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ORIGINAL ARTICLE

The fate of ^{15}N-labelled urea in an alkaline calcareous soil under different N application rates and N splits

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Abstract We aimed to quantitatively investigate the effects of rate and timing of nitrogen (N) application on fate of ^{15}N-labelled urea in an alkaline calcareous soil during a winter wheat (WW) and summer maize (SM) seasons. The treatments consisted of conventional N application (i.e., WN300-2T or MN240-2T, 300 or 240 kg N ha^{-1} with two N splits to WW or SM), reduced N application (i.e., WN210-2T or MN168-2T, 210 or 168 kg N ha^{-1} with two N splits to WW or SM), recommended N application (i.e., WN210-3T or MN168-3T, 210 or 168 kg N ha^{-1} with three N splits to WW or SM), and control (N0). The result showed that the fate of ^{15}N fertilizer was significantly influenced by rate and timing of the applied N. Compared with the conventional N treatment, crop ^{15}N recovery in the recommended N treatment increased significantly by 16.7 % for WW and 17.2 % for SM, but total ^{15}N losses reduced significantly by 12.3 and 13.5 %, respectively. Residual ^{15}N in 100–200 cm soil layer was the lowest in recommended N treatment, preventing leaching of much $^{15}NO_3{}^{-}$–N to deeper soil layers. Our results indicated that the recommended N treatment at rate of 210 or 168 kg N ha^{-1} with three N splits to WW or SM would maintain crop yields but significantly increase N recovery efficiency and reduce the risk of environmental pollution caused by N losses.

Keywords ^{15}N-labelled urea · Alkaline calcareous soil · Nitrogen application rate · Nitrogen splits · Nitrogen recovery efficiency · Nitrogen losses

Introduction

China is now facing the unprecedented challenge of agricultural production to meet the food demands of the large and growing population. By 2030, its grain demand is expected to increase by 40 % to 680 Mt (Zhao et al. 2008). Application of mineral fertilizers, especially nitrogen (N), is a main component for increasing grain yield. To achieve high yields, high rates of N fertilizer are often applied (Zhu et al. 2000; Wang et al. 2011; Valkama et al. 2013). Current practices of applying 550–600 kg N ha^{-1} year^{-1} to winter wheat (*Triticum aestivum* L., WW) and summer maize (*Zea mays* L., SM) rotation, which account for

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50 % of WW and 35 % of SM production on the North China Plain (NCP) (Du et al. 2010), is well above the national average of N applied. Recent studies have showed that the conventional nutrient management practices, characterized by excessive N inputs, and inefficient application methods do not significantly increase crop yields, but lead to low N recovery efficiency (NRE) and high N losses (Zhu and Chen 2002; Ju et al. 2009), which can cause many environmental problems, such as the surface and groundwater contamination, the release of greenhouse gases, and soil quality degradation (Cassman et al. 2002; An et al. 2015). Therefore, efficient N management has been widely concerned due to its close relation with NRE and environmental problems (Ju et al. 2009).

The major factor that determines NRE is N application rate (Dobermann et al. 2006), as N losses increase rapidly when applied N fertilizer exceed the crop assimilation capacity (Meisinger et al. 2008; Kaizzi et al. 2012). The effects of other management practices, i.e., N application timing, source and placement are also important on NRE (Power and Schepers 1989). Previous field experiments have showed that grain yield, crop N uptake and NRE increase with increasing top-dressed N fertilizer (López-Bellido et al. 2005; Cui et al. 2008). To a certain degree, the crop yield and N uptake can indirectly reflect the status of soil available N. Ju et al. (2002) indicated that the contribution of fertilizer N to crop N uptake accounted for about 45 % with the wheat yield of 4.68–5.43 t ha^{-1}. However, Wang et al. (2011) showed that the contribution of fertilizer N to crop N uptake accounted for 15.5–30.5 % with the wheat yield levels of 8.0–9.0 t ha^{-1}. These variations may be ascribed to crop yield level and soil fertility (Cao and Yin 2015).

Under field conditions N losses are mainly due to ammonia volatilization (AV), denitrification and nitrate leaching (Zhu and Chen 2002; Basso and Ritchie 2005; Cameron et al. 2013). The amount of AV is a variable percentage of the applied N in farmland, depending on soil and climatic conditions as well as N fertilizer management (Sommer et al. 2003; Han et al. 2014). N fertilization is considered the primary source of the greenhouse gas N_2O due to the mineral N available for nitrification and denitrification (Mosier and Kroeze 2000). Many field measurements of N_2O emissions induced by N fertilizer have been performed and the influencing factors (e.g., crop type and fertilizer rate/timing) have been analyzed, suggesting that conventional N management practices result in increases in N_2O emissions (Phillips et al. 2009; Shi et al. 2012; Linquist et al. 2012). Some studies have reported that nitrate loss through leaching can be alleviated by adjusting the N application rate and the ratio of basal to topdressed fertilization (Ottman and Pope 2000; Ju et al. 2007). Therefore, efficient N management practice is important to mitigate adverse environmental effects.

The NCP is one of the most productive regions for growing WW and SM in China. Recent studies on the fate of fertilizer N in NCP have mainly focused on N application rate and fertilizer type (Ju et al. 2009), but few studies have been conducted to investigate the application of N to ensure that the best management practice is used when considering the application amount and time based on the crop N requirements. We hypothesized that efficient N management practice (recommended N application), which was developed by increasing ratios of top-dressing fertilizer and split application based on crop N uptake, would enhance NRE and reduce N loss. The specific objectives for this study were to examine whether efficient N management practice affects crop yield and NRE, and quantify the fate of ^{15}N-labelled urea applied to an alkaline calcareous soil in NCP. Our study compared three distinct N application practices (i.e., the conventional N application, reduced N and recommended N application) in the WW and SM cropping system.

Materials and methods

Study site

The field experiment was conducted on a cropland (37°5′N and 115°10′E) with rotation of WW and SM from October 2007 to October 2008 in Shenzhou City, Hebei Province, China. The experimental site is located in the Experimental Station of Dryland Farming Institute, Hebei Academy of Agriculture and Forestry Sciences, in NCP, where the altitude is 31 m and the climate is warm-temperate, sub-humid continental monsoon, with cold winters and hot summers. The annual frost-free period averages 188 days. The soil at the study site is an alkaline calcareous soil. Total precipitation was 95.1 mm in

WW season (from October 2007 to July 2008) and 364.6 mm in SM season (from July 2008 to October 2008), of which 60–70 % fell in summer (June–September; Fig. 1). Before the WW planting in 2007, soil samples were collected for routine soil analysis. Selected physical and chemical properties of the soil are shown in Table 1. Soil samples (0–20 cm depth) were collected before N application using an open-faced bucket auger (5 cm diameter) from the study area, mixed evenly, and analyzed. Soil organic matter and Total N contents were determined using a total organic C/N analyzer (Multi N/C 3100/HT1300, Analytik Jena AG, Germany). Inorganic N (NH_4^+–N and NO_3^-–N) was extracted with 2 M KCl and determined by flow injection analysis (TRAACA-2000, Germany). Olsen extractable P (Frank et al. 1998) was determined by colorimetry using phosphomolybdate reduction. Available K was determined with an atomic absorption spectrometer (NovAA300, Analytik Jena). Soil pH was measured with a compound electrode (PE-10, Sartorius, Germany) using a soil to water ratio of 1:2.5. Soil bulk density was determined on a 5 cm diameter intact core and field moisture holding determined by the Wilcox method (Marshall and Holmes 1988).

Experimental design

The experiment includes both macro-plot and ^{15}N-labelled micro-plot experiments. The macro-plot experiment was designed as a complete randomized block with three replications. To monitor the fate of ^{15}N fertilizer in seasonal crop, there were two ^{15}N-labelled micro-plots established within the fertilized macro-plots, which were bordered by a zinc galvanized iron retainer wall (1.2 m × 0.8 m × 0.45 m) inserted into the soil to a depth of 0.40 m. For both macro-plot and ^{15}N-labelled micro-plot experiments, four treatments included no N fertilizer (N0) as the control, conventional N application (i.e., WN300-2T, 300 kg N ha^{-1} with 2 splits to WW; MN240-2T, 240 kg N ha^{-1} with 2 splits to SM), reduced N application (i.e., WN210-2T, 210 kg N ha^{-1} with 2 splits to WW; MN168-2T, 168 kg N ha^{-1} with 2 splits to SM), and recommended N application (i.e., WN210-3T, 210 kg N ha^{-1} with 3 splits to WW; MN168-3T, 168 kg N ha^{-1} with 3 splits to SM). The timing and rate of N fertilizer application are given in Table 2. Wheat cultivar 'hengguan 35' and maize cultivar 'zhendan 958' were used. In the WW season, each plot was supplied with 150 kg P_2O_5 ha^{-1} (calcium superphosphate, P_2O_5, 12 %) and 90 kg K_2O ha^{-1} (potassium chloride, K_2O, 60 %) at sowing. In the SM season, each plot was supplied with 90 kg K_2O ha^{-1} (potassium chloride, K_2O, 60 %) at sowing. ^{15}N-enriched (10 % at.% excess ^{15}N) urea was applied in both the WW and SM micro-plots. All basal and topdressing N fertilizers were evenly spread by hand and immediately irrigated with 600 m^3 ha^{-1} (60 mm), the total mounts of irrigation were all 180 mm for WW and SM. The WW and SM were sown directly into the soil at a row width of 15 × 20 cm and a spacing of 25 × 50 cm. Field management was uniform in a local high-yield field.

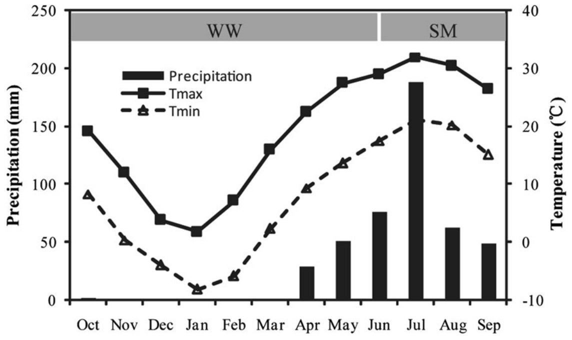

Fig. 1 Monthly precipitation and mean maximum and minimum temperatures in the WW and SM seasons. WW and SM denote the winter wheat and summer maize

Collection and measurement of ammonia volatilization (AV)

AV rates from ^{15}N fertilizer were measured using a dynamic chamber method (Hargrove et al. 1977; Cao et al. 2013). The collection device of AV was made of the closed plexiglass chamber (15 cm × 25 cm), PVC tube (4 cm × 250 cm), absorbable bottle (250 mL), and a Vacuum pump (5500 rpm). AV rates were measured twice per day and the sampling period was 3 h, from 7:00 to 10:00 a.m. and from 3:00 to 6:00 p.m. After 3 h with airflow at a rate of −8 L min, the chambers were removed to eliminate the differences in conditions between the inside and outside of the chamber. AV rates were continually measured for 7–14 days after each application of fertilizer, which depended on the timing and rate of fertilizer N, and until there were no significant differences in AV rates

Table 1 Selected physical and chemical properties of the soil

Items	Value
Organic matter (g kg^{-1})	11.70
Total N (g kg^{-1})	0.81
NO_3^-–N (mg kg^{-1})	12.14
NH_4^+–N (mg kg^{-1})	0.76
Available P (Olsen method, 0.5 mol L^{-1} $NaHCO_3$, mg kg^{-1})	5.80
Available K (1 mol L^{-1} neutral NH_4OAc, mg kg^{-1})	103.00
Soil pH	8.60
Field moisture holding (%)	31.20
Soil bulk density (g cm^{-3})	1.40

Table 2 Rate and timing of N fertilizer in the ^{15}N-labelled micro-plots during the WW and SM seasons

Treatment	N rate in the WW season (kg N ha^{-1})				Treatment	N rate in the SM season (kg N ha^{-1})			
	Basal	Elongating	Booting	Total		Basal	Bell	Silking	Total
N0	0	0	0	0	N0	0	0	0	0
WN300-2T	150	150		300	MN240-2T	80	160	0	240
WN210-2T	105	105		210	MN168-2T	56	112		168
WN210-3T	70	70	70	210	MN168-3T	33.6	100.8	33.6	168

WW winter wheat, *SM* summer maize, *N0* no N fertilization, *N300-2T and N240-2T* the conventional N application (300 kg N ha^{-1} with 2 splits to WW; MN240-2T, 240 kg N ha^{-1} with 2 splits to SM), *N210-2T and N168-2T* the reduced N application (210 kg N ha^{-1} with 2 splits to WW; 168 kg N ha^{-1} with 2 splits to SM), *N210-3T and N168-3T* the recommended N application (210 kg N ha^{-1} with 3 splits to WW; MN168-3T, 168 kg N ha^{-1} with 3 splits to SM), the same as below

between the N treatment and control. Daily AV rates were calculated by the average of the rates measured on each day. Cumulative AV losses were calculated by the sum of the daily emission over the observation period. The concentration of $^{15}NH_4^+$–N in the acid trap was measured by an improved ZHT-03 mass spectrometer (Beijing Analysis Instrument Factory, Beijing, China) equipped with an ionization source of 1200 V, and a single inlet. The AV rates from micro-plot were calculated according to the following equation:

$$A = \frac{M}{S} \times \frac{1}{t} \times 24 \times 10^{-2}, \text{ the cumulative AV losses} = \sum A_i \times T_i \quad (1)$$

where A is the flux of AV from ^{15}N fertilizer (kg N ha^{-1} day^{-1}), M is total content of ammonia in absorbing liquid (mg N), S is the area of the base of the closed chamber (m^2), t is the duration of collection (h), and T is time interval of two sampling (d).

In order to estimate the response of AV losses to environmental factors, the relationship between AV losses and environmental factors (i.e., air temperature, precipitation, soil moisture and N rate) was calculated by as the following regression equations:

$$Y_1 = a_0 + a_1T_1 + a_2M_1 + a_3P_1 + a_4N_1 \quad (2)$$

where, Y_1 is cumulative AV losses within 14 days after the applied ^{15}N fertilizer. T_1, M_1, P_1 and N_1 are the code of cumulative average temperature, soil moisture, precipitation and ^{15}N rates within 14 days, respectively. a_0 is a constant, a_1, a_2, a_3 and a_4 are the linear coefficients of T_1, M_1, P_1 and N_1, respectively.

Collection and measurement of denitrification losses

Denitrification was measured using the acetylene inhibition method and intact soil core incubation (Mahmood et al. 1998). Within the micro-plots, three soil samples were taken at random with a PVC tube

(3.2 cm × 15 cm), which were placed in a 2500 mL incubation pot buried in a 20 cm depth soil. Acetylene gas was injected into the incubation pot with 10 % (v/v) headspace volume. After a 24 h incubation, 20 mL of gas samples were collected in 10 mL vacuum bottles through the three-way value. Sampling events occurred every 14 days during crop growing period under no fertilizer application. However, gas samples were collected by increased frequency and were sampled on the 3rd, 7th and 14th days after N fertilizer application. A total of 312 gas samples were collected (192 samples for WW season, 120 samples for SM season). Nitrous oxide (N_2O) produced by denitrification was analyzed using isotopic mass spectrometers with a fully automated interface for pre-gas chromatograph concentration (Thermo Finnigan MAT-253 isotopic mass spectrometers, Germany). The slope of the changes in N_2O concentration was used to estimate the denitrification rate. Denitrification losses were calculated according to Mahmood et al. (1998).

In order to estimate the response of denitrification losses to environmental factors, the relationship between denitrification losses and environmental factors (i.e., soil temperature, soil moisture and N rate) was calculated by as the following regression equations:

$$Y_2 = a_0 + a_1T_2 + a_2M_2 + a_3N_2 \tag{3}$$

where, Y_2 is denitrification loss After a 24 h incubation, T_2, M_2 and N_2 are the code of average daily temperature, moisture and NO_3–N content in soil, respectively. a_0 is a constant, a_1, a_2 and a_3 are the linear coefficients of T_2, M_2 and N_2, respectively.

Soil and plant sampling and analyses

A total of 240 soil samples were collected from 0 to 200 cm soil layer and separated into 20 cm depth increments after crop harvest. Soil samples were dried at room temperature, crushed and sieved through a 2 mm screen for chemical analysis. The entire aboveground biomass was removed from micro-plots at harvest and partitioned into the different organs to determine crop dry matter, yield and total N uptake. Natural abundance of crop and soil in the unfertilized macro-plot and the at.% ^{15}N in micro-plot were detected using an improved ZHT-03 mass spectrometer at the Agro-forestry Academy, Chemical Institute of Hebei Province, China. The percentage of N derived from fertilizer-N (Ndff, %) was calculated from the equation as followed (Cookson et al. 2001).

$$\mathrm{Ndff}(\%) = (c-b)/(a-b) \times 100 \tag{4}$$

where c is the at.% ^{15}N in the fertilized crop or soil, a is the at.% ^{15}N in the fertilizer, and b is the at.% ^{15}N in the unfertilized crop or soil (at maturity, ^{15}N natural abundance in crop is 0.382 %, ^{15}N natural abundance is 0.366 %). N fertilizer accumulation and recovery by wheat or maize were calculated by the following equations:

$$\text{Crop uptake N}\left(\text{kg N ha}^{-1}\right) = \text{Crop dry matter} \times \text{N concentration} \times 10^{-3} \tag{5}$$

$$\text{N from fertilizer} - \text{N}\left(\text{Ndff, kg N ha}^{-1}\right) = \left(\text{Ndff}_{\text{crop}} \times \text{crop uptake N}\right) \times 10^{-2} \tag{6}$$

$$\text{Soil indigenous N}\left(\text{Ndfs, kg N ha}^{-1}\right) = (1) - (2)$$

$$\text{Residual N in soil}\left(\text{kg N ha}^{-1}\right) = \text{soil thickness(cm)} \times \text{soil bulk density}\left(\text{g cm}^{-3}\right) \times \text{N concentration} \times \text{Ndff}_{\text{soil}} \tag{7}$$

$$\text{Fertilizer N loss}\left(\text{kg N ha}^{-1}\right) = \text{Total fertilizer N} - (2) - (3) \tag{8}$$

$$\text{N recovery efficiency}(\%) = (2)/\text{Total fertilizer N} \times 100$$

$$\text{N residual efficiency}(\%) = (3)/\text{Total fertilizer N} \times 100$$

$$\text{N loss efficiency}(\%) = (4)/\text{Total fertilizer N} \times 100.$$

Statistical analysis

All statistical analyses were performed using the SAS version 8.0 (SAS Institute, Inc., Cary, NC, USA). The data were statistically assessed through One-way ANOVA between means within the factors (treatments or depth of soil layer). The least significant difference (LSD) test was used to detect the differences among treatment means at $P < 0.05$. Standard deviations were computed by root mean square errors.

The significances of the regression coefficients were tested by F-test to check the significance of each coefficient.

Results

Yield, N uptake and NRE by crop

All N treatments were significantly different than the control treatments ($P < 0.05$), showing an increase in grain yield, total biomass, and total N uptake in the WW and SM seasons (Table 3). In the WW and SM seasons, grain ^{15}N uptake in the recommended N treatment was significantly higher than the conventional N and reduced N treatments ($P < 0.05$), while N uptake from soil had no significant differences ($P > 0.05$). The total N uptake by WW consisted of 28.1–33.5 % of fertilizer ^{15}N and 66.5–71.9 % of soil N, with the WW yield level of 6.27–6.43 t ha^{-1}. The total N uptake by SM consisted of 27.0–29.4 % of fertilizer ^{15}N and 70.7–73.1 % of soil N, with the SM yield level of 8.37–8.56 t ha^{-1}. The NRE significantly increased with the decrease of N application rate ($P < 0.05$), with the recommended N treatment showing the largest increase at 16.7 and 17.2 % higher than the conventional N treatment for WW and SM, respectively.

Losses of AV and denitrification from ^{15}N fertilizer

In the WW season, AV losses from ^{15}N fertilizer mainly occurred in three N application period. For basal N and topdressing N at the jointing stage, the dynamic changes in AV losses were similar for different N treatments, and cumulative AV losses at the jointing stage were higher than basal for each N treatment. For topdressing N at the booting stage, cumulative AV losses in the WN210-3T treatment were significantly decreased compared with the WN300-2T and WN210-2T treatments ($P < 0.05$; Fig. 2a). Total AV losses decreased significantly with the reducing N rate ($P < 0.05$), with the WN210-3T treatment showing the greatest decrease at 55.2 % lower than the WN300-2T treatment ($P < 0.05$; Table 4). The sum of AV losses in three N application periods accounted for 83.5, 77.1 and 86.8 % of total AV losses throughout WW season, respectively.

In the SM season, AV losses from ^{15}N fertilizer also mainly occurred in three N application periods. For basal N and topdressing N at the bellbottom stage, the dynamic changes in AV losses were similar for different N treatments, and cumulative AV losses at the bellbottom stage were higher than basal for each N treatment (Fig. 2b). For topdressing N at the silking stage, cumulative AV losses for MN168-3T were significantly lower compared to MN240-2T and MN168-2T ($P < 0.05$), respectively (Fig. 2b). Total AV losses decreased significantly with the decrease of N application rate ($P < 0.05$), with the MN168-3T treatment showing the greatest decrease at 51.9 % lower than the MN240-2T treatment ($P < 0.05$; Table 4). The sum of AV losses in three N application periods accounted for 94.9, 93.4 and 96.7 % of total AV losses throughout SM season, respectively.

During the WW and SM seasons, denitrification losses significantly decreased with the decrease of N rate ($P < 0.05$). For the WW season, denitrification losses in WN210-3T treatment were significantly lower than those in the WN300-2T and WN210-2T treatments by 47.0 and 20.3 %, respectively ($P < 0.05$; Table 4). For the SM season, denitrification losses in MN168-3T treatment were significantly lower than those in the MN240-2T and MN168-2T treatments by 61.8 and 43.7 %, respectively ($P < 0.05$). Throughout the monitoring period, denitrification losses in all N treatments accounted for <0.7 % of total N inputs for the WW season, while accounted for 1.3–2.4 % of total N inputs for the SM season.

Measured AV data were used to estimate the coefficients for regression equations to predict the effects of N rate, air temperature, soil moisture and precipitation on the cumulative AV losses. The coefficient of determination ($R^2 = 0.897$) of the regression equations was significant ($P < 0.01$) throughout the monitoring period (Table 5), indicating that the models accurately predicted AV losses in this field experiment. The coefficients of ^{15}N rates, cumulative air temperature and precipitation for cumulative AV losses were positive, while the coefficient of soil moisture was not significant. Measured denitrification data were used to estimate the coefficients for regression equations to predict the effects of NO_3–N content, temperature and moisture in soil on the denitrification losses. The coefficient of determination ($R^2 = 0.764$) of the regression equations was significant ($P < 0.01$) throughout the monitoring

Table 3 Crop yield, ^{15}N uptake and NRE under different N fertilizer treatments

Crop	Treatment	Dry matter (t ha^{-1})	N uptake (kg N ha^{-1})	Ndff (kg N ha^{-1})	NRE (%)	Ndfs (kg N ha^{-1})	Ndff/N uptake (%)	Ndfs/N uptake (%)
Winter wheat	Grain							
	N0	5.85 ± 0.72 b	113.10 ± 7.32 b	–	–	113.10 ± 7.32 a	–	–
	WN300-2T	6.37 ± 0.44 a	141.02 ± 12.17 a	48.22 ± 2.69 b	16.07	92.80 ± 9.76 b	34.19	65.81
	WN210-2T	6.27 ± 0.62 a	137.11 ± 11.36 a	47.61 ± 2.14 b	22.67	89.50 ± 8.52 b	34.72	65.28
	WN210-3T	6.43 ± 0.53 a	144.93 ± 14.11 a	54.71 ± 3.13 a	26.05	90.22 ± 7.12 b	37.75	62.25
	Total biomass							
	N0	11.88 ± 1.25 b	188.41 ± 8.26 b	–	–	188.41 ± 8.26 a	–	–
	WN300-2T	14.68 ± 1.47 a	247.52 ± 13.21 a	73.12 ± 1.96 b	24.37	174.50 ± 6.23 b	29.54	70.46
	WN210-2T	14.36 ± 1.79 a	243.21 ± 11.24 a	68.41 ± 2.28 c	32.57	174.80 ± 5.65 b	28.13	71.87
	WN210-3T	15.06 ± 1.68 a	257.43 ± 14.58 a	86.33 ± 4.52 a	41.10	171.2 0 ± 7.31 b	33.54	66.46
Summer maize	Grain							
	N0	7.54 ± 0.64 b	90.51 ± 9.24 b	–	–	90.51 ± 9.24 a	–	–
	MN240-2T	8.47 ± 0.71 a	106.59 ± 8.37 a	48.92 ± 1.38 b	20.38	57.67 ± 6.12 b	45.90	54.10
	MN168-2T	8.37 ± 0.53a	106.93 ± 6.74 a	48.97 ± 2.01 b	29.15	57.96 ± 4.97 b	45.80	54.20
	MN168-3T	8.56 ± 0.81 a	109.38 ± 8.21 a	54.21 ± 2.84 a	32.27	55.17 ± 3.21 b	49.56	50.44
	Total biomass							
	N0	14.96 ± 1.01 b	199.60 ± 7.39 b	–	–	199.60 ± 7.39 a	–	–
	MN240-2T	17.73 ± 1.22a	256.00 ± 11.34 a	68.98 ± 1.67 b	28.74	187.02 ± 3.68 b	26.95	73.05
	MN168-2T	17.46 ± 1.34 a	252.60 ± 10.68 a	70.72 ± 2.12 b	42.10	181.88 ± 7.10 b	28.00	72.00
	MN168-3T	18.15 ± 1.19a	263.10 ± 13.49 a	77.23 ± 3.24 a	45.97	185.80 ± 6.04 b	29.35	70.65

Value (mean ± standard deviation, $n = 3$) followed by different lowercase letters between N treatments within a column are significantly different at $P < 0.05$, the same as below

Ndff denotes N from ^{15}N-labelled urea; Ndfs denotes N derived from soil (Total N uptake − Ndff); NRE, N recovery efficiency

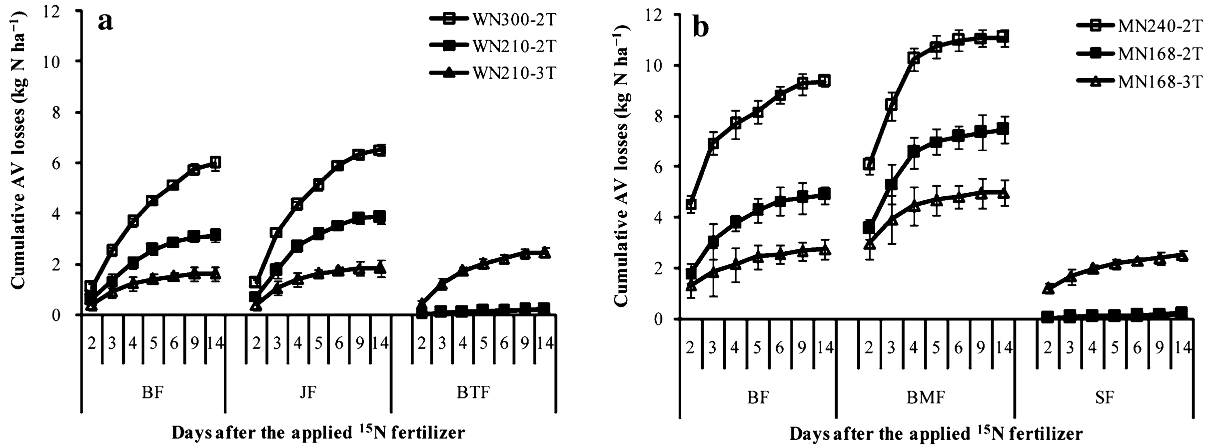

Fig. 2 Cumulative AV in three N application periods; **a** winter wheat (WW); **b** summer maize (SM). *Vertical bars* represent the standard error ($n = 3$). *BF* basal fertilizer, *JF* jointing fertilizer, *BTF* booting fertilizer, *BMF* bellbottom fertilizer, *SF* silking fertilizer, *AV* ammonia volatilization

Table 4 ^{15}N losses by AV or denitrification under the different N fertilizer treatments

Crop	Treatment	Cumulative AV losses		Cumulative denitrification losses	
		Amount (kg N ha^{-1})	AV loss efficiency (%)	Amount (kg N ha^{-1})	Denitrification loss efficiency (%)
WW	N0	–	–	–	–
	WN300-2T	15.24 ± 1.68 a	5.08	1.85 ± 0.29 a	0.62
	WN210-2T	9.29 ± 1.04 b	4.42	1.23 ± 0.12 b	0.59
	WN210-3T	6.83 ± 1.56 c	3.25	0.98 ± 0.09 c	0.47
SM	N0	–	–	–	–
	MN240-2T	21.79 ± 2.34 a	9.08	5.83 ± 0.42 a	2.43
	MN168-2T	13.43 ± 1.12 b	7.99	3.96 ± 0.64 b	2.36
	MN168-3T	10.48 ± 1.37 c	6.24	2.23 ± 0.36 c	1.33

AV ammonia volatilization

Table 5 The regression equation coefficients of AV and denitrification losses

Dependent variables	Coefficients of independent variables					R^2
	a_0	a_1	a_2	a_3	a_4	
AV losses[a]	−5.724	0.025*	0.161	0.036*	0.058**	0.897
Denitrification losses[b]	−0.157	0.002**	0.223**	/	0.002**	0.764

Where, AV, ammonia volatilization; Y_1, cumulative AV losses within 14 days after the applied ^{15}N fertilizer ($n = 18$); T_1, M_1 and P_1 are the code value for cumulative average temperature, soil moisture and precipitation within 14 days, respectively; N_1, the code value for the ^{15}N rates; Y_2, denitrification losses After a 24 h incubation ($n = 69$); T_2, the code value for average daily temperature; M_2, the code value for average soil moisture; N_2, the code value for soil NO_3–N content; R^2, the coefficient of determination

"/" not measured

* Significant with $P < 0.05$

** Significant with $P < 0.01$

[a] Regression equation of AV losses: $Y_1 = a_0 + a_1T_1 + a_2M_1 + a_3 P_1 + a_4 N_1$

[b] Regression equation of denitrification losses: $Y_2 = a_0 + a_1T_2 + a_2M_2 + a_3N_2$

period (Table 5), indicating that the models accurately predicted denitrification losses in this field experiment. The coefficients of NO_3–N content, temperature and moisture in soil for denitrification losses were positive, and the coefficients for soil moisture were larger than for soil NO_3–N content and temperature.

Distribution of residual ^{15}N fertilizer in the soil profile

In both growing seasons, the top 0–80 cm showed a significant decrease in residual ^{15}N fertilizer with incrementally lower residual ^{15}N fertilizer with depth. After WW harvest, total residual ^{15}N in the 0–200 cm soil profile significantly decreased with the decrease of N application rate ($P < 0.05$), with the WN210-3T treatment showing the lowest N value and lower than the WN300-2T by 37.0 % (Fig. 3a). The residual ^{15}N was mainly distributed in the 0–100 cm soil layer for treatments WN300-2T, WN210-2T and WN210-3T, and accounting for 92.6, 93.7 and 95.8 % of the 0–200 cm soil layer, respectively.

After SM harvest, total residual ^{15}N in the 0–200 cm soil profile significantly decreased with the decrease of N application rate ($P < 0.05$), with the MN168-3T treatment showing the lowest N value and lower than the MN240-2T by 37.4 % (Fig. 3b). For treatments MN240-2T, MN168-2T and MN168-3T, the residual ^{15}N in the 0–100 cm soil layer accounted for 83.1, 88.2 and 94.5 % of the 0–200 cm soil layer, respectively. The residual ^{15}N in 100–200 cm depth of the MN168-3T soil was significantly lower than that in the MN240-2T and MN168-2T soils. Therefore, the MN168-3T treatment can reduce residual ^{15}N fertilizer movement to deeper soil layers.

Fate and proportion of ^{15}N fertilizer

During the WW and SM seasons, the ^{15}N recovery in crop significantly increased with the decrease of N application rate ($P < 0.05$), with the WN210-3T and MN168-3T treatments showing the greatest increase at 16.7 and 17.2 % higher than the WN300-2T and MN240-2T treatments, respectively (Figs. 4, 5). The

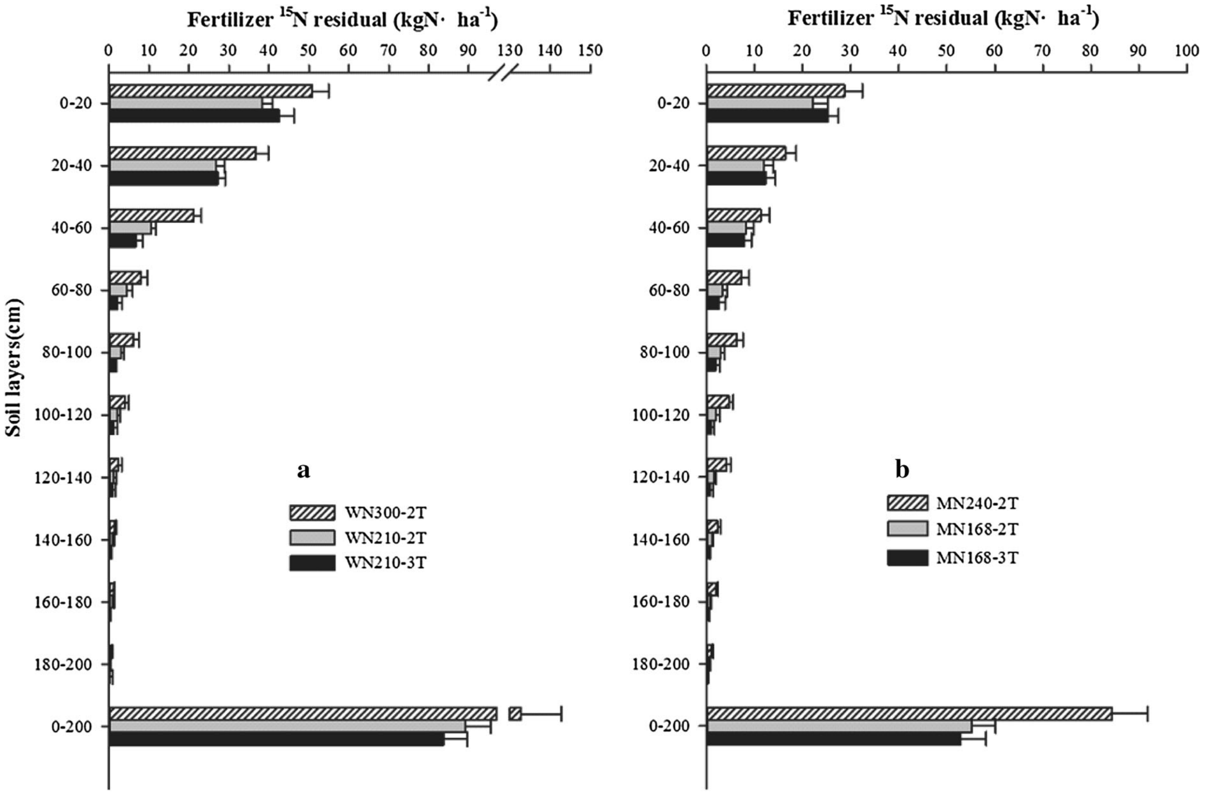

Fig. 3 The distribution of residual ^{15}N in 0–200 cm soil profile after crop harvest; **a** winter wheat (WW); **b** summer maize (SM). *Vertical bars* represent the standard error ($n = 3$)

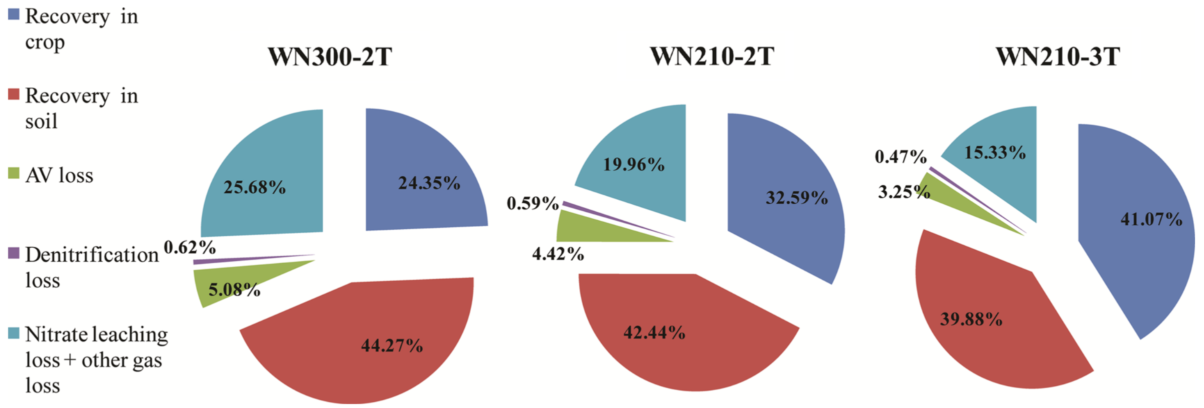

Fig. 4 Effects of different N rates/splits on the fate of ^{15}N fertilizer during the WW growing season. WW denotes the winter wheat

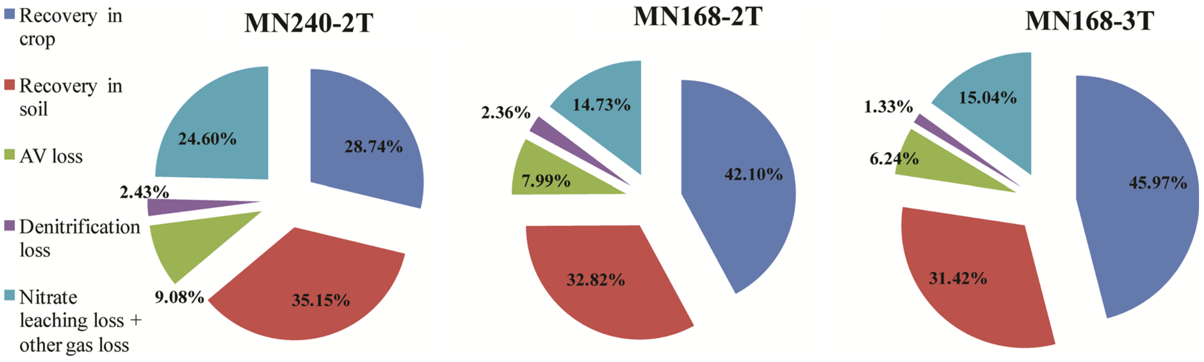

Fig. 5 Effects of different N rates/splits on the fate of ^{15}N fertilizer during the SM growing season. SM denotes the summer maize

^{15}N recovery in soil showed a reduced trend with decreasing N rate, but there was no significant difference between N treatments. However, the total N loss from ^{15}N fertilizer significantly decreased with the decrease of N applied. For the WW season, total N loss in the WN210-3T soil showed the greatest decrease at 12.3 and 5.9 % lower than the WN300-2T and WN210-2T soils, respectively (Fig. 4). For the SM season, total N loss in the WN168-3T soil showed the largest decrease at 13.5 and 2.5 % lower than the WN240-2T and WN168-2T soils, respectively (Fig. 5).

Discussion

Effect of N fertilizer on grain yield and N uptake

Results from this study indicated that grain yield had no significant difference between N treatments, and WW and SM yields attained the level of 6.27–6.43 and 8.37–8.56 t ha^{-1}, respectively (Table 3), which may be ascribed to the relatively high N fertility (total N 0.81 g kg^{-1}) of soil and environmental N inputs in the NCP. An et al. (2015) suggested that inherently more fertile soils produced higher crop yields than the infertile soils irrespective of the management practices employed. Our results also found that soil-derived N was more than two-thirds of the N assimilated by crops under the high N soil (Table 3). This is consistent with the findings of Wang et al. (2016), who found that 67.6–73.2 % of crop N was derived from soil. However, Ju et al. (2002) suggested that about 55 % of total N uptake by WW came from soil with the yield of 4.68–5.43 t ha^{-1}. The difference in results may be attributed to crop yield level and soil fertility. Many studies have found that the contribution of fertilizer N to crop N uptake was significantly influenced by crop yield level and soil fertility (Ju et al. 2002; Wang et al. 2011; Cao and Yin 2015). In this study, the NRE in the recommended N treatment was significantly higher than the conventional N treatment by 16.73 % for the WW and 17.23 % for the SM (Table 3), which is similar to the studies conducted by Shi et al. (2012) and Wang et al. (2016), who found that the higher NRE was obtained when N fertilizer application was divided into an appropriate ratio of basal and topdressing N. This difference may be attributed to better synchronization between N supply and crop demand under the efficient N management.

Losses of AV and denitrification from ^{15}N fertilizer

AV is an important pathway for nitrogen loss in farmland (Harrison and Webb 2001). In the study, total AV losses in the recommended N treatment were significantly lower than the reduced N and conventional N treatments in both growing seasons (Table 4). This is in agreement with the results reported by Lin et al. (2012) and Zhang et al. (2015), who found that increasing N rate resulted in higher NH_3 emissions during the crop growing season. Regression analysis showed that N application rate had a significant positive influence on AV losses. Those results indicated that the AV loss in dry lands was closely related to the rate and timing of N applied, which was mainly due to the transient increase of NH_4^+ produced through urea hydrolysis after addition of N fertilizer into soil (Sommer et al. 2003). Previous studies reported that lower AV 1osses were found for dryer soils than wetter ones (Han et al. 2014; Ferrara et al. 2014). Han et al. (2014) found that increasing irrigation rate resulted in higher AV losses during the SM growing season. Our results also found that total AV losses for the SM season were significantly higher than the WW season, and regression analysis indicated that cumulative air temperature and precipitation had a significant positive influence on cumulative AV losses (Table 5). Cao et al. (2013) reported that the N from the urea applied to paddy fields, is susceptible to NH_3 volatilization under the relatively high temperature of surface water. These variable data were mainly ascribed to the change of environmental factors such as precipitation/irrigation and air temperature.

Our results demonstrated that denitrification losses decreased significantly with decreasing N rate, and the lowest N loss value was in treatments WN210-3T and MN168-3T, which was similar to the findings of Linquist et al. (2012) and Phillips et al. (2009). One explanation for the decreased N losses from denitrification could occur under anoxic conditions with low NO_3–N content (Knowles 1982; Beaulieu et al. 2011). In denitrification, denitrifying bacteria are mostly heterotrophic facultative aerobes that use NO_3^- as an electron acceptor in the absence of O_2 (Knowles 1982). Regression analysis from this study indicated that soil NO_3–N content had a significant positive influence on denitrification losses (Table 5). Furthermore, our results found that denitrification losses for SM season were significantly higher than WW season (Table 4), and regression analysis indicated that temperature and moisture in soil had a significant positive influence on denitrification losses. Those were partly due to temperature and moisture dependency of denitrification process (Hernandez and Mitsch 2007; Song et al. 2014), suggesting that soil temperature and moisture are often the dominant variables controlling denitrification rates (Morse and Bernhardt 2013). because denitrification is an anaerobic process that is favored when O_2 concentrations decrease under more saturated conditions.

Residual amount and distribution of ^{15}N fertilizer

In this study, the rate and timing of the applied N had significant effects on total residual ^{15}N in the 0–200 cm soil profile as indicated by the significantly lower total residual ^{15}N in the recommended treatment compared to the conventional treatment. These results are similar to the findings of Shi et al. (2007) and Ju et al. (2007), who found that residual N significantly increased with the increase of N application rate under an equal ratio of basal fertilizer to topdressing. Conversely, other studies found that there was little difference in the amount of residual N after crop harvest between N rates and N-timing approaches (Ottman and Pope 2000; Kirda et al. 2001; Wang et al. 2016). The difference in results suggested that optimizing the rate and timing of the applied N would be beneficial to improving nitrogen management practices (Wang et al. 2011). In addition, our results also showed that residual ^{15}N after WW harvest was mainly distributed in the 0–100 cm soil layer, without obvious movement below 100 cm soil layer (Fig. 3a). This result is similar to the findings of Ottman and Pope (2000), who found that the N fertilizer rate and timing in irrigated wheat did not influence N fertilizer movement, and most of the residual N fertilizer was distributed in the surface soil. However, the recommended N treatment at rate of 168 kg N ha^{-1} with three N splits to SM was significantly lower than the conventional treatment (Fig. 3b), indicating that the recommended N treatment decreased soil NO_3-N moving down to below 100 cm soil layer in the SM season. This result may be partly due to its close relation with split N application and summer monsoonal precipitation, with the SM season showing 2.83 times higher than the WW season (Fig. 1). the increase in NO_3^- leaching during heavy rain events confirmed that the excess N applied prior to planting had a higher probability of percolating deeper than the root zone with the beginning of the summer monsoon season (Kettering et al. 2013). Similar results were reported by Gehl et al. (2005) and Zeinali et al. (2009), who found that the lower seasonal precipitation resulted in less soil water flux and less leaching losses.

The fate of ^{15}N fertilizer during the WW and SM seasons

The fates of N fertilizer in crop field were uptake by crop, residual in soil, and loss (Zhu and Chen 2002). Many studies showed that the fates of N fertilizer were related to N application, crop rotation, soil fertility, and climatic factors (Richter and Roelcke 2000; Ju et al. 2007; Wang et al. 2016). In this study, the ^{15}NRE in the recommended N treatment significantly increased compared with the conventional N treatment during the WW and SM seasons. This result is similar to the findings of Wang et al. (2016) and Shi et al. (2012), who reported that the topdressed N showed higher ^{15}NRE compared with basal N. Therefore, decreasing N rate or applying N with multiple split applications is a better strategy of N management to improve the synchrony between soil N availability and crop N demand. Shi et al. (2012) found higher N loss with basal N (averaged 37.5 %) than with topdressed N (averaged 11.5 %). In contrast, Yang et al. (2011) reported that total N losses were lower with basal N (1.7–7.1 %) than with topdressed N (3.3–12 %). Our results found that total N loss in recommended N treatment (19.05 % for WW, 22.61 % for SM) was significantly lower than the conventional N treatment (31.38 % for WW, 36.11 % for SM). The different results may be ascribed to the rate and timing of the applied N, soil fertility, rainfall, etc. Our results also found that the losses of N through AV were not more than 10 % during the WW and SM seasons, which were much lower than observed in other experiments with deep placement on the North China Plain (average 14 % loss, Cai et al. 2002a, b; 12 % loss, Zhang et al. 1992). The greater losses in those experiments may have been due to the higher rates of N application, which resulted in higher NH_4^+ concentrations. Other factors such as soil texture or cation exchange capacity may have contributed to the differing AV rates. Denitrification loss of the applied N in the SM season (average 2.04 %) was higher than the WW season (average 0.56 %), which indicating that climatic conditions (e.g., temperature, rainfall) may encourage soil denitrification during the SM season and greatly affect the fate of applied N (Morse and Bernhardt 2013; Song et al. 2014). Generally, the N loss due to AV or Denitrification was very low in this study, the larger ^{15}N losses could be from nitrate leaching and other gas emissions. In addition, our results showed that the N recovery in the 0–200 cm soil profile at SM harvest (average 33.1 %) was significantly lower than at WW harvest (average 42.2 %), suggesting that the lower residual N could be due to the higher the N leaching losses and gaseous losses in the SM season (Ju et al. 2009). Similar results For SM were reported by Shi et al. (2012), who founded a lower residual amount (33 %) of N in the 0–100 cm layer of the Middle and Lower Yangtze River Basin. In contrast, Wang et al. (2016) reported that the N recovery in soil accounted for 49.8 % of the total ^{15}N application at SM harvest, which may be due to the plastic mulching practice and the fertilization methods.

Conclusions

This study clearly demonstrated that the fate and proportion of ^{15}N-labelled urea were significantly influenced by rate and timing of the applied N during the WW and SM seasons. Overall results showed that recommended N treatment, i.e., decreasing conventional N application rate by 30 % and three N splits, significantly increased crop N recovery while reduced total N loss without crop yield decline in the NCP. Those results indicated that the recommended N treatment is a better N management practice to improve the synchrony between soil N availability and crop N demand, and reduce the risk of environmental pollution. In order to meet concerns over food security and environmental quality, further studies are needed to provide a soil Nmin test and a plant tissue nitrate test as well as the rate and timing of the applied N for making N fertilizer recommendations in the NCP.

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