Co-incorporation of manure and inorganic fertilizer improves leaf physiological traits, rice production and soil functionality in a paddy field

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The combined use of organic manure and chemical fertilizer (CF) is considered to be a good method for sustaining high crop yields and improving soil quality. We performed a field experiment in 2019 at the research station of Guanxi University, to investigate the effects of cattle manure (CM) and poultry manure (PM) combined with CF on soil physical and biochemical properties, rice dry matter (DM) and nitrogen (N) accumulation and grain yield. We also evaluated differences in pre-and post-anthesis DM and N accumulation and their contributions to grain yield. The experiment consisted of six treatments: no N fertilizer (T1), 100% CF (T2), 60% CM + 40% CF (T3), 30% CM + 70% CF (T4), 60% PM + 40% CF (T5), and 30% PM + 70% CF (T6). All CF and organic manure treatments provided a total N of 150 kg ha−1. Results showed that the treatment T6 increased leaf net photosynthetic rate ($P_n$) by 11% and 13%, chlorophyll content by 13% and 15%, total biomass by 9% and 11% and grain yield by 11% and 17% in the early and late season, respectively, compared with T2. Similarly, the integrated manure and CF treatments improved post-anthesis DM accumulation and soil properties, such as bulk density, organic carbon, total N, microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN) relative to the CF-only treatments. Interestingly, increases in post-anthesis DM and N accumulation were further supported by enhanced leaf $P_n$ and activity of N-metabolizing enzyme during the grain-filling period. Improvement in $P_n$ and N-metabolizing enzyme activity were due to mainly improved soil quality in the combined manure and synthetic fertilizer treatments. Redundancy analysis (RDA) showed a strong relationship between grain yield and soil properties, and a stronger relationship was noted with soil MBC and MBN. Conclusively, a combination of 30% N from PM or CM with 70% N from CF is a promising option for improving soil quality and rice yield.

Abbreviations

| Abbreviation | Description |
|--------------|-------------|
| N            | Nitrogen    |
| CF           | Chemical fertilizer |
| OM           | Organic manure |
| CM           | Cattle manure |
| PM           | Poultry manure |
| NR           | Nitrate reducates |
| GS           | Glutamine synthetase |
| GOGAT        | Glutamate oxoglutarate aminotransferase |
| SOC          | Soil organic carbon |
| TN           | Total nitrogen |

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et al. reported that organic manure application improved leaf photosynthetic capacity and chlorophyll content. The slow and steady release of mineral nutrients from organic fertilizers during the entire plant growth period would improve leaf physiological activity, post-anthesis DM, and N accumulation, thereby increasing grain yield. Previous research has demonstrated the value of pre-anthesis plant DM accumulation and translocation, which can contribute to higher grain yields. Furthermore, it was shown that 69% of straw N and 84% of non-structural carbohydrates accrued pre-anthesis could be translocated to the grains, although this depends on the sowing conditions and cultivar. Post-anthesis DM production may be a good contributor to cereal grain yield, according to recent evidence. However, due to a lack of information, further study into the relationship between post-anthesis DM production and grain yield is required. In the present study, we hypothesized that the combined use of organic and inorganic fertilizers would improve soil physiochemical and biochemical properties, which would in turn increase plant nutrient uptake and accumulation. We also predicted that the slow and steady release of mineral nutrients from organic fertilizers during the entire plant growth period would improve leaf physiological activity, post-anthesis DM, and N accumulation, thereby increasing grain yield. The objectives of the study were: (1) to determine the effects of organic and inorganic N fertilizer combinations on soil physical and biochemical properties; (2) to assess the effect of integrated fertilization on rice leaf physiology, biomass and grain yield; and (3) to evaluate the difference in pre-and post-anthesis N and DM accumulation and its relationship to rice grain yield.

**Results**

**Soil properties.** The combined application of organic and inorganic fertilizer and the different seasons had significant effects on soil physical and chemical properties such as bulk density (BD), pH, soil organic carbon (SOC), total nitrogen (TN), available nitrogen (AN), available potassium (AK), and available phosphorus (AP) up to 20 cm (Table 1). The treatments showed the same behavior across both seasons. In the CF-only treatment (T1), soil pH (5.92 and 5.90), SOC (14.86 and 14.66 g kg⁻¹), TN (1.45 and 1.43 g kg⁻¹), AN (138.5 and 137.5 mg kg⁻¹), AP (23.5 and 231.2 mg kg⁻¹) and AK (226.5 and 231.2 mg kg⁻¹) were recorded in the early and late seasons, respectively. Compared with T1, the combined treatment T3 increased soil pH by (4.5% and 7.2%), SOC (13% and 19%), TN (14% and 25%), AN (11% and 18%), AP (14% and 22%), and AK (22% and 33%) dur-
ing the early and late seasons, respectively. Treatment T3 also considerably reduced the soil BD relative to T2 by 6% in the early season and 9% in the late season. Soil pH, SOC, TN, AN, AP, and AK in T3 increased by 60%, 46%, 78%, 63%, 57%, and 50%, respectively, in the late season compared with the early season. Soil properties in the treatment T5 did not differ significantly from those in T3. Moreover, treatments T4 and T6 also significantly improved soil physical and chemical properties relative to T2. Lower values for soil parameters were noted in the non N-treated plots.

**Microbial biomass C and N.** Soil MBC and MBN differed significantly among treatments in both seasons, as shown in Fig. 1. The integrated use of organic manure and CF significantly enhanced soil MBC and MBN in both seasons compared with CF-only fertilization. In the both seasons, the treatments showed the same trend. Compared with CF-only (T2), the T3 treatment improved soil MBC by (14% and 26%) and MBN (11% and 19%) in the early and late seasons, respectively. Relative to the early season, MBC and MBN in treatment T3 increased by 85% and 72%, respectively, in the late seasons. However, MBC and MBN did not differ significantly between T3 and T5. The treatments T4 and T6 also significantly improved soil microbial C and N compared with CF-only fertilization and non N-treated plots.

**Net photosynthetic efficiency and chlorophyll content of the flag leaf.** The combination of manure and chemical N fertilizer significantly improved $P_n$, chlorophyll a (Chl a), and chlorophyll b (Chl b) content during the grain filling period in both seasons compared with the CF-only application (Figs. 2, 3). Leaf $P_n$, Chl a, and Chl b declined with increasing days after anthesis (DAA). The treatments exhibited the same trend in both seasons. Averaged across DAA, T4 treatment increased $P_n$ by 11% and 13%, Chl a by 12% and 14%, and Chl b by 14% and 16% in the early and late seasons, respectively, compared with T2. However, T4 was

**Table 1.** Changes in soil physical and chemical properties under the combined organic and inorganic N fertilization. Note: T1, no N fertilizer; T2, 100% CF; T3, 60% CM + 40% CF; T4, 30% CM + 70% CF; T5, 60% PM + 40% CF; T6, 30% PM + 70% CF; BD, bulk density; SOC, soil organic carbon; SOM, soil organic matter; TN, total nitrogen; AP, available phosphorous; AK, available potassium. Values followed by the same letters, within column, are not significantly different at $p \leq 0.05$. Mean values (n = 3) ± SE. ns: non significant, $^* p < 0.05$, $^** p < 0.05$. 

| Treatment | BD (g cm$^{-3}$) | pH (water) | SOC (g kg$^{-1}$) | SOM (g kg$^{-1}$) | TN (g kg$^{-1}$) | AN (mg kg$^{-1}$) | AP (mg kg$^{-1}$) | AK (mg kg$^{-1}$) |
|-----------|-----------------|------------|------------------|------------------|-----------------|-----------------|-----------------|-----------------|
| Early season | | | | | | | | |
| T1       | 1.36 ± 0.01 a  | 5.97 ± 0.32 c | 13.53 ± 0.85 d | 25.0 ± 2.05 d | 1.40 ± 0.08 e  | 132.62 ± 6.53 d | 23.23 ± 1.54 c | 225.22 ± 10.55 c |
| T2       | 1.36 ± 0.02 a  | 5.95 ± 0.24 c | 14.10 ± 1.01 d | 25.01 ± 1.52 d | 1.41 ± 0.03 c  | 138.53 ± 8.05 c | 23.54 ± 2.00 c  | 226.54 ± 12.36 c |
| T3       | 1.24 ± 0.02 c  | 6.10 ± 0.34 a | 16.81 ± 0.44 a | 29.20 ± 0.85 a | 1.63 ± 0.02 a  | 154.38 ± 10.11 a | 26.59 ± 1.05 a  | 276.53 ± 11.50 a |
| T4       | 1.29 ± 0.04 b  | 6.00 ± 0.50 b | 15.41 ± 0.81 b | 28.15 ± 1.58 c | 1.54 ± 0.07 b  | 148.87 ± 9.44 b | 24.81 ± 2.15 b  | 266.80 ± 15.50 b |
| T5       | 1.25 ± 0.01 c  | 6.11 ± 0.30 a | 16.69 ± 1.44 a | 28.60 ± 2.87 a | 1.65 ± 0.10 a  | 155.40 ± 5.05 a | 26.87 ± 1.22 a  | 274.28 ± 7.55 a  |
| T6       | 1.30 ± 0.06 b  | 6.03 ± 0.41 b | 15.01 ± 0.28 c | 26.42 ± 1.81 c | 1.52 ± 0.04 b  | 149.60 ± 8.57 b | 24.59 ± 1.88 b  | 265.60 ± 9.33 b  |
| Average  | 1.30 a         | 6.02 b      | 14.80 b          | 27.17 b          | 1.53 b         | 146.60 b        | 24.94 b         | 255.83 b         |
| Late season | | | | | | | | |
| T1       | 1.36 ± 0.04 a  | 5.96 ± 0.21 c | 13.44 ± 1.08 d  | 24.85 ± 1.74 d  | 1.39 ± 0.02 d  | 133.14 ± 12.01 d | 24.28 ± 3.01 b  | 226.42 ± 15.57 d |
| T2       | 1.35 ± 0.03 a  | 5.90 ± 0.12 d | 14.66 ± 0.35 c  | 25.22 ± 2.35 c  | 1.43 ± 0.05 c  | 137.55 ± 5.25 c | 24.80 ± 2.43 c  | 231.21 ± 8.88 c  |
| T3       | 1.17 ± 0.01 d  | 6.28 ± 0.62 a | 17.68 ± 0.42 a  | 29.10 ± 1.85 a  | 1.83 ± 0.04 a  | 167.79 ± 6.78 a | 30.31 ± 2.55 a  | 308.24 ± 10.76 a |
| T4       | 1.21 ± 0.05 b  | 6.19 ± 0.40 b | 16.21 ± 0.85 b  | 28.74 ± 0.95 b  | 1.71 ± 0.03 b  | 156.56 ± 11.05 b | 27.78 ± 1.42 b  | 286.52 ± 6.55 b  |
| T5       | 1.20 ± 0.07 c  | 6.26 ± 0.84 a | 17.56 ± 0.70 a  | 31.92 ± 3.05 a  | 1.81 ± 0.02 a  | 168.80 ± 10.56 a | 28.65 ± 2.77 a  | 303.25 ± 16.20 a |
| T6       | 1.23 ± 0.02 b  | 6.18 ± 0.33 b | 16.31 ± 1.22 b  | 28.62 ± 2.11 b  | 1.72 ± 0.04 b  | 158.25 ± 5.33 b | 30.54 ± 3.11 b  | 284.52 ± 9.30 b  |
| Average  | 1.25 b         | 6.12 a      | 15.98 a          | 28.08 a          | 1.65 a         | 162.68 a        | 27.69 a         | 273.36 a         |

ANOVA

| Treatment (T) | * * * * * * * * |
| Season (S)    | * * * * * * * * |
| T × S         | ns ns ns ns ns ns ns ns |

$^{**} p < 0.05$
Activity of N-metabolizing enzymes. The activity of N-metabolizing enzymes such as NR, GS, and GOGAT during the grain filling period were significantly affected by the combined application of manure and chemical fertilizer and different seasons (Fig. 4). Higher activity of N-metabolizing enzymes was noted in N-treated plants, whereas lower activity was observed in non-N-treated plants during both seasons (Fig. 4A–F). N-metabolizing enzyme activity showed the same behavior in both seasons. NR activity decreased during the grain filling period; it reached a maximum at 4 DAA, then slowly decreased and was lowest at 24 DAA (Fig. 4A, B). Averaged across DAA, the NR activity of T6 was 10% and 14% higher than that of T2 in the early and late seasons, respectively. NR activity did not differ significantly among T4 and T6. Similarly, T3 and T5 also improved NR activity, and the lowest values were observed in non-N-treated plots.

By contrast, GS and GOGAT activities first increased and then decreased during the grain filling period; highest at 14 DAA and lowest at 24 DAA (Fig. 4C–F). Across the grain filling period, GS activity was (13% and 17%) and GOGAT was (11% and 16%) higher in T6 than in T2 in the early and late seasons, respectively. However, no significant differences were observed among T4 and T6. The combined fertilization treatments T3 and T5 had significantly higher values of GS and GOGAT activity than the non-N-treated plots.

Accumulation and translocation of DM and N. Dry matter and N accumulation reflect plant growth and metabolic capacity and ultimately control the economic yield. In the present study, the accumulation of DM and N increased progressively with plant growth and attained maximum values at plant maturity. DM and N accumulation differed significantly among fertilizer treatments and seasons, as shown in Table 2. DM accumulation was 9% and 11% higher in T6 than in T2 at maturity in the early and late seasons, respectively, and N accumulation was 10% and 12% higher in T6. DM and N accumulation in T6 were 22% and 20% higher in the late season than in the early season. DM and N did not differ significantly between T4 and T6. The combined treatments T3 and T5 also improved DM and N accumulation but did not differ significantly from T2. The lowest values of DM and N accumulation were observed in non-N-treated plots.

The combination treatments also showed the highest translocation rates of DM and N accumulated pre-anthesis (Table 5). Relative to non-N-treated plots, the combination treatments improved DM and N translocation significantly in both seasons.

Post-anthesis DM and N accumulation. The combination of manure and synthetic fertilizer significantly improved post-anthesis DM and N accumulation (Table 2). Post-anthesis DM accumulation in the CF-only treatment (T2) was 482 and 436 (g m⁻²) in the early and late seasons, respectively, and post-anthesis N accumulation in T2 was 4.18 and 4.06 (g m⁻²). Post-anthesis DM accumulation was 9% and 14% higher in the T6 combination treatment than in T2 in the early and late seasons, respectively, and post-anthesis N accumulation was 10% and 13% higher. T6 did not differ significantly from T4 in post-anthesis DM and N accumulation. Treatments T3 and T5 also improved post-anthesis DM and N accumulation, and the lowest post-anthesis values were observed in non-N-treated plots.

Rice yield and yield components. Rice grain yield and yield attributes were significantly improved by the combination of organic manure and inorganic N fertilization (Table 3). However, there was no significant difference effects of different season on grain yield and yield components, with the exception of panicles number and 1000 grain weight. The T6 treatment produced significantly higher panicle number (11% and 14%), grain filling percentage (5% and 7%), 1000-grain weight (6% and 9%), and grain yield (11% and 17%) compared with T2 in the early and late seasons, respectively. These parameters did not differ significantly between T4 and T6.
T3 and T5 combination treatments also had higher yields and yield components than non-N-treated plots. The lowest yield and yield components were observed in non-N-treated plots.

**Relationships between leaf physiological traits and grain yield.** Changes in leaf physiological traits significantly affect the grain yield of rice. In the present study, linear regression analysis revealed highly significant and strong relationships between leaf physiological attributes and grain yield, as shown in Fig. 5. Flag leaf Pn ($R^2 = 0.98^{**}$, Fig. 5A), and the activities of the N-metabolizing enzymes NR ($R^2 = 0.94^{**}$, Fig. 5B), GS ($R^2 = 0.96^{**}$, Fig. 5C), and GOGAT ($R^2 = 0.98^{**}$, Fig. 5D) were strongly positively associated with grain yield. Therefore, higher leaf physiological activity during the grain filling period contributed significantly to rice grain yield.

**Relationships of pre- and post-anthesis DM and N accumulation with grain yield.** The grain yield of cereal crops highly dependent on pre- and post-anthesis accumulation of DM and N. In the current study, linear regression analysis showed strong positive relationships of post-anthesis DM (DMA, $R^2 = 0.81^{**}$, Fig. 6A) and N accumulation (NA, $R^2 = 0.73^{**}$, Fig. 6C) with grain yield. Moreover, translocation of DM (DMT, $R^2 = 0.71^{**}$, Fig. 6B) and N (NT, $R^2 = 0.80^{**}$, Fig. 6D) accumulated pre-anthesis were also positively related to grain yield. Finally, linear regression confirmed that post-anthesis DM accumulation was strongly positively correlated with grain yield. Therefore, improvements in post-anthesis DM accumulation contributed significantly to higher grain yield in rice.

**Relationships of soil properties with N-metabolizing enzyme activities and grain yield.** Changes in above-average plant yields are highly dependent on fluctuations in soil quality and can be helpful in soil sustainability and stability. In this study, RDA revealed strong positive relationships of N metabolism enzyme activities and grain yield with soil properties and microbial activity (Fig. 7). Soil properties such as pH, SOC, TN, AP, MBC, and MBN showed strong correlations with plant biomass accumulation, rice grain yield and N metabolism enzyme activities, and photosynthetic efficiency during the grain-filling period. However, the correlation of MBN with N metabolism enzyme activity, plant N, and biomass accumulation was significantly higher under organic manure fertilization, presumably as a result of improved soil fertility.

**Discussion**

Soil physical and chemical properties were significantly improved by the combination of organic and inorganic N fertilization compared with the application of urea fertilizer alone (Table 1). The combination treatment also resulted in lower soil BD. Reductions in soil BD in the combination treatments were due to the bulky nature of the organic manure, which prevented the soil from separating	extsuperscript{34}. Moreover, the use of organic manures has been shown to promote soil aeration and enhance soil aggregation, which leads to a decline in soil BD	extsuperscript{35}. Our outcomes are in consistent with Franzluebbers et al.	extsuperscript{35}, who concluded that variation in SOC not only directly affects soil BD but also increases soil aggregation and healthy pore growth due to improved soil physicochemical and biological properties.

Soil chemical properties such as soil pH, SOC, TN, AN, AK, and AP increased significantly with the combined application of organic and inorganic fertilizers. We noted that the decomposition of organic manure gradually

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**Figure 2.** Changes in leaf net photosynthesis rate at (4, 14, and 24 DAA) during grain filling period both early (A) and late season (B) under the combined organic and inorganic N fertilization. Vertical bars represent the standard error of mean. Different letters above the column indicate significance at the ($P<0.05$). ns: non significant, *$p<0.05$, **$p<0.05$. For treatment details please see Table 1.
released nutrients into the soil, and increasing the amount of organic fertilizer from 30 to 60% improved soil chemical properties. The use of CF alone reduced soil pH, whereas the combination treatment increased soil pH considerably. Chemical N fertilization may have significantly reduced the exchangeable base cations in the soil, ultimately leading to a decline in soil pH. The use of synthetic N has also been shown to shift soils into the Al³⁺ buffering stage. Another possible explanation for increased soil pH in the combination treatments is that organic fertilizer contains enough basic cations and carbonate ions to neutralize the acidification effect.

Soil C is an important parameter for the evaluation of soil quality and fertility. The substantial improvements in SOC, TN and AN observed in this study may have resulted from both direct C and N inputs from the organic manure and indirect C and N inputs associated with greater crop biomass, such as roots and crop residues. Soil organic C at any specific location depends mainly on the seasonal return and recycling of organic materials, roots, and shoot stubbles. Our results are in agreement with Purakayastha et al., who reported that organic manure integrated with chemical fertilizer increased soil TN by 56–90% and SOC by 11–80% in the upper soil layer. The application of organic fertilizers may also have increased soil nutrient availability because the manure absorbed more leachate, improving soil water holding capacity, decreasing nutrient leaching, and ultimately increasing the availability of N, P, and K in the soil.

Higher soil available P in the combination treatments is consistent with the results of P addition from chemical fertilizers, as plants typically use only a portion of the applied P. Likewise, organic manure often provides a large amount of P to the soil and reduces the fixation of added P, resulting in enhanced competition of organic molecules with PO₄³⁻ ions for P retention sites in the manure treatments. Higher available K in the combination treatments relative to the urea-only treatment may reflect exudation of organic acids during the decomposition process, which releases negative ions that have a preference for di- or trivalent cations (e.g., Al³⁺, Ca²⁺, and Mg²⁺), leaving K⁺ to be absorbed by negatively charged soil colloids. This phenomenon can help to minimize K fixation and improve soil K availability.

Soil MBC and MBN reflect characteristics of the soil microbial community structure. In the present study, organic manure in combination with CF significantly enhanced soil MBC and MBN (Fig. 1). Increases in MBC and MBN may have occurred because organic fertilizer improved the physicochemical and biological properties of the soil, leading to increased absorption and uptake of mineral N by the crop. In addition, manure may have facilitated the conversion of mineral N to MBC and other N forms. Another possible explanation is that the combination treatments may have improved soil fertility and rice biomass production (Tables 4, 5), leading to an increase in crop residues. Such residues are beneficial for the propagation of soil microbes and may therefore facilitate the conversion of C and N. Furthermore, the integration of organic fertilizer with synthetic N is widely accepted as an efficient means of improving soil microbial activity, soil structure, aggregation, and water retention capacity.

Leaf chlorophyll (Chl) content is widely used to assess plant photosynthetic health. Chl synthesis and protein are also associated with leaf N concentration, and higher photosynthetic rates promote stem elongation, enhance leaf area expansion, and delay leaf senescence. Leaf Chl content and Pn are directly related to N uptake. Photosynthetic rate responds readily to N and water supply and is the key driver of plant production by enhancing

![Figure 3](https://doi.org/10.1038/s41598-021-89246-9)
growth and biomass\textsuperscript{55,56}. In this work, leaf \( Pn \) and Chl content were highest in the integration treatments relative to the CF-only application during the grain-filling (Figs. 3 and 4). This increase may have been due to the fact that the combined application of manure and synthetic fertilizer increased soil fertility and quality (Table 4), decreasing the leaching of mineral elements from the upper soil layer and improving the physical structure of the soil, thereby increasing plant nutrient absorption\textsuperscript{57}. Another possible reason for improvement in \( Pn \) and Chl content during the grain-filling period is the quicker release of nutrients from synthetic fertilizer and accompanied by the slow and steady release of nutrients from organic fertilizer across the entire growth period\textsuperscript{20}.

\( Pn \) and Chl \( a \) were always highest in T6 and lowest in T1 (Figs. 3 and 4). These findings highlight the importance of Chl \( a \), as it is the primary photosynthetic pigment.

Several key enzymes such as NR, GS, and GOGAT play an important role in plant N assimilation\textsuperscript{27}. In this study, higher N-metabolizing enzyme activity was observed in response to the combined application of manure and synthetic fertilizer (Fig. 4). This may have resulted from improved soil quality under the combination treatment (Table 5). Our findings indicated that the combination of organic manure and chemical fertilizer improved N accumulation in leaves more effectively than the traditional urea-only application, and this was necessary to provide adequate grain-filling substrate and promote superior grain yield\textsuperscript{58}. Similarly, Gupta et al.\textsuperscript{59} also reported a strong relationship of soil N availability and N absorption with the activities of key N assimilatory enzymes during grain filling. Our outcomes are consistent with those of Sun et al.\textsuperscript{58}, who concluded that GS and GOGAT activities in flag leaves during the grain-filling period were strongly positively associated with grain yield and crop quality.

Delayed leaf senescence supports comparatively high photosynthetic activity and promotes maximum DM production and grain yield; it may be achieved by synchronizing soil N availability and plant N uptake during the grain-filling period\textsuperscript{24,25}. In the present study, delayed leaf senescence, high photosynthetic efficacy, and enhanced N-metabolizing enzyme activity were observed during the grain-filling period in the combination treatments (Figs. 3 and 4). These treatments improved N uptake and post-anthesis DM accumulation, and ultimately improved grain yield. This was further supported by the significant and highly positive correlations of grain yield with \( Pn (R^2=0.98^{**}, \text{Fig. 5A}), NR (R^2=0.94^{**}, \text{Fig. 5B}), GS (R^2=0.96^{**}, \text{Fig. 5C}) \) and GOGAT (\( R^2=0.98^{**}, \text{Fig. 5D} \)), indicating that greater \( Pn \) and N metabolism activities during grain formation promoted the formation of a superior sink (as quantified by panicle length and spikelet number), and consequently led to superior rice grain.
Table 2. Pre-and post-anthesis dry matter and nitrogen accumulation and translocation under the combined organic manure and inorganic N fertilizer. DMA dry matter accumulation, DMT dry matter translocation, NA nitrogen accumulation, NT nitrogen translocation, Ant anthesis, Mat maturity, Post-a anthesis accumulation. Values followed by the same letters, within column, are statistically non-significant at ($p < 0.05$). Mean values ($n = 3$) ± SE. ns: non significant, $^* p < 0.05$, $^{**} p < 0.05$. For treatment details please see Table 1.

| Treatment | DMA (g m$^{-2}$) | PH (cm) | PN (hill$^{-1}$) | PL (cm) | SSP (panicle$^{-1}$) | FGP (%) | TGW (g) | GY (kg ha$^{-1}$) |
|-----------|------------------|---------|----------------|---------|---------------------|---------|---------|-----------------|
| Early season | | | | | | | | |
| $T_1$ | 648 ± 14.52 d 956 ± 16.02 d 308 ± 14.20 c | 214 ± 8.82 c 6.27 ± 0.85 c 8.84 ± 0.82 c | 2.64 ± 0.32 c 4.42 ± 0.44 b | 956 ± 16.02 d 308 ± 14.20 c | 214 ± 8.82 c 6.27 ± 0.85 c 8.84 ± 0.82 c | 2.64 ± 0.32 c 4.42 ± 0.44 b | 956 ± 16.02 d 308 ± 14.20 c | 214 ± 8.82 c 6.27 ± 0.85 c 8.84 ± 0.82 c | 2.64 ± 0.32 c 4.42 ± 0.44 b |
| $T_2$ | 998 ± 17.21 b 1480 ± 20.52 b 482 ± 11.08 b | 249 ± 11.52 a 10.12 ± 1.12 b 14.30 ± 1.44 b | 4.18 ± 0.82 b 6.28 ± 0.30 a | 1480 ± 20.52 b 482 ± 11.08 b | 249 ± 11.52 a 10.12 ± 1.12 b 14.30 ± 1.44 b | 4.18 ± 0.82 b 6.28 ± 0.30 a | 1480 ± 20.52 b 482 ± 11.08 b | 249 ± 11.52 a 10.12 ± 1.12 b 14.30 ± 1.44 b | 4.18 ± 0.82 b 6.28 ± 0.30 a |
| $T_3$ | 969 ± 15.64 b 1399 ± 18.22 c 430 ± 14.54 b | 241 ± 13.84ab 10.45 ± 0.92 a 14.78 ± 0.82 b | 4.33 ± 0.22 b 6.24 ± 0.34 a | 1399 ± 18.22 c 430 ± 14.54 b | 241 ± 13.84ab 10.45 ± 0.92 a 14.78 ± 0.82 b | 4.33 ± 0.22 b 6.24 ± 0.34 a | 1399 ± 18.22 c 430 ± 14.54 b | 241 ± 13.84ab 10.45 ± 0.92 a 14.78 ± 0.82 b | 4.33 ± 0.22 b 6.24 ± 0.34 a |
| $T_4$ | 1092 ± 11.52 a 1598 ± 16.50 a 506 ± 12.58 a | 266 ± 13.00 a 11.40 ± 0.58 a 16.11 ± 1.12 a | 4.71 ± 0.70 a 6.63 ± 0.80 a | 1598 ± 16.50 a 506 ± 12.58 a | 266 ± 13.00 a 11.40 ± 0.58 a 16.11 ± 1.12 a | 4.71 ± 0.70 a 6.63 ± 0.80 a | 1598 ± 16.50 a 506 ± 12.58 a | 266 ± 13.00 a 11.40 ± 0.58 a 16.11 ± 1.12 a | 4.71 ± 0.70 a 6.63 ± 0.80 a |
| $T_5$ | 909 ± 18.00 c 1370 ± 19.82 c 461 ± 18.52 b | 236 ± 15.10 b 10.75 ± 0.82 b 14.71 ± 0.62 b | 3.96 ± 0.14 b 6.78 ± 0.34 a | 1370 ± 19.82 c 461 ± 18.52 b | 236 ± 15.10 b 10.75 ± 0.82 b 14.71 ± 0.62 b | 3.96 ± 0.14 b 6.78 ± 0.34 a | 1370 ± 19.82 c 461 ± 18.52 b | 236 ± 15.10 b 10.75 ± 0.82 b 14.71 ± 0.62 b | 3.96 ± 0.14 b 6.78 ± 0.34 a |
| $T_6$ | 1094 ± 16.12 a 1618 ± 21.30 a 524 ± 10.12 a | 251 ± 9.85 ab 11.50 ± 1.10 a 16.18 ± 1.02 a | 4.68 ± 0.38 a 6.26 ± 0.30 a | 1618 ± 21.30 a 524 ± 10.12 a | 251 ± 9.85 ab 11.50 ± 1.10 a 16.18 ± 1.02 a | 4.68 ± 0.38 a 6.26 ± 0.30 a | 1618 ± 21.30 a 524 ± 10.12 a | 251 ± 9.85 ab 11.50 ± 1.10 a 16.18 ± 1.02 a | 4.68 ± 0.38 a 6.26 ± 0.30 a |
| Average | 952 a | 1493 a | 480 | 242 a | 10.08 a | 15.20 a | 4.37 a | 6.10 a |

ANOVA

| Treatment (T) | * | * | * | * | * | * | * |
| Season (S) | ns | ns | ns | * | * | ns | ns |
| T × S | ns | ns | ns | ns | ns | ns | ns |

Table 3. Changes in rice growth, yield and yield components under organic and inorganic N fertilizer application. PH plant height, PN panicle number, PL panicle length, SSP spikelet number per panicle, FGP filled grain percent, TGW thousand grain weight, GY grain yield. Values followed by the same letters, within column, are not significantly different at ($p \leq 0.05$). Mean values ($n = 3$) ± SE. ns: non significant, $^* p < 0.05$, $^{**} p < 0.05$. For treatment details please see Table 1.
yield. Consistent with our results, many authors have reported that increased N uptake leads to increases in \( Pn \) overall photochemical efficiency of PSII, and leaf physiological activity; this delays leaf senescence in the late growth period and eventually enhances photosynthetic production during the grain-filling stage\(^{59,60}\).

Cereal crop yields are strongly dependent on post-anthesis DM production and the translocation of DM accumulated prior to anthesis to grain\(^{30}\). Pal et al.\(^{30}\) also concluded that the contribution to grain yield of DM production prior to anthesis ranged from 22 to 69%, depending on rice cultivar and the sowing time. Moreover, Wu et al.\(^{32}\) stated that variation in rice yield between the early and late growing seasons could be explained primarily by the difference in post-anthesis DM production. In the current study, the combined treatments \( T_4 \) and \( T_6 \) had the highest values of DMT and accumulated pre-anthesis and post-anthesis DM production, as shown in Table 2. Highest DM and N accumulation under the combination of 30% manure and 70% inorganic fertilizer could be attributed to a high and continuous supply of nutrients due to improved soil fertility (Table 1). The constant and steady release of N from the cattle and poultry manure, particularly during the grain-filling period, would have encouraged their use by the plant\(^{26}\).

**Figure 5.** Linear relationships of grain yield with net photosynthetic rate (\( Pn \)) (A), Nitrate reductase (NR) (B), glutamine synthetase (GS) (C), and glutamine oxoglutarate aminotransferase (GOGAT) (D). **\( p < 0.01 \). \( n = 6 \).

**Figure 6.** Linear relationships of grain yield with post-anthesis DMA (A), dry matter translocation (B), post-anthesis NA (C), and nitrogen translocation (D). Note: DMA- dry matter accumulation, DMT- dry matter translocation, NA-nitrogen accumulation, NT- nitrogen translocation. **\( p < 0.001 \). \( n = 6 \).
Linear regression analysis showed that post-anthesis DM accumulation was more strongly positively correlated with grain yield ($R^2 = 0.81^{**}$, Fig. 6A) than was DMT ($R^2 = 0.71^{*}$, Fig. 6B). This finding underscored that both processes are important, but post-anthesis DM production plays a more important role in higher grain yield. Similarly, pre- and post-anthesis N accumulation were also highly positively correlated with grain yield: post-anthesis NA ($R^2 = 0.73^{**}$, Fig. 6C) and NT ($R^2 = 0.80^{**}$, Fig. 6D). The current study confirms that plants rely primarily on post-anthesis DM production and N accumulation for grain filling. Higher post-anthesis DM production and N accumulation in the combination treatments were due mainly to adequate availability of nutrients, which delayed leaf senescence and increased N remobilization.

Rice grain yield is determined by yield components, including the number of tillers, panicle length, and spikelets per panicle$^{32,61}$. In the present study, the combination of manure and synthetic N fertilizer significantly improved rice growth, yield, and yield components compared to urea fertilization alone (Table 3). The higher rice growth, yield, and yield traits under the combination treatments could be attributed mainly to a balanced and continuous supply of nutrients due to enhanced soil fertility (Table 4), which ultimately improved plant nutrient uptake and growth. The continued and slow release of N from organic manure, particularly during the grain-filling period, may have enabled its efficient utilization by the crop$^{41,62}$. Moreover, the RDA showed that the x-axis explained 96.3% of the variation, and the y-axis explained 0.03%. It revealed significant positive correlations of grain yield, leaf physiological traits, N metabolism enzyme activities, and dry matter accumulation with all soil properties (Fig. 7).

**Conclusion**

Application of a combination of organic manure and chemical fertilizer enhanced soil physical and biochemical traits, leaf physiological activity, and rice yields compared with chemical fertilizer alone. The co-incorporation of manure and synthetic fertilizers significantly improved pre- and post-anthesis DM production and N accumulation compared with the application of urea alone. Improvements in DM production and N accumulation were due primarily to improved soil fertility and leaf physiological activity, including $P_n$, Chl, and the activities of N-metabolizing enzymes, which further increased DM production and N uptake. RDA revealed positive relationships between grain yield and soil properties (i.e., SOC, TN, AN, and AP), and a significantly higher correlation was observed between grain yield and soil MBC and MBN. The combination of organic manure and synthetic fertilizer in a 30:70 ratio is a beneficial and sustainable practice for rice production and soil quality improvement.

**Materials and methods**

**Experimental site.** The experiment was conducted at the experimental farm of Guangxi University, China (22° 49′ 12″ N, 108° 19′ 11″ E; 75 m) in the early season (March to July) and late season (July to November) of 2019. The region is characterized by a subtropical, monsoon climate with average annual rainfall of 1080 mm. The average maximum and minimum temperatures are 32.5 °C and 24.2 °C in the early season and 31.2 °C and 22.0 °C in the late season (Table 4). The soil is classified as a ultisol based on USDA classification. It is slightly acidic with pH 5.94, soil organic carbon (SOC) 14.56 g kg$^{-1}$, total N (TN) 1.41 g kg$^{-1}$, available phosphorous (P) 23.12 mg kg$^{-1}$, available potassium (K) 233.33 mg kg$^{-1}$, and a high bulk density (BD) of 1.36 g cm$^{-3}$ (Table 5).

**Experimental design and field management.** The field experiment was performed in a randomized complete block design (RCBD) with three replicates and a plot size of 3.9 m × 6 m (23.4 m$^2$). Cattle manure (CM) and poultry manure (PM) were the organic fertilizers and urea was the chemical fertilizer (CF). The experiment
consisted of six treatments, i.e., no N fertilizer (T1); 100% CF (T2); 60% CM + 40% CF (T3); 30% CM + 70% CF (T4); 60% PM + 40% CF (T5), and 30% PM + 70% CF (T6). The noodle rice cultivar “Zhenguiai” was used as the test crop. Rice seeds were sown in an open field in plastic seedling trays, and urea was applied to the nursery at the time of preparation. The 25 day-old seedlings were transplanted to the field, and two seedlings per hill. The plant-to-plant distance was 10 cm, the row-to-row distance was 30 cm, and the total number of plants in each plot was 780. The recommended dose of NPK was 150:75:150 (kg ha\(^{-1}\)), and every plot received 175.5 g of P\(_2\)O\(_5\) from superphosphate, 365 g of KCl from potassium chloride, and 351 g of N from PM or CM and CF (urea) after proper N estimation. The net N, P, and K contents in the urea, superphosphate, and potassium chloride was 46%, 20%, and 60%, respectively. The chemical composition of the organic manure and the nutrient content and quantity for each treatment are shown in Tables 5 and 6. N and KCl were applied in three splits as a basal dose (60%), at the early tillering stage (20%), and at panicle initiation (20%). P was delivered as a basal dose one day before transplantation (Table 6).

Organic fertilizer, such as CM and PM were obtained from the cattle and poultry farms, located in the local area. Organic manure applied to plots 20 days prior to transplantation. The T1 treatment received no N fertilizer but received P and K fertilizers at rates equal to those in N-treated plots. Standard flood water was provided at a depth of approximately 5 cm from transplant to physiological maturity. Normal agricultural practices were used for all treatments, including irrigation (about 5 cm flood water), insecticide application (chlorantraniliprole formulations sprayed at the recommended rate of 150 mL a.i. per ha), and herbicide application (paraquat at 10 gallons per acre).

**Sampling and analysis.** Sampling and analysis of soil and manure. Subsamples of initial soil and organic fertilizers (CM and PM) were dried at room temperature and passed through a 2-mm sieve. In addition, three replicate soil samples were taken from the 0–20 cm depth after harvest in the early and late seasons, to assess changes in soil properties. Soil bulk density (BD) was determined by the core method as described by Grossman\(^{20}\), and used to calculate soil total porosity using Eq. (1) recommended by Hillel\(^{21}\):

\[
\text{Porosity} = (1 - (BD/PS)) \times 100
\]
where BD is soil bulk density and PS is particle density, assumed to be 2.65 mg m⁻³. Soil moisture content was measured as described by Ledieu et al.⁶². Air-dried soil was passed through a 0.5-mm sieve, and the weight of the tin (g) was taken as W₁. A 1 g soil sample was taken along with the tin and weighed as W₂. The soil samples were dried in an oven for 2 h at 105 °C to obtain a constant weight as W₃. Soil moisture content (%) was determined using Eq. (2):

\[
MC\%\ = \frac{W_2 - W_3}{W_3 - W_1} \times 100
\]

(2)

Soil organic carbon was measured using the oxidation method. Soil subsamples (0.5 g) were digested with 5 ml of 1 M K₂Cr₂O₇–H₂SO₄ and 5 ml of concentrated H₂SO₄ and boiled at 175 °C for 5 min, accompanied by titration of FeSO₄ digests according to the method of Bao.⁶⁶ To measure total soil N content, 200 mg of soil was digested using the salicylic-acid sulfuric-acid hydrogen peroxide method of Ohyama et al.⁶⁷, and TN was analyzed using the micro-Kjeldahl method of Jackson⁶⁸. Total P was determined using the ascorbic acid described by Murphy⁶⁹. Total K was measured by preparing a standard stock solution by dissolving KCl in distilled water and measuring TK at 7665 R wavelength with an atomic absorption spectrophotometer (Z-5300; Hitachi, Tokyo, Japan) after sample digestion. Soil organic matter (SOM) was calculated by multiplying the SOC by 1.72.

AN was estimated using the methods of Kostechkas and Marcinkeviciene⁷⁰ and Dorich and Nelson⁷¹. Soil AP was measured by the NaHCO₃ extraction method and analyzed by the Mo–Sb colorimetric procedure using a spectrophotometer (UV 2550, Shimadzu, Japan) by method of Bao⁶⁶. Soil AK was assessed by the method of Knudsen et al.⁷², using normal 1 M NH₄OAc. Soil pH was measured with a digital pH meter (Thunderbolt PHS-3C, Shanghai, China) after mixing the soil and organic manure with distilled water at a 1:2.5 (w/v) ratio for 1 h.

Soil microbial biomass. The fumigation extraction technique was used to determine MBC as described by Brookes et al.⁷³, and MBN according to the procedure of Vaince et al.⁷⁴. From the composite soil samples, we took 10 g of soil and divided it into similar halves. In a vacuum desiccator, 25 ml of ethanol-free CHCL₃ was put in petri dish to disinfect first half of the soil (5 g) for 24 h at room temperature (25 °C). The samples were placed in warm water at 80 °C after fumigation to eliminate fumes, and 20 ml of K₂SO₄ (0.5 M) was then used to remove C and N from both the fumigated and non-fumigated soils. The filtrated samples were then processed on a TOC Analyzer (TNM1; Shimadzu) and subjected to Kjeldahl digestion in order to calculate total C (TC) and TN. Equation (3) was used to estimate MBC and MBN:

\[
MBC\ or\ MBN = \frac{TN\ or\ TC_{fu} - TN_{or\ TC_{nfu}}}{kEN\ or\ kEC}
\]

(3)

where TNfu and TNnfu are the total N in fumigated and non-fumigated samples, and TCfu and TCnfu are the TC concentrations in fumigated and non-fumigated samples. A kEC coefficient of 0.45 was used to estimate MBC according to the method of Jenkinson et al., and a kEN coefficient of 0.54 was used to estimate MBN according to the method of Joergensen and Mueller.⁷⁵

Accumulation and translocation of DM and N. Three replicate plants were collected at anthesis and at physiological maturity to measure DM and N accumulation. The rice plant was divided into leaves (leaf blade + leaf sheath), stems, and panicles, and then oven-dried to constant weight at 65 °C. Rice plant sub samples were ground to powder, and total N was estimated using the micro-Kjeldahl method as described by Jackson⁶⁸. The DM of leaves, stems, and chaff at maturity. NTa is the total aboveground N accumulation at anthesis, and NTstem,m, NTleaf,m, and NTchaff,m are the total N accumulation of stems, leaves, and chaff at physiological maturity. Assuming that all DM and N losses from the vegetative organs of the plant were transferred to the grains, N translocation (NT) and DM translocation (DMT) during the grain filling stage were measured according to the equations of Papakosta and Gagianas⁷⁶:

\[
DMT = DMa - (DM_{leaf,m} + DM_{stem,m} + DM_{chaff,m})
\]

(4)

\[
NT = NTa - (NT_{leaf,m} + NT_{stem,m} + NT_{chaff,m})
\]

(5)

where DMa is the total aboveground DM accumulation at anthesis and DM_{leaf,m}, DM_{stem,m}, and DM_{chaff,m} are the DM of leaves, stems, and chaff at maturity. NTa is the total aboveground N accumulation at anthesis, and NT_{stem,m}, NT_{leaf,m}, and NT_{chaff,m} are the total N accumulation of stems, leaves, and chaff at physiological maturity.

Rice leaf net photosynthetic efficiency and chlorophyll content. To assess the process of leaf senescence during the reproductive phase, flag leaf chlorophyll content, photosynthetic rate (Pn), and days to maturity were also determined. Flag leaf Pn and Chl content (Chl a and Chl b) were measured at the grain-filling stage. Pn was measured on the completely expanded flag leaf using a portable photosynthesis instrument (LI-6400, LI-COR, Lincoln, NE, USA). Measurements were made on a sunny day from 10:00 a.m. to 12:00 p.m. The sampling conditions were light intensity 1200 μmol m⁻² s⁻¹, air humidity 70%, CO₂ 375 μmol mol⁻¹, and leaf temperature 28 °C.

To measure leaf chlorophyll content, 1 g of fresh leaf tissue was cut into small pieces, placed in a volumetric flask that contained 10 mL of 80% acetonitrile solution as described in Porra et al.⁸⁻⁵, and stored in the dark for 24 h. The absorbance of the extracted solution was measured at 663 and 645 nm using a UV spectrophotometer (Infinite M200, Tecan, Männedorf, Switzerland) to estimate chlorophyll a and b content (mg g⁻¹) using the equations described by Arnon.⁷⁸
where \( C(\text{Chl a}) \) and \( C(\text{Chl b}) \) are the content of Chla or Chb; \( D_{663} \) and \( D_{645} \) are the absorbance at 663 and 645 nm, respectively, using spectrophotometer (Model-1800, Tecan-infinite M200, Switzerland).

**Nitrogen metabolizing enzyme activities.** Five flag leaves were collected from each treatment during the grain-filling period, immediately frozen in liquid N and stored at \(-80^\circ C\) for estimation of the activities of the N-metabolizing enzymes such as Nitrate reductase (NR), Glutamine synthetase (GS), and Glutamate synthase was extracted and measured using a Glutamate Synthase (GOGAT).

NR was extracted and measured using a Nitrate Reductase (NR) assay Kit (BC0080, Solarbio, Beijing, China). Briefly, 0.1 g leaf samples were extracted in 1 ml extraction solution and the mixture was centrifuged at 4000 g for 10 min. The resulting supernatant was collected for further analysis. The absorbance at 520 nm was used for the calculation of NR activity. GS was extracted and measured using a Micro Glutamine Synthetase (GS) assay Kit (BC0915, Solarbio, Beijing, China). Briefly, 0.1 g leaf samples were thoroughly ground in liquid nitrogen and extracted with 1 mL extraction buffer. The mixture was centrifuged at 8000 g at 4 °C for 10 min. The supernatant after centrifuging was collected for activity measurement. The absorbance at 520 nm was used for the calculation of GS activity.

Glutamate synthase was extracted and measured using a Glutamate Synthase assay Kit (BC0070, Solarbio, Beijing, China). Briefly, 0.1 g leaf samples were extracted in 1 mL extraction buffer. The extraction mixture was centrifuged at 10,000 g at 4 °C for 10 min. The resulting supernatant was harvested and the absorbance at 340 nm was measured for the calculation of GOGAT activity.

**Rice yield and yield attributes.** Five central rows from each plot were selected at physiological maturity to measure rice growth, yield, and yield traits. Three hills were randomly selected at physiological maturity to measure plant height, panicle number, panicle length, spikelet number per panicle, grain filling percentage, and thousand-grain weight. The crop was harvested when almost all the heads showed a complete loss of green color. Grain yield (kg/ha) was measured from five central rows in each treatment and adjusted to 14% moisture content.

**Statistical analysis.** Analysis of variance (ANOVA) was performed with Statistics 8.1 software to examine variations in soil physical and biochemical properties, leaf physiological traits, rice grain yield, and yield components. Percentage data were arcsine transformed prior to analysis. Data from both seasons were used in the analysis in order to detect differences between seasons as well as fertilizer treatments. Treatment was considered to be a fixed effect, and season was considered to be a repeated measures factor and a fixed effect. The interaction between fertilizer treatment and season was also taken as a fixed effect, but the interaction of season and treatment with replication was taken as a random effect. Mean separation was performed using the least significant difference (LSD) method at \( p < 0.05 \). Linear regression analysis was performed to evaluate the relationship between grain yield and \( P_n \), N-metabolizing enzyme activities, pre-and post-anthesis DM, and N accumulation. Redundancy analysis (RDA) was performed using Canoco version 5 (Cambridge University Press, Cambridge, UK).

Received: 15 May 2020; Accepted: 15 April 2021
Published online: 11 May 2021

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Acknowledgements
This research was financially supported by the National Natural Science Foundation of China (Grant No. 31771712). We wish to thanks the Guangxi University, Agriculture Station staff for their cooperation in helping to conduct and manage this experiment.

Author contributions
A.I and L.J conceived the main idea of research, designed and performed the experiment and analyzed the data. A.I wrote the manuscript. L.H, S.U A.K, K.A, S.F and R.K reviewed drafts of the paper and provided suggestions. In addition, L.A, and S.W were assessed and data collection. All authors discussed the results and commented on the manuscript.
Competing interests
The authors declare no competing interests.

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