Field Crops Research 214 (2017) 273–282

Contents lists available at ScienceDirect

Field Crops Research

journal homepage: www.elsevier.com/locate/fcr

Duckweed (*Spirodela polyrhiza*) as green manure for increasing yield and reducing nitrogen loss in rice production

Yuanlin Yao[a,b], Min Zhang[a,b], Yuhua Tian[a], Miao Zhao[c], Bowen Zhang[a,b], Meng Zhao[a,b], Ke Zeng[a], Bin Yin[a,*]

[a] State Key Laboratory of Soil and Sustainable Agriculture, Institute of Soil Science, Chinese Academy of Sciences, Nanjing 210008, China
[b] University of Chinese Academy of Sciences, Beijing 100049, China
[c] College of Resources and Environment, Chengdu University of Information Technology, Chengdu 610225, China

ARTICLE INFO

Keywords:
Green manure
Duckweed
N use efficiency
Ammonia volatilization
^{15}N recovery

ABSTRACT

Increasing rice production to feed the world's growing population while protecting the environment requires more optimal use of fertilizers. In China, the current high input, high output and high reliance on synthetic nitrogen (N) fertilizer in agriculture has resulted in high N losses, especially ammonia (NH_3) emission. Urea combined with green manure (GM) might be a promising approach to improve N fertilizer management. However, few studies have evaluated duckweed in this manner. Duckweed does not require arable land for cultivation and thus avoids competition with food crops. Therefore, a field experiment was conducted for three years with five treatments (CK, no N-fertilizer; CT, conventional practice, urea alone at 300 kg N ha^{-1}; CTD, urea combined with duckweed at 300 kg N ha^{-1}; RN, urea alone at 225 kg N ha^{-1}; and RND, urea combined with duckweed at 225 kg N ha^{-1}) in an intensive rice cropping system in the Taihu Region of China. The results for two years showed that urea combined with duckweed cover reduced NH_3 loss by 36–52% over CT. This reduction was attributed primarily to the formation of a physical barrier and the uptake of NH_4^+ by duckweed. The ^{15}N recovery for ^{15}N balance conducted for one year was 38% higher and the ^{15}N loss was 16% lower for CTD than that of CT. Furthermore, urea combined with duckweed increased N accumulation in the aboveground plants by 14–25% over CT for the 3 years. As a result, urea combined with duckweed achieved higher rice yield by 9–10%, and higher net economic benefit by 10–11% over CT for the 3 years; however, using the conventional rate of 300 kg N ha^{-1} did not increase rice yield over using the reduced N rate of 225 kg N ha^{-1}, with or without duckweed. Thus, duckweed as GM combined with chemical fertilizer application provided an approach for increasing the rice yield without increasing inputs of N fertilizer and thereby provided a financially attractive option for farmers to achieve environmental integrity and ensure food security in rice production.

1. Introduction

Globally, agriculture is currently facing unprecedented challenges for nourishing the increasing population without devastating the environment (Chen et al., 2014; Zhang et al., 2015). On the one hand, crop production needs to be doubled by 2050 to meet global food demand. On the other hand, anthropogenic reactive N (Nr) loss to the environment has already exceeded a proposed planetary boundary (Zhang et al., 2015). China is the largest N fertilizer producer and consumer in the world and plays a major role in global food security (Galloway et al., 2008). The annual application of N fertilizer reached 27 Tg yr^{-1} for crop production during 2001–2010 in China (Yan et al., 2014). Current Chinese agriculture is highly fertilized and depended on synthetic fertilizer, which results in high Nr emissions and adverse effects of soil fertility (Chen et al., 2016). Inevitably, the overuse and misuse of fertilizer makes China the world's largest Nr producer (Gu et al., 2015).

Ammonia (NH_3) emission is a major component among Nr loss and agriculture is the main source of NH_3 emission. The total health damage related to atmospheric Nr emissions accounted for US$19-62 billion in 2008 in China, and 52–60% were derived from NH_3 emission (Gu et al., 2012). NH_3 volatilization from agriculture represents the inefficient use of fertilizers and causes the formation of particulate matter, the eutrophication of aquatic systems, soil acidification, and indirect N_2O emission (Saggar et al., 2013). Therefore, improving N fertilizer management is crucial for feeding the growing population while reducing adverse environment and health impacts.

Paddy rice fields account for 33% of the total arable land in China,

* Corresponding author.
E-mail address: byin@issas.ac.cn (B. Yin).

http://dx.doi.org/10.1016/j.fcr.2017.09.021
Received 15 April 2017; Received in revised form 7 September 2017; Accepted 12 September 2017
0378-4290/

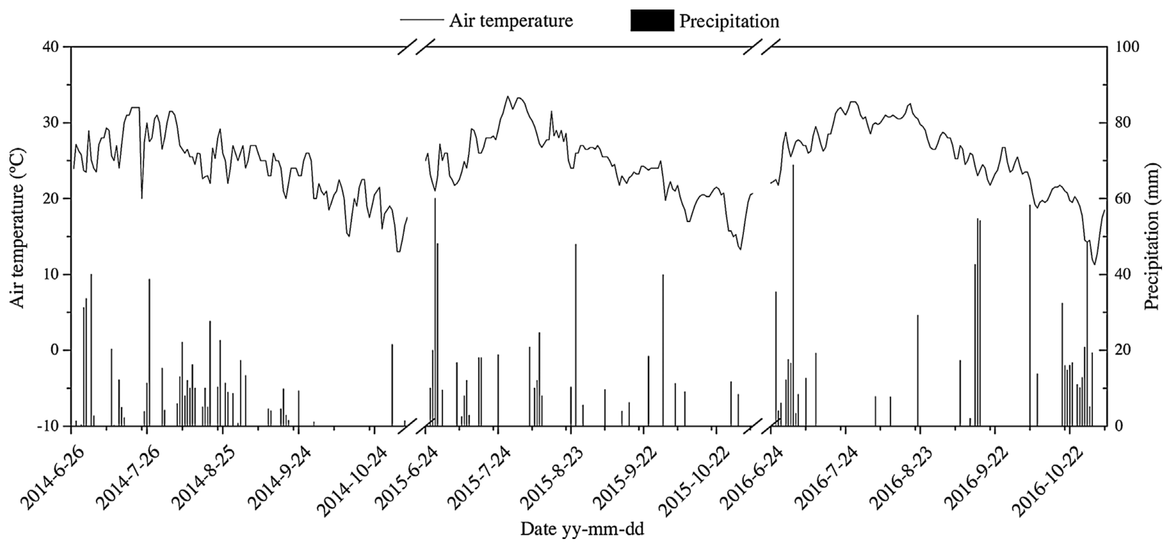

Fig. 1. Mean daily air temperature and precipitation during 2014–2016 rice seasons.

which occupy 29% of global rice output and supply food for more than 65% of the Chinese people (Zhao et al., 2015a). NH_3 volatilization from paddy fields is considered the major N loss pathway and is greater than from other crop systems (Zhu, 1997). The average NH_3 volatilization rate was 17% in Chinese rice fields (Wu et al., 2015). To make matters worse, grain yields in China have suffered from stagnation in 79% of the national rice area, but the consumption of N fertilizer will continue to increase by 25% in 2030 due to population growth and diet changes (Ray et al., 2012; Shen et al., 2013). Fortunately, 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 environmental damage costs while simultaneously increasing crop yields without a further increase of fertilizer use (Liu et al., 2016). Therefore, it is imperative to reduce NH_3 loss and improve rice yields without increasing N inputs. Many efforts have been made to achieve these goals such as applying site-specific N management, optimizing N application methods (increasing splitting frequency and adjusting the N rate and time), using enhanced efficiency of N fertilizers (controlled-release fertilizer and urease inhibitors) and combining synthetic N fertilizer with organic fertilizer or biochar. However, more labor or knowledge requirements or higher prices in the enhanced efficiency of N fertilizers have restricted the expansion of these approaches (Linquist et al., 2013; Chen et al., 2016).

The application of N chemical fertilizer in combination with green manure (GM) is an alternative approach to enhance crop production, N use efficiency (NUE) and soil fertility with less chemical inputs (Li et al., 2015). GM is generally eco-friendly, economically viable and renewable for sustainable agriculture.

Previous studies of GM are mainly focused on leguminous plants; however, very little information is available regarding duckweed as GM (the widespread floating aquatic plant), which includes 37 species in 5 genera (*Spirodela*, *Lemna*, *Landoltia*, *Wolffia* and *Wolffiela*) within the family Lemnaceae and with doubling times of 1.34–4.54 days and yield ranges between 8.8–117 t dry weight ha^{-1} $year^{-1}$ (Cheng et al., 2002; Forni and Tommasi, 2016).

Over the last 40 years, research regarding duckweed mainly focused on phytoremediation and nutrient recovery from wastewater and for animal feedstock and the production of biofuels, due to its high growth rate, high biomass yield, excellent nutrient uptake ability, and tolerance to high nutrient levels (Cheng et al., 2002; Mohedano et al., 2012). It is worth noting that duckweed can grow well in paddy fields (Wu et al., 2012); however, the performance of duckweed in paddy rice agriculture has been rarely studied. So far, few studies have examined the influence of duckweed on NH_3 volatilization (Zimmo et al., 2003; Li et al., 2009; Sun et al., 2015), and only Li et al. (2009) reported that duckweed cover combined with urea could effectively increase rice yield at 90 and 180 kg N ha^{-1}. Meanwhile, the current Chinese agriculture systems are highly fertilized, few studies have comprehensively accessed the agronomic and economic benefits and ^{15}N balance of urea combined with duckweed in current intensive rice cropping systems.

The Taihu Region is one of the five major rice growing regions in China and is considered to be the most typical rice production area in China because of a long rice cultivation history (> 7000 years) and is one of the most densely populated and intensive agricultural areas in China. This area is located in the middle and lower reaches of the Yangtze River paddy soil region of China (Zhao et al., 2012). This region covers 36,500 km^2 and 75% of its total land area is under rice cultivation. The average N application rate in this region reached 300 kg ha^{-1}, which is the highest among the rice growing regions (Hofmeier et al., 2015; Wu et al., 2015), but the rice yield has already plateaued (7.5 t ha^{-1}) (Ju et al., 2009; Zhao et al., 2015a). The NH_3 loss can be as high as 40% of the total applied N, due to the strong sunlight and high temperatures in the summer (Cai et al., 1988). Therefore, a field experiment was conducted for three years in the Taihu Region with the aims of assessing the benefits of urea combined with duckweed on NH_3 volatilization, crop yield and net economic benefit (NEB), and ^{15}N balance in paddy rice fields.

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, located in the Taihu Region of China. The rice-wheat crop rotation has been cultivated in this region for thousands of years. The climate is characterized by humid subtropical monsoon with an average air temperature of 15.5 °C and a mean annual precipitation of 1038 mm. The soil is classified as Gleyi-Stagnic Anthrosol developed from lacustrine sediments. The pH (H_2O) of the topsoil (0–20 cm) is 7.35, the soil contains 35 g kg^{-1} organic matter; 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 mean daily air temperature and precipitation during the experimental period from 2014 to 2016 are shown in Fig. 1.

Table 1
Date and rate of urea applications and water management regimes.

Code	N rate (kg N ha^{-1})	Duckweed (t fresh weight ha^{-1})	Basal fertilization (kg N ha^{-1})	First topdressing (kg N ha^{-1})	Second topdressing (kg N ha^{-1})
CK	0	0	0	0	0
CT	300	0	120	60	120
CTD	300	2	120	60	120
RN	225	0	90	45	90
RND	225	2	90	45	90
Application date			Basal fertilization	First topdressing	Second topdressing
2014			Jun 26	Jul 5	Aug 15
2015			Jun 24	Jul 7	Aug 14
2016			Jun 24	Jul 7	Aug 8
Water management			Pre-flooding irrigation	Mid-season aeration	Final drainage
2014			Jun 19	Jul 23–Aug 1	Oct 21
2015			Jun 17	Jul 20–Aug 3	Oct 20
2016			Jun 17	Jul 21–Aug 4	Oct 20

2.2. Experimental design

Field experiments were conducted for 3 consecutive rice cropping seasons from 2014 to 2016. The five treatments were CK (a control following local practice with no N-fertilizer without duckweed), CT (the current traditional practice without duckweed), CTD (the current traditional practice with duckweed cover), RN (a reduced N dose of 25% without duckweed) and RND (a reduced N dose of 25% with duckweed cover). The rates and timing of fertilizer application for the five treatments are shown in Table 1. The experimental design was an unbalanced split plot design with four blocks and with N rates (0, 225 and 300 kg N ha^{-1}) as the main plot; the N sources (urea alone and urea combined with duckweed) were used as subplots.

The dimension for the main plot was 6 m × 7 m, and it was 2 m × 2 m for the split plot (CTD and RND). Each main plot was separated by a 30 cm wide earthen ridge. The CTD and RND plots were bounded by polyvinyl chloride plastic frames. The frames were buried 23 cm deep and protruded 10 cm above the soil to prevent any runoff or run-on water from removing or adding N fertilizer. Water pipes were installed in the frames for irrigation. The rice seedlings (30 days of age) were transplanted into well-puddled soils at a spacing of 20 cm × 20 cm for all treatments. The rice cultivar was *Oryza sativa* L., cv. *Nanjing* 46. The synthetic fertilizers used were prilled urea (N, 46%), superphosphate (P_2O_5, 12%), and potassium chloride (K_2O, 60%). Pre-flooding irrigation for each plot was performed one week prior to rice transplanting. Floodwater was continuously maintained at a depth of 3–5 cm in all plots, except during the mid-season aeration and the final drainage before crop harvest (Table 1). All irrigation events were coordinated with precipitation events, and in the 2015 and 2016 rice seasons, the same irrigation practices were followed. Pesticide and herbicide applications were the same for each plot. Rice was harvested on November 5, 2014, November 5, 2015 and November 3, 2016.

As shown in Table 1, N fertilizer was applied as a basal application (40%), first topdressing (20%) and second topdressing (40%) in the CT, RN, CTD and RND plots, and the urea was homogeneously broadcast onto the surface water. P_2O_5-fertilizer (90 kg ha^{-1}) and K_2O-fertilizer (120 kg ha^{-1}) were broadcast as basal fertilizers in all treatments. The duckweed (*Spirodela polyrhiza* (L.) Schleid.) was collected from ponds near the experimental station and applied to CTD and RND plots as a dual crop along with rice before basal fertilizer application, with 200 g fresh weight m^{-2} (about 80% coverage of surface water), the water content of the duckweed was 97.1% and its total N content was 52.5 g kg^{-1} (dry weight).

2.2.1. Experiment I: the measurement of NH_3 volatilization, rice yield, crop N and net economic benefit

The NH_3 volatilization was monitored by a dynamic chamber method, which was composed of a dynamic chamber, a vacuum pump, and an acid solution to capture gaseous NH_3 (Cao et al., 2013; Zhao et al., 2015a). The cylindrical chamber was made from poly-methyl methacrylate, with an inner diameter of 20 cm and a height of 15 cm. Ambient air located at 2.5 m above the surface water was pumped to complement the inner air in the chamber. When collecting NH_3 volatilization, the chamber was inserted into the surface water and soil to a depth of approximately 10 cm, and the NH_3 in the chamber was then trapped in a glass bottle containing 60 ml of sulfuric acid solution (0.05 mol L^{-1}). The air flow rate through the chamber was set to 15–20 headspace min^{-1}. NH_3 volatilization was measured twice per day in the morning (7:00–9:00) and afternoon (15:00–17:00). The volatilization of NH_3 was continually measured until NH_3 was no longer detected. The concentration of NH_4^+-N in the acid trap was measured by the indophenol blue method (Novozamsky et al., 1974). The cumulative NH_3 volatilization flux was the sum of the NH_3 volatilization fluxes on sampling days. The surface floodwater of each plot was collected and filtered when the NH_3 volatilization was sampled. Floodwater NH_4^+-N concentration, NO_3^--N concentration and pH were measured by the indophenol blue method, the ultraviolet spectrophotometer method (HJ/T 346–2007) and a portable pH meter (Germany Sartorius PB-10), respectively.

At crop maturity, above-ground plant biomass was harvested from a 3 m^2 sample area away from the plot boundaries in all of the plots. Straw and grain were separated, air-dried, and weighed to calculate the dry matter yield (Cao et al., 2013; Zhao et al., 2015a). Grain and straw were oven-dried at 80 °C for 24 h, and then powdered by a grinder and passed through a 150-μm screen to determine the N concentration by the Kjeldahl method. The crop N accumulation was calculated depending on the N concentration and the oven-dried weight. The net economic benefit (NEB) was calculated as the difference between the value of the harvest grain and costs of N, P and K fertilizers and duckweed inputs. The NUE in terms of recovery efficiency of N fertilizer (RE_N) and agronomic N use efficiency (AE_N) and the N surplus were calculated only for CT and RN treatments (Ladha et al., 2005).

$$NEB = P_{yield} - C_{input}$$

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

$$AE_N = \frac{\text{Grain yield in fertilized treatment} - \text{Grain yield in CK}}{N_{input}}$$

$$\text{N surplus} = N_{input} - (N_{yield} \text{ in fertilized treatment} - N_{yield} \text{ in CK})$$

where P_{yield} is value of the grain (3 ¥ kg^{-1} rice grain); and C_{input} is input cost (4 ¥ kg^{-1} urea-N; 3 ¥ kg^{-1} P_2O_5; 4 ¥ kg^{-1} K_2O; 400 ¥ ha^{-1} $time^{-1}$ for fertilizer broadcast application, 800 ¥ ha^{-1} $time^{-1}$ for duckweed collection and application) (Xia et al., 2016); N_{input} is total fertilizer N input; N_{yield} is crop N accumulation.

2.2.2. Experiment II: the ^{15}N balance under duckweed cover

Microplots were established in the CT and CTD plots during the 2014 rice season. The polyvinyl chloride plastic columns were inserted to a soil depth of 23 cm and protruded 10 cm above the soil; the inner diameter of the columns was 60 cm with four rice hills. The ^{15}N-labeled urea (^{15}N abundance was 10%) was provided by the Shanghai Research Institute of Chemical Industry and was applied at the same times and rates as the urea in the larger plots. The rice plant, duckweed and soil samples were collected after crop maturation. The duckweed and all rice roots from the 0–20 cm soil layer of each microplot were collected and washed, and the rice plant samples were divided into straw, root and grain. Four soil cores from 0–5 and 5–20 cm were collected from each microplot using a steel auger and mixed well into a single soil sample. Straw, root, grain, duckweed and soil were oven-dried for 24 h at 80 °C, and then powdered and passed through a 150-μm screen to determine the N concentration by the Kjeldahl method. The ^{15}N abundance was analyzed using an isotope ratio mass spectrometer (Flash EA Delta V Advantage, Thermo Fisher Scientific Inc., Waltham, MA, USA).

All ^{15}N was expressed as the atom percent excess and corrected for the background abundance (i.e., 0.366%). The percentage of ^{15}N recovery were calculated according to Hauck and Bremner (1976).

$$^{15}\text{N recovery (\%)} = \frac{B - A}{C - A} \times 100$$

where A is the natural abundance of ^{15}N; B is the ^{15}N atom percent in the plant or soil; and C is the ^{15}N atom percent in the fertilizer N.

2.3. Data analysis

Before conducting the statistical analysis, all of the data were tested for normality using the Shapiro-Wilks test and homogeneity of variances using Bartlett's test. Differences in the main effects of years (2014, 2015 and 2016), treatments (CK, CT, CTD, RN and RND), N rates (0, 225 and 300 kg N ha^{-1}), N sources (urea alone and urea combined with duckweed) and block, and their interactions were tested using general linear model in SPSS 19.0 (SPSS China, Beijing, China). In the model, the years, treatments, N rates and N sources were fixed factors, and the block was the random factor; and Duncan method was applied for multiple comparisons. The *t*-test was applied to test for the significance of the effects of duckweed on the recovery of ^{15}N in the crop and soil as well as ^{15}N loss. The correlation between daily NH_3 flux and daily floodwater NH_4^+-N concentration and pH was analyzed by Pearson correlation. The graphs were prepared with Origin 8.5 software (Origin Lab Ltd., Guangzhou, China).

3. Results

3.1. Seasonal cumulative NH_3 volatilization, and daily NH_3 fluxes, floodwater NH_4^+-N and NO_3^--N contents and pH

Urea combined with duckweed cover (CTD and RND) significantly decreased the cumulative NH_3 loss and NH_3 intensity over the 2 years (Table 2). The CTD and RND lowered the cumulative NH_3 loss by 36% and 52% over CT ($p < 0.05$) for the 2 years, respectively. In contrast, the RN only significantly reduced the NH_3 loss by 37% over CT in 2014 ($p < 0.05$), and there was no significant difference between them in 2015 ($p > 0.05$). NH_3 volatilization mainly occurred during the basal fertilization (BF) period in 2015 for all of the fertilized treatments, whereas it primarily occurred during the first topdressing (T1) period (except CTD and RND) in 2014. Urea combined with duckweed cover significantly decreased the NH_3 loss of the T1 period compared to the urea application alone ($p < 0.05$) over the 2 years. The CTD remarkably lowered the NH_3 loss of the second topdressing (T2) periods for the 2 years and only reduced the NH_3 loss of BF in the 2014 rice season over CT, whereas the RND only significantly reduced the NH_3 loss of the T2 periods in the 2015 rice season over RN ($p < 0.05$). Moreover, urea combined with duckweed cover remarkably decreased the NH_3 intensity by 44% at 300 kg N ha^{-1} and 59% at 225 kg N ha^{-1} compared with CT over the 2 years ($p < 0.05$). In contrast, the RN only significantly reduced the NH_3 intensity in 2014 over CT.

The daily NH_3 fluxes peaked 1–3 days after each fertilizer application, lasted approximately one week, and then dropped to relatively low levels (Fig. 2a–b). Urea combined with duckweed cover could significantly lower the peak values of daily NH_3 fluxes compared with the urea application alone (CT and RN) ($p < 0.05$). Meanwhile, the average daily NH_3 fluxes of the 2 rice seasons in the CTD and RND treatments were lowered by 36% and 52% over the urea application alone treatments, respectively.

Similar to daily NH_3 fluxes, the daily floodwater NH_4^+-N contents exhibited the same pattern for the 2 years (Fig. 2c–d). The floodwater NH_4^+-N contents peaked 1–3 days after each fertilizer application. Compared with the urea application alone, urea combined with duckweed cover resulted in similar and even lower floodwater NH_4^+-N concentration at the date of the peaks of daily NH_3 flux. After the peaks of daily floodwater NH_4^+-N concentration, the urea combined with duckweed cover resulted in the similar and even higher floodwater NH_4^+-N contents over the urea application alone.

The daily floodwater pH varied during the rice growing season for all of the treatments (Fig. 2e–f). The trends in floodwater pH were comparable across treatments, and the effect of duckweed on floodwater pH was relatively small, the average floodwater pH were only reduced by 0.1–0.2 unit at 300 kg N ha^{-1} and 0.2–0.3 unit at 225 kg N ha^{-1} over CT for the 2 years. The daily floodwater NO_3^--N concentrations exhibited seasonal variation in duckweed cover treatments (Fig. 2g–h), the significantly higher floodwater NO_3^--N contents in duckweed cover treatments mainly occurred at T1 period, with average values of 2.4 (2014) and 2.8 (2015) mg N L^{-1} at 300 kg N ha^{-1}

Table 2
Cumulative NH_3 volatilization and NH_3 intensity from the different treatments during the 2014–2015 rice growing seasons.

Code	N rate (kg N ha^{-1})	Cumulative NH_3 loss (kg N ha^{-1})				Decrease in NH_3 loss relative to CT (%)	NH_3 intensity (kg NH_3-N t^{-1} grain)[a]
		BF	T1	T2	Total		
2014							
CK	0	0.7c	1.7c	0.7c	3.1e		0.7d
CT	300	18.4a	20.1a	10.9a	49.4a		6.4a
CTD	300	18.3a	12.5b	7.9b	38.7b	22	4.4b
RN	225	10.7b	12.9b	7.4b	30.9c	37	4.0b
RND	225	11.9b	4.4c	7.0b	23.3d	53	2.7c
2015							
CK	0	0.9c	0.6c	0.4d	1.9c		0.4c
CT	300	11.4a	9.4a	8.0a	28.7a		3.2a
CTD	300	7.1b	3.1b	4.2bc	14.4b	50	1.4b
RN	225	8.2b	8.0a	6.3ab	22.4a	22	2.5a
RND	225	7.5b	3.0b	3.4c	13.9b	52	1.3b

CK, no N-fertilizer; CT, urea broadcast alone at 300 kg N ha^{-1}; CTD, urea broadcast combined with duckweed at 300 kg N ha^{-1}; RN, urea broadcast alone at 225 kg N ha^{-1}; RND, urea broadcast combined with duckweed at 225 kg N ha^{-1}.
BF, basal fertilization period; T1, first topdressing period; T2, second topdressing period.
The values followed by the different letters are significantly different at the level of LSD0.05 within the same column for a given year.
[a] NH3 intensity is the total NH3-N loss divided by the rice yield.

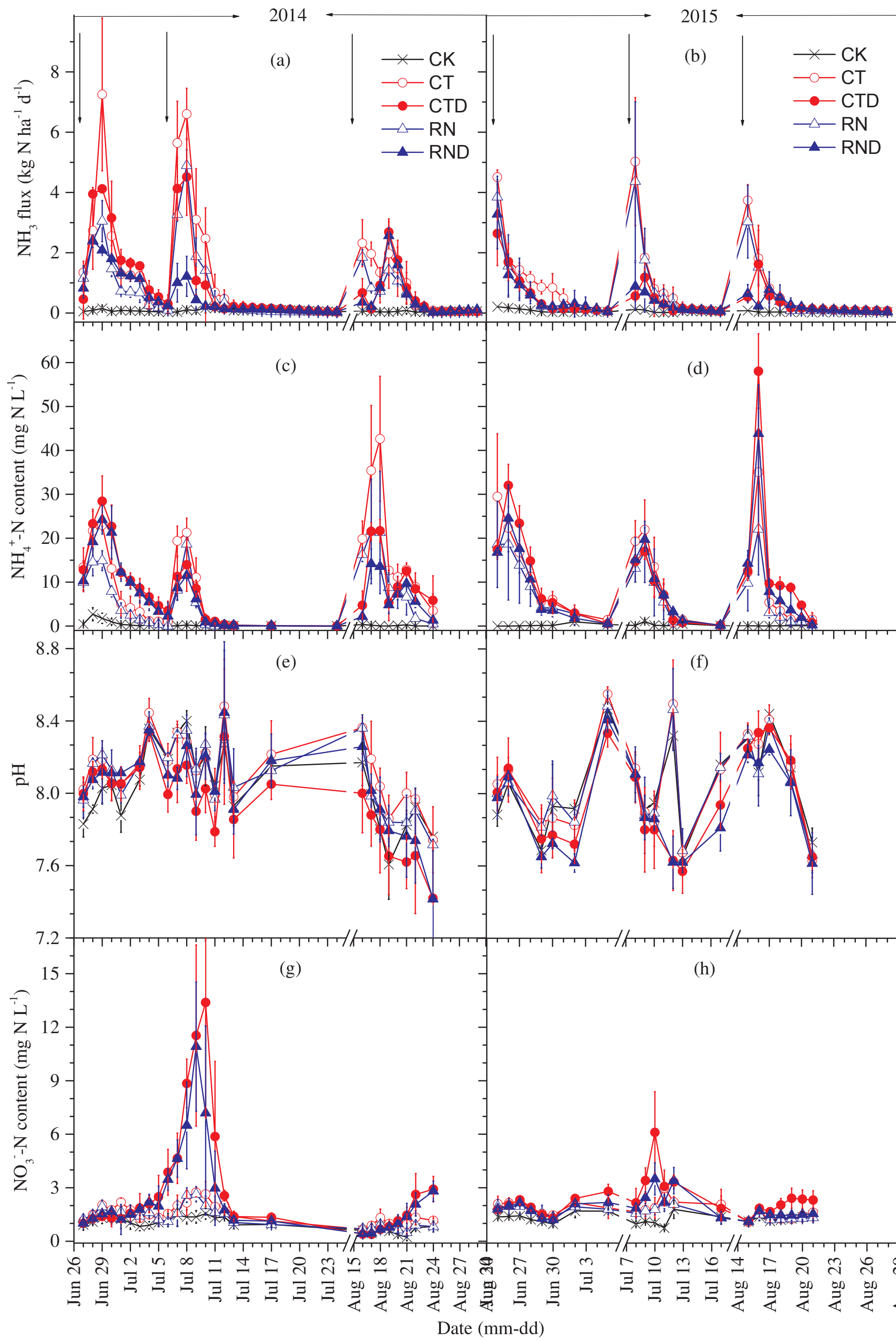

Fig. 2. Seasonal changes in NH_3 fluxes (a–b), floodwater $NH_4{}^+$-N contents (c–d), pH (e–f) and $NO_3{}^-$-N contents (g–h) from different treatments in rice seasons of 2014 and 2015. The arrows denote fertilizer application. The values are the means (n = 4) with their standard deviations (vertical lines).

and 1.9 (2014) and 2.4 (2015) mg N L^{-1} at 225 kg N ha^{-1}. In contrast, the floodwater $NO_3{}^-$-N contents in urea application alone treatments remained stable, no more than 3 mg N L^{-1} for the 2 years, with average values of 1.5 (2014) and 1.8 (2015) mg N L^{-1} at 300 kg N ha^{-1} and 1.3 (2014) and 1.6 (2015) mg N L^{-1} at 225 kg N ha^{-1}.

The daily floodwater $NH_4{}^+$-N concentrations was significantly positive correlated with daily NH_3 fluxes for each fertilized treatment ($p < 0.05$) (Table 3), and daily floodwater pH was positive correlated with daily NH_3 fluxes in the urea alone treatments (CT and RN), but there was no correlation between the daily floodwater pH and daily NH_3 fluxes for the duckweed cover treatments.

3.2. Yield, NEB, crop N, NUE and N surplus at harvest

During the 3 rice seasons, the years, treatments, N rates and N sources had significant influences on rice yield, NEB, straw biomass, crop N, RE_N, AE_N and N surplus at harvest (Tables 4–6).

The interactions of N rates × N sources × years, N rates × N sources, and N sources × years were not significant. The only significant interactions were N rates × years and treatments × years for yield and NEB (Tables 4 and 5). For the N rates × years interaction, the yield and NEB increased with N rate up to 225 kg N ha^{-1} for each year, and their responses to N rate were the greatest in 2015 (Table 4); as for treatments × years interaction, the yield and NEB for fertilized treatments increased under duckweed cover, and urea combined with

Table 3
The relationship between NH_3 volatilization and floodwater NH_4^+-N content and pH.

Code	Correlation between NH_3 volatilization and NH_4^+-N		Correlation between NH_3 volatilization and pH	
	2014	2015	2014	2015
CK	0.16	−0.06	0.47*	0.13
CT	0.49*	0.70**	0.47*	0.54*
CTD	0.59**	0.71**	0.50*	0.53
RN	0.59**	0.71**	0.58**	0.61*
RND	0.64**	0.41*	0.37	0.34

CK, no N-fertilizer; CT, urea broadcast alone at 300 kg N ha^{-1}; CTD, urea broadcast combined with duckweed at 300 kg N ha^{-1}; RN, urea broadcast alone at 225 kg N ha^{-1}; RND, urea broadcast combined with duckweed at 225 kg N ha^{-1}.
* Significant correlation at the 0.05 level (two-tailed).
** Significant correlation at the 0.01 level (two-tailed).

Table 4
N rates × years interaction for rice yield and NEB at harvest.

N rate (kg N ha^{-1})	0	225	300
Yield (t ha^{-1})			
2014	4.88	8.23	8.24
2015	4.58	9.67	9.62
2016	4.91	8.92	9.10
LSD (0.05)[a]	0.93		
NEB (×1000 ¥)[b]			
2014	13.5	21.9	21.8
2015	12.6	26.2	26.0
2016	13.6	24.0	24.4
LSD (0.05)	3.23		

[a] The least significant difference for N rates × years interaction at the 0.05 probability level.
[b] NEB is the difference between the value of the harvest grain and the costs of N, P and K fertilizers and duckweed inputs.

Table 5
Treatments × years interaction for rice yield and NEB at harvest.

Treatment	CK	RN	RND	CT	CTD
Yield (t ha^{-1})					
2014	4.88	7.80	8.66	7.68	8.80
2015	4.58	8.99	10.35	8.99	10.25
2016	4.91	8.32	9.51	8.75	9.44
LSD (0.05)[a]	0.53				
NEB (× 1000 ¥)[b]					
2014	13.5	21.0	22.8	20.6	23.1
2015	12.6	24.6	27.9	24.5	27.4
2016	13.6	22.6	25.6	23.8	25.0
LSD (0.05)	2.87				

CK, no N-fertilizer; CT, urea broadcast alone at 300 kg N ha^{-1}; CTD, urea broadcast combined with duckweed at 300 kg N ha^{-1}; RN, urea broadcast alone at 225 kg N ha^{-1}; RND, urea broadcast combined with duckweed at 225 kg N ha^{-1}.
[a] The least significant difference for treatments × years interaction at the 0.05 probability level.
[b] NEB is the difference between the value of the harvest grain and the costs of N, P and K fertilizers and duckweed inputs.

duckweed cover treatments had higher yield and NEB than urea alone treatments. However, there were no significant differences in yield and NEB between CT and RN and between CTD and RND ($p > 0.05$); and the yield and NEB in 2015 was the greatest for the fertilized treatments (Table 5).

The CTD and RND achieved significantly higher rice yield by 10 and 9% compared to CT ($p < 0.05$) (Table 5), respectively. Accordingly, the CTD and RND resulted in higher NEB by 10 and 11% over CT ($p < 0.05$), respectively.

The highest straw biomass and crop N occurred in 2016 ($p < 0.05$) (Table 6). The duckweed cover treatments had higher straw biomass over the urea alone treatments ($p < 0.05$). The CTD and RND gave higher crop N by 25 and 14% over CT ($p < 0.05$). In addition, the RN produced higher RE_N and AE_N and gained lower crop N and N surplus over CT ($p < 0.05$).

The biomass and N accumulation of duckweed had similar trends during the rice season (Fig. 3). The N accumulation by duckweed increased rapidly from 3.1 kg N ha^{-1} at the initial stage to 18.3 and 18.1 kg N ha^{-1} for CTD and RND at the BF period (Fig. 3b), respectively, After the mid-season aeration (from July 20 to August 1), most of the duckweed died and the total N content of duckweed declined to 5.1 and 5.2 kg N ha^{-1} for CTD and RND on August 23, respectively. The N accumulation by duckweed increased again after the second topdressing and gradually declined to 2.4 kg N ha^{-1} at harvest.

3.3. The ^{15}N balance

The ^{15}N balance at harvest in the 2014 rice season was shown in Table 7. The ^{15}N recovery by aboveground rice plants was 38% higher and the ^{15}N loss was 16% lower in CTD than that of CT, respectively ($p < 0.05$). For CTD, the ^{15}N accumulation in the aboveground rice plants reached 33% of applied N (11% in the straw, 22% in the grain) at harvest. The soil residual ^{15}N was mainly found in the 0–5 cm soil depth and was similar in the CTD and CT ($p > 0.05$). The ^{15}N accumulation by duckweed only accounted for 1% of the applied N.

4. Discussion

4.1. NH_3 volatilization during the rice seasons

The daily NH_3 fluxes peaked within 1–3 days after urea broadcasting in this study due to the high NH_4^+-N content in the floodwater resulting from strong sunlight as well as high temperature and high pH in the field (Cai et al., 1988; Cao et al., 2013). The NH_3 volatilization was more likely to occur during the BF period in 2015, which was similar to previous studies (Cao et al., 2013; Zhao et al., 2015a), whereas it mainly occurred during the T1 period in 2014 (except CTD and RND). This discrepancy was mainly attributed to the different weather conditions (Fig. 1), more frequent rainy days occurred during the BF period over the T1 period in 2014.

There have been limited studies that have documented the reduction of NH_3 loss by duckweed cover in the paddy fields. It was clearly indicated that the urea combined with duckweed cover could substantially reduce NH_3 loss at 225 and 300 kg N ha^{-1} compared with urea alone (Table 2), and as a result, the NH_3 intensity were remarkably decreased. Li et al. (2009) have reported that the duckweed (*Lemna minor*) cover could obviously reduce NH_3 loss at 90 and 180 kg N ha^{-1} compared with urea alone. In contrast, only reducing the N dose (RN) could not effectively reduce the total NH_3 loss (Table 2). In 2015, the RN only significantly reduced the NH_3 loss during the BF period compared with CT; however, there were no significant differences for the NH_3 loss during the T1 and T2 periods between them, which might be derived from the similar floodwater pH values between them (Fig. 2h) and the floodwater pH determined the transformation of NH_4^+ to NH_3.

In the present study, we adopted the enclosure chamber method for measuring NH_3 loss because it is more useful for comparing NH_3 losses from several different fertilizer treatments since only small plot areas are required for each treatment. However, the errors are inevitable for measuring NH_3 volatilization by the enclosure chamber method, previous studies also suggested the exchange fluxes of NH_3 above emitting surfaces are stable when air exchange rates are assured with air flow rates of 15–20 headspace min^{-1} to replacement volumes (Li et al., 2008; Cao et al., 2013). In our study, the vacuum pump assured the air velocity at a rate of 15–20 headspace min^{-1} through the chamber; in this way, the errors can be minimized.

Table 6
Straw biomass, crop N, NUE and N surplus for the different treatments at harvest in 2014– 2016 rice seasons.

Variable		Straw biomass ($t\ ha^{-1}$)	Crop N ($kg\ N\ ha^{-1}$)	RE_N (%)[a]	AE_N ($kg\ kg^{-1}$)[b]	N surplus ($kg\ N\ ha^{-1}$)[c]
Year	2014	6.8c	144b	26b	11c	195b
	2015	7.7b	143b	30b	17a	188b
	2016	8.4a	179a	39a	14b	167a
Treatment	CK	5.1c	76e	–	–	–
	CT	8.0b	163c	30b	12b	213a
	CTD	8.8a	203a	–	–	–
	RN	7.6b	148d	44a	16a	173b
	RND	8.7a	186b	–	–	–

CK, no N-fertilizer; CT, urea broadcast alone at 300 kg N ha^{-1}; CTD, urea broadcast combined with duckweed at 300 kg N ha^{-1}; RN, urea broadcast alone at 225 kg N ha^{-1}; RND, urea broadcast combined with duckweed at 225 kg N ha^{-1}.
Values followed by the different letters are significantly different at the level of $LSD_{0.05}$ within the same column for a given variable.
[a] The RE_N is apparent N recovery efficiency for CK, CT and RN.
[b] The AE_N is agronomic N use efficiency for CK, CT and RN.
[c] The N surplus is the difference between the total fertilizer N input and fertilizer N accumulation by rice plant for CK, CT and RN.

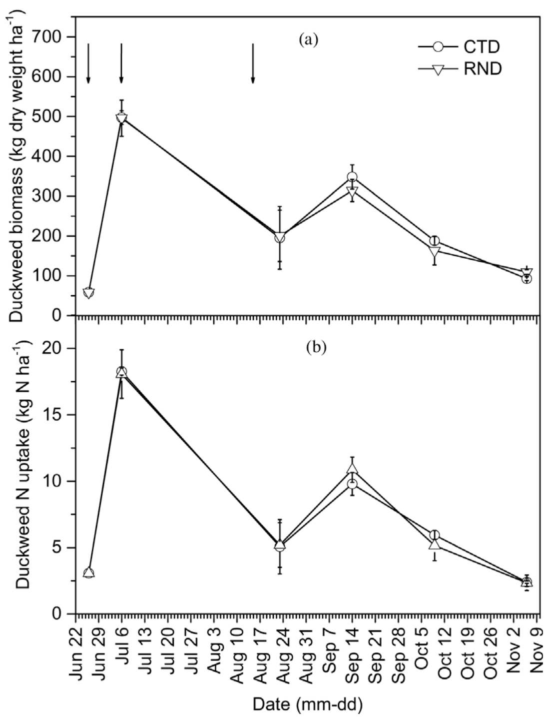

Fig. 3. Seasonal changes in duckweed biomass (a) and duckweed N accumulation (b) in 2014 rice season. The arrows denote fertilizer application. The values are the means with their standard deviations (n = 4).

4.2. NH_3 mitigation effects under duckweed cover

In this study, the reduced NH_3 volatilization under duckweed cover treatments was mainly attributed to the formation of a physical barrier and the uptake of NH_4^+ by duckweed. Urea combined with duckweed cover effectively reduced the NH_3 loss in the T1 period for 2 years. During the T1 period, the surface water was fully covered by duckweed in CTD and RND; thus, duckweed formed a densely physical barrier, which could inhibit the NH_3 escaping from floodwater (Li et al., 2009). Meanwhile, the uptake of NH_4^+ by duckweed was also responsible for decreasing the NH_3 volatilization. The maximum N assimilation by duckweed reached 18 kg N ha^{-1} in the late BF period in this study (Fig. 3), and 70–73% was derived from fertilizer N (data not shown). Duckweed has a preferential uptake of NH_4^+-N over other source of N as its assimilation requires less energy (Zimmo et al., 2004). It has been proven that duckweed can show tolerance to high nutrient levels and has great NH_4^+ uptake efficiency over a short period of time in wastewater (El-Shafai et al., 2007; Ansal et al., 2010; Zhao et al., 2014; Forni and Tommasi, 2016). Meanwhile, duckweed also shows NH_3 preference uptake and it has the ability of direct absorption of NH_3 from the water. This is because the un-dissociated NH_3 molecule is lipid-soluble and thus easily enters plant cells of duckweed through its membrane (Landolt, 1997; Mohedano et al., 2012; Selvarani et al., 2015). Furthermore, it has been recorded that the maximum NH_3 removal efficiency of *L. minor* achieved 96% in wastewater (Selvarani et al., 2015). For the BF period, the reduction of NH_3 loss in duckweed cover treatments were not significant compared with urea alone, which might be attributed to the highest NH_3 volatilization rate at the first 1–3 days accompanied by the weak uptake of NH_4^+-N of duckweed during the first 2 days after broadcasting urea. It was found that duckweed had very little uptake of NH_4^+-N during the first 2 days, and after this lag period, the duckweed performed a rapid rate for NH_4^+-N uptake (Zhao et al., 2014). Meanwhile, maintaining fully coverage of the water surface by duckweed is critical to achieve a high N recovery efficiency (Alaerts et al., 1996; El-Shafai et al., 2007; Xu and Shen, 2011). Xu and Shen (2011) noted that 80% surface coverage will take 3 days for the duckweed to fully cover the wastewater. The initial surface coverage was approximately 80% in this study and thus it made NH_3 escape possible at the BF period. Therefore, the pre-culture of duckweed in paddy fields before rice transplantation or the initial full coverage of the surface water might be more efficient for reducing the NH_3 loss of the BF period. In addition, duckweed cover treatments lowered the NH_3 loss of the T2 period (Table 2). Although a large amount of duckweed died after the mid-season aeration event, duckweed could survive dry conditions through turion (a small dormant bud) formation and renewed vegetative growth after re-flooding (Landolt, 1997; Lam et al., 2014), which resulted in its re-covering of

Table 7
Percent recovery of applied N (^{15}N-labeled urea) and ^{15}N loss at harvest in the 2014 rice season.

Code	Straw	Grain	Root	Duckweed	Soil (0–5 cm)	Soil (5–20 cm)	Total in aboveground plants	^{15}N loss
CT	8b	16b	1a	–	17a	1a	24b	58a
CTD	11a	22a	1a	1	17a	1a	33a	48b

CT, urea broadcast alone at 300 kg N ha^{-1}; CTD, urea broadcast combined with duckweed at 300 kg N ha^{-1}. The different letters within the same column indicate significant differences among the different treatments for a given year, at $p < 0.05$.

the water surface at the initial T2 period; thus, the rapid N uptake of the rice plant in combination with NH_4^+-N recovery by duckweed reduced the risk of NH_3 loss of the T2 period.

The floodwater pH determined the NH_3/NH_4^+ ratio and lower pH could prevent NH_4^+ dissociation in the floodwater (Fillery et al., 1984). Abundant nutrient supplies in floodwater favored photosynthetic activity by algae under the urea application alone, which could result in depletion of dissolved CO_2 and an elevated pH of floodwater (Buresh et al., 2008), and thereby, higher NH_3 loss occurred in the urea application alone treatments. In contrast, duckweed cover could absorb NH_4^+ and intercept the incoming light, which made it difficult for algae growth and survival (Forni and Tommasi, 2016), and thereby, the lower floodwater pH trends appeared under duckweed cover (Fig. 2e–f). To prevent the growth of algae, a minimum initial duckweed coverage of 60% is recommended by Xu and Shen (2011). Similarly, it was also found by previous studies that duckweed could effectively lower the floodwater pH (Zimmo et al., 2003; Li et al., 2009). In addition, the duckweed cover could lower the floodwater temperature by 1–2 °C due to less light energy that penetrated into the floodwater (Alaerts et al., 1996; Zimmo et al., 2003; Li et al., 2009). In the presence of duckweed cover, the rapid heating of floodwater from morning until midday was prevented. A decrease of temperature in duckweed-covered plots might have decreased the potential of NH_3 loss.

The average floodwater NO_3^--N contents in duckweed cover treatments were higher than those of the urea alone treatments, especially during the T1 period (Fig. 2g–h), which might be attributed to the establishment of nitrification microbial community and excessive NH_4^+-N in floodwater, and as a result, the nitrification process was enhanced under duckweed cover (Srivastava et al., 2016). Enhanced nitrification efficiency under duckweed cover might be driven by several factors including the attachment of epiphytic microorganisms (especially ammonia-oxidizing bacteria of *Nitrosomonas*) at the root and abaxial fronds of duckweed (Zhao et al., 2015b), aerobic zones always being present through duckweed root radial oxygen loss, the availability of root exudation for microbes, and optimal pH and temperature (Mohedano et al., 2012; Srivastava et al., 2016). However, the magnitude of nitrification-denitrification under duckweed cover is not clear so far.

The ^{15}N loss reached 58 and 48% in CT and CTD, respectively, of which denitrification and NH_3 volatilization might be the primary N loss pathways. A field experiment conducted by Wang et al. (2017) at the same experiment site showed that the N loss caused by denitrification could reached 13.3–21.1% of the applied N for 270 kg N ha^{-1} during the rice season. Therefore, further studies are needed to explore the influence of duckweed cover on nitrification-denitrification processes.

4.3. Improvement of the rice yield by duckweed cover

Urea combined with duckweed cover treatments effectively enhanced rice yield over urea alone treatments (Table 5). There is limited information on the effects of duckweed in rice production, and only Li et al. (2009) have reported that the duckweed cover could increase grain yield by 9.8 and 9.4% at 90 and 180 kg N ha^{-1} over urea alone. It was found that the frames had no influence on the rice yield (an unpublished experiment conducted in RN plots). The higher rice yields in the duckweed cover treatments were mainly attributed to the reduced N loss (Tables 2 and 7) and enhanced crop N accumulation (Table 6).

Urea combined with duckweed cover effectively reduced ^{15}N loss (Table 7) and NH_3 loss (Table 2), and thereby, more N was available for rice plants under duckweed cover. Moreover, the NH_4^+-N uptake by duckweed and its release was also responsible for the enhanced crop N accumulation under duckweed cover treatments. Duckweed can act as an excellent "nutrient sink" for harvesting nutrients over a short period of time when a large amount of NH_4^+-N is available (Cheng et al., 2002; El-Shafai et al., 2007; Ansal et al., 2010). The N accumulation by duckweed increased rapidly during the early rice growing stage (Fig. 3b) due to its high multiplication rate under sufficient nutrient supply in floodwater and the open rice canopy. Despite the duckweed cover treatments obtained similar and even lower floodwater NH_4^+-N concentrations at the date of the peaks of daily NH_3 flux (Fig. 2c–d), they gave higher trends of floodwater NH_4^+-N content after the date of the peaks of daily NH_3 flux, which might be attributed to the NH_4^+-N release from decomposing duckweed. As a result, duckweed cover remarkably promoted the development of rice roots in the soil surface layer and increased tiller numbers by 18–21% compared to those of urea alone (data not shown). The total N content of duckweed declined during the mid rice season due to the large amount of duckweed death caused by the mid-season aeration event (Fig. 3b). This indicated that the duckweed had a rapid decomposition rate and that a considerable amount of the N accumulated by duckweed was released into floodwater or soil; thus, more N is available for rice plant in the duckweed cover treatments. An unpublished duckweed decomposition experiment showed that the mass loss and N content of duckweed decreased to approximately half of the initial values after 7 days due to its low fiber content of 6–16% and high protein content of 15–45% (Timmerman and Hoving, 2016). Meanwhile, duckweed cover treatments produced higher trends of floodwater NH_4^+-N content at the late T2 period; and the total N in duckweed gradually declined during the late rice growing season (Fig. 3), which implied that N was released again from decomposing duckweed, which were beneficial for increasing pre-heading N accumulation and post-heading dry matter accumulation, and hence, the rice yield was enhanced. The ^{15}N experiment revealed that only 1% of applied ^{15}N was retained in duckweed for CTD at harvest (Table 7). A considerable N accumulation by duckweed at the early rice growing stage and the small N retained in duckweed at the late rice growing stage (Fig. 3b) and the higher rice yield under duckweed cover (Table 6) suggested that the benefits of duckweed conserving N fertilizer outweighed competition for the applied N. Therefore, duckweed appears to act as slow release fertilizer and the duckweed cover exhibited positive effects on improving the crop N accumulation and hence increasing rice yield.

In addition, a large amount of the biomass accumulation by duckweed may improve soil properties due to its carbon fixation though photosynthetic activity. The seasonal changes in duckweed biomass were similar to the seasonal changes of N accumulation of duckweed (Fig. 3a). It was found that the maximum carbon fixation of *S. polyrhiza* could achieve 5.4 g m^{-1} d^{-1} (Wang et al., 2015); thus, duckweed growth can promote carbon sequestration in paddy fields and increase the soil organic carbon pool which can significantly improve soil properties through improvement in soil microbial activities. Meanwhile, duckweed cover can reduce the use of N by weeds due to the suppression of weed growth through the interception of light by duckweed cover and can decrease the sheath blight disease of rice plants (Huang et al., 2003). Furthermore, duckweed-microbe interaction may have significant effects on N transformation in paddy fields (Srivastava et al., 2016), and the higher floodwater NO_3^--N contents in duckweed cover treatments provided another form of N for rice plants (Fig. 2g–h). As a result, urea combined with duckweed could better synchronize the crop N demand and N supply.

The N rate of 300 kg N ha^{-1} did not increase rice yield and NEB compared to the N rate of 225 kg N ha^{-1} (Table 4). The yield potential in this region is 11.7 t ha^{-1} (Zhao et al., 2015a), whereas the CT treatment only achieved 72% of the yield potential, although N rates with an application of urea in CT already exceed crop needs for N. The continuous increasing input of synthetic fertilizer N cannot promise a proportionate increase in crop productivity because of diminishing returns. The low crop N accumulation at harvest and high N loss in CT implied that the N supply and crop N demand was not better synchronized. The similar yield and NEB in RN and CT implied that only reducing the N dose could not promote rice production and did not result in more financial benefit to farmers and hence it is unlikely to be sufficiently attractive to farmers. However, the use of duckweed, with a reduction of 25% of the N dose, was financially attractive and was able to increase rice yield and reduce N loss, which could achieve the “Zero Increase Action Plan” announced by the Ministry of Agriculture in China. The responses of rice yield and NEB to N rate were the greatest in 2015 (Table 4) due to the favorable weather condition during the rice growing season (Fig. 1). In 2015, less rainfall occurred at the late rice growing stage, which could beneficial for rice filling. In addition, inducing turion formation of duckweed by abscisic-acid at the late stage of the first rice season may be an alternative strategy to keep duckweed survival until the next rice season (Smart and Trewavas, 1983), which can lower the input cost of duckweed application.

5. Conclusions

Despite concerns regarding the detrimental effects of excess fertilizer N on the environment and the ability to achieve comparable rice yields with less N (Table 6), the reduction in urea use and potential benefits to the environment do not significantly increase the financial benefits for farmers and is not enough to ensure food security. An increase in crop yield with little or no added cost is required to increase the financial benefits for farmers. The use of duckweed was able to substantially increase rice yield, and was financially attractive despite the added costs for collection and application. The aim of the Chinese government is to lower the N rate while ensuring food security and reducing environmental harms, and farmers are willing to accept financially attractive practices. Notably, the RND has the ability to lower N fertilizer use by 25% while simultaneously increasing rice yield and reducing NH_3 loss. The ability to achieve higher yield with duckweed cover mainly attributed to reduced N loss and enhanced crop N accumulation. The reduced N loss under duckweed cover, especially NH_3 volatilization, mainly resulted from the formation of a physical barrier and the uptake of NH_4^+ by duckweed. The enhanced crop N accumulation with duckweed cover was mainly derived from the recycling of N by duckweed, temporarily recovering a considerable amount of NH_3 and NH_4^+ and later releasing it for rice plants, and improving soil properties, reducing the use of N by weeds, and providing another form of N (NO_3^--N) for rice plants through the interaction of duckweed-microbe.

An attractive feature of duckweed is its capability to adapt to diverse climate conditions and it is an excellent “nutrient sink”. In addition, it does not require arable land for cultivation and can be easily found and collected in ponds or creeks. Inducing turion formation of duckweed may be an alternative strategy for self-reproduction until the next rice season, which can reduce the cost of collection and application of duckweed. In addition, Chinese paddy fields are distributed in a variety of climatic zones; thus, it is needed to select proper duckweed species adapted to different growing conditions.

Acknowledgments

This research was financially supported by the National Key Research and Development Project of China (2017YFD0200104) and the National Key Research and Development Project of China (2016YFC0207906). We sincerely thank field management assistance from the Changshu Agroecosystem Experimental Station for the help of managing the field experiments. We sincerely thank Elizabeth Humphreys, the Co-Editor-in-Chief of the journal, and anonymous referees for their valuable comments and critical suggestions improving the original manuscript.

References

Alaerts, G.J., Mahbubar, R., Kelderman, P., 1996. Performance analysis of a full-scale duckweed-covered sewage lagoon. Water Res. 30, 843–852.
Ansal, M., Dhawan, A., Kaur, V., 2010. Duckweed based bio-remediation of village ponds: an ecologically and economically viable integrated approach for rural development through aquaculture. Livest. Res. Rural Dev. 22.
Buresh, R.J., Reddy, K.R., van Kessel, C., 2008. Nitrogen transformations in submerged soils. In: Schepers, J.S., Raun, W.R. (Eds.), Nitrogen in Agricultural Systems. ASA, CSSA, and SSSA, Madison, WI, pp. 401–436.
Cai, G.X., Freney, J.R., Humphreys, E., Denmead, O.T., Samson, M., Simpson, J.R., 1988. Use of surface films to reduce ammonia volatilization from flooded rice fields. Aust. J. Agr. Res. 39, 177–186.
Cao, Y.S., Tian, Y.H., Yin, B., Zhu, Z.L., 2013. Assessment of ammonia volatilization from paddy fields under crop management practices aimed to increase grain yield and N efficiency. Field Crops Res. 147, 23–31.
Chen, X., Cui, Z., Fan, M., Vitousek, P., Zhao, M., Ma, W., Wang, Z., Zhang, W., Yan, X., Yang, J., Deng, X., Gao, Q., Zhang, Q., Guo, S., Ren, J., Li, S., Ye, Y., Wang, Z., Huang, J., Tang, Q., Sun, Y., Peng, X., Zhang, J., He, M., Zhu, Y., Xue, J., Wang, G., Wu, L., An, N., Wu, L., Ma, L., Zhang, W., Zhang, F., 2014. Producing more grain with lower environmental costs. Nature 514, 486–489.
Chen, M., Sun, F., Shindo, J., 2016. China’s agricultural nitrogen flows in 2011: environmental assessment and management scenarios. Resour. Conserv. Recycl. 111, 10–27.
Cheng, J., Bergmann, B.A., Classen, J.J., Stomp, A.M., Howard, J.W., 2002. Nutrient recovery from swine lagoon water by Spirodela punctata. Bioresour. Technol. 81, 81–85.
El-Shafai, S.A., El-Gohary, F.A., Nasr, F.A., Peter van der Steen, N., Gijzen, H.J., 2007. Nutrient recovery from domestic wastewater using a UASB-duckweed ponds system. Bioresour. Technol. 98, 798–807.
Fillery, I.R.P., Simpson, J.R., De Datta, S.K., 1984. Influence of field environment and fertilizer management on ammonia loss from flooded rice. Soil Sci. Soc. Am. J. 48, 914–920.
Forni, C., Tommasi, F., 2016. Duckweed: a tool for ecotoxicology and a candidate for phytoremediation. Curr. Biotechnol. 5, 2–10.
Galloway, J.N., Townsend, A.R., Erisman, J.W., Bekunda, M., Cai, Z., Freney, J.R., Martinelli, L.A., Seitzinger, S.P., Sutton, M.A., 2008. Transformation of the nitrogen cycle: recent trends, questions, and potential solutions. Science 320, 889–892.
Gu, B., Ge, Y., Ren, Y., Xu, B., Luo, W., Jiang, H., Gu, B., Chang, J., 2012. Atmospheric reactive nitrogen in China: sources, recent trends, and damage costs. Environ. Sci. Technol. 46, 9420–9427.
Gu, B., Ju, X., Chang, J., Ge, Y., Vitousek, P.M., 2015. Integrated reactive nitrogen budgets and future trends in China. Proc. Natl. Acad. Sci. 112, 8792–8797.
Hauck, R.D., Bremner, J.M., 1976. Use of tracers for soil and fertilizer nitrogen research. Adv. Agron. 28, 219–266.
Hofmeier, M., Roelcke, M., Han, Y., Lan, T., Bergmann, H., Böhm, D., Cai, Z., Nieder, R., 2015. Nitrogen management in a rice–wheat system in the Taihu region: recommendations based on field experiments and surveys. Agric. Ecosyst. Environ. 209, 60–73.
Huang, S.W., Yu, L.Q., Duan, G.F., Li, D., Yuan, Y.F., Yu, M.L., Yu, J.X., Luo, K., 2003. Control of weeds and rice sheath blight disease in paddy fields by rice chaff and duckweeds (lemna spp.). Plant Prot. 29, 22–26.
Ju, X.T., Xing, G.X., Chen, X.P., Zhang, S.L., Zhang, L.J., Liu, X.J., Cui, Z.L., Yin, B., Christie, P., Zhu, Z.L., Zhang, F.S., 2009. Reducing environmental risk by improving N management in intensive Chinese agricultural systems. Proc. Natl. Acad. Sci. 106, 3041–3046.
Ladha, J.K., Pathak, H.J., Krupnik, T., Six, J., van Kessel, C., 2005. Efficiency of fertilizer nitrogen in cereal production: retrospects and prospects. Advances in Agronomy. Academic Press, pp. 85–156.
Lam, E., Appenroth, K.J., Michael, T., Mori, K., Fakhoorian, T., 2014. Duckweed in bloom: the 2nd International conference on duckweed research and applications heralds the return of a plant model for plant biology. Plant Mol. Biol. 84, 737–742.
Landolt, E., 1997. How do Lemnaceae (duckweed family) survive dry conditions? Bull. Geobot. Inst. Eth 63, 25–31.
Li, H., Liang, X., Chen, Y., Tian, G., Zhang, Z., 2008. Ammonia volatilization from urea in rice fields with zero-drainage water management. Agric. Water Manage. 95, 887–894.
Li, H., Liang, X., Lian, Y., Xu, L., Chen, Y., 2009. Reduction of ammonia volatilization from urea by a floating duckweed in flooded rice fields. Soil Sci. Soc. Am. J. 73, 1890–1895.
Li, F., Wang, Z., Dai, J., Li, Q., Wang, X., Xue, C., Liu, H., He, G., 2015. Fate of nitrogen from green manure, straw, and fertilizer applied to wheat under different summer fallow management strategies in dryland. Biol. Fert. Soils 51, 769–780.
Linquist, B.A., Liu, L., van Kessel, C., van Groenigen, K.J., 2013. Enhanced efficiency nitrogen fertilizers for rice systems: meta-analysis of yield and nitrogen uptake. Field Crops Res. 154, 246–254.
Liu, X., Vitousek, P., Chang, Y., Zhang, W., Matson, P., Zhang, F., 2016. Evidence for a historic change occurring in China. Environ. Sci. Technol. 50, 505–506.
Mohedano, R.A., Costa, R.H.R., Tavares, F.A., Belli Filho, P., 2012. High nutrient removal rate from swine wastes and protein biomass production by full-scale duckweed ponds. Bioresour. Technol. 112, 98–104.
Novozamsky, I., Eck, R., van Schouwenburg, J.C., van Walinga, I., 1974. Total nitrogen determination in plant material by means of the indophenol-blue method. Neth. J. Agric. Sci. 22, 3–5.
Ray, D.K., Ramankutty, N., Mueller, N.D., West, P.C., Foley, J.A., 2012. Recent patterns of crop yield growth and stagnation. Nat. Commun. 3, 1293.
Saggar, S., Singh, J., Giltrap, D.L., Zaman, M., Luo, J., Rollo, M., Kim, D.G., Rys, G., der Weerden, T.J.v., 2013. Quantification of reductions in ammonia emissions from fertiliser urea and animal urine in grazed pastures with urease inhibitors for agriculture inventory: New Zealand as a case study. Sci. Total Environ. 465, 136–146.
Selvarani, A.J., Padmavathy, P., Srinivasan, A., Jawahar, P., 2015. Performance of

duckweed (Lemna minor) on different types of wastewater treatment. Int. J. Fish. Aqua. Stud. 2, 208–212.
Shen, J., Cui, Z., Miao, Y., Mi, G., Zhang, H., Fan, M., Zhang, C., Jiang, R., Zhang, W., Li, H., Chen, X., Li, X., Zhang, F., 2013. Transforming agriculture in China: from solely high yield to both high yield and high resource use efficiency. Global Food Secur. 2, 1–8.
Smart, C.C., Trewavas, A.J., 1983. Abscisic-acid-induced turion formation in Spirodela polyrrhiza L. I. Production and development of the turion. Plant Cell Environ 6, 507–514.
Srivastava, J.K., Chandra, H., Kalra, S.J.S., Mishra, P., Khan, H., Yadav, P., 2016. Plant–microbe interaction in aquatic system and their role in the management of water quality: a review. Appl. Water Sci. 1–12.
Sun, H.J., Zhang, H.L., Min, J., Feng, Y.F., Shi, W.M., 2015. Controlled-release fertilizer, floating duckweed, and biochar affect ammonia volatilization and nitrous oxide emission from rice paddy fields irrigated with nitrogen-rich wastewater. Paddy Water Environ. 14, 105–111.
Timmerman, M., Hoving, I.E., 2016. Purifying manure effluents with duckweed. Wageningen UR Livestock Research.
Wang, W., Yang, C., Tang, X., Zhu, Q., Pan, K., Cai, D., Hu, Q., Ma, D., 2015. Carbon and energy fixation of great duckweed Spirodela polyrhiza growing in swine wastewater. Environ. Sci. Pollut. R. 22, 15804–15811.
Wang, S., Shan, J., Xia, Y., Tang, Q., Xia, L., Lin, J., Yan, X., 2017. Different effects of biochar and a nitrification inhibitor application on paddy soil denitrification: a field experiment over two consecutive rice-growing seasons. Sci. Total Environ. 593, 347–356.
Wu, X., Lu-jia, L., Han, M., Yang, L., Ming-yao, Z., Xiao-qing, Q., 2012. Species of duckweeds in summer in Jiangsu province and water environments they grow in. J. Ecol. Rural Environ. 5, 554–558.
Wu, L., Chen, X.P., Cui, Z.L., Wang, G.L., Zhang, W.F., 2015. Improving nitrogen management via a regional management plan for Chinese rice production. Environ. Res. Lett. 10, 095011.
Xia, L., Ti, C., Li, B., Xia, Y., Yan, X., 2016. Greenhouse gas emissions and reactive nitrogen releases during the life-cycles of staple food production in China and their mitigation potential. Sci. Total Environ. 556, 116–125.
Xu, J., Shen, G., 2011. Growing duckweed in swine wastewater for nutrient recovery and biomass production. Bioresour. Technol. 102, 848–853.
Yan, X., Ti, C., Vitousek, P., Chen, D., Leip, A., Cai, Z., Zhu, Z., 2014. Fertilizer nitrogen recovery efficiencies in crop production systems of China with and without consideration of the residual effect of nitrogen. Environ. Res. Lett. 9, 095002.
Zhang, X., Davidson, E.A., Mauzerall, D.L., Searchinger, T.D., Dumas, P., Shen, Y., 2015. Managing nitrogen for sustainable development. Nature 528, 51–59.
Zhao, X., Zhou, Y., Min, J., Wang, S.Q., Shi, W.M., Xing, G.X., 2012. Nitrogen runoff dominates water nitrogen pollution from rice-wheat rotation in the Taihu Lake region of China. Agric. Ecosyst. Environ. 156, 1–11.
Zhao, Z., Shi, H., Liu, Y., Zhao, H., Su, H., Wang, M., Zhao, Y., 2014. The influence of duckweed species diversity on biomass productivity and nutrient removal efficiency in swine wastewater. Bioresour. Technol. 167, 383–389.
Zhao, M., Tian, Y.H., Ma, Y.C., Zhang, M., Yao, Y.L., Xiong, Z.Q., Yin, B., Zhu, Z.L., 2015a. Mitigating gaseous nitrogen emissions intensity from a Chinese rice cropping system through an improved management practice aimed to close the yield gap. Agric. Ecosyst. Environ. 203, 36–45.
Zhao, Y., Fang, Y., Jin, Y., Huang, J., Ma, X., He, K., He, Z., Wang, F., Zhao, H., 2015b. Microbial community and removal of nitrogen via the addition of a carrier in a pilot-scale duckweed-based wastewater treatment system. Bioresour. Technol. 179, 549–558.
Zhu, Z.L., 1997. Nitrogen balance and cycling in agroecosystems of China. Nitrogen in Soils of China, Netherlands, pp. 323-338.
Zimmo, O.R., van der Steen, N.P., Gijzen, H.J., 2003. Comparison of ammonia volatilisation rates in algae and duckweed-based waste stabilisation ponds treating domestic wastewater. Water Res. 37, 4587–4594.
Zimmo, O.R., van der Steen, N.P., Gijzen, H.J., 2004. Nitrogen mass balance across pilot-scale algae and duckweed-based wastewater stabilisation ponds. Water Res. 38, 913–920.