertilization, and time of fertilization, significantly affect rice grain physicochemical traits [30–32]. In this study, the combined manure and synthetic N fertilization treatment significantly increased grain SC, AC, and PC compared to Pos-CF (Table 3). The observed improvement in PC and AC under the integrated amendments suggests that both fertilizer types delivered sufficient macro and micronutrients, mainly N, which are essential for the growth and development of rice. Kumar et al. [17] reported that co-application of inorganic and organic fertilizers enhances grain quality and increases AC by 7% compared to inorganic fertilizer alone. Moreover, the manure-coupled mineral fertilizers did not significantly affect physical traits, such as the starch dissolving value, expansion rate, or evenness (Table 3). In general, the physical quality of the rice grain does not vary during the short-term uptake of manure

fertilizer. However, the combined organic and inorganic amendment improved the physical quality of the rice grains, which was supported by a previous study [27].

The RVA is a physical index that estimates the viscosity properties of rice starch as a precursor of cooking and processing qualities [64,65]. In this study, the combined application of manure and CF significantly affected the starch RVA profile (Table 4). RVA is closely correlated with the taste quality of rice, in which higher peak viscosity and the breakdown and a smaller setback value improve grain quality. In the current study, applying the organic amendments significantly improved the RVA profile properties compared to chemical N fertilization alone. The cause of the change in the RVA profile characteristics was mainly genotypic; however, environmental factors played an active role. Tamaki et al. [66] reported that rice grain quality and cooking features improve significantly after fertilization with organic manure. Cultivating rice organically enhances eating quality by improving starch viscosity (increases starch stickiness), as described by the higher breakdown and maximum viscosity values of brown rice flour [67]. In contrast, earlier studies reported that synthetic N fertilization alone, which is utilized in conventional farming, causes a deterioration in the cooking quality of rice [29,65].

The soil chemical properties and leaf physiological activities significantly affect the nutritional quality and cooking characteristics of rice grains [68]. In the present study, a strong correlation was detected between soil properties, leaf physiological traits, and the grain nutritional and starch RVA profile features (Figure 8). The analysis revealed a positive correlation among soil properties (SOC, TN, and AN), leaf physiological traits (*Pn* and SPAD values), grain nutritional attributes (SC, AC, and PC), and the starch RVA profile features (PV, TV, FV, SB, and PT). Similar findings were reported by Xuan et al. [69] that amylose content is strongly positively correlated with FV, TV, and SB. In addition, Shi et al. [70] and Ali et al. [71] showed that RVA is significantly correlated with amylose content, suggesting that amylose content may affect the RVA traits to alter the taste of rice.

5. Conclusions

In this study, the combined application of manure and synthetic fertilizers significantly improved soil chemical attributes, such as SOC, TN, and AN as well as leaf physiological activities. The integrated application of synthetic with organic manure led to higher rice grain SC, AC, and PC. Moreover, linear regression analysis showed that the chemical properties positively correlated with the leaf net photosynthetic rate and the SPAD values. Enhancements in the net photosynthetic rate and SPAD values improved the SC and AC of rice. The correlation heat map analyses revealed a positive correlation between the RVA profile and soil nutrients and leaf physiology. Altogether, the findings of this study show that the integrated application of synthetic and cattle or poultry manure in rice fields could be useful for enhancing soil health and rice quality on a sustainable basis.

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Abbreviations

C—carbon; CM—cattle manure; PM—poultry manure; CF—chemical fertilizer; SOC—soil organic carbon; SOM—soil organic matter; TN—total nitrogen; *Pn*—net photosynthetic rate; SC—starch content; AC—amylose content; PC—protein content; RAV—Rapid Visco-Analyzer.

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