Do high nitrogen use efficiency rice cultivars reduce nitrogen losses from paddy fields?

Gui Chen^{1,2,3}, Ying Chen⁴, Guohua Zhao⁵, Wangda Cheng³, Shiwei Guo⁶, Hailin Zhang⁷,

Weiming Shi^{1,*}

¹State Key Laboratory of Soil and Sustainable Agriculture, Institute of Soil Science, Chinese Academy of Sciences, Nanjing 210008, China

²Graduate School of Chinese Academy of Sciences, Beijing 100081, China

³Development of Agricultural Ecological Environment, Jiaxing Academy of Agricultural Science, Jiaxing 314016, China

⁴College of Forest Resources and Environment, Nanjing Forestry University, Nanjing 210037, China

⁵College of Biological, Chemical Sciences and Engineering, Jiaxing University, Jiaxing 314001, China

⁶Institute of Food Crops, Jiangsu High Quality Rice R & D Center, Jiangsu Academy of Agricultural Sciences, Nanjing 210014, China

⁷Department of Plant and Soil Science, Oklahoma State University, Stillwater, OK 74078, USA

***Corresponding author: Weiming Shi**

Tel: 86-25-86881566; Fax: 86-25-86881000; E-mail: wmshi@issas.ac.cn

Abstract

Several high nitrogen use efficiency (high-NUE) rice cultivars have been developed to meet the increased food demand and urgent environmental concerns, but the effect of these cultivars on reducing N losses from paddy fields is not well documented. A two-year field experiment was conducted in the Taihu Lake region of China to evaluate the advantages of high-NUE rice on yields and N losses in 2011 and 2012. Two “high-NUE” rice cultivars and a conventional cultivar were compared at the same fertilizer N input of 200 kg N ha⁻¹. Ammonia (NH₃) volatilization, N₂O emission, N leaching and runoff losses were monitored during the entire rice growing season within a rice-wheat rotation. The high-NUE rice cultivars Wuyunjing 23 (W23) and Zhendao 11 (Z11) achieved higher grain yields, and took up more N with a higher NUE than the conventional cultivar Wuyujing 3 (W3). There was a trend towards smaller N losses with the high-NUE cultivars W23 and Z11 (N leaching, N₂O emissions and NH₃ volatilization decreased by 12-23%, 5-10% and 2-8%, respectively), but the total reductions for the growing season compared to W3 were not statistically significant. Even though the high-NUE cultivars did not significantly decrease the absolute losses of N from the field, they did show lower N losses expressed on a yield-scaled basis than the conventional cultivar due to higher grain yields. Cultivars W23 and Z11 produced 21-27% less NH₃ volatilization, 23-26% less N₂O emission, 23-33% less N leaching, and 13-24% less N in runoff per ton of rice grain harvested than those of W3. Another cultivar Aoyusi 386 (A386), known to have a higher N uptake at early growth stages than W23 and Z11 was also planted in 2012. This cultivar significantly reduced N losses compared with the conventional

variety. It was concluded that improving N uptake rate of high-NUE cultivars in the early stages of growth would be most effective in decreasing N losses and that the main part of N fertilizer applications should be later in the growing season than is currently practiced in the region.

Keywords: high-NUE rice cultivars, N uptake, N losses, environmental pollution

1. Introduction

China is one of the major rice producers globally. Approximately, 26% of its total cultivated land is utilized for growing rice (Zhao et al., 2009). However, with the expansion of infrastructure, industrialization, and urban encroachment, the area of arable lands has shrunk rapidly in recent years. Meanwhile, the population is steadily increasing and living standards are expected to improve further. Hence, the demand for food, feed and fiber is expected to continuously increase in the future (Zhu and Chen, 2002). Many farmers in China apply more chemical nitrogen (N) fertilizer than needed to ensure high grain yields. It has been reported that grain production and fertilizer N consumption reached 502 million and 32.6 million tons nationally in 2007, increases of 54 and 191%, respectively, as compared with 1980, when fertilizer N production was 12.3 million nationally (Zhu and Chen, 2002; Guo et al., 2010). Over-applying N fertilizer in some regions has resulted in decreased N use efficiency (NUE) (Zhang et al., 2009; Qiao et al., 2012; Sun et al., 2012) and increased N

1 losses to water bodies through nitrate leaching and surface runoff and to the atmosphere
2 through ammonia (NH₃) volatilization and nitrous oxide (N₂O) emission (Vlek and Byrnes,
3 1986; De Datta and Buresh, 1989; Min et al., 2011; Min et al., 2012; Shang et al., 2013).
4 Those losses will eventually contribute to air pollution, climate change, biodiversity loss, and
5 eutrophication of water systems (Kronzucker et al., 2003; Shi et al., 2009; Shen et al., 2010;
6 Liu and Zhang, 2011; Qiao et al., 2012). In recent years, environmental pollution related to N
7 fertilizer applied to paddy fields has attracted great attention. As a result, many methods have
8 been proposed to reduce N losses, including N application models, site-specific N
9 management (SSNM), split application to match N needs of crops, and other water and
10 nitrogen management improvements (Pampolino et al., 2007; Wang et al., 2007; Ju et al.,
11 2009; Sun et al., 2012; Sun et al., 2013). However, some of these approaches are difficult for
12 farmers to implement, especially in China where farm sizes are very small. Farmers have
13 been following traditional farming practices, and have challenges in adapting modern
14 agricultural techniques.

15 Technological developments in rice breeding have allowed breeders to develop rice
16 cultivars classified as high-nitrogen use efficiency (high-NUE) because they produce higher
17 grain yields than traditional varieties with the same rate of N application (Moll et al., 1982;
18 De Macale and Velk, 2004; Zhang et al., 2009). Jiang et al. (2004) showed that high-NUE
19 rice cultivar Liangyoupeiiju (hybrid rice) produced 10-27% higher grain yield than low-NUE
20 rice cultivar Baguixiang (inbred rice) under the same N input level during a two-year field
21 experiment. Gueye and Becker (2011) also indicated that high-NUE rice cultivar Farox
22 239-3-3-2 (conventional *Japonica*) had 38% greater yield than low-NUE IR 31851

(conventional *Japonica*) under the same N application rate. Other studies have also demonstrated high-NUE rice cultivars responded well to N fertilizers and generated higher yields compared to conventional low-NUE cultivars (Ladha et al., 1998; Wang et al., 2004; Li et al., 2012). The cultivation of high-NUE rice cultivars is believed to offer significant potential for alleviating N losses to water bodies and the atmosphere whilst concurrently addressing the increasing demand for food (Guindo et al., 1994; Wu and Tao, 1995; Raun and Johnson, 1999; Glass et al., 2002; Lian et al., 2006; Shi et al., 2010).

The characteristics of high-NUE rice cultivars have been studied in recent years. Zhang et al. (2009) suggested that high-NUE rice cultivars (conventional *Japonica*) accumulated more N and exhibited higher physiologically-based NUE than low-NUE rice cultivars (conventional *Japonica*). Fan et al. (2010) indicated that high-NUE conventional *Japonica* rice cultivars possessed greater roots length and surface area compared with low-NUE rice cultivars (conventional *Japonica*) and a higher roots exudation rate indicating stronger roots. Shi et al. (2010) found that high-NUE rice cultivars (conventional *Japonica*) almost invariably showed higher N uptake efficiency than low-NUE varieties at the seedling stage in conditions of low N, which was mainly attributed to 20-30% higher activities of the N metabolic enzymes (GS and NADH-GOGAT) in the roots of high-NUE cultivars. Chen et al. (2013) demonstrated that the high-NUE was partially attributed to higher tolerance to ammonium toxicity. It is proposed that these attributes enable high-NUE rice cultivars to alleviate the negative environmental impacts of using urea or ammonium-based N fertilizers in paddy fields. However, this remains to be validated in the field.

We conducted a two-year field experiment near Yixing City in the Taihu Lake region, a

highly developed and densely populated region of southeast China. Nitrogen losses via NH_3 volatilization, N_2O emission and N losses through leaching and runoff from paddy fields cultivated with high- and low-NUE rice cultivars at the recommended N rate (200 kg N ha^{-1}), which was suggested by agronomists in view of balances between rice grain yield and environmental impacts in the Taihu Lake region (Zhu et al., 2010), were monitored. The objectives were to investigate whether high-NUE rice cultivars in the Taihu Lake region can play an effective role in alleviating N losses from paddy fields, and to identify the N loss pathways. The results of this study can provide useful information for understanding how high-NUE rice cultivars alleviate N losses in paddy fields and how to guide local farmers to implement best management practices.

2. Materials and methods

2.1. Experimental design

Field experiments were conducted in Yixing City, Jiangsu Province, China, in 2011 and 2012. Yixing is located in the Taihu Lake region ($30^\circ 5' - 32^\circ 8' \text{N}$, $119^\circ 8' - 121^\circ 5' \text{E}$) and is within the northern subtropical humid climatic zone. Yixing has an average annual temperature of 16°C and annual average rainfall of 1250 mm. The soil at the experimental site was a Gleyic-Stagnic Anthrosol developed on a lacustrine deposit. Summer rice and winter wheat double cropping is practiced in this region. Soil samples for site characterization

were collected from the top 20 cm of the soil profile before rice cultivation and showed the following analysis: organic carbon, 13.2 g kg⁻¹; total N, 1.45 g kg⁻¹; alkali-hydrolyzable N, 148.9 mg kg⁻¹; Olsen P, 27.4 mg kg⁻¹; NH₄OAC-extractable K, 39.3 mg kg⁻¹; and pH (1:1 soil to water), 5.4.

The experiment was conducted with a split-plot design with three rice cultivars as the main plots and two N application rates, namely 0 (CK) and 200 (recommended input) kg N ha⁻¹, as subplots (in triplicate). Each main plot was 60 m² in area and the subplots were 20 m². Each cultivar in the subplot contained approximately 480 hills of rice plant transplanted by hand.

Urea was applied as N fertilizer in the following proportions: 40% as basal fertilizer prior to transplanting, 30% as top-dressing fertilizer before the tillering stage, and another 30% as the second top-dressing fertilizer before the ear differentiation stage. Superphosphate (75 kg P₂O₅ ha⁻¹) and potassium chloride (130 kg K₂O ha⁻¹) were broadcast and incorporated into the plow layer before planting. The two top-dress fertilizations were surface-applied without incorporation. Mid-season aeration (water drained for approximately one week) was used to inhibit ineffective tillering and improve rice root growth. Final drainage was practiced for maturing and harvesting of rice. The floodwater was maintained in the plot at a depth of 3-5 cm during the rice season except for the mid-season aeration or final drainage periods.

2.2. Rice cultivars and experimental treatments

Three rice cultivars (conventional *Japonica*), namely Wuyunjing 23 (W23), Zhendao 11 (Z11), and Wuyujing 3 (W3), were selected in the 2011 rice season based on grain yield level

1 according to the China Rice Data Center (<http://www.ricedata.cn/variety/>), where W23, Z11
2 and W3 were approved in 2010, 2010 and 1992 by the Department of Agriculture Jiangsu
3 Province with grain yields of 659 kg ha⁻¹, 660 kg ha⁻¹ and 550 kg ha⁻¹, respectively. Cultivars
4 W23 and Z11 achieved higher grain yields with the same fertilizer N input, hence, were
5 classified as high-NUE cultivars. W3 had relatively lower grain yield, thus, was classified as
6 a low-NUE cultivar. The selected rice cultivars were all developed in the Taihu Lake region.
7 W3 was a traditional cultivar that had been cultivated widely for many years, while W23 and
8 Z11 were newly developed and not yet adopted by farmers. In addition to the three rice
9 cultivars described earlier, another rice cultivar (conventional *Japonica*), Aoyushi 386 (A386),
10 was added in the 2012 rice season. A386 was chosen by an earlier hydroponic experiment.
11 A386 accumulated 370 mg dry matter and 2.05 mg N in one plant after 25 days' hydroponic
12 cultivation, which was obviously larger than 316 mg and 1.54 mg, respectively for W23
13 (unpublished).

15 2.3. Sampling and measurements

17 Rice grain yield was determined by harvesting 4 m² from each subplot by hand. The grain
18 yield was adjusted to 140 g kg⁻¹ moisture content and expressed as t ha⁻¹ by conversion with
19 yield of 4 m². The above-ground rice plants of five representative hills in the inner rows were
20 collected at the elongation-heading and maturity stages. The plant samples were oven-dried at
21 75°C until a constant weight was reached for measurements of N uptake for the whole
22 growth season, before elongation-heading stage and after elongation-heading stage. Nitrogen

1 content of the above-ground biomass was determined using the Kjeldahl method.

2 Ammonia volatilization was measured by a continuous airflow enclosure method (Kissel et
3 al., 1977). A chamber was set in each subplot (20 m²). The volume of the chamber with
4 200 mm in diameter and 130 mm in height, could be adjusted by inserting the chamber into
5 soil or water at different depths. The rate of airflow was set to 15-20 times that of the
6 chamber volume per minute using a pump. Ammonia volatilization was measured once in the
7 morning (8:00 to 10:00) and once in the afternoon (14:00 to 16:00) immediately after each
8 urea application for about 1 week, until ammonia was no longer detected. Air was
9 continuously pumped for 2 h and allowed to flow through an NH₃ absorbent containing 2%
10 H₃BO₃ and mixed indicator comprising methyl red, bromocresol green and ethanol for each
11 measurement. This adsorbent solution was then titrated with a predetermined acid solution to
12 determine the amount of trapped ammonia. After the measurement, these chambers were
13 removed from the measurement area to prevent them from affecting the environmental
14 conditions. Total NH₃ volatilization flux was calculated as the sum of daily volatilization rates
15 divided by the period of time. This method of ammonia volatilization measurement is very
16 widely used in paddy field experiments in China (Tian et al., 2001; Zhao et al., 2009; Sun et
17 al., 2015).

18 The N₂O samples were usually collected using closed chambers (Terry et al., 1981), made
19 of plexiglass with the size of 50×50×60 or 50×50×120 cm depending on the crop height,
20 and the chambers were placed on a fixed PVC frame. Gas samples were collected in the
21 morning (8:00 to 10:00) with an open-end needle connected to evacuated vial bottles stopped
22 with a butyl rubber septa, and usually at an interval of 7 days but more frequently after

fertilizer application and during mid-season aeration as well as during the subsequent re-flooding periods during the rice season. Four gas samples were withdrawn from each chamber at an interval of 15 min. The gas samples were analyzed using a gas chromatographer (HP 5890 II, USA; analytical error $\pm 0.3\%$) equipped with a ^{63}Ni electron capture detector (ECD) and back flush controlled by a 10-port valve. The carrier gas was 95% argon and 5% methane at the flow rate of 30 mL min^{-1} and oven temperature at 85°C , inject temperature at 100°C , and detector temperature at 320°C . Compressed air was used as a standard gas with the value of 313 ppbv demarcated by the National Institute of Japanese Agricultural Environment. The variance coefficient for the standard samples was within 0.1-0.4% in 10 h (Xiong et al., 2002). N_2O flux was calculated by using a temporal increase in N_2O concentration in the air of the chamber with time. Average N_2O flux during the crop season is expressed as the average of their individual measurements weighted by measurement intervals. Cumulative N_2O emissions were calculated from average N_2O flux and observation days (Zhao et al., 2011; Min et al., 2012; Sun et al., 2015).

PVC tubes porous at one end were installed in each subplot at a depth of 80 cm to collect percolation water since the groundwater depth was about 1 m in this region (Zhu et al., 2000). The depth of 80 cm was below the rice root zone and N losses including ammonium ($\text{NH}_4^{+}\text{-N}$), nitrate ($\text{NO}_3^{-}\text{-N}$) and soluble organic N were at risk of leaching into groundwater in the Taihu Lake region (Zhao et al., 2012). The percolating water was pumped out several times with a hand pump before samples were taken. A continuous flow analyzer (Skalar Analytical B.V., the Netherlands) was utilized to determine total N concentration in the percolation water. Nitrogen leaching loss was calculated from the N concentration of each

sampling and the volume of percolation water over the study period. The rate of vertical percolation was 2 mm every 24 h determined with a slightly modified rapid-response percolation meter (Zhao et al., 2012).

The runoff water was sampled at the drainage outlet of each sub-plot whenever a drainage event occurred. An electromagnetic flow meter was placed at the water outlet to record water flow rate, which was used to calculate the runoff volume from each subplot. Runoff events occurred 7 times with 5 times by heavy rainfall and 2 times by water management in the field in 2011 and 5 times with 4 times by heavy rainfall and 1 times by water management in 2012. The total N concentration in the runoff water samples was also analyzed with a continuous flow analyzer after digestion (Skalar Analytical B.V., the Netherlands). Runoff N loss was calculated from N concentration and the volume of runoff water over the whole growing season.

2.4 Statistical analysis

The data were statistically analyzed with SPSS version 17.0. One-way analysis of variance with an LSD post hoc test was performed to determine the differences among the rice cultivars during the two-year field experiment. Significant differences at $p < 0.05$ are indicated by different letters. The figures were plotted with Sigma Plot version 10.0.

3. Results

3.1. Grain yields and total nitrogen uptake

The rice cultivars W23 and Z11 produced significantly higher grain yields than W3 in both 2011 and 2012 rice season at 200 kg N ha⁻¹. The two-year average yields of W23 and Z11 were 20 and 27% higher than that of W3, respectively (Table 1). This further confirms that the newly developed rice cultivars are definitely superior to the widely used older rice cultivar at the same N input. Cultivars W23 and Z11 accumulated 15-25% more N than W3 (mean of 2 years, Table 1), indicating that they had a high N use efficiency.

Table 1

3.2. Nitrogen use efficiency

As shown in Table 2, cultivars W23 and Z11 had significantly higher NUE than W3 in both years when expressed as kg grain per kg available N (applied fertilizer + available N from the soil). Furthermore, it was found that N uptake efficiencies (NupE) of W23 and Z11 were significantly higher than that of W3. However, differences in physiological N use efficiency (PE) for grain (PE-grain) or biomass (PE-bio) between W23, Z11, and W3 were not statistically significant, implying that only high NupE contributed to the higher NUE of W23 and Z11. In addition, W23 and Z11 showed obviously higher apparent crop recovery of fertilizer N than W3, which suggests the possibility of reduced N losses from the paddy fields planted with W23 or Z11.

Table 2

3.3. Ammonia volatilization

In our study, the N fertilizer was split into 3 applications. Ammonia volatilization mostly occurred within about 7 days after each fertilization event. As shown in Table 3, W23 and Z11 had significantly lower amounts of NH_3 volatilization than W3 at the second top-dressing in both years. However, total ammonia loss for the whole season did not differ significantly among the cultivars.

Table 3

3.4. Nitrous oxide emission, nitrogen leaching and runoff losses

Nitrous oxide emission, nitrogen leaching and runoff losses in 2011 and 2012 are presented in Table 4. The high NUE cultivars gave no significant decrease in N_2O emissions in either year.

Percolation water samples were collected at the depth of 80 cm, which was close but above the groundwater table at the study site. We also detected reduced N loss via leaching in the field with W23 and Z11 compared with W3. Although on average W23 and Z11 had 16% less N leaching loss than that of W3 during the two-year field experiment, they were not

significant (Table 4).

There were no consistent trends among W23, Z11 and W3 in N runoff loss (Table 4), indicating that high-NUE rice cultivars W23 and Z11 may have no effect on alleviating N losses via runoff.

Table 4

3.5. Nitrogen losses via NH_3 volatilization, N_2O emission, leaching and runoff on a unit grain basis

Table 5 shows the different N losses expressed on a yield-scaled basis (van Groeningen et al, 2010), i.e. as kg N lost per t grain produced. Because the high-NUE varieties W23 and Z11 gave higher grain yields than the conventional variety W3, the yield-based N losses are significantly smaller for the high-NUE varieties. In most cases, when expressed in this way, N losses from these varieties were about 20% less than with the conventional variety W3.

Table 5

3.6 Nitrogen losses from the field planted with A386 in 2012

In the 2012 rice season, we added a rice cultivar A386 which was characterized as having high N uptake capability at early growing stage to test whether it was more beneficial

1 compared to rice cultivar W3. A386 accumulated 32% more N than W3 before
2 elongation-heading stage (Table 6), which was more than the corresponding increases for
3 W23 and Z11 in 2012 (Table 1). A386 appeared to be superior to the other two high-NUE
4 cultivars in that the N losses from A386, expressed in absolute terms (kg N ha^{-1}), were
5 significantly smaller than from W3. Losses through NH_3 volatilization, N_2O emission, N
6 leaching and runoff were, respectively, 21, 22, 38 and 15% less than from W3 (Table 6).

8 Table 6

10 4. Discussion

12 4.1. High-NUE rice cultivars reducing N losses from paddy fields

14 Part of the fertilizer N applied to paddy fields is almost inevitably lost through a
15 combination of NH_3 volatilization, nitrification-denitrification, leaching and runoff (Freney et
16 al., 1990; Zhu and Chen, 2002). Unfortunately, there is still no direct method to quantify total
17 gaseous N losses ($\text{N}_2+\text{N}_2\text{O}$) associated with nitrification-denitrification in paddy fields (Zhao
18 et al., 2009). Because N_2O is a major greenhouse gas, approximately 310 times more potent
19 than CO_2 and state over about 120 years in the atmosphere (Pisante et al., 2010), we
20 monitored N_2O emission in addition to other N loss pathways.

21 In our study, high-NUE rice cultivars W23 and Z11 took up significantly more N than
22 low-NUE rice cultivar W3 during the rice season in both years (Table 1). Specifically, W23

1 and Z11 took up 7-12% more N than W3 before elongation-heading stage (Fig. 1), and
2 accumulated 30-62% more N from elongation-heading stage to maturity (Fig. 2) with the
3 same fertilizer N input. Zhang et al. (2009) also found similar outcomes from other
4 high-NUE rice cultivars (Wuyunjing 7 and Nanguang, conventional *Japonica*).

5 During the field experiments, W23 and Z11 showed a slight trend of decreased N losses
6 compared to W3 but because of the expected variability of field data the overall decreases
7 over the season were not statistically significant (Table 3 and Table 4). W23 and Z11 did
8 significantly reduced NH_3 volatilization after the second topdressing compared to W3 (Table
9 3). We found that W23 and Z11 had larger accumulation of leaf dry matter, leaf area and root
10 exudation rate than W3, and the leaf area showed a good relationship with NH_3 volatilization,
11 although other indexes also appeared negative correlation after the second topdressing
12 (unpublished). This suggests that the obviously lower NH_3 volatilization after the second
13 topdress in W23 and Z11 was probably due to larger leaf area of these cultivars. It has been
14 reported that more sunlight intensity could cause larger amount of NH_3 volatilization
15 (Sudhakara and Prasad, 1986; Tian et al., 2001). Therefore, larger leaf area could block more
16 sunlight to paddy water and then lead to less NH_3 volatilization. In addition, W23 and Z11
17 probably generated greater canopy boundary layer, which possibly reduce air movement and
18 mixing with 'ambient' air flow because they all affect NH_3 volatilization in paddy fields
19 (Jayaweera and Mikkelsen, 1991; Vlek and Craswell, 1981). A likely reason for the minor
20 impact of these varieties on N losses is that the period of increased N uptake by these
21 cultivars is not synchronized with the period of largest N losses. Although W23 and Z11
22 absorbed 7-12% more N than W3 before elongation-heading stage a large part of N losses

took place before this stage. Despite the failure of the two high-NUE cultivars to decrease N losses when expressed in absolute terms (i.e. as kg N ha⁻¹ lost from the paddy field), they can be regarded as beneficial for two reasons. First, they produced higher grain yields compared with the conventional variety for the same fertilizer N input (increases of at least 1 t ha⁻¹; Table 1). Second, N losses in absolute terms were equal to those from the conventional variety (in fact slightly less, though not statistically different), so the yield-scaled N losses (kg N lost t⁻¹ grain produced) were significantly smaller. Therefore, these cultivars use N inputs from fertilizer more efficiently and produce the same amount of grain with less land area.

The majority of NH₃ volatilization occurred within one week of N application. Similar observation has also been reported by other studies (Hayashi et al., 2006; Pacholski et al., 2006). The second topdressing took place about 10 days before elongation-heading stage. Therefore, most of NH₃ volatilization had been completed by the heading stage (Table 7). High N₂O fluxes were observed after fertilization and during the mid-season aeration; but very low fluxes were observed during the final drainage before the rice harvest in our study which is consistent with previous studies (Xu et al., 1997; Xing et al., 2002; Gupta et al., 2007). As shown in Table 7, 86% of N₂O was emitted from the paddy fields before the elongation-heading stage. This is because all 3 fertilizations and the mid-season aeration were far before the elongation-heading stage.

Methane (CH₄) is another important greenhouse gas emitted from paddy field. However, CH₄ and N₂O emission in paddy fields showed a trade-off effect (Cai et al., 1997). Studies showed that more than 90% of CH₄ fluxes to the atmosphere from paddy soils were through

the rice plant (Schutz et al., 1989; Aulakh et al., 2000). Other studies also indicated different amount of CH₄ emissions from paddy fields planted with different rice cultivars. Watanabe et al. (1995) reported rice cultivar did not influence the pattern of the seasonal variation in CH₄ emission rates, but the amount of CH₄ emitted differed among rice cultivars. The number of tillers, shoot length, shoot weight and root weight were not correlated with the CH₄ emission rates at the tillering and reproductive growth stages. Shao et al. (1997) suggested that fivefold differences of CH₄ emission could be reached among rice cultivars in paddy fields. Increased root exudation and greater litter production would increase CH₄ emission, but increased root mass largely growing in the oxidized soil layer could help mitigate CH₄ emission. In the present study, W23 and Z11 showed similar physiological traits such as root dry matter, root volume and root exudation rate, but relatively higher leaf area before elongation-heading stage (not shown). However, most of CH₄ emission happened before mid-aeration period and then decreased rapidly (Cai et al., 1997). Li et al. (2008) found high-NUE rice cultivar secreted more oxygen to the rhizosphere due to stronger aerenchyma organ, which would inhibit CH₄ production. Therefore, it needs to be further tested if high-NUE rice cultivars can inhibit CH₄ emission in paddy fields. The 3 cultivars had similar growing time (about 155 days) and irrigation managements. Therefore, differences in CH₄ emission among rice cultivars affected by irrigation were probably minimal.

Previous studies showed N leaching occurred more readily after fertilization events and at the early stages of rice growth due both to the large proportion of N fertilizer input and the relatively slow N uptake by plants at the early stages (Peng et al., 2011; Zhao et al., 2012). In our study, it was found that 71% of leaching loss occurred before elongation-heading stage

(Table 7). However, runoff N loss generally occurred during drainage and rainfall events. As reported, approximately 75 to 90% of the annual rainfall events occur during the rice season in the Taihu Lake region, indicating that rainfall is the main cause for N loss through runoff (Qiao et al., 2012). During our study, only one rainfall event resulted in runoff in 2011 and only a small amount of runoff was monitored in 2012. In addition, the N concentration in runoff water of each runoff event was very low. This may explain why N runoff loss from this experiment was not a significant pathway.

4.2. Strengthening management during earlier growth stage of rice

The variety A386, only tested in 2012, showed significantly greater N uptake than W3 before the elongation-heading stage (Table 6). It also showed significant reductions in N losses via NH_3 volatilization, N_2O emission, leaching and runoff compared with W3 (Table 6). Therefore a key target for crop breeders is the development of rice varieties with the ability to uptake more N at the early growth stage. Another key factor for reducing N losses from paddy fields is more rational timing of N fertilizer applications. Currently, too much N fertilizer is applied as basal fertilization especially in the Taihu Lake region, which is more than the N requirement at the early stage increasing the risk of N losses. A change in management to match N application with crop requirements needs to be promoted by advisory agencies. In addition, other measurements should be applied to improve fertilizer N use efficiency in paddy fields, especially during the period of earlier growth. For example, deep placement of urea, applying slow-release and controlled release N fertilizer would be

effective in minimize N losses (Cassman et al., 1995). Nitrification or urease inhibitors could also have effect on improving NUE. Sun et al. (2015) demonstrated that nitrification inhibitor increased rice yield by 10-17% under the same fertilizer N application rate of 180 kg N ha⁻¹, which indicated that nitrification inhibitor increased N use efficiency of rice. Ammonia volatilization in the paddy field was increased from 7.6-8.5% to 10.2-13.0%, although N₂O losses were reduced from 0.38-0.88% of fertilizer N to 0.19-0.44%. Soares et al. (2012) showed urease inhibitor delayed the peak of NH₃ losses and reduced NH₃ volatilization between 54% and 78% when compared with the control. Therefore, combining nitrification inhibitors with urease inhibitors would have much better effect on improving NUE for rice. However, it is hard for farmers to use additional fertilizer amendments without government subsidies. Therefore, much easy approaches that are practicable for farmers should be developed, recognizing their social and economic constraints as many farmers are also engaged in more profitable off-farm work such as high-NUE rice cultivars with earlier growth N uptake ability.

Fig.1 and Fig. 2

Table 7

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Tables

Table 1. Grain yield and nitrogen uptake by rice cultivars in 2011 and 2012 season

Rice season	Rice cultivar	Gain yield (t ha ⁻¹)	Total N uptake (kg ha ⁻¹)
2011	W3	7.41±0.50 c	151±7 c
	W23	8.93±0.38 ab	173±8 b
	Z11	8.87±0.93 b	179±7 ab
2012	W3	7.70±0.07 c	149±6 c
	W23	9.79±0.49 a	187±13 a
	Z11	9.58±0.13 ab	187±5 a

The data are presented as means ± SD of three replicates. The different letters (s) in the same column indicate a significant difference ($P<0.05$) among treatments.

Table 2. Nitrogen use efficiency (NUE) by rice cultivars in 2011 and 2012 season

Rice season	Rice cultivar	NUE (kg kg ⁻¹ N)	NupE (kg kg ⁻¹ N)	PE-bio (kg kg ⁻¹ N)	PE-grain (kg kg ⁻¹ N)	Apparent crop recovery of fertilizer N (%)
2011	W3	24.9±1.66 c	0.51±0.02 c	94.4±6.01 a	49.1±3.26 a	26.3±1.22 d
	W23	30.0±1.26 ab	0.58±0.03 b	96.8±8.02 a	51.7±3.89 a	33.3±1.26 c
	Z11	29.8±3.12 b	0.60±0.02 ab	97.2±2.02 a	49.6±3.85 a	32.8±2.76 c
2012	W3	25.9±0.25 c	0.50±0.02 c	97.8±6.32 a	51.6±1.54 a	39.4±2.43 b
	W23	32.9±1.63 a	0.63±0.04 a	101±4.94 a	52.5±3.46 a	49.4±3.56 a
	Z11	32.2±0.44 ab	0.63±0.02 a	97.7±5.11 a	51.3±0.62 a	50.1±2.15 a

NUE: grain yield (kg ha⁻¹) divided by the sum of avail-N (the sum of N in the forms of ammonium and nitrate) in the soil and N applied (kg ha⁻¹); NupE: accumulated N in the rice plant (kg ha⁻¹) divided by the sum of avail-N in the soil and N applied (kg ha⁻¹); PE-bio: rice biomass (kg ha⁻¹) divided by the total N-accumulated in the rice plant (kg ha⁻¹); PE-grain: grain yield (kg ha⁻¹) divided by the total N-accumulated in the rice plant (kg ha⁻¹). Apparent crop recovery of fertilizer N: the difference of N taken up by fertilized crop and N taken up by the control divided by fertilizer N. The data are presented as means ± SD of three replicates; the different letters (s) in the same column indicate a significant difference ($P<0.05$) among treatments.

Table 3 Ammonia (NH₃) volatilization from the paddy field in 2011 and 2012 season

Rice season	Rice cultivar	NH ₃ volatilization of three fertilizations (kg N ha ⁻¹)			The total (kg N ha ⁻¹)
		Base-fertilization	Topdressing-1	Topdressing-2	
	W3	13.4±2.2 a	7.34±1.57 b	3.38±0.36 ab	24.2±1.39 ab
2011	W23	12.8±1.7 a	7.06±0.29 b	2.30±0.54 c	22.2±1.10 b (8.26%)
	Z11	13.1±2.3 a	7.58±1.97 b	2.10±0.63 c	22.9±4.96 b (3.96%)
	W3	7.26±2.30 b	17.2±2.19 a	4.34±0.78 a	28.8±1.01 a
2012	W23	6.74±1.64 b	16.6±1.18 a	3.32±0.49 b	26.7±3.29 ab (7.45%)
	Z11	7.48±1.92 b	17.6±0.48 a	3.10±0.46 bc	28.2±2.40 a (2.07%)

The data are presented as means ± SD of three replicates. The values of NH₃ volatilization are equal to the difference between the N-treated and the control. The data in parentheses are reductions compared with W3. The different letters (s) in the same column indicate a significant difference ($P<0.05$) among treatments.

Table 4. Nitrogen leaching and runoff losses, and N₂O emission from the paddy field during 2011 and 2012 season

Rice season	Rice cultivar	N ₂ O emission (kg N ha ⁻¹)	Leaching N (kg Total-N ha ⁻¹)	Runoff N (kg Total-N ha ⁻¹)
	W3	0.46 ± 0.05 a	4.82 ± 0.61 ab	6.60 ± 0.90 a
2011	W23	0.43 ± 0.03 ab (7.18%)	4.17 ± 0.56 ab (13.5%)	6.08 ± 0.16 a
	Z11	0.42 ± 0.09 ab (9.99%)	3.71 ± 0.62 b (22.9%)	6.87 ± 0.71 a
	W3	0.37 ± 0.04 b	5.26 ± 0.64 a	4.16 ± 0.05 b
2012	W23	0.35 ± 0.02 b (5.54%)	4.46 ± 1.00 ab (15.3%)	4.24 ± 0.48 b
	Z11	0.35 ± 0.05 b (5.15%)	4.62 ± 0.58 ab (12.3%)	4.17 ± 0.33 b

The data are presented as means ± SD of three replicates. The values of N₂O emission, leaching N and runoff N are equal to the differences between the N-treated and the control. The data in parentheses are reduction compared with W3. The different letters (s) in the same column indicate a significant difference ($P < 0.05$) among treatments.

Table 5. Nitrogen losses on a unit rice grain basis from the paddy field in 2011 and 2012 season

Rice season	Rice cultivar	NH ₃ volatilization (kg N t ⁻¹)	N ₂ O emission (kg N t ⁻¹)	Leaching N (Total-N) (kg N t ⁻¹)	Runoff N (Total-N) (kg N t ⁻¹)
2011	W3	3.26 ± 0.19 b	0.062 ± 0.006 a	0.65 ± 0.10 a	0.89 ± 0.12 a
	W23	2.48 ± 0.12 d	0.048 ± 0.004 b	0.51 ± 0.04 b	0.68 ± 0.02 b
	Z11	2.57 ± 0.12 d	0.047 ± 0.010 bc	0.46 ± 0.06 b	0.77 ± 0.08 b
2012	W3	3.74 ± 0.13 a	0.048 ± 0.006 b	0.68 ± 0.08 a	0.54 ± 0.01 c
	W23	2.72 ± 0.33 cd	0.036 ± 0.002 c	0.46 ± 0.10 b	0.43 ± 0.05 c
	Z11	2.95 ± 0.25 bc	0.037 ± 0.005 c	0.48 ± 0.06 b	0.44 ± 0.03 c

The data are presented as means ± SD of three replicates. The values equal to amount of N loss divided by grain yield. The different letters (s) in the same column indicate a significant difference ($P < 0.05$) among treatments.

1 Table 6. Nitrogen uptake and N losses in paddy fields of W3 and A386 in the 2012 season

Rice season	Rice cultivar	N uptake before elongation-heading stage (kg ha ⁻¹)	NH ₃ volatilization (kg N ha ⁻¹)	N ₂ O emission (kg N ha ⁻¹)	Leaching N (kg Total-N ha ⁻¹)	Runoff N (kg Total-N ha ⁻¹)
2012	W3	99±4 b	28.8±1.0 a	0.37±0.04 a	5.26±0.64 a	4.16±0.05 a
	A386	131±10 a	22.9±2.3 b	0.29±0.02 b	3.27±0.69 b	3.52±0.24 b

2 The data are presented as means ± SD of three replicates. The different letters (s) in the
3 same column indicate a significant difference ($P<0.05$) among treatments.

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1 Table 7. Proportion of N loss before rice elongation-heading stage (%) of the total loss in
2 each rice season

Rice season	Rice cultivar	NH ₃ volatilization	N ₂ O emission	Leaching N (Total-N)	Runoff N (Total-N)
	W3	100	80.5	77.7	98.1
2011	W23	100	84.3	76.8	98.7
	Z11	100	80.9	75.3	98.6
	W3	100	89.7	62.7	99.6
2012	W23	100	91.1	67.4	99.5
	Z11	100	87.5	66.5	99.6
Overall average		100	85.7	71.1	99.6

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Figures

Fig. 1. Nitrogen uptake of rice cultivars before elongation-heading stage at the recommended N input in 2011 (a) and 2012 (b) season. The error bars represent standard deviation and different small letter indicates a significant difference ($P<0.05$) among treatments.

Fig. 2. Nitrogen uptake of rice cultivars after elongation-heading stage at the recommended N input in 2011 (a) and 2012 (b) season. The error bars represent standard deviation and different small letter indicates a significant difference ($P<0.05$) among treatments.

Fig. 1

Fig.2