

Amendment with controlled release urea increases leaf morpho-physiological traits, grain yield and NUE in a double-cropping rice system in southern China

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Abstract

BACKGROUND: Understanding of mechanisms that underpin high-yielding cropping systems is essential for optimizing management practices. Currently, the contribution of plant traits such as leaf area, chlorophyll content and intercepted photosynthetically active radiation (PAR_i) to yield and nitrogen use efficiency (NUE) are not fully understood. In addition, the understanding of how canopy traits are affected by nitrogen (N) management practices is unclear. The present study aimed to determine the effect of amendment with controlled release urea (CR), common urea or no urea on NUE and plant eco-physiological characteristics in a 2-year field study in a double rice cropping system.

RESULTS: Regulation of N release through amendment with CR significantly increased grain yield, NUE and leaf morpho-physiological attributes. CR coupled with common urea (at comparable total N rates) increased leaf area index (LAI), relative chlorophyll content index (CCI) and PAR_i, leading to higher grain yield and NUE (increased 24.4% and 25.3% in early and late rice, respectively) compared to local farming practice. Structural equation model (SEM) analysis showed that differences in N application, between CR and common urea, directly accounted for differences observed in soil nutrient, PAR_i and NUE rather than yield components. Additionally, compared to traditional yield determinants, LAI and PAR_i (between booting and filling stage) are capable of predicting and explaining grain yield by 0.69 and 0.92 of R² in early and late rice, respectively.

CONCLUSION: Leaf morpho-physiological traits are important for developing N management practices to increase NUE and improve food security for paddy agriculture in southern China.

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Supporting information may be found in the online version of this article.

Keywords: leaf morpho-physiological traits; yield determinants; grain yield; soil property; controlled release urea

INTRODUCTION

Global food demand is increasing, with climate change, urbanization and growing populations presenting a challenge to production.^{1,2} Increasing crop yield to meet food demand at the same time as maintaining or improving nitrogen use efficiency (NUE) is a critical step towards sustainable agricultural production.^{3,4} Plant physical traits can impact yield through limiting potential for photosynthesis, and an increase in canopy, leaf size and chlorophyll content could directly enhance intercepted photosynthetically active radiation (PAR_i) in cereal crops.^{5,6} Optimizing agronomic practices through use of new varieties to improve canopy development for high-yielding agricultural production has been explored in a previous study.⁷ Moreover, much of the literature in this area has focused on the responses of grain yield to optimized nitrogen (N) management, particularly with separating urea applications into multiple topdressing over the reproductive

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period.^{8,9} Few studies have investigated the contributions of N type and application to soil properties, canopy traits and grain output in paddy fields concurrently. Tropical and subtropical areas of the globe account for approximately 25% of global rice production through irrigated double cropping, continuous rice systems. Therefore, improving the resilience of food production within these systems is of critical importance to meet the requirements of increasing populations.¹⁰ Thus, further assessment and analysis of crop canopy responses to N type in paddy soil are needed to support local policy decision and achieve efficient productivity.

Plant leaf development and senescence are associated with a permanently fluctuating nutrient availability in soil, which plays a critical role in N and carbohydrate redistribution to grain yield over the reproductive period.^{11,12} N addition increases ammonium and nitrate content in soil and subsequently N content in the leaf and stem. This leads to an increase in leaf area and enhances in the active area of photosynthesis for capturing light energy.^{1,13} Sunlit leaves in the canopy account for about 30% of the total leaf area, absorbing approximately 70% of solar radiation energy, and contributing approximately 53% of total canopy photosynthesis.¹⁴ Controlled release N fertilizer has advantages with respect to maintaining and increasing leaf greenness as a result of providing continuous N supplement, which indirectly increases carbon dioxide assimilation for the remobilization of non-structural carbohydrates to grain organs.^{5,15} Both controlled and field experiments suggest that supplementation with controlled release N fertilizer increases cereal crop morphology traits, gas exchange attributes, and soil inorganic N and organic C content over the reproductive period.^{16–18} Short-term straw returning fertilization effects on crop yields are likely to reduce because of the uncertainty in the quality and quantity of straw decomposition to nutrients over the changing growing season.^{19,20}

Conventional N urea application practices (such as multiple application and subsurface placement) enhance leaf development, N allocation to harvest organs and decrease the N losses to environment.²¹ However, lack of effective labor and appropriate machinery often limit the application of these practices in paddy soil. Recently, controlled release urea (CR) as a novel, effective and environmentally friendly fertilizer, has been applied to cereal crops such as rice, wheat and maize. Compared to the required multiple applications of conventional urea, CR saves labor and time because of the one-time basal application.²² A number of studies have demonstrated that CR increases NUE and leaf N remobilization, as well as reduces N losses to water and atmosphere, as a result of a lower ammonium concentration

in soil over the rice growing period.^{8,9,23} CR has potential to support the development of sustainable agriculture through balancing economic benefits and minimizing environmental cost. These aims underpin the goal of implementing China's zero growth plan which aims to eliminate excessive fertilizer use.

Reliable estimates of the combined contribution of soil properties, leaf characteristics, NUE and yield components to grain yield require methods that can consider a number of factors together. However, few studies have considered all the four classes of drivers together, and those focused on one or two classes showed that the predictive power of them to the variability of grain yield in rice was relatively low. To overcome this difficulty, a structural equation model (SEM) was adopted to test whether associations between multiple factors and grain yield exist. Additionally, the SEM is effectively able to identify the direct and indirect effect pathways of driving factors to yield components and grain yield. Specifically, the present study aimed (i) to investigate the impacts of fertilizer practices with N type on leaf chlorophyll content index (CCI), leaf area index (LAI) and PAR_i, NUE, grain yield, and yield components, and (ii) to identify the underlying mechanism of how soil properties, leaf characteristics, NUE and yield components contribute to grain yield in rice.

MATERIALS AND METHODS

Site description and experimental design

A 2-year field study in a double rice cropping system was conducted at Wangcheng (28°20'7.8"N, 112°48'3.6"E) in the middle of the Yangtze river basin of China during 2016–2017. The experimental site has a typical subtropical monsoon climate with soil type Hapli-stagnic anthrosols.⁵ The mean daily minimum and maximum air temperature at the study site were 14.6 °C and 21.8 °C. The average annual rainfall and daily radiation were 1695 mm and 12.5 MJ (see Supporting information, Fig. S1). The initial soil pH, organic matter, available N, available phosphorus and available potassium at the beginning of the experiment were 6.6, 36.33 g kg⁻¹, 118.65 mg kg⁻¹, 10.19 mg kg⁻¹ and 107.03 mg kg⁻¹, respectively.

Fertilizer treatments were designed to test the impact of nine N management regimes on crop productivity and agronomic traits in early rice and late rice. Treatments are outlined in Table 1 and were replicated in triplicate.²⁴ N was applied as basal fertilizer (70%) and the rest was top-dressing applied at tillering (30%). All the crops were local cultivars of *Oryza sativa* ('Fengyuanyou 272' and 'Shenyou 9586' for early rice and late rice, respectively) and native field management practices were employed, including

Table 1. Details of fertilizer practices for N, P₂O₅ and K₂O over the early rice and late rice season

Crop	Chemical fertilizer (kg ha ⁻¹)	Treatment								
		CK	CR1	CR2	CR2SR	N1	N2	N3	N2SR	NE
Early rice	N	0	150	150	150	120	150	180	150	144
	P ₂ O ₅	75	75	75	75	75	75	75	75	46
	K ₂ O	120	120	120	120	120	120	120	120	97
Late rice	N	0	150	150	150	120	150	180	150	161
	P ₂ O ₅	75	75	75	75	75	75	75	75	87
	K ₂ O	120	120	120	120	120	120	120	120	94

Note: CK, none N treatment; CR, controlled release urea; SR, returning straw; NE, recommended fertilization by nutrient expert system.

conventional tillage, weed and pest control, as described in Liu *et al.*²⁵ Calcium superphosphate (75 P₂O₅ kg ha⁻¹) and potassium chloride (120 K₂O kg ha⁻¹) were applied as basal fertilizer for each plot. All straw in the previous crop at harvest was incorporated with soil ploughing at a depth of 15 cm. Early rice was transplanted in late April and harvested in mid-July, whereas late rice was transplanted in late July and harvested in mid-October. Plots (4 m × 5 m) were designated treatments in a randomized complete block design.

Samples and analyses

LAI, CCI and PAR_i were measured at key growing stages: tillering, booting, filling and maturity for both early and late rice season. All fresh leaf material was separated from the rice plant after sampling and washing, and then the Canon scanner (MF113; Canon Inc., Tokyo, Japan) was used to obtain a JPG format picture for analyzing leaf area using ImageJ (National Institute of Mental Health, Bethesda, MD, USA).^{26,27}

LAI calculation was obtained by multiplying leaf area by the plant density (225 000 plants ha⁻¹) [i.e. LAI = leaf area (m² per plant) × 225 000 (plants ha⁻¹)/10 000 (m² ha⁻¹)]. The CCM-200 (Opti-Sciences Inc., Hudson, NH, USA) was adopted to measure CCI *in situ* from the first fully expanded functional leaf in each plant.²⁸

The estimation of PAR_i was determined by total incident solar radiation (*R*) and LAI using²⁹:

$$PAR_i = \Sigma 0.5R (1 - e^{-kLAI})$$

where *R* is the incoming total solar radiation (MJ m⁻² day⁻¹); *k* is the light extinction coefficient, which equals 0.60 for rice and LAI is the leaf area index (m² leaf m⁻² ground).⁵

NUE was the ratio of grain yield response between with and without fertilization to the amount of applied N.³⁰

$$NUE \text{ (kg kg N}^{-1}\text{)} = \frac{\text{Yield}_{(\text{fertilized})} - \text{Yield}_{(\text{control})}}{\text{Quantity of N applied.}}$$

Soil samples at a depth of 0–20 cm were collected using a 2 cm diameter stainless steel sample auger with an S-shape at harvest in rice field and were mixed to determine soil properties after removing stone and visible roots manually. The soils were air-dried and then sieved with a 0.25 mm to measure soil available N (AN), organic matter (OM) and total soil N content (STN). Soil AN were determined with the Alkali N proliferation method and OM content was quantified using the K₂C₂O₇-H₂SO₄ oxidation method. STN was measured using the Kjeldahl digestion-distillation method.⁵

Statistical analysis

To examine how different N type (i.e. urea as N1, N2, N3 and NE; CR as CR1 and CR2; and straw as CR2SR and N2SR) affected rice production increased by yield components [effective panicles (EEP), 1000-grain weight and grain yield (KGW)], canopy eco-physiological traits (LAI, CCI and PAR_i), soil properties (AN, OM and STN) and NUE, the SEM was adopted to quantify the relative importance of potential direct and indirect pathways,³¹ which was constructed using the R package 'lavaan'. Path coefficients were standardized by the variance ratio of the two variables forming the path. Significant paths were retained with solid line in SEMs, whereas non-significant paths marked with dashed lines.

The following statistical indices were used to assess whether the model suitably fitted our dataset: (i) the ratio of chi-squares to the freedom degree (smaller than 2 and *P* > 0.05 is considered better); (ii) the root mean square error of approximation and non-normed fit index (RMSEA < 0.06, NNFI > 0.95), which favor higher parsimony; (iii) standardized root mean square residual (SRMR < 0.05), which measures deviations of residuals from a hypothesized covariance model; and (iv) indices that correct for sample size (CFI) (larger than 0.95 is considered better). These diagnostics are a subset of that proposed by Hooper *et al.*³² for evaluating the SEM model.

To identify the relative importance of traditional yield components (KGW and EEP) and photosynthetic factors (LAI, CCI and PAR_i) to grain yield, the stepwise regression model was adopted using 'leaps' package in R (<https://cran.r-project.org>). To test whether there was a difference between N fertilizer treatments, we used one-way analysis of variance and calculated least significant difference (LSD) values (*P* < 0.05). The analyses were conducted in SPSS, version 20.0 (IBM Corp., Armonk, NY, USA).

RESULTS

Grain yield

Rice yield significantly increased with fertilization management compared with that in CK (*P* < 0.01) (Fig. 1; see also Supporting information, Table S1). Average grain yield varied from 2812 to 5969 and 6092 to 8022 kg ha⁻¹ for early rice and late rice, respectively. Early rice yield was significantly increased by N addition with the highest (6083 kg ha⁻¹ in 2016) under CR1 compared to CK and NE treatments (Fig. 1a). The highest yield for late rice was obtained under CR2 in 2017 (Fig. 1b).

N use efficiency

Crop NUE varied significantly among the fertilizer treatments, and an average value of NUE under CR2 in both 2016 and 2017 is higher than that under other treatments for the early rice, whereas, for the late rice, a higher NUE in 2016 and 2017 was found under CR1 (*P* < 0.01) (Fig. 2; see also Supporting information, Table S1). The average NUE under CR2 (38.2%) for early rice in both 2 year was higher than that under local farm practice (N2, 30.7%) and recommended fertilization (NE, 31.5%) (Fig. 2a). Similarly, partial or full substitution of common urea by CR was superior with respect to increasing NUE for late rice in 2017 (Fig. 2b) compared to other treatments, and NUE under CR2 (62.1%) was slightly higher than that under NE treatment (61.0%) in 2016 (Fig. 2a).

Relative leaf chlorophyll content

Measurements of CCI under different N regimes for early rice and late rice over the growing period are shown in Table 2. CR plus urea treatments can significantly increase the CCI values in early rice and late rice over the growing period (*P* < 0.05). The peaks of CCI with year tend to be found at the booting (B) stage in the early rice but at the filling (F) stage in the late rice. Compared with local farming practice (N2), the average CCI values under partial and full substitution of CR increased by 27.3% (B stage) and 13.6% (F stage) for early rice and late rice in 2017, respectively. Moreover, the correlation results revealed that the maximum contribution of CCI to grain yield were B and F stages for early (*R*² = 0.70) and late rice (*R*² = 0.64), respectively (see Supporting information, Fig. S3).

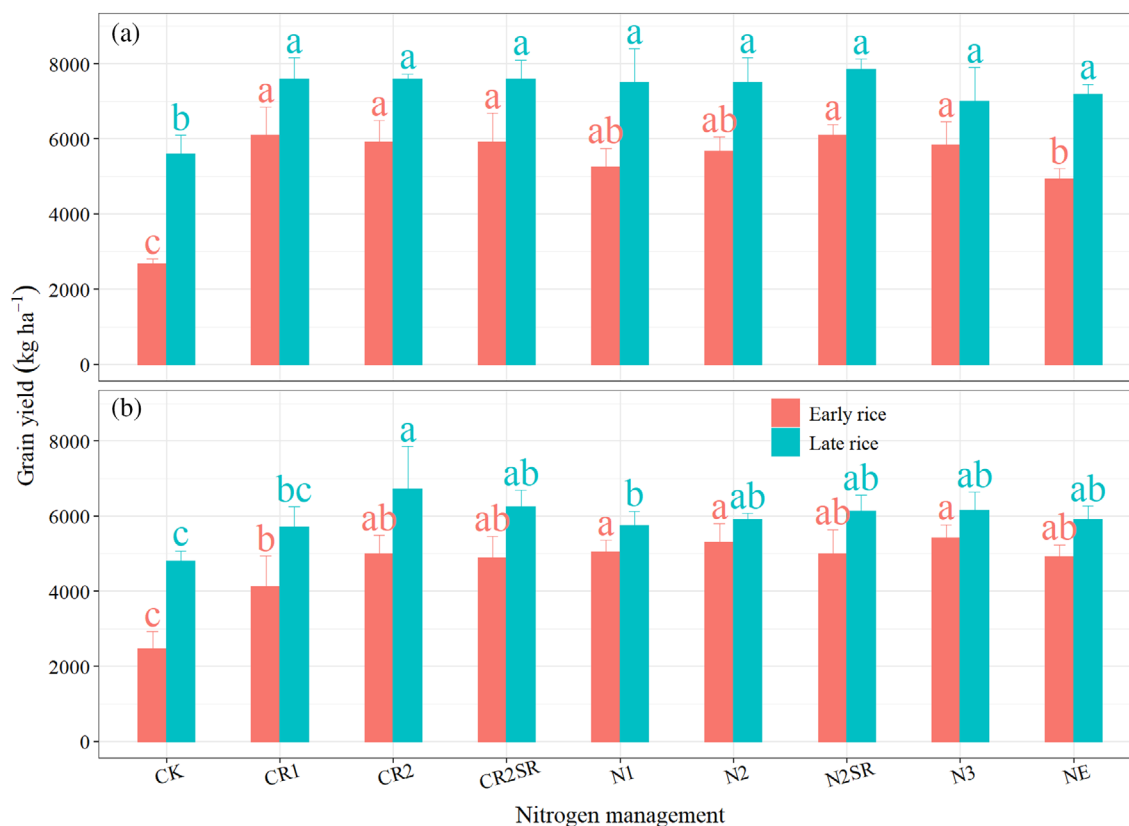


Figure 1. Variations of grain yield for early rice and late rice under different N management from 2016 (a) to 2017 (b). Different lowercase letters represent significant differences ($P < 0.05$, LSD) under different N treatments in each rice season.

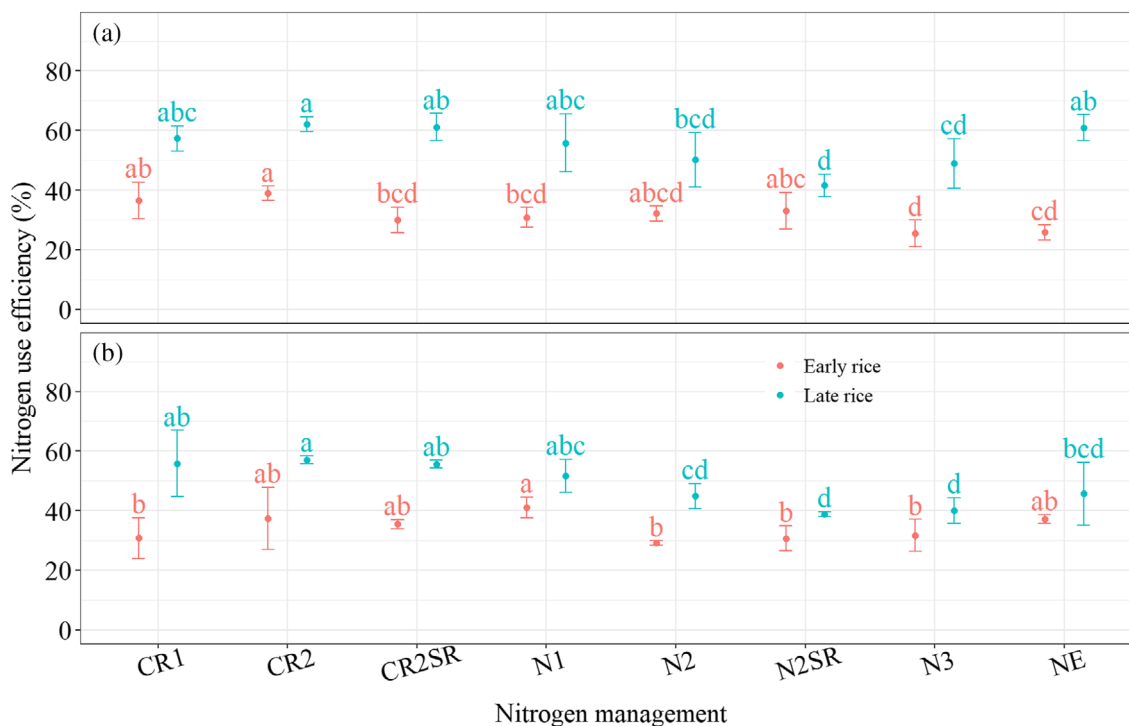


Figure 2. Variations of N use efficiency for early rice and late rice under different N management from 2016 (a) to 2017 (b). Different lowercase letters represent significant differences ($P < 0.05$, LSD) under different N treatments in each rice season.

Table 2. Changes of CCI values of early and late rice season under different N managements in 2016 and 2017

Year	Treatments	Early rice					Late rice				
		T	B	F	M	M	T	B	F	M	
2016	CK	17.8 ± 3.8 c	25.2 ± 4.0 e	36.6 ± 4.3 d	15.9 ± 5.2 c	24.6 ± 5.7 d	34.3 ± 4.5 c	21.4 ± 3.5 d	9.5 ± 2.1 c		
	CR1	35.0 ± 4.7 a	54.0 ± 5.5 a	58.5 ± 7.7 a	37.5 ± 5.5 a	32.5 ± 3.3 bc	55.8 ± 9.0 a	38.9 ± 5.4 a	32.3 ± 5.9 a		
	CR2	32.7 ± 3.3 ab	48.0 ± 8.1 abc	56.3 ± 10.2 ab	25.6 ± 9.6 b	36.4 ± 5.1 b	53.3 ± 4.6 ab	39.9 ± 5.6 a	31.8 ± 7.2 a		
	CR2SR	34.4 ± 3.4 a	49.0 ± 8.1 ab	59.9 ± 11.3 a	35.6 ± 8.5 a	42.1 ± 4.2 a	56.3 ± 7.2 a	38.4 ± 3.5 a	34.3 ± 5.3 a		
	N1	33.5 ± 5.3 ab	42.5 ± 9.3 bcd	50.0 ± 7.9 bc	22.9 ± 7.2 bc	32.1 ± 3.3 c	54.0 ± 7.3 ab	27.8 ± 3.4 c	18.4 ± 3.3 b		
	N2	29.0 ± 5.3 b	38.7 ± 7.3 d	47.1 ± 8.0 c	22.6 ± 5.0 bc	32.9 ± 5.0 bc	50.7 ± 6.1 ab	29.5 ± 3.1 bc	19.5 ± 3.5 b		
2017	N2SR	28.3 ± 8.2 b	40.5 ± 5.8 cd	45.8 ± 7.7 c	25.9 ± 6.2 b	30.7 ± 4.8 c	49.1 ± 5.0 b	32.8 ± 3.5 b	22.8 ± 4.8 b		
	N3	31.6 ± 5.8 ab	44.7 ± 6.6 bcd	49.2 ± 8.9 bc	19.8 ± 3.8 bc	33.9 ± 4.8 bc	51.5 ± 6.4 ab	29.7 ± 2.9 bc	20.1 ± 3.5 b		
	NE	33.4 ± 5.3 ab	39.0 ± 8.6 d	45.2 ± 9.8 c	18.1 ± 5.0 bc	34.7 ± 4.3 bc	47.9 ± 5.5 b	31.3 ± 4.9 bc	22.4 ± 5.0 b		
	CK	9.8 ± 2.0 d	32.6 ± 4.1 b	22.3 ± 4.3 b	3.9 ± 0.9 d	29.1 ± 3.4 c	45.8 ± 4.3 e	42.5 ± 3.5 e	19.3 ± 0.7 c		
	CR1	14.3 ± 4.0 cd	44.8 ± 7.5 a	38.6 ± 6.8 a	21.7 ± 7.5 b	37.2 ± 3.3 a	62.2 ± 5.0 ab	65.4 ± 7.0 a	41.1 ± 4.0 a		
	CR2	15.8 ± 4.2 bcd	42.3 ± 5.5 a	37.5 ± 6.9 a	29.9 ± 6.0 a	37.0 ± 4.6 a	59.6 ± 9.9 abc	59.8 ± 3.7 ab	37.7 ± 6.8 a		
	CR2SR	14.5 ± 4.0 bcd	41.5 ± 5.9 a	37.9 ± 6.1 a	22.3 ± 6.4 b	34.2 ± 3.7 ab	62.6 ± 4.0 a	64.1 ± 7.7 a	41.0 ± 9.0 a		
	N1	14.6 ± 5.5 bcd	47.7 ± 4.4 a	36.4 ± 6.3 a	7.5 ± 1.6 cd	35.4 ± 4.0 a	49.3 ± 3.3 de	47.1 ± 3.2 de	27.0 ± 5.3 b		
	N2	18.2 ± 6.0 abc	47.4 ± 7.1 a	37.2 ± 5.8 a	9.1 ± 3.9 c	36.8 ± 4.3 a	53.4 ± 8.6 cde	51.8 ± 3.7 c	29.5 ± 2.6 b		
	N2SR	18.0 ± 4.0 ab	41.2 ± 7.0 a	36.2 ± 5.4 a	5.3 ± 1.5 cd	31.8 ± 3.8 bc	50.2 ± 4.9 de	53.4 ± 5.1 c	29.7 ± 6.0 b		
2017	N3	21.4 ± 4.1 a	44.7 ± 7.7 a	36.2 ± 4.6 a	7.3 ± 2.5 cd	35.9 ± 2.5 a	54.8 ± 5.8 abcd	57.0 ± 5.5 bc	30.3 ± 5.6 b		
	NE	17.8 ± 3.9 abc	45.5 ± 4.8 a	33.7 ± 4.8 a	7.6 ± 2.1 cd	36.1 ± 3.9 a	53.9 ± 5.5 bcde	53.4 ± 4.5 c	30.7 ± 3.9 b		

Note: The same lowercase letters are not significantly different between treatments at the 5% level of the LSD test. Letters are shown only when there were significant differences among the treatments. T, B, F and M represent tillering, booting, filling and mature in rice growing stages, respectively.

Table 3. Dynamics of LAI of early and late rice season under different N managements in 2016 and 2017

Year	Treatments	Early rice				Late rice			
		T	B	F	M	T	B	F	M
2016	CK	0.6 ± 0.2 b	1.5 ± 0.1 e	1.3 ± 0.1 d	0.8 ± 0.4 b	1.7 ± 0.5 b	3.6 ± 1.2 c	1.5 ± 0.3 d	0.8 ± 0.1 c
	CR1	1.0 ± 0.3 ab	3.2 ± 0.5 bcd	3.4 ± 0.5 abc	1.2 ± 0.2 ab	3.7 ± 1.1 a	5.4 ± 1.3 abc	5.7 ± 0.6 a	2.5 ± 0.4 a
	CR2	1.1 ± 0.2 a	3.6 ± 0.8 abc	3.5 ± 0.7 ab	1.0 ± 0.1 ab	3.5 ± 0.7 a	4.2 ± 0.3 bc	5.4 ± 0.6 ab	2.1 ± 0.7 a
	CR2SR	0.7 ± 0.1 ab	4.1 ± 0.8 a	4.0 ± 0.1 a	1.1 ± 0.3 ab	3.7 ± 1.1 a	5.9 ± 2.9 ab	5.9 ± 1.3 a	2.3 ± 0.3 a
	N1	0.9 ± 0.4 ab	2.8 ± 0.1 d	2.9 ± 0.3 bc	0.8 ± 0.2 b	2.9 ± 0.4 ab	6.8 ± 0.3 a	5.1 ± 0.8 ab	1.4 ± 0.4 b
	N2	0.8 ± 0.0 ab	2.9 ± 0.3 cd	2.5 ± 1.1 c	1.4 ± 0.2 a	3.4 ± 0.5 a	3.4 ± 0.1 c	4.1 ± 1.0 bc	1.5 ± 0.3 b
	N2SR	0.9 ± 0.3 ab	3.8 ± 0.3 ab	2.9 ± 0.6 bc	1.0 ± 0.4 ab	3.0 ± 0.7 a	4.9 ± 1.8 abc	4.2 ± 1.3 bc	1.3 ± 0.4 bc
	N3	0.9 ± 0.2 ab	4.0 ± 0.2 a	3.3 ± 0.7 abc	0.9 ± 0.1 b	3.5 ± 0.6 a	5.2 ± 1.0 abc	3.1 ± 0.3 c	1.2 ± 0.4 bc
	NE	0.9 ± 0.4 ab	3.1 ± 0.2 bcd	2.9 ± 0.2 bc	0.9 ± 0.0 b	3.7 ± 0.3 a	4.6 ± 1.0 abc	4.2 ± 0.1 bc	1.4 ± 0.4 b
2017	CK	0.4 ± 0.1 b	1.6 ± 0.5 c	1.0 ± 0.2 b	0.4 ± 0.1 b	1.2 ± 0.2 c	2.9 ± 0.4 c	1.5 ± 0.1 b	1.1 ± 0.0 d
	CR1	0.6 ± 0.1 a	2.4 ± 0.3 bc	2.9 ± 1.1 a	1.6 ± 0.3 a	1.6 ± 0.1 bc	7.3 ± 0.6 a	3.1 ± 0.2 a	1.3 ± 0.1 cd
	CR2	0.7 ± 0.3 a	2.5 ± 0.4 bc	2.7 ± 0.2 a	1.3 ± 0.7 a	1.7 ± 0.3 bc	5.4 ± 1.3 b	4.2 ± 1.4 a	2.3 ± 0.4 a
	CR2SR	0.5 ± 0.0 ab	2.8 ± 0.5 bc	2.9 ± 0.3 a	1.4 ± 0.5 a	1.9 ± 0.7 ab	6.1 ± 0.5 ab	4.1 ± 1.6 a	1.5 ± 0.1 bc
	N1	0.5 ± 0.1 ab	3.8 ± 1.2 ab	3.3 ± 1.1 a	0.5 ± 0.4 b	1.8 ± 0.2 ab	5.3 ± 1.0 b	2.8 ± 1.0 ab	1.2 ± 0.0 cd
	N2	0.6 ± 0.1 a	3.6 ± 1.1 ab	3.7 ± 1.1 a	0.5 ± 0.1 b	1.6 ± 0.3 bc	5.3 ± 0.6 b	3.2 ± 0.1 a	1.9 ± 0.6 ab
	N2SR	0.6 ± 0.1 a	3.6 ± 0.4 ab	2.8 ± 0.5 a	0.4 ± 0.1 b	1.5 ± 0.2 bc	6.2 ± 1.2 ab	3.7 ± 0.7 a	1.7 ± 0.2 bc
	N3	0.7 ± 0.1 a	4.5 ± 1.6 a	3.5 ± 1.4 a	0.7 ± 0.3 b	2.2 ± 0.3 a	5.2 ± 1.4 b	3.3 ± 0.9 a	2.0 ± 0.1 ab
	NE	0.5 ± 0.1 ab	3.1 ± 0.7 ab	3.5 ± 0.9 a	0.6 ± 0.1 b	1.6 ± 0.3 bc	4.7 ± 0.5 b	3.4 ± 0.7 a	2.3 ± 0.6 a

Note: The same lowercase letters are not significantly different between treatments at the 5% level of the LSD test. Letters are shown only when there were significant differences among the treatments. T, B, F and M represent tillering, booting, filling and mature in rice growing stages, respectively.

Table 4. Intercepted photosynthetically active radiation (PAR) in the double rice-cropping system during 2016–2017 as affected by different N management

Year	Treatments	Early rice						Late rice					
		T	B	F	M	T	B	F	M	T	B	F	M
2016	CK	62.2 ± 3.6 e	137.5 ± 5.9 e	196.0 ± 13.2 e	232.7 ± 29.8 d	124.1 ± 26.6 b	246.2 ± 42.5 b	383.6 ± 56.2 b	445.4 ± 61.2 b	124.1 ± 26.6 b	246.2 ± 42.5 b	383.6 ± 56.2 b	445.4 ± 61.2 b
	CR1	101.0 ± 5.5 bcd	222.7 ± 13.2 bcd	323.7 ± 20.9 bcd	406.3 ± 31.9 abc	167.2 ± 24.7 a	320.2 ± 30.5 a	493.4 ± 31.7 a	585.0 ± 30.4 a	167.2 ± 24.7 a	320.2 ± 30.5 a	493.4 ± 31.7 a	585.0 ± 30.4 a
	CR2	111.9 ± 13.8 ab	241.3 ± 24.3 ab	348.5 ± 32.0 abc	436.9 ± 38.4 ab	171.8 ± 16.5 a	324.4 ± 19.8 a	496.0 ± 22.0 a	586.0 ± 23.2 a	171.8 ± 16.5 a	324.4 ± 19.8 a	496.0 ± 22.0 a	586.0 ± 23.2 a
	CR2SR	114.9 ± 9.0 a	248.3 ± 14.3 a	358.2 ± 17.7 ab	447.5 ± 19.4 a	177.7 ± 35.1 a	331.2 ± 46.9 a	504.1 ± 52.9 a	594.9 ± 53.2 a	177.7 ± 35.1 a	331.2 ± 46.9 a	504.1 ± 52.9 a	594.9 ± 53.2 a
	N1	97.5 ± 6.4 cd	215.9 ± 10.2 cd	314.2 ± 12.8 cd	393.9 ± 14.6 bc	174.4 ± 5.3 a	331.4 ± 5.5 a	503.9 ± 5.9 a	588.9 ± 7.9 a	174.4 ± 5.3 a	331.4 ± 5.5 a	503.9 ± 5.9 a	588.9 ± 7.9 a
2017	N2	88.9 ± 12.0 d	198.0 ± 25.5 d	289.0 ± 37.0 d	363.3 ± 48.7 c	167.6 ± 7.9 a	313.3 ± 10.2 a	478.0 ± 13.7 a	563.1 ± 16.6 a	167.6 ± 7.9 a	313.3 ± 10.2 a	478.0 ± 13.7 a	563.1 ± 16.6 a
	N2SR	107.9 ± 3.4 abc	233.7 ± 9.4 abc	335.6 ± 16.7 abc	412.8 ± 30.7 abc	169.9 ± 5.2 a	321.3 ± 3.2 a	489.9 ± 4.8 a	575.1 ± 6.1 a	169.9 ± 5.2 a	321.3 ± 3.2 a	489.9 ± 4.8 a	575.1 ± 6.1 a
	N3	117.3 ± 2.8 a	250.5 ± 6.0 a	359.8 ± 10.2 a	448.2 ± 18.0 a	180.2 ± 14.9 a	331.9 ± 18.8 a	499.0 ± 20.0 a	578.1 ± 16.1 a	180.2 ± 14.9 a	331.9 ± 18.8 a	499.0 ± 20.0 a	578.1 ± 16.1 a
	NE	99.9 ± 0.6 bcd	220.5 ± 1.2 bcd	320.3 ± 1.9 cd	401.4 ± 3.1 abc	176.4 ± 10.1 a	327.2 ± 15.3 a	494.3 ± 18.4 a	576.2 ± 16.8 a	176.4 ± 10.1 a	327.2 ± 15.3 a	494.3 ± 18.4 a	576.2 ± 16.8 a
	CK	64.3 ± 10.2 d	153.3 ± 22.4 d	199.7 ± 29.1 c	289.4 ± 54.6 b	105.4 ± 14.0 b	249.1 ± 24.8 b	305.6 ± 29.4 b	427.3 ± 49.1 b	105.4 ± 14.0 b	249.1 ± 24.8 b	305.6 ± 29.4 b	427.3 ± 49.1 b
	CR1	90.7 ± 18.7 c	216.8 ± 38.4 c	283.7 ± 46.6 b	434.1 ± 58.6 a	147.0 ± 16.1 a	338.8 ± 29.5 a	411.5 ± 33.4 a	552.6 ± 27.4 a	147.0 ± 16.1 a	338.8 ± 29.5 a	411.5 ± 33.4 a	552.6 ± 27.4 a
	CR2	91.6 ± 19.6 c	218.1 ± 34 c	284.5 ± 39.2 b	424.5 ± 53.3 a	139.8 ± 10.9 a	327.6 ± 24.8 a	399.9 ± 29.0 a	564.9 ± 38.9 a	139.8 ± 10.9 a	327.6 ± 24.8 a	399.9 ± 29.0 a	564.9 ± 38.9 a
	CR2SR	95.5 ± 3.3 bc	228.5 ± 9.2 bc	298.4 ± 14.1 b	450.8 ± 45.0 a	155.5 ± 6.1 a	352.0 ± 1.5 a	426.3 ± 0.8 a	578.6 ± 20.4 a	155.5 ± 6.1 a	352.0 ± 1.5 a	426.3 ± 0.8 a	578.6 ± 20.4 a
	N1	113.1 ± 18.5 abc	258.7 ± 35.6 abc	331.1 ± 44.0 ab	458.0 ± 64.8 a	143.0 ± 12.8 a	328.0 ± 24.0 a	398.1 ± 28.3 a	532.8 ± 50.1 a	143.0 ± 12.8 a	328.0 ± 24.0 a	398.1 ± 28.3 a	532.8 ± 50.1 a
	N2	117.9 ± 20.5 ab	268.0 ± 39.5 ab	343.6 ± 48.8 ab	492.9 ± 72.8 a	150.6 ± 17.6 a	340.1 ± 21.9 a	412.5 ± 23.7 a	569.6 ± 45.1 a	150.6 ± 17.6 a	340.1 ± 21.9 a	412.5 ± 23.7 a	569.6 ± 45.1 a
N2SR	113.7 ± 5.0 abc	259.2 ± 7.3 abc	331.8 ± 8.1 ab	462.7 ± 16.2 a	142.5 ± 4.7 a	334.1 ± 11.2 a	407.0 ± 13.5 a	554.4 ± 17.9 a	142.5 ± 4.7 a	334.1 ± 11.2 a	407.0 ± 13.5 a	554.4 ± 17.9 a	
N3	127.5 ± 12.6 a	284.8 ± 23.4 a	363.4 ± 29.0 a	516.0 ± 47.6 a	152.4 ± 15.6 a	341.9 ± 23.5 a	414.1 ± 25.6 a	567.4 ± 20.3 a	152.4 ± 15.6 a	341.9 ± 23.5 a	414.1 ± 25.6 a	567.4 ± 20.3 a	
NE	110.0 ± 14.1 abc	254.2 ± 29.4 abc	327.2 ± 37.4 ab	471.4 ± 62.1 a	135.1 ± 9.1 a	318.5 ± 15.6 a	390.0 ± 17.5 a	564.0 ± 22.5 a	135.1 ± 9.1 a	318.5 ± 15.6 a	390.0 ± 17.5 a	564.0 ± 22.5 a	

Note: The same lowercase letters are not significantly different between treatments at the 5% level of the LSD test. The alphabets are shown in table only when there were significant differences among the treatments. T, B, F and M represent tillering, booting, filling and mature in rice growing stages, respectively.

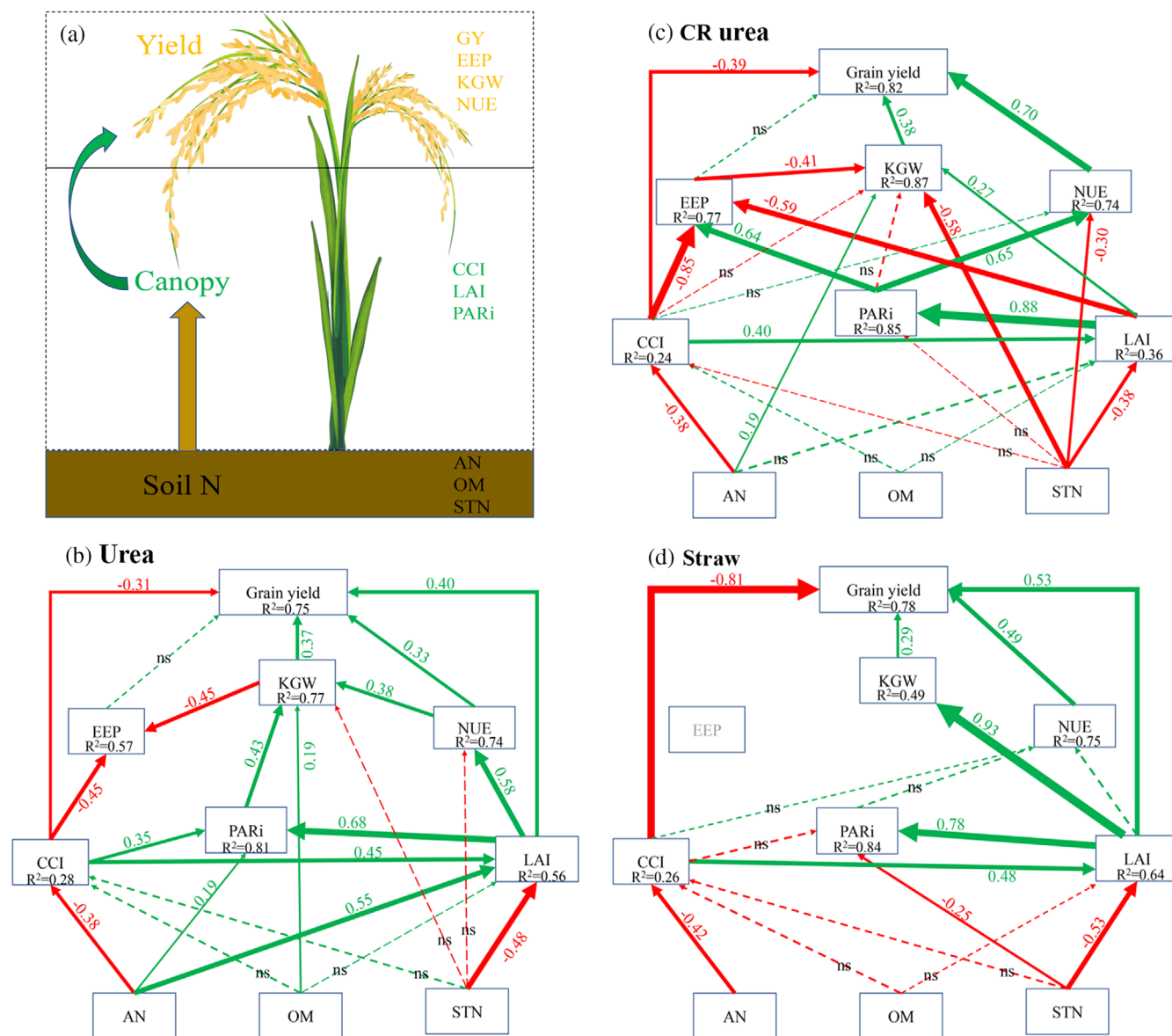


Figure 3. Structural equation models (a) show the causal effects of soil property [available nitrogen (AN), organic matter (OM) and total nitrogen content (STN)], canopy characteristics [leaf area index (LAI), relative chlorophyll content (CCI), intercepted photosynthesis active radiation (PAR_i), N use efficiency (NUE), yield components (effective panicles per plant (EEP), 1000-grain weight (KGW) and grain yield under urea (b), controlled release urea (c) and straw addition (d). Green and red solid line denote significant paths for positive and negative relationship, respectively, while dashed line with green and red denote non-significant (ns) paths that combined with other paths to produce significant total effects. Path values are standardized coefficients. R² value indicates the explained variance proportion of each variable.

LAI

The average LAI for early rice and late rice is shown in Table 3. N addition increased LAI at each growing stage compared to the CK treatment (Table 3; see also Supporting information, Table S1) ($P < 0.05$). Compared to local farming practice (N2), the leaf area per unit in both early and late rice significantly increased under partial or full substitute by CR over the reproductive period (especially at the F and M stages). The higher value of average LAI across all straw returning treatments was found at the B stage in both early and late rice. The maximum LAI occurred under N3 (4.25 m² m⁻²) and CR1 (6.35 m² m⁻²) for early rice and late rice, respectively. In addition, the correlation results showed that the highest R² values for early and late rice were observed at the filling stage, which indicated that enhancing LAI at the

filling stage could be helpful to increase grain yield at harvest (see Supporting information, Fig. S4).

PAR_i

Photosynthetically active radiation intercepted by crop canopy for early rice and late rice over the vegetative and reproductive stage significantly increased with N addition (Table 4; see also Supporting information, Table S1) ($P < 0.05$). Compared with other treatments, higher N with 180 kg N ha⁻¹ (N3) over the early rice growing period was able to obtain the highest PAR_i for 2016 and 2017 with 448 and 516 MJ m⁻², respectively. Partial and full substitute of CR in early rice significantly reduced PAR_i compared with comparable N rates of urea over the vegetative period in 2017. The correlation results revealed that the maximum

Table 5. Structural equation model fit statistics for urea, CR and straw addition

Index	Nitrogen management		
	Urea	CR urea	Straw addition
χ^2	14.8	10.2	5.7
d.f.	19	18	15
$\chi^2/\text{d.f.} < 2$	0.78	0.57	0.38
P value > 0.05	0.74	0.93	0.99
RMSEA < 0.06	0	0	0
SRMR < 0.05	0.045	0.032	0.033
CFI > 0.95	1.0	1.0	1.0
NNFI > 0.95	1.04	1.10	1.15

Abbreviations: CFI, bentler comparative fit index; NNFI, non-normed fit index; RMSEA, root mean square error of approximation; SRMR, standardized root mean square residual.

Table 6. Stepwise regression model for the relationship between grain yield and agronomic traits (* $P < 0.05$, ** $P < 0.01$)

Crop	Agronomic traits	Multiple linear regression model	Model R^2	F-value	P-value
Early rice	Traditional	GY = 3031 + 6.1 EPP**	0.65	25.64	< 0.01
	Photosynthetic	GY = 5448 + 120 PAR _T ** - 53.9 PAR _B **	0.69	14.44	< 0.01
Late rice	Traditional	GY = -8380 + 11.9 EPP** + 487.2 KGW**	0.81	28.5	< 0.01
	Photosynthetic	GY = 6815 - 17.8 PAR _T * + 10.7 PAR _B ** + 236.5 LAI _F *	0.92	49.1	< 0.01

Note: GY (kg ha⁻¹), grain yield; KGW (g), kernel grain weight; EEP (number per plant), effective panicles per plant; PAR_i (MJ m⁻²), intercepted photosynthetic active radiation; LAI (m² m⁻²), leaf area index; T, B and F represent tillering, booting and filling in rice growing stages.

contribution of PAR_i to grain yield (see Supporting information, Fig. S5) was different from the contribution of CCI to grain yield (see Supporting information, Fig. S3) and the highest R^2 values between early and late rice were obtained at F and B stages for early ($R^2 = 0.81$) and late rice ($R^2 = 0.61$), respectively (see Supporting information, Fig. S5).

Overall evaluation of N type on crop productivity

The SEM results demonstrated that 82% and 75% of the variation in grain yield under CR and common urea could be accounted for by the combination of NUE, KGW, CCI, PAR_i, AN and STN, respectively (Fig. 3b,c and Table 5). The combinations of NUE, KGW, LAI, CCI, AN and STN together contributed to 78% of the variation in grain yield under the incorporated straw treatment (Fig. 3d). The increase in AN content mediated grain yield responses mainly through the changes in LAI, CCI, PAR_i, KGW and NUE under common urea (Fig. 3b), and LAI, CCI, EEP, PAR_i, KGW under CR (Fig. 3c) and CCI, LAI and KGW under incorporated straw (Fig. 3d), respectively. Compared to straw returning treatment, the complicated pathways under CR and common urea were observed. N addition with CR strongly increased the correlation coefficient between NUE and grain yield, as well as that between PAR_i and NUE compared to common urea (Fig. 3c). Moreover, the LAI and PAR_i over the vegetative and reproductive period can explain the variations of grain yield for early and late rice by the average values of 0.69 and 0.92, respectively (Table 6).

Additionally, the stepwise correlation result agreed well with that in the SEM analysis. The traditional explanation ratio of yield components on grain yield in early and late rice (average $R^2 = 0.73$, $P < 0.01$) was smaller than that with leaf morpho-physiological attributes over the key growing period (average

$R^2 = 0.81$, $P < 0.01$) (Table 6). High PAR_i at tillering stage and low PAR_i at booting stage in early rice strengthened the explanation of grain yield response to increased common urea and CR by regulating KGW and NUE (Fig. 3c and Table 6). By contrast, in late rice, decreased PAR_i at tillering stage and increased PAR_i at booting stage could result in the positive effects on grain yield (Table 6). The above observations strongly indicate that the increase in response to N addition could be primarily attributed to leaf morpho-physiological traits at key stage rather the changes of yield components, which could have potential in adjusting N management practices over the growing period and predicting grain yield at harvest.

DISCUSSION

Variations of grain yield and its controlling factors

Numerous studies have demonstrated that seasonal variability of fertilizer application and soil properties play a critical role in regulating leaf growth and development, yield components and grain yield.³³⁻³⁶ In addition, there is also increasing evidence that leaf morpho-physiological attributes are key drivers of grain component response in breeding progress and modelling crop growth and development.^{5,37} Changes of leaf morpho-physiological attributes can affect harvest organs by altering the quality and quantity of N translocation and redistribution between the leaf and stem. In the present study, the increase in NUE mediated grain yield responses mainly through available N in soil and leaf LAI, CCI and PAR_i under CR and common urea (Fig. 3b,c).

Irrespective of the widely accepted correlation of grain yield with yield components and soil properties, shifts in content of AN and STN in soil can stimulate leaf CCI, PAR_i and NUE in plant

and thus drive the changes of grain yield and its components in CR treatment. This may be because CR increased N uptake and utilization in aboveground organs for increasing litter to arable soil as a result of sustained N supply, thus leading to an increase in soil nutrient.^{38,39} The findings are consistent with those of this study, which have found an increase in leaf photosynthesis traits and yield component as relative chlorophyll content and KGW under CR treatment (Table 3; see also Supporting information, Fig. S2). Increased KGW between early and late rice could be associated with an increase in soil available N but not NUE under the CR (Fig. 3; see also Supporting information, Figs S2 and S6). This is in agreement with previous study that N addition significantly increased N content in soil, strengthened the remobilization of rhizosphere nutrients to aboveground leaf and stem organs, thus increasing root C:N ratio, kernel C uptake and plant N uptake in cereal crop.⁴⁰ Furthermore, leaf morpho-physiological attributes shifts can enhance grain yield and KGW beyond that expected from the direct impacts of soil nutrient on NUE alone, further highlighting the critical role of CR in regulating the variation of grain yield in rice.

In addition to conventional evaluation method, the single objective (i.e. NUE, LAI and grain yield) responded to agronomic practices could have insignificant effects as shown in the present study. For example, NUE and LAI between treatments from both years of 2016 and 2017 was large skew because of the changing microclimate and weather conditions such as temperature, precipitation and radiation in the field (see Supporting information, Fig. S1). Thus, the SEM could have potential in assessing the relationship of grain yield and its components to soil, fertilizer practices and leaf traits because of the advantages of a quantitative evaluation method of interaction effects among multiple indicators.^{31,41} As for the CR, increased grain yield and KGW were regulated by changes in soil AN content but not the leaf PAR_i under the partial or full substitute by CR (Fig. 3c), also supporting the above argument. These results confirmed that the conventional single indicator evaluation method could not detect prevalingly indirect pathway impacts of soil AN on rice grain yield, further highlighting the privilege in assessing the interaction effect of complicated variables. In the future, more significant elements should be measured for supporting the conventional evaluation.

Implication of N addition on canopy traits and grain yield

Nitrogen addition plays an essential role in increasing grain yield through enhancing N availability in plant growth.¹⁵ Controlled release fertilizer provides sufficient N for plant growth and development, which contributes to enhance leaf photosynthesis and increase N uptake and remobilization over the vegetative and reproductive period.^{1,25,42} In the present study, compared with local farming practice (N2), partial or full substitution of common urea by CR increased leaf CCI by 18.6–26.2% and 20.3–26.6% for the reproductive stage of early rice and late rice, respectively (Fig. 2 and Tables 2 and 4). This is agreement with the slow-release N of CR slowing down leaf senescence processes as a result of N translocation in the aging leaf to the fresh leaf and stem after anthesis.^{43,44} Continuous N addition could increase leaf chlorophyll that is a biochemical photosynthetic component, which is able to activate electrons in photosynthesis for releasing chemical energy and enhancing light interception.⁵ In addition, leaf CCI, LAI and PAR_i between early and late rice season were positively correlated with grain yield from booting to grain filling stages (see Supporting information, Figs S3–S5). Accordingly, the stepwise

analysis revealed that PAR_i was a key driver of grain yield response between tillering and booting stage (Fig. 3., average $R^2 = 0.81$, $P < 0.01$). High CCI and LAI lead to increase accumulated aboveground biomass and leaf photoassimilate partitioning to root over the vegetative period, and thus indirectly enhancing the N redistribution and remobilization to economic organs over the reproductive period (Tables 2 and 3; see also Supporting information, Figs S3 and S4).^{1,45,46}

Negative N effects on grain yield and NUE under chemical fertilizer plus straw in paddy soil agree with the results of previous studies,⁴⁷ but differ from those in long-term straw management that reported increased grain yield and NUE.⁴⁸ This might be because differences in grain yield and NUE responses to the mixture of chemical N source to long-term straw may largely result in increasing soil organic N compared to short-term straw management as in the present study (Figs 1 and 2).⁴⁸ In addition, this differential result could be associated with N immobilization from incorporated straw and the lack of synchrony of N release with crop demand over the growing period.⁴⁹ Therefore, to require reliable effects of crop residues on N mobilization between soil and plant, more observations and model estimations need to be made in the future.

CONCLUSIONS

Improved N management through amendment with CR significantly increased the grain yield, NUE and leaf morpho-physiological attributes between early and late rice. The SEM analysis highlighted that enhanced soil nutrient, especially increases in AN and STN, as well as increased KGW, PAR_i and NUE, but not EEP, accounted for such differences between the CR and common urea. Compared to the traditional yield components, leaf morpho-physiological attributes as LAI and PAR_i between tillering and booting stage could be capable of predicting and explaining grain yield at harvest by 0.69 and 0.92 of R^2 for the early and late rice, respectively. These findings suggest that leaf morpho-physiological traits are important for developing N management practices to balance tradeoffs between high N use efficiency and food security for paddy agriculture in southern China.

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SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

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