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Rice dry matter and nitrogen accumulation, soil mineral N around root and N leaching, with increasing application rates of fertilizer

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ABSTRACT

Rice morphology and N leaching, together with mineral N in the soil and soil solution around root, were determined at different growth stages in a 3-year experiment located in the Taihu Lake region, China. The results showed that the N application rates had little impact on the soil mineral N around root, but increased the dry matter and N accumulation aboveground in the high fertility soil (55.3 mg kg^{-1} of soil mineral N before rice season in 2008). However, no significant difference in grain yield was observed in all N treatments in these 3 years. Path analysis showed that spikelet per panicle made the greatest direct contribution (0.781) and total contribution (0.309) to grain yield compared to other yield components. And a higher panicle per m^2 and dry matter accumulation resulted in yield decline later in the season due to a decline in the percentage of filled grains.

No significant increases in plant N uptake, regardless of N application rates, were observed at the seedling stage, which indicated that lower N application rates could suffice during the rice early growing stages. Nitrate contents, in spite of high N rates input, in the percolation water were all below 1.0 mg L^{-1} throughout the rice growing season. The increased N rates showed an increment of total N leaching through the percolation water, but not significant. The cumulative total N leaching only accounted for 1.86–4.96% of N fertilizer input, which suggested the N leaching should not be considered as main pollution resources in paddy filed in summer rice season. However, the evaluation of N leaching in different stages indicated that N leaching at seedling stage was larger in dominant (averaged 39.8% of total N leaching) than other stages. For the lower absorbing ability of rice seedling and more N leaching risk, suggestions on N fertilizer reduction should be made at rice early growing stage in this region.

1. Introduction

Rice is one of the major crops in China and makes up 43.7% of the total national grain production. Areas planted to rice, more than 125 million hectares, account for 9% of the total arable land area in the world (Aboulsmail et al., 2004; Duan et al., 2007). An increasing world population is also associated with a decrease in cultivable land, which makes intensive rice cropping necessary if future rice production needs are to be met (Dawe et al., 2000).

Since chemical N fertilizer was introduced into China in the 1950s, improvements in grain yield have mainly relied on increases in N fertilizer application rates. The mean N fertilizer input has approached 300 kg ha^{-1} for rice monoculture (Cui et al., 2000) and was over 350 kg ha^{-1} in one paddy field in the Taihu Lake region (Huang et al., 2007). However excess nitrogen fertilizer, not utilized by the plant directly or indirectly, has been identified as major contamination to the ground water and surrounding environment (Conrad and Fohrer, 2009) and made up 59% of the total N in Taihu Lake region, which has led to water eutrophication (Toshisuke et al., 2008; Zhu and Wen, 1992; Song et al., 2004; Zhu et al., 2004; Xie et al., 2007, 2008; Li and Yang, 2004). Nitrate, which can be easily leached through percolation water into the ground water, is a potential risk for human health (Mkandawire, 2008; Liang et al., 2011). World Health Organization has proposed the admissible nitrate–nitrogen content in drinking water for the bottle-fed infants, which should be below 11.0 mg L^{-1} (WHO, 2011). And the European Union Water Framework set a maximum admissible content of 50 mg L^{-1} for nitrate in ground water (GWD; European Parliament and Council, 2006). The saturation condition in the paddy field guarantee the continuous N leaching through the percolation water as the field was submerged. Furthermore, overuse of nitrogen fertilizer can cause grain yield decline (Wang et al.,

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Abbreviations: NA, nitrogen accumulation; AN, additional amount N taken up by rice plant over control; PM, panicle per m^2; SP, spikelet per panicle; SM, spikelet per m^2; PFG, percentage of filled grains; TGW, thousand grain weight; DMA, dry matter accumulation; TDMA, total dry matter accumulation.

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2009a,b) and the optimal nitrogen fertilizer rate for the rice growing season could be reduced to 200 kg N ha^{-1} in the Taihu Lake region (Ju et al., 2009; Deng et al., 2012). Hence, it is urgent to find the nitrogen balance between plant requirements and supply, together with the N leaching in order to reduce the severe environmental impacts from agricultural activities in the Taihu Lake region.

Many studies have been undertaken into the nutrient supply needed to produce high yields (Feng et al., 2006; Zhang et al., 2006, 2007). However, most research focused on the final yield and N use efficiency. Few studies were concerned about plant requirements and soil supply at different growth stages during plant development in the paddy field. Studies into rice plant growth and nutrient status are critical if rice yield and grain quality is to be predicted, and recommendations for nitrogen topdressing at panicle initiation stage are to be made (Nguyen and Lee, 2006). As the plant growth reflects the total N supply from all sources, plant N status should be an indispensable indicator for N availability to crops at any given time (Balasubramanian et al., 1999). Plant nitrogen uptake is also an integrative determinant for rice growth and yield as it is interactively associated with biomass accumulation, leaf area, root development and spikelet formation (Yoshida and Horie, 2010; Aguirre and Johnson, 1991; Stitt and Krapp, 1999). The morphology of roots involved in the absorption of soil mineral nutrients changes with plant nutritional status and consequently influences plant growth, nitrogen accumulation and yield (Lynch, 1995; Zhang et al., 2009a,b). Studies have shown that soil mineral nutrient supply in the rhizosphere strongly affects root growth and morphology (Wang et al., 2009a,b). Thus, it is necessary to investigate soil mineral N around root and plant morphology at different rice growth stages, if the recommendation for N fertilizer management on the balance between grain yield and environment are to be made.

Rice yield was also determined by the yield components such as spikelet per m^2 (SM), filled grains and grain weight. The spikelet per m^2 (sink size) has been considered as an indicator of high yield (Sheehy et al., 2001; Yoshida et al., 2006). The sink size can be further decomposed into spikelet per panicle (SP) and panicle per m^2 (PM). Hence, the sink size increase could increase SP or PM. Many researches had been conducted to clarify the influence of PM and SP to the grain yield (Lu et al., 2008; Huang et al., 2011; Wang et al., 2007; Yan et al., 2010), and the contributions of these yield components to yield varied simultaneously with the climate and N management (Ao et al., 2008; Katsura et al., 2008; Yoshida et al., 2006). As the PM was influenced by tiller number, which was influenced by the N input, water management and rice genes, the determination of contributions to the grain yield seems to be important for N management.

The objectives of this research were to: (a) Investigate the changes in rice N uptake and soil mineral N around root at rice important growth stages; (b) characterize the N leaching with the increasing N rates; and (c) use path analysis to clarify the contribution of the yield component to the grain yield.

2. Materials and methods

2.1. Site location, soil analysis and current agricultural practice

The site, materials and methods have been described previously by Xue et al. (2009) and Qiao et al. (2012). The field experiment was located at Yixing, Taihu Lake region (31°17.49′ N, 119°54.02′ E), China, on gleyed-stagnant anthrosols (FAO soil taxonomy in 1974) and began in 2008. Current agricultural practice in this region is an intensive double-cropping system growing summer rice and winter wheat. The soil analyses of the top soil horizon (0–15 cm) indicated the following: organic matter, 12.6 g kg^{-1}; total N, 0.64 g kg^{-1}; total P, 0.39 g kg^{-1}; total K, 13.9 g kg^{-1}; mineral N, 53.5 mg kg^{-1}; Olsens-P, 42.6 mg kg^{-1}; NH_4OAc-extractable K, 49.6 mg kg^{-1} and pH, 6.23.

2.2. Field trial design

The plots were arranged randomly with three replications for each treatment and had an individual area of 5.5 m × 5.5 m. The seven nitrogen fertilizer treatments in summer rice were as follows: N_0 (control, 0 kg N ha^{-1}) N_1 (135 kg N ha^{-1}), N_2 (189 kg N ha^{-1}), N_3 (216 kg N ha^{-1}), N_4 (243 kg N ha^{-1}), N_5 (270 kg N ha^{-1}) and $N_6{}^1$ (405 kg N ha^{-1}). For the two previous years, rice in $N_6{}^1$ (405 kg N ha^{-1}) at harvest continuously presented lodging effect and consequently the lower grain yield, compared to other N treatments. Therefore, we adjusted the $N_6{}^1$ to 0 kg N ha^{-1} for the winter wheat and summer rice in 2010, and the yield did not show a significant difference with the control (0 kg N ha^{-1}) set up before. With regard to the summer rice in 2011, the $N_6{}^1$ was readjusted as $N_6{}^2$ with 81 kg N ha^{-1} application.

The three key fertilization stages were a basal fertilizer application at transplanting, two topdressings at tillering and heading, respectively. The N fertilizer distribution ratio was 3:3:4 for basal, tillering and heading dressings, respectively. The basal dressing was NPK compound fertilizer (N 15%, P_2O_5 15% and K_2O 15%) for N treatments and was mixed with soil puddle before the rice was transplanted. P and K fertilizer, from superphosphate (P_2O_5 12%) and potassium chloride (KCl 60%), were 81 kg ha^{-1} and used as a basal dressing. Supplemental applications at the tillering and heading stages consisted of urea were applied in the paddy field.

2.3. Water management and meteorological data of the rice season in 2008, 2009 and 2011

The temperature and precipitation of rice season in these 3 years experiments were showed in Fig. 1. The averaged temperature in rice season was 24.7 °C, 24.9 °C and 24.2 °C for these 3 years, respectively. And the precipitations of rice season were 797.9 mm, 747.8 mm and 1255.4 mm for 2008, 2009 and 2011, respectively. The paddy field was pre-flooded for 3–4 days before the field was fertilized and then transplanted. After the rice seedlings were transplanted, the field was sustained a water layer with 8 cm. For the aim of controlling the rice tillering number, the drainage of the paddy field, which called mid-season aeration, was implemented 10 days after the tillering stage dressing and last at least for 5 days. Then the field was re-submerged until the end of heading stage, where after no water layer was left in the field till the rice harvest.

2.4. Sampling and analysis

2.4.1. Plant sampling and analysis

The rice (*Oryza sativa* L. cv. zhèn dào 10#) was selected for these years. In 2008, the seed was sown on the 20th May and transplanted on the 13th June at a density of 20–25 hills m^{-2} and three seedlings per hill. Tillering and head dressings were applied on the 3rd and 28th July, respectively. In 2009, zhèn dào 10# seed was sown on the 18th May and transplanted on the 15th June at a density of 20–25 hills m^{-2} and four seedlings per hill. The tillering and head dressings were applied on the 10th July and the 1st August, respectively. In 2011, seed was sown on the 20th May and transplanted on the 24th June with a density of 20–25 hills m^{-2} and three seedlings per hill. The top dressings were applied on 24th July and 8th August. Seven hills of plants were sampled 15 days in 2009 and 10 days in other 2 years after the fertilizer was applied and the dry weights of the straw and roots were measured.

At harvest, the above ground part of the plant was collected, and the straw and grain weights were measured separately. Grain

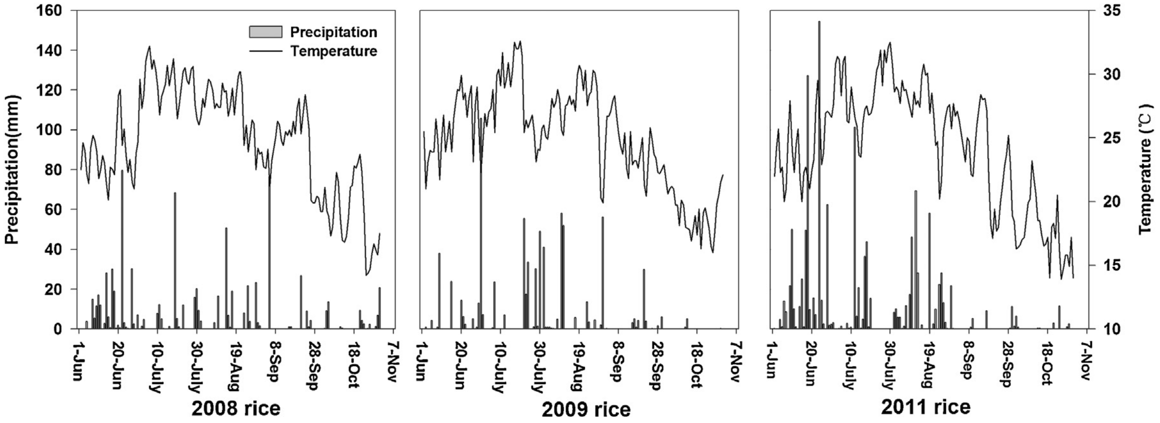

Fig. 1. Precipitation and temperature at rice cultivation in 2008, 2009 and 2011.

and straw subsamples were oven-dried at 70 °C for 24 h after heat-processed at 105 °C for 30 min, they were then ground and sieved to pass through a 0.5 mm sieve for chemical analysis. The N in the plant samples was determined using the micro-Kjeldahl method (Bremner, 1960). N uptake was calculated by multiplying the concentration of nitrogen by the plant oven dry weight, expressed in kg ha^{-1}.

Theoretic yield indicated no yield loss through the process of rice harvest, which measure was: panicles per m^2 × spikelet per panicle × filled grains × grain weight.

Path analysis is used to describe the directed dependencies among a set of predictors, and also the interaction between these predictors. The direct contribution was indicated the statistic effect, not the causal effect. The indirect effect in the path model indicated a predictor has no main effect but is only involved in an interaction (Ducan, 1996).

X_1, X_2, X_3 and X_4 of path analysis represent panicles per m^2 (PM), spikelet per panicle (SP), percent of filled grains (PFG) and thousand grain weight (TGW).

2.4.2. *Soil sampling and analysis*

The soil samples were collected from plant roots, while the plants were pulled out from the paddy field at seedling, tillering, heading and harvest periods. The soil samples were immediately taken to the laboratory. In 2008, the soil samples were air-dried, ground and sieved to pass a 2 mm sieve for chemical analysis. In 2009 and 2011, the fresh soil samples were mixed with four replications and centrifuged at 5000 rpm at 24 °C for 30 min for each plot (Elkhatib et al., 1987). Then the resulting suspensions, as the soil solution samples, were refrigerated at 4 °C after filtering, and the solid soil without suspensions were extracted with 2 M KCl for 1 h at 24 °C and refrigerated at 4 °C after filtering. The moisture content for the solid soil was also determined. The concentrations of NH_4^+-N and NO_3^--N, both from the soil solution and from the soil solid components, were determined using a continuous-flow auto analyzer (Traacs 800, Bran & Luebbe, Hamburg).

2.4.3. *Percolation water sampling and analysis*

The devices for percolation water sampling were set up at each plot in 2011. The percolation water was collected by porous polyvinyl chloride pipes (PVC columns). As rice root cannot penetrate the plow pan, the percolation water below the plow pan could be considered as the leachate in the rice season (Ringrose-Voase et al., 2000). Therefore, the PVC columns, 0.05 m in diameters and 0.70 m in length, were vertically inserted into soil at depths of 0.4 m in each plot. In accordance with sampling depth, the PVC columns were cut into appropriate length and sealed. To prevent sediment into the column, the bottom of PVC column was surrounded with fine quartz sand and covered by nylon net with 0.147 mm mesh-size. The upper part of PVC column was surrounded with dried clay powder to prevent water flow from the upper soil into the pores section (Tian et al., 2007). For the aim of collecting N leaching, another 0.003 m PVC pipe was inserted into bottom though the top of each PVC column. There are 0.3 m extra length above the ground of each column after incorporating into the soil. As soon as N fertilizer was applied, the percolation water was collected with a portable vacuum pump for the sampling frequency every other day for 1 week and then every 10 days. The percolation water samples were immediately taken to the laboratory and refrigerated at 4 °C after filtering. The total N, NH_4^+-N and NO_3^--N in the percolation water were later determined colorimetrically using a continuous-flow auto analyzer (Traacs 800, Bran & Luebbe, Hamburg).

To determine the leaching speed, we set up a plot with no fertilization input, which received the same filed management simultaneously with the other plots. The improved rapid response percolation water meter (Zhao et al., 2011) to measure the percolation rate in the rice season. In our study, the rate of vertical water percolation was 0.0025 m d^{-1} based on the three replication measurement, which was close to the result in the Taihu Lake region (Wang et al., 2010; Zhao et al., 2011).

The leaching volume (LV) (m^3 ha^{-1}) was: leaching speed (mm d^{-1}) × flooded periods (d) × $10^{-3} \times 10^4$, and then the cumulative N leaching was calculated as (Zhao et al., 2011):

$$\text{Cumulative N leaching}\quad(\text{kg N ha}^{-1}) = \text{TIN}\quad(\text{mg N L}^{-1}) \times \text{LV}\quad(\text{m}^3\,\text{ha}^{-1}) \times 10^{-3}$$

where TIN was the time – interval – weighted N concentration which was the sum of [individual N concentration × intervals of two adjacent time]/total growth time.

2.5. *Statistics*

Data were analyzed using statistical program SPSS 16.0 for the analysis of variance (ANOVA) for all parameters, followed by Duncan (New Multiple Range Method) to identify differences between the growth stages. Pearson correlation test was used to test for significant differences amongst the parameters. Path analysis is used to describe the directed dependencies among a set of variables, and in this paper was used for identifying components that contributed to higher yield (Norusis, 2008).

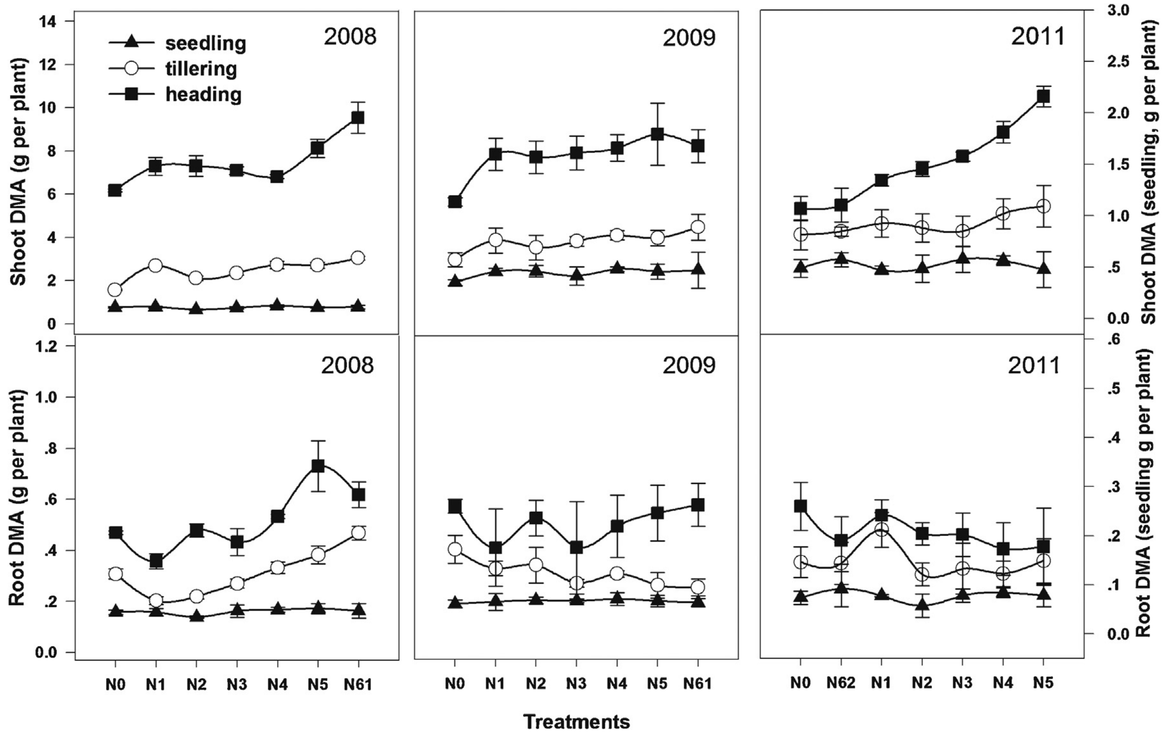

Fig. 2. Shoot and root day matter accumulation of rice at seedling (triangle), tillering (circle) and heading (square) periods under different N treatments N_0 to N_5: 0, 135, 189, 216, 243 and 270, respectively. The N application for $N_6{}^1$ was 405 kg N ha^{-1} in previous 2 years and $N_6{}^2$ was 81 kg N ha^{-1} in 2011. Error bars presented standard error.

3. Results

3.1. Plant dry matter, nitrogen content and nitrogen accumulation at rice critical stages

The dry matter accumulation (DMA) of the shoots and roots at different growth stages is shown in Fig. 2. There was no significant difference in shoot and root DMA among the treatments at the seedling stage. However, after the seedling growth stage, shoot and root DMA per plant were significantly affected by nitrogen (N) application rate. In the control, there was a significant lower shoot DMA compared to the other treatments at the heading stages. There was a large increase in shoot DMA between the tillering and heading stages. The increase in shoot biomass was 4.60, 4.60, 5.20, 4.75, 4.09, 5.42 and 6.51 g per plant, corresponding to N_0, N_1, N_2, N_3, N_4, N_5 and $N_6{}^1$, respectively, in 2008, while in 2009 and 2011, the average increase was 3.93 and 2.78 g per plant. Root DMA rose with increasing N application rate in 2008, while in the next 2 years the trend was not so evident. The treatment without N fertilizer showed a constrain effect on the aboveground DMA, while promoted the DMA below ground.

No significant difference was found in plant N concentration among N treatments at the seedling stage in these 3 years (Fig. 3). Generally, plant N concentration was significantly lower in the control than in the other N treatments after the seedling stage, and significant differences were also observed between lower (N_1) and higher N application (N_4 and N_5). A rapid decrease in N concentration was also found between the seedling and tillering stages in these 3 years. As the field experiment was newly set up in 2008, the original soil fertility may compromise the dilution effect on plant N concentrations at heading stages when compared with tillering stage. However, this effect was showed both in 2009 and 2011, as the lower slopes of N rates and plant N concentrations presented at heading stages (Fig. 3). After initiate of rice tillering, plant N concentration was positively affected by N rates, especially in heading stage in these 3 years ($R = 0.80$ in 2008; $R = 0.89$ in 2009; $R = 0.88$ in 2011; $p < 0.01$ $n = 21$).

There were significant correlations between N rates and N accumulation at the tillering stage, and stronger correlations at the heading stage ($R = 0.79$, in 2008; $R = 0.89$, in 2009; $R = 0.95$, in 2011; $p < 0.01$ $n = 21$) (Fig. 4). In general, the differences in NA were significant between the lower N (N_1, N_2 and N_3) and higher N (N_5) application rates after the seedling stage. Obviously, the NA at seedling stage only accounted for a small portion of that at rice harvest in these years. At the initiate of tillering, above ground NA increased considerably. With regard to the result in 2008, NA had increased 7.50, 10.3, 8.86, 10.1, 12.7, 11.1 and 12.7 times than that of seedling stage, corresponding to N_0, N_1, N_2, N_3, N_4, N_5 and $N_6{}^1$, respectively. Compared to tillering stage, NA of each treatment also experienced a rapid increase at heading stage, which were averaged 40.7, 60.9 and 60.4 kg N ha^{-1} and made up averaged 78.3%, 84.0% and 69.4% of total nitrogen uptake at harvest in 2008, 2009 and 2011, respectively. There were significant differences in NA between N_1 and N_5 application rate at harvest period in 3 years.

Additional N (AN) taken up by rice plant over the treatment without N input under different N treatments is shown in Table 1. Generally, no significant difference was observed in AN of all N treatments at seedling stage in these 3 years, and the AN was increased as the increment of N rates after the seedling stage. Notably, the continuous experiment made the indigenous N supply (present as N uptake in control) present a decrease trend, which was 99.2, 88.8 and 85.1 kg N ha^{-1} at the rice harvest for 2008, 2009 and 2011, respectively. Therefore, N fertilizer application gave greater contribution to the AN in the later years when compared to 2008.

3.2. Mineral nitrogen in soil and soil solution around root

In 2008, no significant differences were found in mineral N among the N application treatments. Mineral N in the soil was higher at the seedling and tillering stages, and the lowest at the

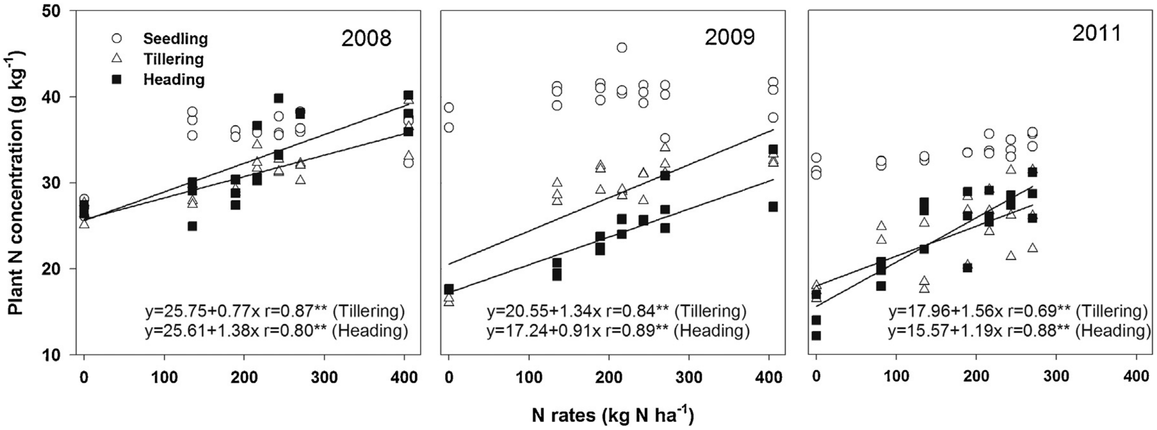

Fig. 3. Plant N concentrations at seedling (hollow circle), tillering (hollow up triangle) and heading (black square) stages under different N applications ($n = 21$).

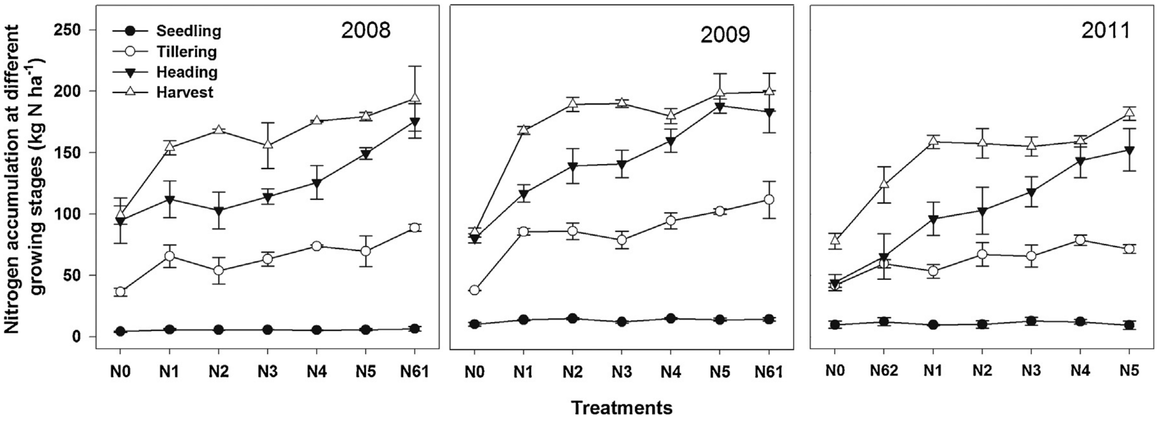

Fig. 4. Nitrogen accumulation aboveground at rice different growing stages. The symbols in figures represent for seedling stage (solid circle), tillering stage (hollow circle), heading stage (down solid triangle) and harvest stage (hollow up triangle). N_0 to N_5: 0, 135, 189, 216, 243 and 270 kg N ha^{-1}, respectively. The N application for $N_6{}^1$ was 405 kg N ha^{-1} in previous 2 years and $N_6{}^2$ was 81 kg N ha^{-1} in 2011. Error bars presented standard error.

harvest stage. Between the tillering and heading stage, a rapid decrease was observed in soil mineral N. The rates of decrease were 52%, 42%, 38%, 33%, 49%, 42% and 32%, from N_0 to $N_6{}^1$ in 2008 (Table 2).

For the results in 2009 and 2011 (Table 2), soil mineral N obtained the greatest value at the seedling stage, and then decreased with rice growing season, the content slightly increased at harvest period. And also, the soil mineral N varied annually. The average value for soil mineral N was 17.01 mg kg^{-1}, 12.08 mg kg^{-1}, 9.22 mg kg^{-1} and 9.37 mg kg^{-1}, respectively, from seedling to harvest stage in 2009. Compared to 2009, however, soil N was significant lower at seedling stage, but higher at later growing periods, especially at harvest stage. The average value for soil mineral N was 14.37 mg kg^{-1}, 11.75 mg kg^{-1}, 11.34 mg kg^{-1} and, 13.80 mg kg^{-1}, respectively, in 2011. $NH_4{}^+$-N was the dominated form, making up 98.6%, 96.5%, 100% and 55.6% in the soil mineral N at the seedling, tillering, heading and harvest stages, respectively, in 2009; and in 2011, the percentage of $NH_4{}^+$-N was 100%, 98.1%, 98.4% and 60.2%, respectively.

Mineral N in the soil solution was higher at the seedling stage than at the tillering and heading stages and there were significant differences between lower (N_1, N_2 and N_3) and higher (N_4, N_5 and $N_6{}^1$) N application rates at the seedling stage in 2009, however, the differences were weaker in 2011 (Table 3). Again, mineral N in the soil solution was also predominated in the form of $NH_4{}^+$-N, which made up 89.8%, 79.7% and 84.0%, respectively, in the mineral N at the seedling, tillering and heading stages in 2009; and in 2011 the mineral N was completely in the form of $NH_4{}^+$-N.

Table 1
Additional N (AN) taken up by rice plant over control under different N treatments at rice critical growing stages in 2008, 2009 and 2011.

Treatment	Seedling (kg N ha^{-1})			Heading (kg N ha^{-1})			Harvest (kg N ha^{-1})		
	2008	2009	2011	2008	2009	2011	2008	2009	2011
Indigenous N supply	4.3 ± 0.5	11.6 ± 1.6	9.0 ± 1.6	94.6 ± 8.4	83.7 ± 3.8	63.9 ± 9.1	99.2 ± 7.3	88.8 ± 3.8	85.1 ± 6.5
N_1	1.5a	3.6a	-0.2a	17.5b	38.0d	52.0c	54.8a	75.5c	81.1b
N_2	1.2a	4.5a	0.3a	8.3b	61.1c	58.6c	68.5a	97.6ab	79.8b
N_3	1.4a	3.9a	2.8a	19.6b	62.9c	74.0bc	56.6a	98.4ab	77.4b
N_4	1.1a	4.7a	2.4a	31.1ab	82.5b	99.3ab	76.5a	87.7bc	81.5b
N_5	1.5a	3.7a	-0.4a	54.7a	111.8a	108.3a	80.1a	106.8 a	104.2a
Average	1.3	3.7	1.0	26.2	71.3	78.4	67.3	93.2	84.8

Means followed by different letters are significantly different at 0.05 probability level according to Duncan's New Multiple Range Test.

Table 2
Mineral N in soil at rice critical growing periods in 2008, 2009 and 2011.

Treatment	Mineral N in soil (mg kg^{-1}) of different years			
	Seedling	Tillering	Heading	Harvest
Mineral N in soil (mg kg^{-1}) of 2008				
N_0	59.43 ± 6.01b (25.9%)	41.31 ± 5.23c (13.7%)	19.81 ± 2.32c (34.3%)	24.53 ± 2.4b (44.5%)
N_1	69.51 ± 6.50ab (17.7%)	50.29 ± 5.78bc (30.3%)	29.33 ± 3.17b (49.1%)	25.58 ± 2.29b (41.7%)
N_2	57.66 ± 6.78b (20.3%)	52.82 ± 5.16b (23.5%)	32.86 ± 2.98b (45.0%)	26.31 ± 2.84b (47.6%)
N_3	60.94 ± 5.34b (9.40%)	56.51 ± 5.45ab (30.8%)	37.62 ± 4.12ab (40.8%)	25.77 ± 0.99b (45.1%)
N_4	73.79 ± 4.71a (20.3%)	64.49 ± 4.92a (25.9%)	33.10 ± 3.01ab (35.3%)	22.53 ± 2.87b (36.2%)
N_5	58.33 ± 5.05b (29.7%)	55.89 ± 4.39ab (30.4%)	36.16 ± 2.64b (20.0%)	27.40 ± 0.94b (40.5%)
$N_6{}^1$	72.01 ± 5.50a (9.50%)	56.84 ± 5.86ab (9.40%)	38.58 ± 2.58a (32.9%)	33.37 ± 2.49a (40.4%)
Average	55.04	54.02	32.49	26.50
Mineral N in soil (mg kg^{-1}) of 2009				
N_0	11.37 ± 1.41b (94.9%)	11.19 ± 1.27c (96.4%)	7.35 ± 0.88c (100%)	9.05 ± 0.89b (77.6%)
N_1	17.76 ± 0.48a (95.5%)	10.48 ± 0.78c (97.2%)	7.50 ± 0.49bc (100%)	9.88 ± 1.47ab (64.8%)
N_2	18.34 ± 0.76a (96.8%)	15.00 ± 0.85a (96.0%)	8.55 ± 0.45c (100%)	8.82 ± 0.83b (50.5%)
N_3	17.78 ± 0.83a (97.7%)	14.00 ± 0.13b (97.6%)	8.23 ± 0.31bc (100%)	9.37 ± 0.72ab (50.0%)
N_4	17.91 ± 1.30a (97.5%)	10.34 ± 0.58c (94.7%)	9.52 ± 0.67b (100%)	9.12 ± 1.24ab (50.8%)
N_5	18.92 ± 0.95a (98.0%)	11.48 ± 1.43c (98.6%)	14.17 ± 0.17a (100%)	9.98 ± 1.42ab (50.6%)
$N_6{}^1$	19.82 ± 1.74a (97.3%)	9.60 ± 0.85c (95.3%)	13.68 ± 1.12a (100%)	10.50 ± 0.54a (45.1%)
Average	17.01	12.08	9.22	9.37
Mineral N in soil (mg kg^{-1}) of 2011				
N_0	10.50 ± 0.61c (100%)	11.87 ± 1.72ab (100%)	8.94 ± 2.61b (97.7%)	12.52 ± 3.49a (64.6%)
$N_6{}^2$	12.50 ± 1.91c (100%)	11.92 ± 3.22ab (100%)	8.61 ± 0.71b (97.8%)	14.85 ± 4.41a (71.5%)
N_1	13.27 ± 2.89ab (100%)	12.13 ± 0.51ab (100%)	9.09 ± 1.31b (100%)	14.54 ± 3.70a (57.5%)
N_2	13.37 ± 1.70bc (100%)	9.93 ± 3.06b (96.1%)	11.53 ± 2.09b (100%)	14.57 ± 0.70a (53.1%)
N_3	13.80 ± 0.30abc (100%)	11.71 ± 2.11ab (96.4%)	9.04 ± 0.46b (97.6%)	14.21 ± 2.15a (52.3%)
N_4	13.27 ± 2.58bc (100%)	9.61 ± 3.49b (97.0%)	11.23 ± 1.18b (97.4%)	11.92 ± 0.25a (61.0%)
N_5	17.03 ± 1.80a (100%)	15.22 ± 1.90a (96.9%)	18.19 ± 2.22a (98.6%)	15.09 ± 2.21a (61.5%)
Average	13.94	11.75	11.34	13.81

Values in the parentheses were percentage of $NH_4{}^+$-N in total mineral N. Means followed by different letters are significantly different at 0.05 probability level according to Duncan's New Multiple Range Test. N_0 to N_5: 0, 135, 189, 216, 243 and 270 kg N ha^{-1}, respectively. The N application for $N_6{}^1$ was 405 kg N ha^{-1} in previous 2 years and $N_6{}^2$ was 81 kg N ha^{-1} in 2011.

3.3. *Total nitrogen, nitrate, and ammonia in percolation water*

For all treatments, the total N and $NH_4{}^+$ in percolation water all peaked at the 1st after seedling fertilization and deceased rapidly, and the next two topdressings (tillering and heading) had little impact on total N and $NH_4{}^+$ concentration in percolation water. However, the $NO_3{}^-$ in percolation water remained at a low level at the seedling and tillering fertilization period, and for the heading dressing, the $NO_3{}^-$ presented relatively higher value and a fluctuant trend. Furthermore, the N fertilizer rates significantly increased the total N in percolation water ($p < 0.05$, $n = 7$), and the averaged concentration throughout the rice growing season was 4.38, 3.85, 3.36, 2.95, 2.96, 3.27 and 2.52 mg L^{-1}, respectively, from the highest N application to the control. However, the positive relationship between the N rates and the total N in percolation water was not observed at the later topdressing. Notably, at the 3rd day after seedling fertilization the total N decreased rapidly, by the rate of 38.3%, 72.4%, 73.9%, 67.1%, 58.9%, 51.5% and 78.3% for N_5, N_4, N_3, N_2, N_1, $N_6{}^2$ and N_0, respectively. The total N, $NH_4{}^+$ and $NO_3{}^-$ in the percolation water varied between 1.33 and 13.57 mg L^{-1}, 0.46 and 3.57 mg L^{-1} and 0.00 and 0.81 mg L^{-1}, respectively. The mean concentrations of total N, $NH_4{}^+$ and $NO_3{}^-$ in percolation water were 3.33, 1.41 and 0.14 mg L^{-1}, which indicated $NO_3{}^-$ was only 10% of $NH_4{}^+$. The total N was occupied by the dissolved organic N (deduct the $NH_4{}^+$ and $NO_3{}^-$ from the total N), averaged 53% in the percolation water.

Table 3
Mineral N in soil solution around root at rice critical growing periods in 2009 and 2011.

Treatment	Seedling	Tillering	Heading
Mineral N in soil solution (mg L^{-1}) of 2009			
N_0	1.59 ± 0.19d (88.7%)	0.95 ± 0.13a (81.1%)	0.38 ± 0.12c (76.3%)
N_1	2.82 ± 0.30cd (90.3%)	1.03 ± 0.09a (78.6%)	0.51 ± 0.13bc (86.3%)
N_2	3.84 ± 0.16c (94.5%)	0.92 ± 0.05a (76.1%)	0.43 ± 0.06bc (83.7%)
N_3	4.40 ± 0.64bc (95.2%)	0.90 ± 0.16a (77.8%)	0.37 ± 0.10c (83.8%)
N_4	6.30 ± 1.00ab (87.1%)	0.87 ± 0.08a (86.2%)	0.52 ± 0.13bc (76.9%)
N_5	6.65 ± 1.50ab (84.5%)	1.01 ± 0.31a (84.2%)	1.32 ± 1.13b (90.9%)
$N_6{}^1$	7.05 ± 1.61a (88.2%)	0.93 ± 0.13a (74.2%)	2.17 ± 0.25a (90.3%)
Mineral N in soil solution (mg L^{-1}) of 2011			
N_0	2.21 ± 0.75b (100%)	0.95 ± 0.13a (100%)	0.10 ± 0.04a (100%)
$N_6{}^2$	2.23 ± 0.28b (100%)	0.21 ± 0.04a (100%)	0.08 ± 0.00a (100%)
N_1	1.94 ± 0.15b (100%)	0.21 ± 0.04a (100%)	0.05 ± 0.02a (100%)
N_2	1.94 ± 0.20b (100%)	0.48 ± 0.12a (100%)	0.34 ± 0.38a (100%)
N_3	2.40 ± 0.41ab (100%)	0.55 ± 0.65a (100%)	0.11 ± 0.05a (100%)
N_4	2.04 ± 0.13b (100%)	0.40 ± 0.01a (100%)	0.11 ± 0.09a (100%)
N_5	3.49 ± 1.21a (100%)	0.51 ± 0.48a (100%)	0.26 ± 0.48a (100%)

Values in the parentheses were percentage of $NH_4{}^+$-N in total mineral N. Means followed by different letters are significantly different at 0.05 probability level according to Duncan's New Multiple Range Test. N_0 to N_5: 0, 135, 189, 216, 243 and 270 kg N ha^{-1}, respectively. The N application for $N_6{}^1$ was 405 kg N ha^{-1} in 2009 and $N_6{}^2$ was 81 kg N ha^{-1} in 2011.

Table 4
Cumulative leaching of total N, NH_4^+-N, NO_3^--N and dissolved organic N (DON) through percolation water in rice growing season in 2011.

Treatments	Total N (kg N ha^{-1})	NH_4^+-N (kg N ha^{-1})	NO_3^--N (kg N ha^{-1})	DON (kg N ha^{-1})
N_5	5.03 ± 0.78a	2.18 ± 0.34a (43.3%)	0.30 ± 0.20a (5.96%)	2.55 ± 0.33a (50.7%)
N_4	5.02 ± 1.03a	2.06 ± 0.72a (41.0%)	0.18 ± 0.11ab (3.59%)	2.78 ± 0.20a (55.4%)
N_3	4.38 ± 0.78ab	1.59 ± 0.2ab (36.3%)	0.23 ± 0.11a (5.25%)	2.56 ± 0.61ab (58.5%)
N_2	3.94 ± 0.97ab	1.86 ± 0.79a (47.2%)	0.09 ± 0.01ab (2.28%)	1.99 ± 0.47b (50.5%)
N_1	3.79 ± 0.37ab	1.85 ± 0.37a (51.5%)	0.15 ± 0.08ab (3.96%)	1.69 ± 0.11b (44.6%)
N_6^2	4.02 ± 0.29ab	1.69 ± 0.19ab (42.0%)	0.20 ± 0.10ab (4.98%)	2.13 ± 0.45ab (53.0%)
N_0	3.35 ± 0.45b	0.85 ± 0.16b (25.4%)	0.03 ± 0.00b (0.90%)	2.48 ± 0.40ab (73.7%)
Average	4.22	1.74 (41.0%)	0.17 (3.84%)	2.36 (55.2%)

Means followed by different letters are significantly different at 0.05 probability level according to Duncan's New Multiple Range Test. N_0 to N_5: 0. 135, 189, 216, 243 and 270 kg N ha^{-1}, respectively. The N application for N_6^2 was 81 kg N ha^{-1} in 2011. The number in the parentheses after NH_4^+, NO_3^- and DON denote the percentage of total N. DON was calculated from the total N deduction the NH_4^+-N and NO_3^--N.

Table 3 shows the cumulative leaching of total N, NH_4^+, NO_3^- and DON through percolation water in rice growing season in 2011. In general, the total N and NH_4^+ leaching through percolation water were increased with the increasing N rates, while NO_3^- and DON showed no response to N fertilizer rates. The significant differences were observed both in total N and NH_4^+ among control (N_0) and higher N application treatments (N_4 and N_5). Notably, DON taken a predominant proportion of the total N leaching, and the NH_4^+-N was larger in dominant than NO_3^--N, which were averaged 55.6%, 40.6% and 3.80%, respectively, for the three N forms. In the rice growing season, the total N leaching account for only 1.86–4.96% of N fertilizer input. Among the three N application stages, the seedling stage was predominant by 39.8% of total N leaching, secondly the tillering stage was dominant by 34.1%, and finally the heading stage was only 26.0%.

3.4. Grain yield, yield components and yield contributors

Generally, no significant differences in grain yield were observed among different N treatments (from N_1 to N_5) in these 3 years, respectively, even though the annual grain yields varied considerably (Table 5). Notably, N_6^1 (405 kg N ha^{-1}) presented a decline trend in previous 2 years, which indicated negative effect of overuse N application on grain yield. The theoretical yield (TY) showed a similar trend with the real grain yield. The number of panicles per m^2 (PM) and spikelet per m^2 (SM) were positively correlated with the augment of N rates for these 3 years, respectively ($p < 0.05$, $n = 7$). In contrast to the indexes above, the percentage of filled grains (PFG) and thousand grain weight (TGW) presented the opposite trend, which decreased with N increasing rates. The total day matter accumulations (TMDA) seem to have no response to the N rates among N applied treatments.

Results of the path analysis for yield and its components are shown in Table 6. For grain yield, spikelet per panicle showed the greatest positive direct contribution (0.781), and then the panicles per m^2 (0.522). Percent of filled grains (−0.240) and thousand grain weight (−0.518) all presented the negative direct contributions. The high positive direct contributions of panicle per m^2 (0.522) to the grain were masked by the negative indirect contributions via filled grains (−0.980) and thousand grain weight (−0.950). For the grain yield, the total indirect contributions were only positive in spikelet per panicle, and all negative for the other three yield components. The highest indirect contribution (0.675) was showed by percentage of filled grain via thousand grain weight.

Table 5
Grain yield and its components. Theoretical yield (TY), panicle per m^2 (PM), spikelet per panicle (SP), spikelet per m^2 (SM), percent of filled grains (PFG), thousand grain weight (TGW) and total dry matter accumulation (TDMA).

Year/treatment	TY (t ha^{-1})	Grain yield (t ha^{-1})	PM	SP	SM ($\times 10^3$)	PFG (%)	TGW (g)	TDMA
2008								
N_0	6.55c	6.09c	230d	105c	24.2c	97.4a	27.8a	11.15b
N_1	8.97a	8.21a	267c	135b	36.1b	95.9b	25.9b	15.00a
N_2	9.80a	8.43a	263c	163a	45.2b	88.5c	25.7b	14.78a
N_3	8.98a	8.49a	277c	140b	37.0b	90.7c	25.4bc	14.37a
N_4	9.61a	8.21a	311b	141b	46.9b	87.2c	25.0bc	14.48a
N_5	9.15a	8.67a	300b	154ab	46.1ab	81.7d	24.3c	14.77a
N_6^1	8.19b	7.22b	332a	161a	53.4a	61.1e	25.1bc	14.74a
2009								
N_0	6.28c	5.71b	240d	99c	23.8c	96.1a	27.5a	11.74b
N_1	8.77a	7.78a	302c	138a	41.7b	83.2b	25.3b	16.99a
N_2	8.06ab	7.55a	348b	124ab	43.1ab	75.3c	24.8bc	16.87a
N_3	8.99a	7.30a	380a	132a	50.2a	73.3d	24.4c	16.13a
N_4	8.29a	7.32a	348b	131a	45.6ab	74.5cd	24.4c	16.43a
N_5	8.09ab	7.42a	379a	116b	43.9ab	75.8c	24.3c	16.43a
N_6^1	7.54b	7.14a	368ab	135a	49.6a	62.8d	24.2c	17.04a
2011								
N_0	4.99b	4.61b	198b	112b	18.9b	97.2a	27.2a	7.47d
N_6^2	7.37a	6.76a	264a	119ab	30.9a	94.4b	25.3b	11.07cd
N_1	8.05a	7.69a	262a	132a	34.5a	92.1b	25.3b	12.57bcd
N_2	7.79a	7.72a	270a	125a	33.8a	91.5b	25.2b	12.73abcd
N_3	7.74a	6.79a	302a	124a	37.4a	82.5c	25.1b	13.97ab
N_4	7.79a	7.39a	307a	124a	38.1a	81.4c	25.1b	14.00a
N_5	7.87a	7.57a	299a	130a	38.9a	80.9c	25.0b	13.43abc

Means followed by different letters are significantly different at 0.05 probability level according to Duncan's New Multiple Range Test. N_0 to N_5: 0, 135, 189, 216, 243 and 270 kg N ha^{-1}, respectively. The N application for N_6^1 was 405 kg N ha^{-1} in previous 2 years and N_6^2 was 81 kg N ha^{-1} in 2011. Grain yield refer to air-dry weight.

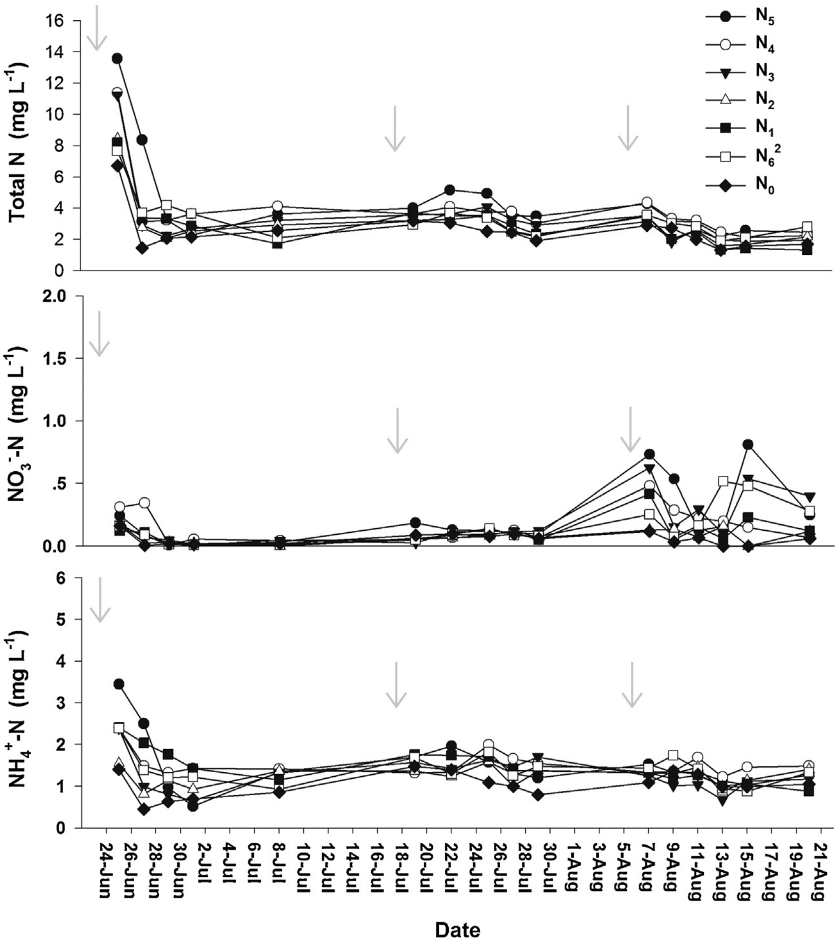

Fig. 5. Changes in total N, NH_4^+-N and NO_3^--N concentration of percolation water during the 2011 rice season. The gray arrow indicated the N application times at seedling, tillering and heading stages. N_0 to N_5: 0, 135, 189, 216, 243 and 270 kg N ha^{-1}, respectively. The N application for $N_6{}^2$ was 81 kg N ha^{-1} in 2011.

4. Discussion

4.1. Response of plant dry matter, plant N uptake, soil mineral N and yield components

Vamerali et al. (2003) found that low available mineral N in the soil improved the development of roots but restrained shoot development. This trend also occurred in the present study, especially in the treatment without N fertilizer. In the control, there was a lower shoot DMA, together with an increased averaged root length (data not shown), which may have absorbed nutrition deeper in the soil profile. However, the increased absorption does not seem to have led to increased shoot growth. In contrast to the results of sugarbeet found by Li and Shao (2003), this study found that there was not always an increase in shoot DMA with increasing application rate in this study. In the control, there was a lower shoot DMA compared to other treatments (Fig. 2). However, no differences were observed in plant N between any of the N application rates at the seedling stage (Fig. 3), which indicated that the lower N application rate (135 kg ha^{-1} in previous 2 years and 81 kg N ha^{-1} in 2011) could maintain normal rice growth during early growing stage in a high indigenous mineral N soil (53.5 mg kg^{-1}).

At the seedling stage, the N accumulation (NA) were no more than 12% of that at harvest in these 3 years (Fig. 4), because the plants were sampled only 10–15 days after transplanting and the nutrient absorption ability of the undeveloped roots would have been weak (Zheng et al., 2007). The fertilizer left in the soil (Yoshida and Padre, 1977) could either benefit the plants during later growth stages or run off and harm the surrounding environment during rainfall (Tian et al., 2007). When the root system and generative organs had developed, the plant showed a rapid accumulation in nutrients and dry matter at the vegetative stage (Fig. 2). Fan et al. (2010) and Zhang et al. (2007) found that the important period for N uptake was from the initiation of tillering to the end of the heading stage and this pattern was also seen in this study (Fig. 4).

Pillai and De (1979) and Wang et al. (2009a,b) found the availability of N for the plant is influenced by total N concentration in the paddy surface water and N fertilizer application rates. When total N concentration rose to 30 mg L^{-1} in the paddy surface water, the mineral N in the soil increased, together with rice plant absorption of mineral N from the soil (Yoshida and Padre, 1977). The papers on the same experiment have showed that the duration of higher N concentrations (over 30 mg L^{-1}) in paddy surface water increased with increasing N application rates (Xue et al., 2009; Qiao et al., 2012). Zheng et al. (2007) also found that soil mineral N around the roots increased with increasing N application rates after basal fertilizer applications. This indicated that more mineral N in the soil was released at higher N application rates and thus the potential available N may resulted in greater N accumulation in the plant (Fig. 4). However, in this study there was not always a significant increase in the soil mineral N around root with increasing N application rates (Tables 2 and 3).

A larger LAI and a lower light extinction coefficient were two important factors that led to greater rice photosynthesis, lower numbers of ineffective tillers and, consequently, an increased grain yield (Anzona et al., 2010). The tiller numbers also showed a significantly positive relationship with N application rates (0.899**, 0.822* and 0.949** in 2008, 2009 and 2011, respectively), and the

Table 6
Contributions of yield components to grain yield of the 3 years experiment.

Yield component	Direct contribution	Indirect contribution via X_i (i = 1,2,3,4)				Total contribution
		X_1	X_2	X_3	X_4	
$X_1(395 \pm 54.4)$	0.522	–	0.339	−0.980	−0.950	−1.068
$X_2(130 \pm 17.9)$	0.781	0.248	–	−0.324	−0.397	0.309
$X_3(84.0 \pm 10.6)$	−0.240	−0.790	−0.356	–	0.675	−0.711
$X_4(25.3 \pm 1.02)$	−0.518	−0.122	−0.070	0.108	–	−0.603

Yield attribute data were obtained from field experiments with seven nitrogen levels in 2008, 2009 and 2011. Data in parenthesis are mean ± SD, $n = 63$ n is sample size. X_1, X_2, X_3 and X_4 represent panicles per m^2 (PM), spikelet per panicle (SP), percent of filled grains (PFG) and thousand grain weight (TGW), respectively.

increase in panicle and spikelet numbers were also achieved by adjusting nitrogen application rates in this experiment, However, the increase in N application rates did not result in an increased grain yield at harvest (Table 5). Grigg et al. (2000) also demonstrated that a larger fertilizer application rate at the seedling stage resulted in a greater N uptake but did not result in a higher grain yield. From a morphological point of view, SM has been shown to be an indicator of high yield by Sheehy et al. (2001). The SM was influenced by tiller number and SP, thus an increase in tiller numbers and SP may lead to an increase in panicle and spikelet number and, potentially, final grain yield. In this experiment SP did show the greatest contribution to grain yield (Table 6). Wang et al. (2007) also found SP variation had more influence on yield than other yield components. Huang et al. (2011) found that PM played a critical role in increasing grain yield in super hybrid rice production. Furthermore, a low number of PM, the result of low tiller emergence and a low percentage of productive tillers, has, to date, been considered to be the main factor constraining grain yield (Yan et al., 2010). However, path analysis on the data produced in this study showed that the number of PM made a negative total indirect contribution to final yield (Table 6) and the greater number of PM in 2009 did not lead to a higher grain yield (Table 5). There are a number of reasons why this may have happened. Firstly, there was insufficient light utilization (Anzona et al., 2010) caused by lodging. With regard to 2009, severe lodging was observed in nearly all treatments, except for the control and N_1. The reduction in grain filling due to lodging resulted in a lower percentage of filled grains (PFG), which showed negative total contributions (−0.711) to the grain yield (Tables 5 and 6). Secondly, excessive N uptake and grain weight (−0.603, presented as total contribution to the yield) under high N application rates (N_5, N_6^{1}) prolonged the rice growing period, and plant N and dry matter that had accumulated remained in the straw at harvest time. Finally, the deterioration in soil mineral N (presented as the decreased indigenous N supply from 2008 to 2011, Table 1), may also account for the decrease in yield. Since SP showed the greater contributions to yield than other yield components, the N applied at the spikelet differentiation could enlarge the spikelet per panicle (Yoshida et al., 2006).

4.2. N leaching risk

In our study, variation of the total N and NH_4^+-N concentration in percolation water well responded to the split N fertilizer management, which increase with the augment of N application rates. However, N rates showed little impact on the NO_3^--N concentration at the early fertilization stage and the NO_3^--N concentration was all below 1.0 mg L^{-1} in percolation water (Fig. 5). Ji et al. (2011) also found a relative lower NO_3^--N concentration in another region. Usually, the paddy field was pre-flooded for rice seedling transplanting, the NO_3^--N concentration in this period was higher at the first few days (Wang et al., 2004), as the high concentration of mineral N (present as NO_3^--N) remained after wheat harvest (Aulakh et al., 2001). In this study, the NO_3^--N concentration was considered after rice transplanting, and the NO_3^--N leaching should be taken into account in the future during the field flooding process before rice transplanting. The fluctuant value in NO_3^--N after the heading topdressing was mainly because the midseason aeration increased soil NO_3^--N, which gave rise to the NO_3^--N leaching through the percolation water as the paddy field reflooded. The concentration of total N and NH_4^+ follow the same pattern with other researches (Tian et al., 2006; Wang et al., 2010).

In our study, the cumulative leaching of total N accounted for 1.86–4.96% of the applied N through the percolation water, which coincided with the research of Tian et al. (2006). The cumulative NO_3^--N leaching varied between 0.03 and 0.30 kg N ha^{-1} in the rice growing season, which consisted with the result of Xie et al. (2007), but was lower than other results found at paddy filed (Tian et al., 2007; Wang et al., 2004; Zhang et al., 2011). A consecutive 3-year experiment (Zhao et al., 2011) showed the NO_3^--N leaching varied largely among years, and Tian et al. (2007) proposed the variable NO_3^--N leaching may be influenced by the field condition and climate. Ji et al. (2011) also found the NO_3^--N leaching was influenced by field conditions, which affected the rate of vertical water percolation, in spite of the comparable NO_3^--N concentration in different soil types. The less cumulative total N leaching and low NO_3^--N concentration in percolation water indicated the N leaching in summer rice growing season was not the predominant way of N losses and should not be considered as a main pollution resource. The predominant form in total N leaching was neither NO_3^--N nor NH_4^+-N, but the dissolved organic N (averaged 55.7%, Table 4).

The additional N (AN) taken up by rice plant over control under all N treatments were low and not significant at seedling stage, and at harvest N rates showed little impact to the AN and yield (Tables 1 and 5). For the morphology view, the lower N rate could maintain the normal rice growth at the early stage (Figs. 2 and 4). Although the N leaching in rice growing season was not the predominant N losses, other ways as ammonia volatilization and runoff in higher N application rate increased the risk of N losses to the environment (Tian et al., 2007; Toshisuke et al., 2008; Zhao et al., 2012). Our results showed that the seedling stage presented the larger in dominant N leaching than other stages, despite the low loss through leaching in rice growing season. Thus an appropriate N reduction at rice early growing stage could decrease the risk of N losses. Furthermore, at the higher N application rates, grain filling was limited by low assimilation of N accumulation post anthesis (Qiao et al., 2012). It is therefore necessary to apply N at or after anthesis to increase DMA and final grain yields (Zhang et al., 2009a,b).

5. Conclusions

The 3 years experiment in paddy field showed that increasing N rates had little impacts on the soil mineral N around root, but enhanced the nitrogen accumulation in the rice plant. The dissolve organic N other than nitrate was dominant in the percolation water. Although the cumulative N leaching in summer rice growing season was not the predominant way of N losses, the relatively more N leaching happened at rice seedling stage. Considering the lower absorbing ability of rice seedling and more N leaching risk, N fertilizer reduction should be made in rice early growing stage. The path analysis showed spikelet per panicle made the greatest direct and total contribution to the grain yield.

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