



## Urea deep placement in combination with Azolla for reducing nitrogen loss and improving fertilizer nitrogen recovery in rice field



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### ABSTRACT

Reducing environmental harm and improving fertilizer use efficiency is critical in Chinese agriculture. Azolla has particular value for paddy rice crop due to its biological nitrogen (N) fixation ability; however, the Chinese rice fields are highly fertilized, which will depress the N fixation of Azolla. Urea deep placement (UDP) reduces floodwater NH<sub>4</sub><sup>+</sup> and ammonia volatilization losses. To date, few studies have explored the effects of UDP combined with Azolla on the N uptake pattern, N recovery and N loss from <sup>15</sup>N-labeled urea. Therefore, a 3-year field experiment was conducted with five treatments (control without urea (CK), urea broadcasting without Azolla (B) or with Azolla (BA), and UDP without Azolla (P) or with Azolla (PA)) in an intensive rice cropping system in the Taihu Region of China. The total <sup>15</sup>N recovery (soil-plant system), crop <sup>15</sup>N uptake (straw and grain) and <sup>15</sup>N loss were assessed, and the ammonia (NH<sub>3</sub>) loss, rice yield, gross return and net economic benefit were measured. The results showed that the UDP treatments had delayed but long-lasting fertilization effects. The PA treatment resulted in the lowest <sup>15</sup>N loss and highest total <sup>15</sup>N recovery at harvest due to the prolonged N availability and negligible NH<sub>3</sub> loss. The PA, P and BA treatments decreased <sup>15</sup>N loss by 47%, 33% and 18% and increased total <sup>15</sup>N recovery by 58%, 40% and 22%, respectively, compared to urea broadcasting alone. Almost all labeled Azolla-N was released within two months of which approximately 40% could be absorbed by the crop. Little labeled fertilizer N was absorbed by Azolla during the rice season. In addition, the PA, P and BA treatments increased rice yield and gross return by 13%, 10% and 9%, respectively, and they produced similar net economic benefit compared to urea broadcasting alone. In conclusion, UDP combined with Azolla is a potential strategy to further reduce fertilizer N loss and improve total fertilizer N recovery while maximizing rice yield if it can be widely adopted by Chinese rice farmers. However, widespread adoption will require improved technologies with lower labour requirement for mechanized deep placement of urea and for the propagation of Azolla.

### 1. Introduction

Rice is a staple food for more than 50% of the world's population, and nitrogen (N) fertilizer use is essential for enhancing rice production; however, the low efficiency of N fertilizer is characteristic of rice cropping systems (Fageria et al., 2010; Lassaletta et al., 2014; Zhang et al., 2015). It is estimated that only 20%–40% of the applied N is recovered by the rice crop (Schnier et al., 1990; Ladha et al., 2005). The poor utilization of N fertilizer by rice is attributed to a large N loss through ammonia (NH<sub>3</sub>) volatilization and denitrification (Zhao et al., 2012). Fertilizer N loss is estimated to range from 10% to 65% of the

applied N (Cassman et al., 1998; Zhao et al., 2012). In China, to pursue a further increase in crop productivity, the rates of N application continue to increase each year, and the fertilizer N is often 2-fold more than what is recovered by the crop. However, the overuse and misuse of N fertilizer may further endanger the environment (Galloway et al., 2008; Lassaletta et al., 2014). For example, the average N application rate in the Taihu Region reached 300 kg N ha<sup>-1</sup>, which is the highest among the rice growing regions (Hofmeier et al., 2015; Wu et al., 2015). However, the N loss has reached approximately 60% of the applied N and the recovery efficiency of fertilizer N (RE<sub>N</sub>) has been no more than 30%, but rice yield has already plateaued (7.5 t ha<sup>-1</sup>) in this region (Ju

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et al., 2009; Zhao et al., 2015). Only reducing the N application rate does not substantially reduce N loss and improve fertilizer N use efficiency (NUE) due to inappropriate methods and timing of the fertilizer application (Zhao et al., 2015). The traditional fertilization pattern is surface broadcasting and is highly dependent on industrially synthetic N fertilizer, which is inefficient for rice production. Therefore, better N management practices are urgently needed to avoid these problems.

Increasing the contribution of biological N fixation (BNF) rather than using industrially synthesized N fertilizer may result in the reduction of high N loss in rice fields due to the reduced  $\text{NH}_3$  volatilization, nitrate leaching and greenhouse gas emissions (Vlek et al., 1995; Ladha and Reddy, 2003; Lassaletta et al., 2014). Among the BNF systems, the water fern *Azolla* (a symbiotic *Azolla*-*Anabaena* association) deserves special attention due to its significant BNF with a range of 30–100 kg N ha<sup>-1</sup> crop<sup>-1</sup>, which is dependent on fertilizer N application rates (Singh and Singh, 1987; Mian, 2002; Cissé and Vlek, 2003; Subedi and Shrestha, 2015). *Azolla* had been used as biofertilizer or green manure in rice fields three decades ago as a cheap source of N in China and Vietnam and is considered a field-fertilizer-factory (Watanabe et al., 1977). At low N rates, *Azolla* grown as a dual crop along with rice can act as a physical barrier on floodwater, which may conserve N, reduce  $\text{NH}_3$  loss, and benefit the rice and long-term soil fertility (Ladha et al., 2000). However, synthetic fertilizer N has largely replaced *Azolla* since the 1960s (Cissé and Vlek, 2003), and current Chinese agriculture is highly fertilized, and a higher  $\text{NH}_4^+$  concentration in floodwater will increasingly depress both the growth and N fixation of *Azolla* (Maejima et al., 2001). Consequently, the development of economically feasible technology to increase the benefits of *Azolla* in intensive rice cropping systems is a major challenge.

To make full use of the benefits of *Azolla* in current intensive rice cropping systems, there is a possible opportunity to combine *Azolla* biofertilizer with urea deep placement (UDP) due to the characteristics of negligible floodwater  $\text{NH}_4^+$  under UDP (Mohanty et al., 1998). However, there is limited information on the use of *Azolla* in association with UDP for improving fertilizer N recovery and reducing fertilizer N loss (Roger et al., 1980; Watanabe et al., 1989; Sisworo et al., 1990). Therefore, a field experiment was conducted in the Taihu Region to assess the crop <sup>15</sup>N uptake pattern, <sup>15</sup>N recovery, and <sup>15</sup>N loss as affected by broadcast and deep-placed urea in combination with *Azolla*. In addition, the  $\text{NH}_3$  loss, rice yield, and net economic benefit (NEB) were quantified for broadcast and deep-placed urea when combined with *Azolla*.

## 2. Materials and methods

### 2.1. Experimental site

The field experiment was conducted at the Changshu Agroecosystem Experimental Station (31°15'15" N, 120°57'43" E), Chinese Academy of Sciences. The station is located in the Taihu Region, which is one of the five major rice growing regions in China. The climate in this region is classified as humid subtropical monsoon with an average annual precipitation of 1038 mm, a mean air temperature of 15.5 °C, and a frost-free period of 224 days. The soil is classified as Gleyi-Stagnic Anthrosol developed from lacustrine sediments with a silt clay loam texture (13.3% sand, 54.8% silt and 31.9% clay). The pH (H<sub>2</sub>O) of the topsoil (0–20 cm) was 7.35, and the soil contained 35 g kg<sup>-1</sup> organic matter, 2.09 g kg<sup>-1</sup> total N, 0.93 g kg<sup>-1</sup> total P, 121.3 mg kg<sup>-1</sup> available K and 17.7 cmol kg<sup>-1</sup> CEC. The mean precipitation and daily air temperature during the experimental period of 2014–2016 were shown in Fig. 1.

### 2.2. Experimental design

Field experiments were carried out over 3 consecutive rice growing seasons from 2014 to 2016. The five treatments were as follows: CK (a

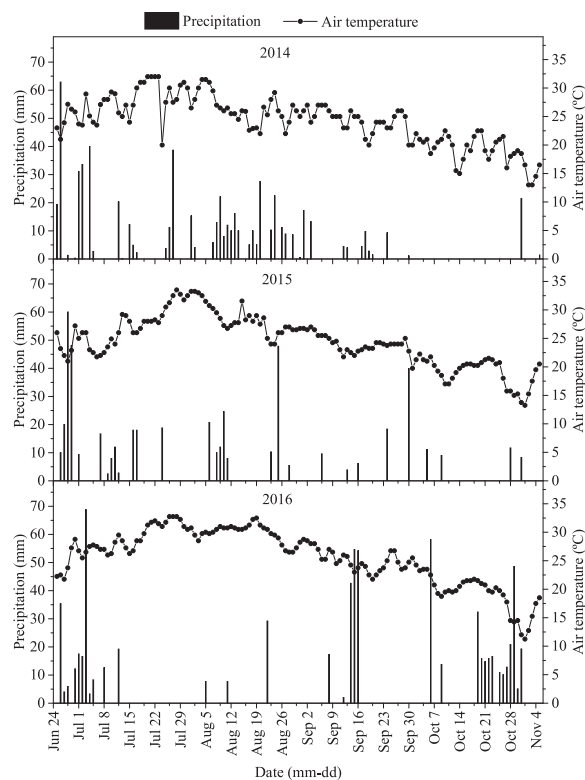


Fig. 1. Mean daily air temperature and precipitation during 2014–2016 rice seasons.

control with no urea), B (the recommended urea-N broadcasting, 225 kg N ha<sup>-1</sup>), BA (the recommended urea-N broadcasting combined with *Azolla*, 225 kg N ha<sup>-1</sup>), P (UDP, 225 kg N ha<sup>-1</sup>) and PA (UDP combined with *Azolla*, 225 kg N ha<sup>-1</sup>). The rates and timing of urea-N fertilizer (N, 46%) application for the five treatments were shown in Table 1. Limited by the labour for urea deep placement and *Azolla* application, we adopted an unbalanced split plot design (Montgomery, 2017). The BA, P and PA were designed as subplots (2 m × 2 m) distributed in B plot (B<sub>v</sub>) and they were used to determine the grain yield without any disturbance. To determine the <sup>15</sup>N uptake pattern with destructive sampling, another batch of BA, P and PA subplots (2 m × 2 m) were set up in another batch of B plot (B<sub>p</sub>). The CK, B<sub>v</sub> and B<sub>p</sub> were arranged in a randomized complete block with four replicates, with a dimension of 6 m × 6.7 m.

The subplots were bounded by polyvinyl chloride plastic frames (23 cm deep in soil and 10 cm above the soil) to prevent any runoff or run-on contamination of fertilizers. Water pipes were installed in the frames for irrigation.

The phosphorus fertilizer (90 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> as triple superphosphate) and potassium fertilizer (120 kg K<sub>2</sub>O ha<sup>-1</sup> as potassium chloride) were broadcast as basal fertilizers for all of the treatments. Urea was homogeneously broadcast onto the surface water as a basal application (40%), a tillering topdressing (20%) and a panicle topdressing (40%) on June 24, July 7 and August 14 for the B and BA plots (Table 1), respectively. For the P and PA plots, urea was one-time point deep-placed (10 cm soil depth, 5 cm away from the seedlings, 1.96 g urea hill<sup>-1</sup>) on June 24, using a steel-pipe tool. The rice seedlings (*Oryza sativa* L., cv. Nanjing 46, 30 days of age) were transplanted into well-puddled soils on June 24, with a spacing of 20 cm × 20 cm in all of the plots. Floodwater was continuously maintained at a depth of 3–5 cm by irrigation except during mid-season aeration (from July 23 to August 3) and final drainage (over 1 week) before rice harvesting. All of the treatments were applied to the same field management practices. Rice was harvested on November 5, 2014, November 5, 2015 and November 3, 2016.

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