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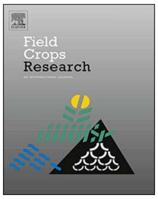

Azolla biofertilizer for improving low nitrogen use efficiency in an intensive rice cropping system

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ABSTRACT

The efficient use of nitrogen (N) in crop production is critical for meeting the challenges of food security and environmental integrity. Azolla biofertilizer may be a promising approach to achieve better N use efficiency (NUE) in paddy rice fields due to its great potential for biological N fixation (BNF). The objective of this study was to determine the efficacy of partially substituting Azolla biofertilizer for synthetic N fertilizer to improve NUE, reduce N loss and enhance rice yield in the currently highly fertilized rice cropping systems of China. Therefore, a 3-year field experiment was conducted with five treatments: CK (control without urea), FN (farmers' N practice), FNA (the farmers' N combined with Azolla biofertilizer), RN (reducing farmers' N by 25%) and RNA (substituting Azolla biofertilizer for 25% farmers' N). The NUE, ammonia (NH_3) volatilization, rice yield and net economic benefit (the difference between the value of the harvest grain and the costs of fertilizer and Azolla inputs) were assessed. The results showed that in the RNA and FNA treatments, Azolla biofertilizer produced higher recovery efficiency of fertilizer N by 69% and 59%, provided higher agronomic N use efficiency by 52% and 31% and achieved higher partial factor productivity of applied N by 43% and 13% than FN for the 3 years, respectively. In addition, the RNA and FNA treatments achieved crop ^{15}N recovery that was 64% and 49% higher than the FN treatment, respectively. The improved NUE under the Azolla biofertilizer treatments were attributed to reduced N loss and enhanced N uptake by rice plants. The RNA and FNA treatments significantly reduced ^{15}N loss by 48% and 26%, as well as lowered NH_3 loss by 42% and 12% over FN, respectively. In addition, Azolla could fix 52 and 44 kg N ha^{-1} crop^{-1} in the RNA and FNA treatments, and thereby, Azolla biofertilizer resulted in higher N uptake that was 17% and 33% higher in the RNA and FNA groups than in FN, respectively. As a result, the RNA and FNA treatments achieved higher rice yield by 8% and 14% over FN, respectively, but they attained similar and higher net economic benefit over FN for the 3 years. Therefore, substituting Azolla biofertilizer for 25% of urea-N provides a financially attractive option for farmers to substantially improve NUE and yield and effectively reduce N loss in intensive rice cropping systems.

1. Introduction

The low nitrogen use efficiency (NUE) in agriculture has led to more than half of the total reactive N (Nr) (60–100 Tg N yr^{-1}) being lost to the environment and has generated adverse environmental and human health impacts (Ladha et al., 2005; Galloway et al., 2008; Lassaletta et al., 2014). The Nr emission to the biosphere has already exceeded a proposed planetary boundary (Steffen et al., 2015). Crop production needs to be doubled by 2050 to meet global food demand. Accordingly, N fertilizer application is expected to increase by approximately threefold in the next 40 years (Tilman et al., 2002). However, it has been suggested that a further increase in N fertilizer application would lead to a disproportionately low increase in crop production with further environmental damage if the crop NUE is not substantially improved (Lassaletta et al., 2014). China has one of the lowest nationally averaged NUE values in the world (Zhang et al., 2015), and has already become the largest Nr emitter, and thus, has the most serious environmental N pollution worldwide (Ladha et al., 2005; Zhang et al.,

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2015). In 2015, the Chinese Ministry of Agriculture announced a "Zero Increase Action Plan" for national fertilizer use by 2020, with the aim of reducing the Nr losses while ensuring food security through improving the NUE (Liu et al., 2016). Therefore, improving crop NUE is crucial and is a huge challenge for Chinese agriculture (Fan et al., 2011; Xu et al., 2012).

It has been found that Azolla has great biological N fixation (BNF) ability (30–100 kg N ha^{-1} $crop^{-1}$) and hence it has particular value for paddy rice crops (Ito and Watanabe, 1985; Singh and Singh, 1987; Roy et al., 2016). In China, Azolla had been widely used as biofertilizer or green manure as a cheap source of N before the 1960s (Lumpkin, 1985). However, the application of synthetic N fertilizer has continuously increased and has largely replaced Azolla since the 1960s (Peoples et al., 1995; Cissé and Vlek, 2003a). As a result, the use of Azolla in rice cultivation has largely been discontinued completely in recent decades (Vlek et al., 2002). Earlier studies found that at low N rates, Azolla has great potential for increasing N recovery and rice yield (Singh et al., 1988; Manna and Singh, 1989). However, it is not clear so far that Azolla biofertilizer can improve NUE, rice yield and financial benefits in highly fertilized rice cropping systems, due to the increased inhibition of both growth and N fixation of Azolla under higher NH_4^+-N concentrations in growth medium (Kitoh and Shiomi, 1991; Maejima et al., 2001; Jampeetong et al., 2016).

The Taihu Region is one of the five major rice growing regions and is considered to be the most typical rice production area in China. This region covers 36,500 km^2 and 75% of its total land area is under rice cultivation (Zhao et al., 2012). The average N application rate in this region has reached 300 kg ha^{-1}, which is the highest among the rice growing regions (Wu et al., 2015). However, NUE has been low ($< 30\%$), rice yield has already plateaued, and the N loss has reached 52% of the total N input, and inevitably, the region has become a hotspot for air and surface water pollution (Ju et al., 2009; Gu et al., 2012; Zhao et al., 2012). Azolla as biofertilizer may be used as a partial substitute for synthetic fertilizer N due to its sustainable supplementation of N to rice crops and the associated improvement of soil fertility. However, a comprehensive assessment of the agronomic and environmental impacts of substituting Azolla biofertilizer for synthetic N fertilizer, which is the most important factor considered by farmers before adopting the practice, is lacking. Therefore, we conducted a 3-year field experiment in the Taihu Region of China with the aim of assessing the benefits of partially substituting Azolla biofertilizer for synthetic N fertilizer on crop NUE, rice yield, net economic benefit (NEB) and N loss, especially NH_3 volatilization.

2. Materials and methods

2.1. Experimental site

The field experiment was carried out 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 of China, which is in the center of the Yangtze River delta. The climate is classified as humid subtropical monsoon with a mean air temperature of 15.5 °C, an average annual precipitation of 1038 mm, and a frost-free period of 224 days. The soil is classified as Gleyi-Stagnic Anthrosol that is developed from lacustrine sediments with a silt clay loam texture (13.3% sand, 54.8% silt and 31.9% clay). The topsoil (0–20 cm) has a pH (H_2O) of 7.35, and contains 26.6 g kg^{-1} organic C, 2.09 g kg^{-1} total N, 0.93 g kg^{-1} total P, 121.3 mg kg^{-1} available K and 17.7 cmol kg^{-1} CEC. The precipitation and mean daily air temperature during the experimental period of 2014–2016 are shown in Fig. 1.

2.2. Experimental design

Field experiments were conducted during the rice growing seasons from 2014 to 2016. In this experiment, an unbalanced split plot design was adopted. The N rates (0, 225 and 300 kg N ha^{-1}) were arranged as the main plot and N sources (urea alone and urea combined with Azolla) were arranged as split plot. The five treatments were as follows: CK (a control with no urea), FN (the farmers' N practice, 300 kg urea-N ha^{-1}), FNA (the farmers' N practice combined with Azolla biofertilizer, 300 kg urea-N ha^{-1}), RN (a reduced urea-N dose of 25%, 225 kg urea-N ha^{-1}) and RNA (substituting Azolla biofertilizer for 25% urea-N, 225 kg N ha^{-1}). Each treatment had four replicates. The dimension of the main plots (CK, FN and RN) was 6 m × 7 m, and it was 2 m × 2 m for FNA and RNA plots. Each main plot was separated by 30 cm-wide earthen banks to prevent lateral water movement. The FNA and RNA plots were bounded by polyvinyl chloride plastic frames of 33 cm high. The frames were pressed 23 cm deep into the soil and protruded 10 cm above the soil to prevent any fertilizer runoff and lateral contamination. Water pipes were installed in the frames for irrigation.

For urea (N, 46%) application, 40% was basally applied (24 June), 20% was topdressed at tillering stage (7 July) and another 40% was topdressed at panicle initiation stage (14 August), which was homogeneously broadcasted onto the surface water. The P fertilizer (90 kg P_2O_5 ha^{-1} as triple superphosphate) and K fertilizer (120 kg K_2O ha^{-1} as potassium chloride) were broadcast as basal fertilizers for all treatments. All treatments were applied to the same rice cultivar (*Oryza sativa* L., cv. *Nanjing* 46) and field management practices. Rice seedlings (30 days of age) were transplanted into well-puddled soils at a spacing of 20 cm × 20 cm in all treatments. During each rice season, the flooding-drainage-reflooding-final drainage mode was adopted, floodwater was continuously maintained at a depth of 3–5 cm from 17 June to 27 July, then a subsequent drainage lasted for 7–10 days before reflooding from 4 August, and a final drainage was followed before 1–2 weeks of rice harvesting.

Azolla was pre-cultured in plastic boxes in the N-free nutrient solution (Watanabe et al., 1977). The water content of the Azolla was 97.3% and its total N content was 47.1 g kg^{-1} (dry weight). Azolla (*Azolla pinnata* R. Brown) was applied as a dual crop along with rice, with 3 t ha^{-1} (fresh weight), to the FNA and RNA plots one day before the basal fertilizer application.

2.2.1. The measurement of NUE, rice yield, NEB, BNF and NH_3 volatilization

Rice was harvested on 5 November, 2014, 5 November, 2015 and 3 November, 2016. A 3 m^2 sample area away from the plot boundaries in each plot was reserved and divided into straw and grain and then air-dried to determine the actual grain yield and straw biomass for each plot (Cao et al., 2013; Zhao et al., 2015). Grain and straw were oven-dried at 80 °C to a constant weight, and then powdered and passed through a 150-µm screen to determine the N concentration using the Kjeldahl method, and the N uptake was calculated based on the N concentration and the oven-dried weight. The NUE was calculated in terms of RE_N, PFP_N and AE_N (Ladha et al., 2005). The N surplus was calculated as the difference between total fertilizer N input and aboveground plant N uptake, and the NEB was the balance between the value of harvest grain and the costs of N, P, K fertilizers and Azolla inputs.

$$RE_N = \frac{N_{yield}\ in\ treatment - N_{yield}\ in\ CK}{N_{input}}$$

$$PFP_N = \frac{Yield}{N_{input}}$$

$$AE_N = \frac{Yield\ in\ treatment - Yield\ in\ CK}{N_{input}}$$

$$\text{N surplus} = \text{N}_{\text{input}} - \text{N}_{\text{yield}}$$

$$\text{NEB} = \text{P}_{\text{yield}} - \text{C}_{\text{input}}$$