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Precise delivery of nitrogen at tillering stage enhances grain yield and nitrogen use efficiency in double rice cropping systems of South China

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ABSTRACT

Nitrogen fertilizer management substantially affects the growth, productivity, and nitrogen use efficiency (NUE) of rice production systems. To assess the effects of precise application of nitrogen fertilizer at tillering stage as 'tillering-fertilizer' on grain yield and nitrogen use efficiency, field trials were conducted in the late season of 2019 and 2020, in addition to a radioisotope ^{15}N tracer experiment. The experiment was comprised of four N fertilizer treatments i.e., no fertilizer application as a control (T1), traditional surface broadcast fertilization (90 kg N/ha as basal fertilizer and 60 kg N/ha as tillering fertilizer, T2), deep placement of tillering fertilizer (surface broadcast at 90 kg N/ha as basal fertilizer and 45 kg N/ha as tillering fertilizer at 10 cm depth, T3), deep placement of tillering fertilizer (surface broadcast at 90 kg N/ha as basal fertilizer and 30 kg N/ha as tillering fertilizer at 10 cm depth, T4) and two rice cultivars i.e., *Wufengyou 615* and *Yuxiangyouzhan*. Results showed that grain yield remained 18.8% and 12.4% higher owing to increase in productive panicles and grain filling, 42.2% and 38.9% higher nitrogen agronomic efficiency, 4.6% and 10.4% higher nitrogen recovery efficiency, and 10.2% and 7.8% higher nitrogen physiological efficiency in T3 than T2 for *Wufengyou 615* and *Yuxiangyouzhan*, respectively. Moreover, nitrogen derived from fertilizer in T3 was increased by 8.0% and 4.4% compared to T2 for *Wufengyou 615* and *Yuxiangyouzhan*, respectively whereas the residual rate of T3 was increased by 31.5%. In addition, the T3 treatment substantially enhanced the leaf area index and leaf chlorophyll content that led to improved photosynthesis and the activities of enzymes related to nitrogen metabolism. Overall, 45 kg N/ha as tillering fertilizer at 10 cm depth and surface broadcast at 90 kg N/ha as basal fertilizer can improve grain yield as well as nitrogen use efficiency in late season rice production system of South China.

1. Introduction

Rice is one of the three major cereal crops in the world, providing food for more than half of the world's population (He et al., 2019; Liu et al., 2019). In China, rice is the main food for two-thirds of the population (Pan et al., 2016; Chen et al., 2018). The area under rice cultivation in China is quite extensive, however, yield per unit area is relatively lower than in other advanced countries, thus there is a huge development potential that is yet to be achieved (Wang et al., 2021a). Rice production is crucial to ensure food supply in China and the rest of the world (Ashraf et al., 2018, 2022a). Ensuring the supply of nitrogenous fertilizer is the prerequisite in achieving a high yield of rice (Hazra et al., 2021). China uses higher nitrogenous fertilizer than the rest of the world, however, the use efficiency of nitrogenous fertilizers is quite low. Thus, the excessive loss of nitrogenous fertilizers is a major problem in cropping systems, especially rice production systems (An et al., 2018) and needs efficient and precise delivery of fertilizers (Ashraf et al., 2022b). Excessive application of nitrogenous fertilizer can cause soil degradation and increase greenhouse gas emissions (Zhang et al., 2019; Li et al., 2021a). With the increase in nitrogen runoff, a large amount of nitrogen fertilizer could potentially pollute natural water resources and cause eutrophication. The degree of nitrate pollution in ground and surface water is generally related to the excessive use of nitrogenous fertilizers (Wang et al., 2021b), which is causing serious problems (Zhu

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et al., 2016).

Rice cultivation in China is still dominated by traditional transplanting methods, however, with the development of the economy and technology and the outflow of rural labor, traditional planting methods can no longer meet the needs of agricultural development. Therefore, the mechanical transplanting of rice is being popular among farmer community (Knight et al., 2011; Tao et al., 2016). Deep and/or precise delivery of nitrogen fertilizer has been proved to be an effective fertilizer management method (Wu et al., 2017) which shortens the distance between fertilizer and root system, promotes root growth, and increases the activity nitrogen metabolism-related enzymes in rice (Chen et al., 2020). Compared with manual fertilization, the deep fertilization can effectively increase the rice yield and nitrogen use efficiency (Rochette et al., 2013; Rose et al., 2016; Li et al., 2021b) and also reduce runoff, improve fertilizer retention time, and thus reduce water pollution owing to N leaching. Overall, the deep application of nitrogenous fertilizer can achieve higher yields with less application rates and cost of production. The combination of mechanical transplanting and deep placement of nitrogenous fertilizer can substantially improve the production efficiency of rice-based cropping systems.

Currently, the research focus in China is on the application of basal fertilizer by deep fertilization method and its subsequent effects on rice yield and nitrogen utilization efficiency (Pan et al., 2017; Chen et al., 2021). For example, Li et al. (2021b) reported that mechanical deep fertilization could improve the rice root system, enhance nutrient absorption, and ultimately increase rice yield, however, the effects of deep fertilizer at tillering stage on NUE and grain yield are not apparent. The tillering stage is a critical period for the growth and development of rice. During tillering stage, the growth and development of rice and the degree of tillering directly affect the number of effective panicles at maturity and ultimate rice yield. At tillering stage, it is important to ensure sufficient nitrogen availability for rice plants. Therefore, the present study was conducted to assess the efficiency of deep fertilization at tillering stage over traditional fertilizer application methods with the hypothesis that deep N fertilization could improve grain yield, NUE, and related physiological activities in rice.

Moreover, the ^{15}N tracer method is a new method to study the utilization of nitrogenous fertilizer. Using radioisotope ^{15}N to urea can distinguish nitrogen from label urea in the soil. By measuring the abundance of ^{15}N in rice plants and soil, the ability of rice to absorb nitrogen from fertilizer and the degree of loss of nitrogenous fertilizer can be evaluated. Therefore, the present study was conducted to track N and its utilization by its precise delivery in the soil and its effects on yield and nitrogen use efficiency in rice production system of South China.

2. Materials and methods

2.1. Experimental design

The experiments were carried out in the experimental area of South China Agricultural University (SCAU), (113.83 E, 23.15 N, 18 m above sea level), Guangzhou, China, in the late season (July-November) of 2019 and 2020. The soil of the experimental field was loamy, and comprised of 21.46 and 21.82 g kg^{-1} organic C, 1.76 and 1.66 g kg^{-1} total N, 1.22 and 1.36 g kg^{-1} total P, 22.81 and 21.47 g kg^{-1} total K, 77.32 and 78.03 mg kg^{-1} available P, 107.22 and 104.81 mg kg^{-1} available K, pH= 6.55 and 6.67 whereas the total precipitation in Guangzhou was 628.65 and 655.23 mm from July to November, in 2019 and 2020, respectively. The overall experimental area was 640 m^2 with a net plot size of 20 m^2 (6.3 m × 3.2 m). The seeds of two regionally famous and widely cultivated rice cultivars i.e., *Yuxiangyouzhan* (conventional rice) and *Wufengyou 615* (hybrid) were sown on July 13th and 15th. After 10 days of sowing, the fertilizer is manually applied to the developing seedlings at 10 cm soil depth by using furrowing tool developed by the SCAU. The rice seedlings were then transplanted using a rice transplanter (YaMeiKe, Changzhou YaMeiKe mechanical Co., Ltd., Changzhou, China) on August 3th and 5th in 2019 and 2020, respectively, at 25 cm × 15 cm with 3–5 seedlings per hill. The experimental treatments were comprised of four N application treatments and are described in Table 1.

After early-rice harvest, all rice straw was removed from the field and then paddy fields were puddled and kept under shallow water until transplanting. The fertilizers used in the experiment were urea, superphosphate and potassium chloride in both 2019 and 2020. In both years, the strategies for water management were the same as adopted by the local farmers. The field was irrigated about 3 cm water depth after transplanting until the end of the tillering stage, and the water was drained for about 7 days to control the non-productive tillers. Nearly 5–7 cm water was kept at subsequent stages till 10 days before harvest. After 15 days of transplating, 125 ml of 30% cyhalofop-butyl with 20 L of water per 667 m^2 was sprayed to control common weeds. Moreover, 100 g of 20% tricyclazole with 40 L of water per 667 m^2 was sprayed at tillering-stage to control rice blast whereas 120 g 50% carbendazim with 40 L of water per 667 m^2 was sprayed at panicle initiation stage to control sclerotial blight. In addition, 3 g of 70% imidacloprid with 60 L of water was sprayed at grain-filling stage to control rice brown planthopper.

In 2020, the ^{15}N urea labeling tracer test was carried out at the same time as the field experiment. The micro-plots of 1.0 m^2 were set up in the main experimental plots. The micro-plot was separated by 50 cm metal plates inserted 30 cm deep in the soil before the basal fertilizer application. Labeled ^{15}N urea (^{15}N abundance was 10%, provided by Shanghai Chem-Industry Institute, China) was placed at 10 cm depth as topdressing fertilizer with the same N rate as the main plots after 30 days of transplanting. The same rate of P and K fertilizers was applied in micro-plots as the main plots. Irrigation was separately applied with tap water in the micro-plots.

2.2. Grain yield and yield components

Twenty rice plants from each plot were sampled at random and averaged to estimate the number of productive panicles per hill whereas ten plants were sampled to determine the number of spikelets per panicle, seed setting rate and 1000-grain-weight. Finally, a harvested area of 1 m^2 in the middle of each plot was selected to determine the grain yield. The grains were sun-dried to adjust the moisture contents at 14%, estimated by Cereal Crop Moisture Measure Instrument (TDS-1 G), (developed by Zhejiang Tuopu Yunnong Science Technology Limited Company).

2.3. Nitrogen use efficiency

At maturity, all rice plants within 1 m^2 were randomly selected in each plot to estimate the total aboveground biomass. Then six rice plants were divided into leaf, sheath and stem, panicle, respectively, and each part was pulverized to powder and stored to analyze total nitrogen contents. A sample power (0.3 g) was determined with Kjeldahl method. The nitrogen use efficiency and nitrogen harvest index was calculated

Table 1
Experimental design.

Treatments	Total nitrogen rate and its ratio
T1	No nitrogen fertilizer 0
T2	TN 150 kg N ha^{-1} Basal fertilizer 90 kgN ha^{-1} (SB) + Tillering fertilizer 60 kgN ha^{-1}(SB)
T3	TN 135 kg N ha^{-1} Basal fertilizer 90 kgN ha^{-1} (SB) + Tillering fertilizer 45 kgN ha^{-1} (DA)
T4	TN 120 kg N ha^{-1} Basal fertilizer 90 kgN ha^{-1} (SB) + Tillering fertilizer 30 kgN ha^{-1} (DA)

Note: TN, Total nitrogen; SB, Surface broadcast at harrowing; DA, Deep application in ten days after transplanting.

according to Li et al. (2020).

2.4. Nitrogen recovery from fertilizer and the fertilizer loss

The ^{15}N isotope labeling urea was used instead of ordinary urea, and the fertilization ratio and method of the experimental treatment were consistent with the field experiment. At the maturity stage of rice, 5 rice plants were randomly taken from each treatment where ^{15}N labeled urea was applied, and the stems, leaves, and panicles were separated, dried at 60 ℃ for 48 h. The samples were sent to Hebei Academy of Agricultural Sciences to detect the ^{15}N abundance. Combined with ^{15}N abundance in fertilizers, the amount of nitrogen absorbed by rice plants from fertilizers and the fertilizer loss was calculated according to Pan et al. (2012).

2.5. Morpho-physiological traits

2.5.1. Leaf area index (LAI) and total aboveground biomass

Six rice plants were randomly selected at panicle initiation (PI), heading stage (HS), grain-filling stage (GF) and maturity stage (MS), respectively, and separated into sheaths and stem, leaves, and spikes (if existed) after cleaning with tap water thoroughly. The area for all green leaves was measured with a Li-Cor area meter (Li-Cor Model 3100, Lincoln, NE) and leaf area index was then calculated. The separated part of the rice plants was oven-dried at 80℃till constant weight and the above-ground biomass was calculated.

2.5.2. Photosynthetic characteristics

Photosynthetic rate was measured with LI-6400XT portable photosynthesis system (Licor, USA) for ten flag leaves on the main stem from different hills, in the morning from 9:00–11:00 on a sunny day at the heading stage. The light intensity was 1200μmol m^{-2} s^{-1}, and the ambient CO_2 concentration was 350 μL L^{-1}.

2.5.3. Determination of chlorophyl contents

Approximately 25 uppermost plant leaves from different hills from each treatment were randomly collected at PI, HS and GF; then stored at —80 ℃ to determine enzyme activity and total chlorophyll contents. The leaf chlorophyll (Chl) contents were determined according to Li et al. (2022). Fresh leaf samples (0.2 g) were placed in the 10 ml centrifuge tube, and 5 ml of absolute ethanol was added and then stored until the sample turned white. Chl a and b were measured by a UV-Vis spectrophotometer at 663 and 645 nm.

2.5.4. Activities of enzymes related to nitrogen metabolism

The activities of enzymes related to nitrogen metabolism i.e., nitrate reductase (NR), glutamate synthase (GS) and glutamine oxoglutarate aminotransferase (GOGAT) were determined according to (Hou et al., 2019; Zhang et al., 2022). Fresh leaves (0.5 g) were homogenized on ice in 4.0 ml of extraction solution containing phosphate buffer (25 mM, pH 7.5, a mixture of K_2HPO_4 and KH_2PO_4), cysteine (5 mM) and EDTA-Na_2 (5 mM). The homogenate was centrifuged at 10000 rpm at 4 ℃ for 15 min, and the supernatant was collected. An aliquot enzyme (0.4 ml) extract was added to 1.6 ml of reaction reagent (1.4 ml of 0.1 M KNO_3 phosphate buffer and 0.2 ml of 2.0 mg ml^{-1}NADH), and the mixture was incubated at 25 ℃ for 30 min. For the control, 0.2 ml phosphate buffer was used instead of 0.2 ml NADH. The reaction was stopped by adding 1.0 ml of 1% 4-aminobenzene sulfonic acid and 1.0 ml of 0.2% 1-naphthylamine and incubated at room temperature for 15 min; the samples were centrifuged at 4000 rpm for 10 min, and the absorbance of the supernatant was measured at 540 nm.

The glutamine synthetase (GS) activity was determined according to the methods of (Hou et al., 2019; Zhang et al., 2022). Fresh leaves (1.0 g) were homogenized in 3.0 ml of Tris-HCl (0.05 M, pH 8.0) in a pre-cooled mortar in an ice bath, followed by extraction with $MgSO_4$ (2 mM), DTT (2 mM) and sucrose (0.4 M). The homogenate was centrifuged at 15000 rpm at 4 ℃ for 20 min. An aliquot of the crude enzyme solution (0.7 ml) and 0.7 ml of 40 mM ATP were added to 1.6 ml of reaction mixture B containing imidazole-HCl buffer (0.1 M, pH 7.4), sodium hydrogen glutamate (20 mM), $MgSO_4$ (80 mM), Cysteine (20 mM), EDTA (2 mM) and hydroxylamine hydrochloride (80 M). For the control, reaction mixture A without hydroxylamine hydrochloride was used instead of reaction mixture B. The mixture was incubated at 25 ℃ for 15 min. Approximately 1 ml of chromogenic agent (TCA (0.2 M), $FeCl_3$ (0.37 M), HCl (0.6 M)) was added to terminate the reaction. After centrifugation at 5000 rpm for 10 min, the absorbance of the supernatant was measured at 540 nm.

The glutamate synthase (GOGAT) activity was determined according to the methods of (Hou et al., 2019; Zhang et al., 2022). Fresh leaves (0.4 g) were ground in a chilled mortar in 25 mmol/L Tris-HCL buffer (pH 7.6) and the homogenate was centrifuged at 13000 rpm/min for 25 min. The GOGAT reaction mixture consisted of 0.4 ml 20 mmol/L L-glutamine, 0.5 ml 20 mmol/Lα-ketoglutarate, 0.1 ml 10 mmol/L KCl, 0.2 ml 3 mmol/L NADH and 0.3 ml enzyme extract. Through monitoring the oxidation of NADH, GOGAT activity was assayed at 340 nm using a 752UV-Vis spectrophotometer.

2.6. Data analysis

Data were checked for homoscedasticity, then data were analyzed using a generalized linear mixed model under the assumption of a poison distribution. There were no significant effects on parameters between two years, hence, significance of Fertilizer × Variety interaction effects were tested for all parameters. Where two-way interaction effects were observed, Fertilizer and Variety were considered as fixed factor and Replicate as random factor. For each parameter, least-square means of Fertilizer and Variety were computed when appropriate. For parameters for which there was a significant effect, ANOVAs were followed by a comparison of means using the least significant difference (LSD) test at 0.05 probability level. All data was analyzed by using the standard analysis of variance procedure (SAS Institute, 2003).

3. Results

3.1. Grain yield

Yield and its components of both rice cultivars varied significantly ($P < 0.05$) among N application treatments. Compared with T2, the T3 led to the significant improvements in the grain yield and yield components of both rice cultivars. For instance, the highest grain filling rate and 1000-grain weight were recorded in T3 of both rice cultivars. The grain yield was increased by 10.6% and 11.9% in T3 than T2 for *Wufengyou 615* and *Yuxiangyouzhan*, respectively. The productive panicle of T3 was about 9.6% higher than that of T2 for *Wufengyou 615*, whereas for *Yuxiangyouzhan* the productive panicle was 8.4% higher than that of T2. The year and variety interaction was also found significant regarding grain filling rate and 1000-grain-weight whereas interaction between variety and nitrogen treatment was also found significant regarding productive tillers per ha, grain filling rate, 1000-grain-weight and grain yield for both rice cultivars (Table 2).

3.2. Nitrogen use efficiency

At different growth stages, the nitrogen content of T3 treatment was significantly higher than other treatments before maturity stage, e.g., about 15% higher than T2 treatment at heading stage. At maturity stage, the nitrogen content of rice stem for T3 was significantly lower than that of T2 treatment by 5–10%, and the nitrogen content of the leaves was also lower, which was conducive to the accumulation of nitrogen in rice grains (Fig. 1, Figs. S1 to S2).

Note: PI, Panicle initiation stage; HS, Heading stage; GF, Grain-filling stage; MS, maturity stage; The same letter above every bar is not significantly different at the 0.05 probability level according to least

Table 2
Effects of deep placement of fertilizer at tillering stage on grain yield and its components in rice (2-year mean).

Treatments	Productive panicles (10^4 ha^{-1})		Spikelets per panicle		Grain filling rate (%)		1000-grain weight (g)		Grain yield (t ha^{-1})	
WF										
T1	200.5	d	127.5	d	83.2	d	21.9	d	5.2	d
T2	251.1	b	175.8	b	85.4	b	22.4	b	8.5	b
T3	275.2	a	188.5	a	86.1	a	23.2	a	9.4	a
T4	238.6	c	164.9	c	84.3	c	22.3	c	7.9	c
mean	**241.4**		**164.2**		**84.8**		**22.5**		**7.7**	
YX										
T1	163.4	d	139.8	c	81.4	d	19.5	d	5.7	d
T2	251.6	b	165.6	b	82.8	b	20.8	b	8.4	b
T3	272.7	a	179.5	a	83.5	a	21.0	a	9.4	a
T4	221.6	c	167.1	b	82.4	c	20.1	c	7.6	c
mean	**227.3**		**163.0**		**82.5**		**20.3**		**7.8**	
ANOVA										
V	* *		ns		*		* *		* *	
T	* *		*		*		* *		* *	
V×T	* *		ns		*		*		* *	

Note: WF, *Wufengyou 615*; YX, *Yuxiangyouzhan*; V, Variety; T, Treatment; V×T, Interaction between variety and treatment; * and * * represent a significant difference at P < 0.05 and P < 0.01, respectively; ns represents a non-significant.

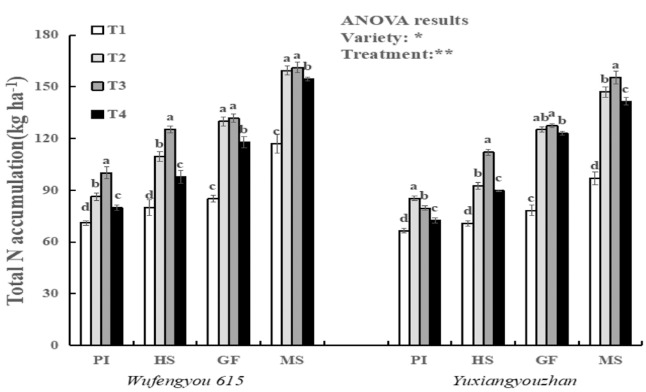

Fig. 1. Effect of deep placement of fertilizer at tillering stage on total N accumulation in rice (2-year mean).

significant different test (LSD0.05). Interaction between variety and treatment; * and * * represent a significant difference at P < 0.05 and P < 0.01, respectively; ns represents a non-significant.

The nitrogen agronomic efficiency of *Wufengyou 615* and *Yuxiangyouzhan* for T3 was 42.4% and 53.3% higher than T2, respectively. Moreover, the nitrogen recovery efficiency and nitrogen physiological efficiency of T3 treatment were also the highest, while the difference in nitrogen harvest index of each treatment was not obvious (Table 3). The effects of interaction between year and variety on both NRE and NPE were significant. Significant interaction effect was also noticed for NRE between year and nitrogen treatments whereas the interaction between variety and nitrogen treatment was also found significant for NAE, NRE, NPE and NHI (Table 3).

3.3. Nitrogen recovery from fertilizer and the fertilizer loss

According to the ^{15}N abundance test, deep application of nitrogen fertilizer at tillering stage significantly increased the absorption of ^{15}N by rice plants. Nitrogen derived from fertilizer (Ndff) in T3 was 8.0% and 4.4% higher than T2 for *Wufengyou 615* and *Yuxiangyouzhan*, respectively. However, Ndff of T4 treatment was lower than the T2 whereas the highest N recovery was found in T3 (Table S1).

According to the ^{15}N abundance test of the soil, the T3 treatment has the least residual fertilizer content in the soil, whilst the nitrogen use efficiency also remained the highest for *Wufengyou 615* and *Yuxiangyouzhan*. The fertilizer loss in T3 was 3.8% and 3.4% lower than T2 (Table S2).

Table 3
Effects of deep placement of fertilizer at tillering stage on nitrogen use efficiency in rice (2-year mean).

Treatments	NAE (kg kg^{-1})		NRE (%)		NPE (kg kg^{-1})		NHI (%)	
WF								
T1	——		——		47.5	d	47.4	d
T2	21.7	b	30.4	b	56.2	b	52.8	b
T3	30.9	a	31.8	a	61.8	a	53.5	a
T4	22.0	b	27.6	c	53.4	c	48.7	c
mean	**24.8**		**30.0**		**54.7**		**50.6**	
YX								
T1	——		——		56.0	d	49.5	d
T2	17.8	b	37.1	b	54.8	b	54.5	b
T3	27.3	a	40.8	a	60.0	a	52.1	a
T4	16.0	c	34.0	c	52.2	c	48.7	c
mean	**20.4**		**37.3**		**55.7**		**51.2**	
ANOVA								
V	* *		* *		*		ns	
T	* *		*		*		*	
V×T	*		*		*		*	

Note: NAE, Nitrogen agronomic efficiency; NRE, Nitrogen recovery efficiency; NPE, Nitrogen physiology efficiency; NHI, Nitrogen harvest index; WF, *Wufengyou 615*; YX, *Yuxiangyouzhan*; V, Variety; T, Treatment; V×T, Interaction between variety and treatment; * and * * represent a significant difference at P < 0.05 and P < 0.01, respectively; ns represents a non-significant.

3.4. Chlorophyll contents and net photosynthetic rate

Different nitrogen management practices affected the photosynthetic pigments and net photosynthetic rates in both rice cultivars. The chlorophyll contents of *Wufengyou 615* and *Yuxiangyouzhan* in T3 were found 21.8% and 48.0% higher than T2, respectively. Moreover, the chlorophyll content in T3 treatment was also substantially higher than T2, while the chlorophyll content in T4 treatment was lower than T2.

At heading stage, the photosynthetic rate of *Wufengyou 615* and *Yuxiangyouzhan* remained 11.9% and 5.0% higher in T3 than T2, respectively. The photosynthetic rate of *Wufengyou 615* and *Yuxiangyouzhan* for T4 was 14.1% and 15.9% higher than T0, respectively. Furthermore, the photosynthetic rate in T3 treatment was also significantly higher than T2, while the photosynthetic rate of T4 treatment was lower than T2 treatment (Table 4). The interaction between year and nitrogen treatments on total Chl at PI was significant whereas the interaction between variety and nitrogen treatment for total Chl contents at PI, HS, GF and net photosynthetic rate at HS was also found significant (Table 4).

Table 4
Effect of deep placement of fertilizer at tillering stage on chlorophyll contents and net photosynthetic rate (Pn) at heading stage in rice (2-year mean).

Treatments	Total Chl (mg·g^{-1})						Net photosynthetic rate (μmol m^{-2} s^{-1})	
	PI		HS		GF			
WF								
T1	3.2	d	1.7	c	2.3	c	17.8	d
T2	3.6	b	3.3	a	2.6	b	22.5	b
T3	3.9	a	3.4	a	2.9	a	24.7	a
T4	3.5	c	2.7	b	2.6	b	20.4	c
mean	**3.6**		**2.8**		**2.6**		**21.4**	
YX								
T1	2.5	d	1.9	d	1.9	d	17.4	d
T2	3.4	b	3.1	b	2.5	b	23.1	b
T3	3.7	a	3.5	a	2.9	a	24.3	a
T4	3.0	c	3.0	c	2.4	c	20.1	c
mean	**3.2**		**2.9**		**2.4**		**21.2**	
ANOVA								
V	*		ns		*		ns	
T	* *		* *		* *		* *	
V×T	*		ns		*		*	

Note: PI, Panicle Initiation stage; HS, Heading Stage; GF, Grain-Filling stage; Chl, chlorophyll; WF, *Wufengyou 615*; YX, *Yuxiangyouzhan*; V, Variety; T, Treatment; V×T, Interaction between variety and treatment; * and * * represent a significant difference at P < 0.05 and P < 0.01, respectively; ns represents a non-significant.

3.5. LAI and total aboveground biomass

Nitrogen management affected the LAI and plant dry biomass in both rice cultivars. The LAI in T3 and T4 treatments was significantly higher than T1 about 30–40%. Moreover, the LAI in T3 was also substantially higher than T2, while the T4 treatment resulted in lower LAI due to the reduction of the total amount of nitrogen fertilizer application rate (Figs. S3).

In addition, the total aboveground biomass of T3 treatment was substantially higher than T1 and T2 for both *Wufengyou 615* and *Yuxiangyouzhan*, respectively. However, the T4 treatment resulted in reduced LAI, which caused reduction in net photosynthetic rate and total aboveground biomass (Figs. S4).

3.6. Nitrogen metabolizing enzymes

Different nitrogen management had a significant impact on the activities of enzymes involved in nitrogen metabolism i.e., NR, GS and GOGAT. The NR activities of T3 and T4 treatments were found 30–50% higher than T1, and the NR activity of T3 was also remained significantly higher than T2 (Fig. 2).

Deep application of nitrogen fertilizer at tillering stage also increased the GS activity of rice plants. The GS activity in T3 treatment was about 30–40% higher than T1 and 8–15% higher than T2 for *Wufengyou 615*. Moreover, the GS activity of T4 treatment was higher than T0 and lower than T2 for both rice cultivars at PI, HS, and GF stages (Fig. 3).

The differences in GOGAT activity in different treatments were not much obvious for *Wufengyou 615*, but the GOGAT activity of *Yuxiangyouzhan* was the highest in T3 treatment whereas lower in T4 treatment (Fig. 4).

4. Discussion

4.1. Grain yield

Nitrogen fertilizer management has significant impact on the growth, productivity and nitrogen use efficiency in rice (Wang et al., 2018). The use of high-efficiency nitrogen fertilizer management can promote the growth and development of rice plants (Liu et al., 2015; Huang et al., 2018). It is also useful to reduce fertilizer rate, production

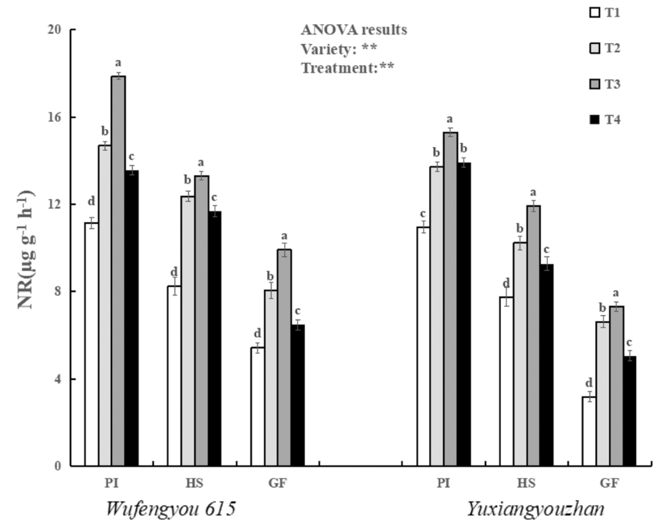

Fig. 2. Effect of deep placement of fertilizer at tillering stage on nitrate reductase (NR) activities for rice (2-year mean). Interaction between variety and treatment; * and * * represent a significant difference at P < 0.05 and P < 0.01, respectively; ns represents a non-significant.

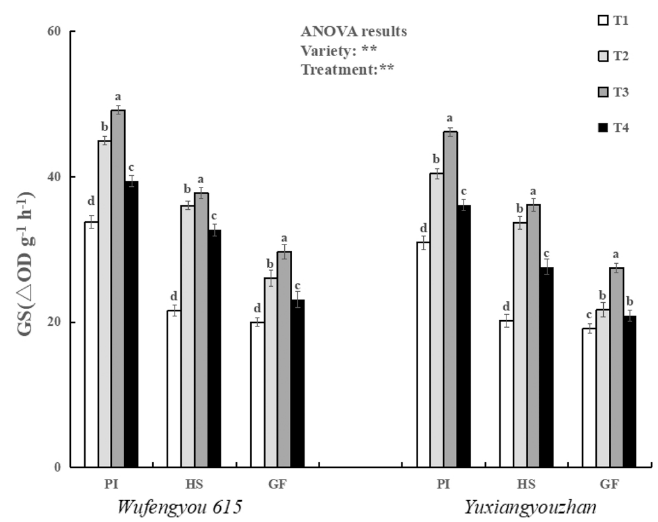

Fig. 3. Effect of deep placement of fertilizer at tillering stage on glutamine synthetase (GS) activities for rice (2-year mean). Interaction between variety and treatment; * and * * represent a significant difference at P < 0.05 and P < 0.01, respectively; ns represents a non-significant.

costs and environmental pollution by improving the N utilization efficiency with minimum loss. Previous studies reported that mechanical deep fertilizer application as basal fertilizer could greatly improve rice yield compared to conventional broadcast fertilizer treatment (Chen et al., 2020; Li et al., 2020). Moreover, Zhang et al. (2021) observed that split application of N fertilizer under deep fertilization increased the direct-seeded rice yield compared to one-time-deep-placement fertilization as basal fertilizer. Our results indicated that a 10% reduction in N fertilizer application rate under deep fertilization (T3) can significantly increase the rice yield compared to other treatments. The yield improvement is attributed to an increase in productive panicles and grain filling percentage. Deep fertilizer application can effectively conserve and provide a continuous supply of nutrients for grain development during late growth stages (Li et al., 2022; Zhong et al., 2021), which promotes productive panicle formation and grain filling, thus resulting in improved grain yield. Deep application of nitrogen fertilizer at tillering stage requires less N dose to get equal or even higher yield

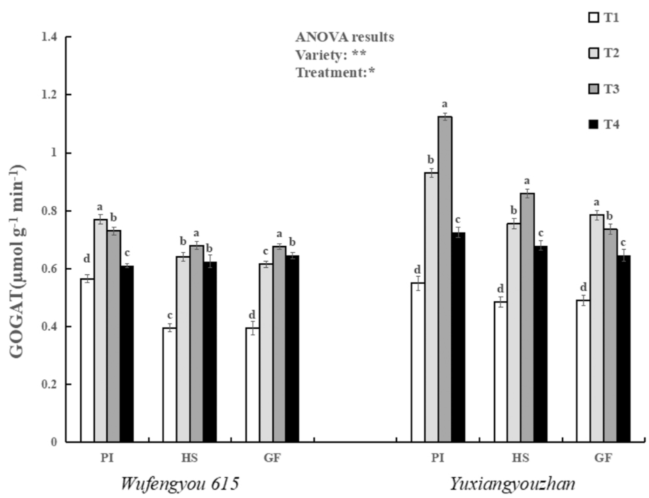

Fig. 4. Effect of deep placement of fertilizer at tillering stage on glutamate synthase (GOGAT) activities for rice (2-year mean). Interaction between variety and treatment; * and * * represent a significant difference at $P < 0.05$ and $P < 0.01$, respectively; ns represents a non-significant.

than traditional surface broadcast fertilization, however, with 20% N reduction under deep fertilization (T4) could lead to yield penalty. It was also found that the interactive effects of year and variety on grain filling rate and 1000-grain-weight were significant whereas variety and nitrogen treatment interaction also affected the productive panicles, grain filling rate, 1000-grain-weight and grain yield significantly. This possibly caused by onset of low temperature at heading stage. The hybrid rice WF was more sensitive to low temperature than inbred rice YX. Thus, optimization of N application rate for deep placement is a prerequisite for field application at a large scale (Li et al., 2021c). Therefore, in order to obtain higher grain yield, the reasonable nitrogen management strategies need to be adopted.

4.2. Nitrogen use efficiency

Our results showed that the nitrogen agronomic efficiency and nitrogen uptake efficiency of the T3 treatment were significantly higher than other treatments. These results agreed with Pan et al. (2017), who found that deep basal fertilization could enhance the NUE, regardless of fertilizer type. The reason might be that deep N fertilization promotes the uptake of total nitrogen by the plant and reduces nitrogen loss in different ways (Zhang et al., 2017; Yao et al., 2018). The present result also observed that the nitrogen derived from T3 fertilizer treatment was significantly higher than other treatments, indicating that rice can absorb more nutrients from the fertilizer to be fully utilized under deep tillering-fertilizer. Moreover, the results of ^{15}N labeling tracer test showed that the deep application of nitrogen fertilizer at tillering stage resulted in close contact of fertilizer with the rice root system, thus improved the nitrogen absorption. The fertilizer-root close contact further helps in regulating the demand and supply relationship between plants and nitrogen fertilizer, thus promoting the absorption of nitrogen by plants (Zhang et al., 2017). In addition, deep application of nitrogen fertilizer at tillering stage may also reduce the nitrogen loss caused by runoff. In this study, the T3 treatment had the highest nitrogen content in the plant compared with other treatments at each stage. It was also found that the interactive effect of year and variety on NRE and NPE was significant, whereas significant effects of variety and nitrogen treatment interaction on NAE, NRE, NPE and NHI was also noticed. This might be ascribed to the differential absorption abilities for both rice cultivars i.e., hybrid rice and inbred rice. Furthermore, nitrogen absorption by plants requires the participation of three nitrogen metabolizing enzymes i.e., NR, GS and GOGAT (Sarker et al., 2002), where NR plays a significant role in limiting the rate of nitrogen assimilation by plants (Masclaux et al., 2000). The deep application of nitrogen fertilizer during tillering period possibly increased the activities of N metabolizing enzymes and thus become the basis for improved nitrogen use efficiency in rice.

4.3. Photosynthesis efficiency and dry biomass

The accumulation of plant dry biomass is the basis for high yield of rice (Hu et al., 2020). Dry matter accumulation is a result of organic matter synthesized due to photosynthesis, an important process of growth and yield formation (Zhao et al., 2015). Compared with traditional fertilization method, the deep application of nitrogen fertilizer at tillering stage can effectively increase the photosynthetic efficiency in rice (Jia et al., 2008). The T3 treatment of topdressing with deep application of nitrogen fertilizer during tillering period resulted in the highest dry matter accumulation. The total chlorophyll content and photosynthesis rate were also found the highest in T3. The higher chlorophyll content might result in improved absorption efficiency of light spectrum, which is the base to get the highest dry matter accumulation in rice. Similarly, deep application of nitrogen fertilizer during tillering period for topdressing also increased the LAI of rice, so a larger leaf area could improve the efficiency of photosynthesis. Hence, the deep application of nitrogen fertilizer at tilling stage can play a vital role to improve the rice productivity.

5. Conclusion

Deep application of nitrogen fertilizer at tillering stage can promote the growth and development of rice and reduce the amount of nitrogen fertilizer application. Overall, application of 45 kg N/ha as tillering-fertilizer by deep placement with traditional surface broadcast at 90 kg N/ha basal fertilizer improved the leaf area index and chlorophyll content, net photosynthesis, and total above-ground biomass. It further improved grain yield owing to an increase in spikelets per panicle and productive panicles, as well as improved nitrogen use efficiency (NUE) compared to traditional surface broadcast fertilizer at 150 kg N/ha (60 kg N/ha as tillering fertilizer and 90 kg N/ha as basal fertilizer).

Author contributions

SG and XR initiated and designed the research, ZZ, YF, ZM, UA, ZW, HT, MY and YQ performed the experiments, ZZ analyzed the data and wrote the manuscript, SG, UA and ZM revised and edited the manuscript, and also provided advice on the experiments. All authors approved the final version of the manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.fcr.2022.108736.

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