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Fates of ¹⁵N-labeled fertilizer in a black soil-maize system and the response to straw incorporation in Northeast China

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Abstract

Purpose Over-fertilization has caused low nitrogen (N) use efficiency and N pollution in China. A better understanding of the fate of fertilizer N is critical for improved appropriate N management practices.

Materials and methods We examined the fate of urea-N applied to a typical black soil-maize system and the response to straw incorporation in Northeast China using the field $15N$ labeling technique. Large plots (25 m^2) were used to reduce artificial disturbance and facilitate multiple samplings in one growing season.

Results and discussion We found that of the applied N (200 kg N ha−¹), 52% was taken up by crops at harvest and 24% was retained in the soil (0–40 cm). The unrecovered 23% was likely lost via gases emission or leaching, which mainly occurred in the early days of maize cultivation. Fertilizer N contributions to the crop N uptake were 42% during vegetative growth and 30% during reproductive growth, which indirectly indicates that native soil N was the dominant N source for maize growth. However, high N uptake by maize resulted in low replenishment of fertilizer N to soil N. As a potential

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nutrient management approach, straw incorporation (2.4 t ha−¹) stimulated N retention and reduced N loss, with 14% unrecovered fertilizer N.

Conclusions To maintain long-term soil N supplies, straw incorporation could be a valid agronomic practice to prevent the degradation of black soil because of long-term N depletion during maize cultivation in Northeast China.

Keywords 15 N labeling \cdot Mollisol \cdot Allocation \cdot Fertilizer-derived N . Nitrogen use efficiency

1 Introduction

With increases in the population and living standards, intensive agricultural activities, such as nitrogen (N) fertilization, play an important role in satisfying the world's food needs (Heffer and Prud'homme [2015](#page-10-0)). However, excessive and inappropriate N fertilization also leads to serious consequences for air and water pollution and causes health problems (Galloway et al. [2008;](#page-10-0) Ju et al. [2009](#page-10-0)). Thus, quantifying the use and loss (fate) of fertilizer N has long been a hot topic in N cycling studies of agroecosystems. By calculating the difference in crop N uptake between N-fertilized and control treatments, Zhang et al. ([2008\)](#page-11-0) evaluated the N use efficiency (NUEdiff) of major cereal crops in China based on the results from 1333 field experiments from 2001 to 2005. They found that the NUE $_{\text{diff}}$ values of maize, wheat, and rice were only 26, 28, and 28%, respectively, implying that > 70% of the fertilizer N accumulated in the soil profile or was lost into the environment. However, numerous researchers have doubted these proportions because of defects in NUE_{diff} concept and calculation method (Yan et al. [2014;](#page-11-0) Ju [2014](#page-10-0)). The NUE $_{diff}$ calculation relies heavily on the N uptake in the control treatment, and the calculation assumes that native soil N plays the

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same role in crop N uptake in control and N-fertilized treatments. However, the native soil N supply is always changed by N fertilization during crop cultivation (Yu et al. [2010\)](#page-11-0).

Alternatively, the $15N$ tracer technique has been widely used to trace the fate of fertilizer or deposited N (Reddy and Reddy [1993](#page-11-0); Rees et al.1997; Stevens et al. [2005](#page-11-0); Pan et al. [2009b](#page-11-0); Rimski-Korsakov et al. [2012;](#page-11-0) Chen et al. [2016](#page-10-0)). The main advantage of the isotopic method is the differentiation of N sources between fertilizer N and soil N and the quantitative evaluation of the fate of fertilizer N. However, considering the high cost of $15N$ –labeled fertilizer in the past, almost all these studies were conducted under strictly controlled conditions with small soil surfaces, such as pot, soil column, or microplot $(\leq 1 \text{ m}^2)$ cultivations, and few studies have been conducted in normal-size plots. Crops growth is frequently limited under human disturbance and environmental stress (Stevens et al. [2005;](#page-11-0) Gardner and Drinkwater [2009\)](#page-10-0); therefore, studies conducted under such conditions may have underestimated crop N uptake and overestimated N losses. Therefore, experiments that simulate field conditions must be designed to evaluate the actual fate of fertilizer N.

Intensive maize (Zea mays L.) cultivation in China primarily occurs in Northeast (40%) and central North (35%) China, where 100.0 and 73.6 million tons (Mt) of corn grain were produced in 2015 (data from [http://data.stats.](http://data.stats.gov.cn) [gov.cn\)](http://data.stats.gov.cn). Because of the variations in soil and climate condition, the fate of fertilizer N in a soil-maize system may be site-dependent (Zhang et al. [2016](#page-11-0)). However, until now, the published data on the fate of fertilizer N in soilmaize systems in China were derived mainly from the Central North (e.g., North China Plain and Chinese Loess Plateau) and less from the Northeast (Zhang et al. [2008](#page-11-0); Cui et al. [2010](#page-10-0)). Compared with the Central North, Northeast China has higher soil organic matter, lower soil pH, and lower soil temperature, which may favor N retention and could reduce N loss during maize cultivation. Therefore, the model-based calculation might have overestimated the N loss in soil-maize system in China without considering the contribution of the Northeast.

The annual production of maize straw in Northeast China is > 100 million tons, which is calculated by grain production and the harvest index (Ma [2008](#page-11-0)). Traditionally, a large proportion $(> 40\%)$ of maize straw was burnt in the open field or in homes, which has a significant impact on the environment and human health via the release of various gases and aerosols (e.g., $CO₂$, NOx, SO₂, CO, VOCs, black carbon, and particulate matter) (Li et al. [2007;](#page-10-0) Gao et al. [2009](#page-10-0); Hong et al. [2016](#page-10-0)). For the past two decades, the Chinese government has advocated reasonable and efficient straw management methods, such as straw incorporation, to maintain and improve soil structure, porosity, and water and nutrient retention without jeopardizing the environment (Liu et al. [2014;](#page-10-0) Zhang et al. [2014\)](#page-11-0). An earlier study by Powlson et al. ([1985\)](#page-11-0) reported that the incorporation of 3 ton ha^{-1} of wheat straw with fertilizer N synchronized the crop N supply with the N demand, and reduced the N losses by 47–60%. However, such conclusions are controversial, for example, crop biomass might be suppressed by nutrient limitation in the early stages of cultivation because of the competitive advantage of microorganisms over roots that is induced by straw incorporation (Kuzyakov and Xu [2013;](#page-10-0) Zhou et al. [2016\)](#page-11-0). Therefore, evaluating the dynamics of N use and N loss by following the fate of fertilizer N under local farmers' management regimes as well as under straw incorporation is of both agricultural and environmental importance.

Although many studies have documented that N use during maize production in China was low and losses were high (Zhu [2008;](#page-11-0) Zhang et al. [2008](#page-11-0); Cui et al. [2010](#page-10-0)), quantitative data on the dynamics and recovery of fertilizer N under field conditions are surprisingly lacking, especially in the black soil region of northeastern China. Many uncertainties remain because of the controversial NUE $_{diff}$ calculation by the difference method and impractical $15N$ experimental methodologies. We adopted a field experiment that took local farmers' management into account (e.g., furrow-ridge cultivation, fertilizer placed in the ridge as a basal dressing before sowing) and used larger plots (25 m^2) than previous studies to simulate realistic field conditions and facilitate multiple samplings in one growing season. The objectives of this study were to (1) investigate the fate of fertilizer N in crops and soils at harvest in Northeast China, (2) explore the temporal dynamics of the fate of fertilizer N during maize cultivation in order to reveal the major stage of N loss, and (3) determine whether straw incorporation can improve N retention and reduce N loss.

2 Materials and methods

2.1 Experimental site

The experiment was conducted in 2015 in a field under longterm maize cultivation at the Jilin Academy of Agricultural Sciences (43° 30′ N, 124° 48′ E). This site was located in Songnen Plain, the largest black soil region and the main corn production base in China. The experimental site has a temperate continental monsoon climate. Average annual precipitation ranges from 500 to 600 mm, annual temperature ranges from 3 to 6 °C, and the frost-free period is from 120 to 150 days. Maize is the staple crop in the study region, and it is sown at the end of April and harvested at the end of September.

The soil is classified as a black soil (Mollisols) according to the genetic classification and Typic Hapludoll according to the US soil taxonomy. Just before the experiment on 25 April 2015, soil at a depth of 0–20 cm was collected to determine the background information. The soil contained 16.1 g kg^{-1} organic C, 1.56 g kg⁻¹ total N, 6.0 mg kg⁻¹ NH₄⁺-N, 47.7 mg kg^{-1} NO₃⁻-N, 21.6 mg kg^{-1} Olsen-P, and 177 mg kg−¹ available K, 16% sand, 45% silt, and 39% clay; and it had a pH of 6.2.

2.2 Experimental design

Three treatments with four replicates of each treatment were set up: (1) Control, without N application; (2) treatment N, applying urea, 200 kg N ha⁻¹; and (3) treatment NS, applying urea and straw, 200 kg urea-N ha⁻¹ and 2400 kg straw ha⁻¹ (dry weight, approximately a quarter of the annual straw production). Each of the 12 plots was 25 m² (3.125 m \times 8 m). These plots were arranged in four blocks, and were surrounded by a buffer strip of approximately 1 m (Fig. 1). Phosphorus and potassium fertilizer applications were the same for all treatments: 90 kg P_2O_5 ha⁻¹ and 90 kg K_2O ha⁻¹. The straw applied was maize straw that had

Fig. 1 Field arrangement and diagram showing the ridgefurrow cropping system, as well as the sampling area in the plot

been harvested in 2014 and crushed to form pieces that were < 2 cm.

All of the fertilizers were in the solid form and applied as basal fertilizer before sowing. To simulate realistic conditions, local practices, such as ridge-furrow cultivation and banded fertilizer application were applied in the experiment. In detail, after plowing and ridging, fertilizer and straw were bandapplied in each ridge. A hand-powered hole-drilling machine was used to sow at the peak of each ridge. Every plot had five ridge furrows and the height of the ridge was 5–8 cm (Fig. 1). The depth of the fertilizer and straw placement was 5 cm.

The 15 N–enriched urea (with 15 N abundance of 5.24%) was mixed with ordinary urea to obtain a final abundance of 1.2% (corresponding to $\delta^{15}N$ 2276‰) prior to field use. A widely used maize hybrid (*Xianyu 335*) was chosen for this study. The planting density was 175 plants per plot (70,000 plant ha⁻¹). To ensure at least one germination per hole, each hole contained two seeds, and one was pulled out if both successfully germinated. Other agronomic management, such as pesticide and herbicide application, was performed

Fig. 2 Daily mean temperature and moisture at a depth of 5 cm during the experiment. Soil and plant samples were collected 68, 94, 131, and 154 days after fertilization at the twelve-leaf (V12), tasseling (VT), dough (R4), and physiological maturity (R6) stages of maize, respectively

according to local practices. No other fertilizer or irrigation was applied during the growth period.

2.3 Sampling

Soils and plants were sampled at four stages during the cultivation: (1) day 68 (V12, twelve-leaf stage), (2) day 94 (VT, tasseling stage), (3) day 131 (R4, dough stage), and (4) day 154 (R6, physiological maturity).

Considering the spatial heterogeneity of fertilizer N, soil was sampled from ridges (0–10, 10–20, 20–30, and 30– 40 cm) and furrows (0–10 and 10–20 cm) separately. Two stainless steel frames (length \times width \times height, $15.5 \times 23.5 \times 20$ and $47 \times 23.5 \times 20$ cm) were inserted into the soil using a hammer at the ridge and the furrow, and then 0–10 and 10–20 cm soils were removed separately. After removing stones and visible fauna, the fresh soil was mixed thoroughly by hand, and a portion was sampled. Soils from the 20–30 and 30–40 cm layers were randomly sampled directly using a soil auger (2.5 cm diameter) within each frame immediately after sampling the 0–20 cm soils. Five soil segments of the same depth from each position (ridge or furrow) of each plot were mixed well to make a composite sample. Undecomposed straws in the NS treatment were also collected as one sample. After soil sampling, the remaining soils were backfilled to the 0–10 and 10–20 cm layers in their corresponding plots. The bulk density of each soil layer was determined at harvest.

Five plants from the central area in each plot were selected as samples (Fig. [1\)](#page-2-0). All five maize plants were

separated into three to six organs according to the sampling stage. In the R4 and R6 stages, plant samples were separated into root, stem, grain, cob, leaf, and other (including leaf sheath, husks, stamens, pistil, and bracts as stem); although, in the V12 and VT stages, only three or four parts (root, stem, leaf, with or without other) were separated. The fresh weights of these organs were determined to record biomass or yield, and the tissue was then cut into < 2-cm pieces by a fodder chopper and mixed thoroughly. Subsamples were taken to the laboratory and dried in an oven (70 °C) to determine their water content, and they were then used to calculate the dry weights of related organs in each plot and to determine the N concentrations and 15N abundance. The root mass was collected in the soil volume at the 0–20 cm layer. The roots were washed thoroughly in a laboratory to remove any adhering soil before drying and weighing. The dried organ samples including roots were kept in separate sealed bags for future chemical analyses.

2.4 Soil temperature and moisture

Soil temperature (°C) and moisture (volumetric water content, %) were determined by six online monitors (Campbell Scientific CS650, North Logan, UT, USA) (Fig. 2). The monitors, three for the N treatment and three for the NS treatment, were embedded into the soil at a depth of 5 cm (ridge). Although the temperature and moisture in the control treatment were not measured, they were assumed to be the same as in the N treatment.

2.5 Chemical and isotope analysis

Dried soil and plant tissue samples were crushed and finely ground in a ball mill for the analysis of the N concentration and 15N abundance using an elemental analyzer (Elementar Vario MICRO cube, Hanau, Germany) coupled with a stable isotope ratio mass spectrometer (Isoprime 100, Stockport, UK). To reduce the likelihood of cross contamination, all soil and tissue samples were ground and determined in the order from lowest ¹⁵N abundance to highest abundance. We ran four standards provided by Sigma (acetanilide, L-histidine, glycine, and D-glutamic; N concentrations are 10.37, 27.10, 18.67, and 9.52%; $\delta^{15}N$ values are + 1.44, - 7.57, 1.57, and − 5.66‰) every ten samples to test the stability and to correct for drift. The standard deviations of the measurements (N concentration and δ^{15} N value) were less than 0.23% and 0.15‰ for the four standards ($n = 15$).

Sub-samples of fresh soil at four stages were extracted with 2 mol l^{-1} KCl (soil: water = 1:5) and shaken for 1 h and filtered through Whatman 42 filter paper. The extracts were used to determine the mineral nitrogen concentrations $(NH_4^+$ -N, NO₃⁻-N) using a continuous chemical analyzer (SmartChem 200, Roma, Italy). The extracts were stored immediately in polypropylene bottles in a -20 °C freezer prior to the analyses.

2.6 Calculation and statistical analysis

We calculated $\delta^{15}N_x$ (sample ¹⁵N enrichment relative to the standard) and ¹⁵N atomic abundance A_x (sample ¹⁵N as a percentage of the total N) as follows:

$$
\delta^{15} \mathcal{N}_{\mathbf{x}} = \left(\frac{R_{\mathbf{x}}}{R_{standard}} - 1\right) \times 1000\%o \tag{1}
$$

$$
A_{\rm x} = \frac{R_{\rm x}}{R_{\rm x} + 1} \times 100\tag{2}
$$

where R_x is the ¹⁵N¹⁴N ratio in the tested sample as measured by the stable isotope mass spectrometer. Atmospheric N_2 is the standard ($R_{\text{standard}} = 0.003676$, δ^{15} N_{standard} = 0‰, $A_{standard} = 0.3663\%$. The fate of fertilizer N in the soilmaize system was calculated based on the principles of $\mathrm{^{15}N}$ mass balance (Stevens et al. [2005](#page-11-0); Kettering et al. [2013\)](#page-10-0).

$$
Ndf_x = \frac{A_x - A_{bg}}{A_U - A_{bg}} \times 100\%
$$
\n(3)

$$
Recovery_{S_x} = \frac{NdfF_x \times N_x \times Bulk\ density \times V \times 10^{-1}}{F_N} \times 100\% \quad (4)
$$

$$
Recovery_{P_x} = \frac{NdfF_x \times N_x \times DW \times 10^{-4}}{F_N} \times 100\% \tag{5}
$$

where Ndf_x is the proportion of soil or plant N derived from fertilizer (%); N_x is the N concentration of soil or each plant component (%); A_x , A_{bg} and A_U represent ¹⁵N abundance of the related N pools, background (in control treatment), and the tracer urea-¹⁵N, respectively; *Recovery_{Sx}* and *Recovery_{Px}* represent the proportions (%) of urea-derived N in soil layers and maize organs, respectively; and F_N is the total rate of fertilizer N applied to soil (200 kg N ha⁻¹). The bulk densities of soils are 1.068 (ridge 0–10 cm), 1.368 (ridge 10–20 cm), 1.365 (ridge 20–30 cm), 1.273 (ridge 30–40 cm), 1.359 (furrow, 0–10 cm), and 1.320 (furrow, 10–20 cm) g cm⁻³. The volume (V) of soil is 1000 m³ ha⁻¹ (0–10 cm layer). DW is the dry weight of related organs (kg ha⁻¹).

The nitrogen stock in the soil was calculated as the sum of the stock in the ridge (areal proportion, 25%) and furrow (areal proportion, 75%). We used two approaches to calculate NUE, as shown in the following equations:

$$
NUE_{15N} = Recovery_{P_{stem}} + Recovery_{P_{log}} + Recovery_{P_{obs}} + Recovery_{P_{other}} + Recovery_{P_{cob}}
$$

$$
+ Recovery_{P_{gwin}}
$$

$$
\left(6\right)
$$

$$
NUE_{diff} = \frac{U - U_0}{F_N} \times 100\% \tag{7}
$$

where NUE determined by the ¹⁵N method (NUE_{15N}) is the sum of $Recovery_{P_x}$ above ground; NUE determined by the difference method (NUE_{diff}) is the ratio between additional N uptake and input fertilizer N; and U and U_0 are the crop N uptake in aboveground parts, including the stem, leaf, cob, grain, and other at the harvest (R6), in the N-fertilized treatments that received urea-N and in the control that received no urea-N, respectively.

Statistical analyses were performed using the software package SPSS 13.0 for Windows (SPSS Inc., Chicago, IL, USA). Differences among the three treatments were examined by one-way ANOVA. Multiple comparisons were performed based on the least significance difference (LSD) test at a confidence level of 95%. Figures were produced using SigmaPlot 12.5 software. Error bars in the figures are standard errors. Standard errors of the integrated variables $(C/N, 15N)$ recovery, proportions of urea-derived N) were calculated by the propagation of error using a Gaussian function (Ku [1966\)](#page-10-0).

3 Results

3.1 Moisture and temperature

Comparing with the N treatment, straw incorporation increased the surface soil moisture to various degrees; from − 1.4 to 1.7% (mean 0.4%, volumetric water content) on days 0–60, and from 1.0 to 8.5% (mean 3.4%) on days 60–154. In addition, straw incorporation decreased the surface soil temperature by 0.2 °C on averagely throughout the experimental

Fig. 3 Temporal patterns of soil mineral N concentrations (left, middle) and their stocks (right) in different layers of soil at background and in four sampling periods during cultivation. "BG," "V12," "VT," "R4," and

" $R6$ " below the x-axis represent background, twelve-leaf, tasseling, dough, and physiological maturity stages, respectively. Error bars in the figure are standard errors $(n = 4)$

period, and the difference was greatest on the summer days when the air temperature was high (Fig. [2](#page-3-0)).

3.2 Soil mineral N dynamics

In the control, the soil mineral N concentrations were low (averaging 1.9 and 14 mg N kg⁻¹ in the 0–10 cm layer as NH_4^+ and NO_3^- , respectively) and changed little over time in either the ridge or furrow soil (Fig. 3). In the N treatment, mineral N concentrations were enhanced in the 0–10 cm soil of the ridge area. Along with the increase of maize growth stages, the enhanced NH_4^+ and NO_3^- concentrations decreased gradually from 74 and 177 mg N kg⁻¹ at the V12 stage to 8.3 and 70 mg N kg^{-1} at the R6 stage. In the NS treatment, soil mineral N concentrations in the 0–10 cm layer were significantly reduced at the V12 stage compared with those in the N treatment, but were the same or greater at the VT, R4, and R6 stages. In the 10–20 cm layer, the soil mineral N concentrations were slightly lower than in the 0–10 cm layer, although the trends were similar over time.

Integrating soil mineral N in different locations (ridge and furrow) and layers $(0-10$ and $10-20$ cm), we calculated the total mineral N stocks in the 0–20 cm layer. Soil NH_4^+ and NO_3^- stocks (0–20 cm) in the control declined rapidly before the VT stage and remained stable at low levels thereafter. However, for the N-fertilized treatments, soil NH_4^+ and NO_3^- stocks decreased more slowly than in the control, from 30 to 38 and 91–103 kg ha^{-1} , respectively, at the V12 stage to 7.9–11 and $36-57$ kg ha⁻¹, respectively, at the R6 stage.

3.3 Soil total N concentration and $\delta^{15}N$

Compared with the control, the N-fertilized treatments increased the soil total N concentrations in the 0–10 and 10– 20 cm layers of the ridge (Fig. [4](#page-6-0)), although the enhancement was significant only at the V12 and R4 stages.

Compared with the control, the N-fertilized treatments increased 15N abundances in almost all soil layers except for the furrow 10–20 cm soil at the R6 stage. From V12 to R6, the soil ¹⁵N abundances (δ^{15} N in ‰) decreased from 287 to 95‰ in the ridge 0–10 cm layer and from 183 to 71‰ in the ridge 10–20 cm layer. Compared with the N treatment, the straw incorporation treatment (NS) increased the soil $15N$ abundance in the ridge 0–10 cm layer, although the difference was only significant at V12 stage ($P < 0.05$).

3.4 Maize biomass, N concentration, N uptake, and $\delta^{15}N$

The biomass of the maize organs (root, stem, leaf, others, cob, and grain) varied with growth stages (Fig. [5](#page-6-0)). The total biomasses at harvest were 21 ± 2 , 22 ± 1 , and 23 ± 1 tons dry weight per hectare for the control, N, and NS treatments, respectively. Of this, the aboveground biomass and grain accounted for 96 and 38–40%, respectively. Significant differences were not observed in the total biomass and grain production among the three treatments $(P > 0.05)$.

Nitrogen fertilization significantly increased the N concentrations in all organs except for the cob at all four growth stages. The N concentrations peaked in the stem and leaf (> 3%) for N-fertilized treatments at the V12 stage. Over time from V12 to R6, the N concentrations

Fig. 4 Soil total N concentrations (left) and $\delta^{15}N$ (right) in different layers of soil in ridge and furrow at four sampling stages during cultivation. "V12," " ∇ T," "R4," and "R6" below the x-axis represent twelve-leaf, tasseling, dough, and physiological maturity stages,

decreased by $48-57$, $77-79$, and $51-59\%$ in the root, stem, and leaf, respectively.

The leaf and grain were the most N-rich organs at harvest, and 74–77% of the absorbed N was allocated to the leaf and grain at the R6 stage (Fig. [6\)](#page-7-0), while only 4.7–5.2% was allocated to the root and cob. In summary, cumulative N uptakes by maize were 215 ± 12 , 280 ± 16 , and 299 ± 11 kg N ha⁻¹ at harvested for the control, N, and NS treatments, respectively (Table [1](#page-7-0)).

Nitrogen labeling significantly increased the $15N$ abundance $(\delta^{15}N$ in ‰) in all plant organs at all four growth stages (Fig. [6](#page-7-0)), from 4 to 13% in the control to 743–

respectively. Different lowercase letters above the columns in the same cluster of the same soil layer and the same sampling indicate significant differences (LSD, $P < 0.05$). Error bars in the figure are standard errors $(n = 4)$

1281‰ in the N-fertilized treatments. Over time from V12 to R6, the enhanced $15N$ abundances in the N and NS treatments decreased gradually from 969 to 1281‰ (root, stem, and leaf) at the V12 stage to 804–992‰ (root, stem, and leaf) and 743–837‰ (others, cob, and grain) at the R6 stage.

3.5¹⁵N recovered by soil and maize and unaccounted loss

Uptake was the main fate of $15N$ when the crops were harvested at the R6 stage, with 52–53% of the fertilizer N recovered (Fig. [7](#page-8-0)). Of this, 34–36% was from vegetative tissue and 17–

Fig. 5 Plant biomass (left) and plant N concentrations (right) in different organs of maize at four sampling stages during cultivation. " $V12$," " VT ," "R4," and "R6" below the x-axis represent twelve-leaf, tasseling, dough, and physiological maturity stages, respectively. "Others" in the figure

includes the leaf sheath, bracts, stamens, and pistil. Different lowercase letters above the columns in the same cluster of the same organ and the same sampling indicate significant differences (LSD, $P < 0.05$). Error bars in the figure are standard errors $(n = 4)$

Fig. 6 Crop N uptake (left) and related δ^{15} N (right) in different organs of maize at four sampling stages during cultivation. " $V12$," " VTT ," " $R4$," and " $R6$ " below the x-axis represent twelve-leaf, tasseling, dough, and physiological maturity stages, respectively. "Others" in the figure

includes the leaf sheath, bracts, stamens, and pistil. Different lowercase letters above the columns in the same cluster of the same organ and the same sampling indicate significant differences (LSD, $P < 0.05$). Error bars in the figure are standard errors $(n = 4)$

18% was from reproductive tissue (Table 1). The total ^{15}N recoveries in the plant organs were as follows: grain (27– 29%) > leaf $(11-12\%)$ > stem (6%) > other (5%) > root (2%) > cob (1%) . Compared with the control, the crop N derived from soil was significantly reduced in the N treatment $(P < 0.05)$ but still accounted for the majority of crop N uptake at the harvest (Table 1). In addition, 36–37% of the crop N at harvest was derived from fertilizer (Ndff) in the N-fertilized treatments.

The remaining proportions of fertilizer N in the soil (ridge 0–40 cm and furrow 0–20 cm) decreased gradually from 65 to 68% at the V12 stage to $25-33\%$ at the R6 stage. Most of the remaining fertilizer N was in the ridge 0–20 cm layer during cultivation (Fig. [7](#page-8-0)). Compared with the N treatment, straw incorporation (NS) resulted in significantly more $15N$ remaining in the 0–10 cm soil layer at the V12 stage $(P < 0.05)$.

The total $15N$ recovery in the soil-maize system at harvest was 77% for the N treatment, which means 23% of the fertilizer N could not be accounted for after one growing season, and it was likely lost via gases or leaching. Straw incorporation (NS) increased the retention of applied fertilizer N and

Table 1 Crop N uptake and its sources (fertilizer N and soil N) as well as N use efficiency evaluated by the ¹⁵N method (NUE_{15N}) and the difference method (NUE_{diff}) during vegetative and reproductive growth stages of cultivation

Treatment	Crop N uptake $(kg ha^{-1})$	Crop N derived from soil $(kg ha^{-1})$	Crop N derived from fertilizer $(kg ha^{-1})$	Ndff [*] $(\%)$	NUE_{15N} (%)	NUE_{diff} (%)
	Vegetative growth stage (days 0–94)					
Control	122 ± 18 a	122 ± 18 a		$\overline{}$	$\overline{}$	
N	159 ± 8 a	$92 \pm 6 a$	67 ± 6 a	42	34	18
NS	184 ± 27 a	113 ± 8 a	71 ± 8 a	39	36	31
	Reproductive growth stage (days 94–154)					
Control	92 ± 19 a	$92 \pm 19 a$		—		
N	122 ± 13 a	$85 \pm 15 a$	37 ± 9 a	30	18	15
NS	$115 \pm 21 a$	$80 \pm 15 a$	$35 \pm 12 a$	30	17	12
	Whole growth period (days $0-154$)					
Control	$215 \pm 12 b$	$215 \pm 12 a$		$\overline{}$		
N	$280 \pm 16 a$	176 ± 11 b	104 ± 11 a	37	52	32
NS	299 ± 11 a	193 ± 5 ab	$106 \pm 5 a$	36	53	42

The same lowercase letters behind standard errors in the same volume indicate no significant difference between the two treatments at the 0.05 significance level

*Ndff represents the proportion crop N derived from fertilizer

Fig. 7 Temporal patterns of $15N$ recovery in different layers of soil and different organs of maize and the unaccounted loss at four sampling stages during cultivation. "V12," "VT," "R4," and "R6" below the x axis represent twelve-leaf, tasseling, dough, and physiological maturity

stages, respectively. "Others" in the figure includes the leaf sheath, bracts, stamens, and pistil. Error bars in the figure are standard errors $(n = 4)$ of total soil pool or total plant pool, calculated by the propagation of error

reduced N loss, although significant differences in plant N uptake were not observed.

4 Discussion

4.1 Fertilizer N recoveries in maize and N use efficiency

This research quantified the fate of urea- 15 N as a basal dressing at four growth stages in a black soil-maize system managed according to local practices. Our results showed that crop N uptake was the main fate of the fertilizer N. The aboveground ¹⁵N recovery (NUE_{15N}) at harvest was 50% for the N treatment in this study, which was in the range of oftenreported NUE_{15N} values (39–65%) of field maize with a similar fertilization rate in the Great Plains of the USA (Nebraska, Illinois, Minnesota, and North Dakota) (Varvel and Peterson [1990;](#page-11-0) Walters and Malzer [1990](#page-11-0); Torbert et al. [1992](#page-11-0); Schindler and Knighton [1999](#page-11-0); Blesh and Drinkwater [2014\)](#page-10-0) and Quebec, Canada (Alkanani and MacKenzie [1996;](#page-10-0) Tran and Giroux [1998\)](#page-11-0). The NUE_{15N} of maize in this study was higher than the reported NUE_{15N} of field maize (18–39%) in the North China Plain (Hebei, Shandong, and Beijing) and the Chinese Loess Plateau (Shannxi and Gansu) (Rees et al. [1997](#page-11-0); Zhong, [2004;](#page-11-0) Pan et al. [2009a](#page-11-0); Wang et al. [2014;](#page-11-0) Xu et al. [2015](#page-11-0); Liu et al. [2015;](#page-10-0) Wang et al. [2016\)](#page-11-0). Variations in soil and climate conditions, fertilizer regimes, and cultivation methods might contribute to the differences in NUE_{15N} among the different regions (Blesh and Drinkwater [2014](#page-10-0); Zhang et al. [2016\)](#page-11-0). The only field study referring to the fate of $15N$ in a maize system in Northeast China was a microplot study $(1.26 \times 0.8 \text{ m}^2)$, which showed that 36% of applied urea N (180 kg N ha^{-1}) was absorbed by maize (Zhang et al. [2010](#page-11-0)). The NUE_{15N} would be even higher (> 50%) if the subsequent use of the

¹⁵N remaining in soils were considered (Blesh and Drinkwater 2013; Yan et al. [2014](#page-11-0)).

NUE can also be assessed by the difference method (NUE_{diff}) according to Eq. (7) (7) (7) . Our study showed that NUEdiff was 32%, which was lower than the 50% obtained by the 15 15 N-labeling method (Table 1). This finding is inconsistent with that of most studies in the literature (Bundy and Andraski [2005;](#page-10-0) Stevens et al. [2005;](#page-11-0) Chen et al. [2016\)](#page-10-0), although several examples similar to our study could also be found (Schindler and Knighton [1999](#page-11-0); Rimski-Korsakov et al. [2012\)](#page-11-0). The lower NUE_{diff} than NUE_{15N} values could be attributed to confounding methodologies, which had described in previous studies (Schindler and Knighton [1999](#page-11-0); Yu et al. [2010\)](#page-11-0). In this study, these values might attributed to (1) the high soil N availability (Fig. [3](#page-5-0)) and low soil temperature (Fig. [1](#page-2-0)) in the early stage of maize cultivation, suppressed the "substitution" or "replenishment" effect between fertilizer N and soil N (negative "added N interaction") (Torbert et al. [1992\)](#page-11-0), and caused less soil N to be absorbed by maize in the N treatment than in the control (Table [1\)](#page-7-0), thereby decreasing the NUE_{diff}; and (2) the ridge fertilization and ridge cultivation (heterogeneous distribution of mineral N and roots, Fig. [3](#page-5-0)) might change the N acquisition strategy of roots in the N treatment, which enhanced the probability of maize N uptake from fertilizer rather than from around soil, and thereby increased NUE_{15N} .

4.2 Fertilizer N recoveries in soil and N losses

The proportion of $15N$ retained in the 0–40 cm soil at harvest was 25% for the N treatment, which fell within the range (7– 36%) of previously reported proportions (Powlson et al. [1986;](#page-11-0) McDonald et al. 1997; Kettering et al. [2013;](#page-10-0) Chen et al. [2016\)](#page-10-0). The main form of the residual fertilizer N (RFN) is organic N,

a stable N reservoir that mineralizes slowly and is utilized poorly by subsequent crops (Kumar and Goh [2002;](#page-10-0) Sebilo et al. [2013](#page-11-0); Zhao et al. [2015a\)](#page-11-0). Because of the low migration ability of organic RFN, most of the RFN was still distributed in the surface layer (0–20 cm) of the fertilization area (Fig. [7\)](#page-8-0).

The corresponding loss of ^{15}N from the soil-crop system was 23% for the N treatment. This proportion was lower than the average N loss in agricultural systems in China (52%) and worldwide (38%) as calculated through meta-analyses (Zhu [2008;](#page-11-0) Gardner and Drinkwater [2009\)](#page-10-0). If the low N loss proportion was widespread in Northeast China, the national N loss proportion during maize production would be overestimated. We found no significant increase in unrecovered 15 N at the four sampling stages (Fig. [7](#page-8-0)), which suggests that N loss mainly occurred in the early growth period before V12 (days 0–68). Low precipitation in the studied region was not conducive to nitrate leaching. Therefore, gaseous N losses, such as NO, N_2O , and N_2 emissions and ammonia volatilization, were likely associated with the unrecovered ^{15}N in the Nfertilized plots. Our study found that the main loss of fertilizer N occurred before the V12 stage, which is in line with previous observations that the N_2O and N_2 emissions peaked shortly after N fertilization (Li et al. [2002;](#page-10-0) Shcherbak et al. [2014\)](#page-11-0).

Despite careful sampling techniques, the spatial heterogeneity of fertilizer N in the soil of each plot as well as the variation in plant biomass and yield in each plot caused a high level of uncertainty when calculating the $15N$ recoveries. Through error propagation in the calculation process, the loss proportion of fertilizer N presented standard errors of up to 5– 10% of applied ¹⁵N (equal to 10–20 kg fertilizer N ha⁻¹). Therefore, it is necessary to reduce variation during the experiment in order to obtain more accurate evaluations of the fate of fertilizer N.

4.3 Contributions of fertilizer N to crop N uptake

Our results show that the recovery of applied $15N$ in the soilmaize system was stage dependent. Maize-absorbed N was mainly stored in the stem and leaves in the early stages of cultivation (Fig. [5](#page-6-0)) and was then reallocated into grain in the late stages of cultivation (Yang et al. [2016\)](#page-11-0). We calculated the sources of maize N (Eq. ([3\)](#page-4-0)) and found that with the depletion of available N derived from fertilizer, the proportions of crop N derived from fertilizer (Ndff) decreased from 42% at the vegetative growth stage to 30% at the reproductive growth stage (Table [1\)](#page-7-0). The utilization of more N from soil than from fertilizer by crops is widely observed in the literature (Reddy and Reddy [1993;](#page-11-0) Rees et al.1997; Stevens et al. [2005](#page-11-0); Wang et al. [2016](#page-11-0)). Summarizing the findings of previous studies, Blesh and Drinkwater ([2014](#page-10-0)) found that 44% of corn N at harvest on average came from fertilizer in spite of wide variation in yields, soil types, and N-management regimes. In this study, we obtained a lower percentage (37%) than the average.

The high reliance of crop N on soil N indicated the importance of soil organic N mineralization as a N source for crops (Schindler and Knighton [1999](#page-11-0)). In this study, we did not observe a significant yield reduction in the control treatment (Fig. [5](#page-6-0)), which could have been due to the high mineral N stock in the field before the experiment began. However, in this study, the high N uptake resulted in a low replenishment of fertilizer N to soil N (59 kg fertilizer-N ha^{-1} retained belowground versus 171 kg soil-N ha^{-1} absorbed aboveground). If we consider soil as a N balance system, soil N in black soil will decrease gradually under long-term N deficits or "N mining." Therefore, exogenous N replenishment and substitution would play a critical role in maintaining long-term N supplies and crop productivity in this region (Chen et al. [2010;](#page-10-0) Liu et al. [2016](#page-10-0)).

4.4 Effects of straw incorporation on N use efficiency and N losses

Of the N uptake by crops in the N-fertilized treatments, 121– $122 \text{ kg N} \text{ ha}^{-1}$ or 41–44% of the N taken up was allocated into straw (including stem, leaf, and others) in this study. Such a large amount of N within the straw indicates that its return to the field is critically important to N cycling in agroecosystems. In addition to feeding livestock and providing industrial materials, the remainder (> 40%) of the harvested maize straw was traditionally burnt in the field or in homes in Northeast China, which significantly pollutes the atmosphere by inducing haze episodes (Gao et al. [2009\)](#page-10-0). To maintain sustainable productivity and eliminate N fertilizer pollution of the environment, re-coupled C and N cycling in arable soil, such as straw incorporation, should be encouraged in intensively cultivated regions (Wang et al. [2014\)](#page-11-0).

Compared with the N treatment, straw incorporation (NS) significantly reduced the soil mineral N concentrations and increased the total $15N$ abundance and recovery in the ridge 0–10 cm layer at the V12 stage (Fig. [3](#page-5-0), Fig. [4\)](#page-6-0). These inverse trends indicated that straw incorporation improved the immobilization of mineral N at the early stage of maize cultivation, which is consistent with previous studies showing that fertilizer N retention was enhanced by straw incorporation in agricultural systems (Powlson et al. [1985](#page-11-0); Malhi et al. [1989](#page-11-0)). The retained N is released during the following cultivations and then indirectly enhances the NUE_{15N} .

Although straw incorporation did not have a significant effect on maize yield and biomass in this study, we should pay attention to the long-term effects of straw incorporation (Liu et al. [2016\)](#page-10-0). Long-term incorporation of straw with chemical N fertilizer can improve both nutrient and water use efficiency and grain yield (Kramer et al. [2002;](#page-10-0) Zhao et al. [2015b](#page-11-0)). Liang et al. ([2013a](#page-10-0), [b\)](#page-10-0) conducted two ^{15}N microplot studies after 19 years of wheat-maize cultivation with and without straw incorporation in Chinese Loess

Plateau, and finally they found that long-term straw incorporation increased the NUE_{15N} from 50 to 58% to 62–65% because of the improved physical, chemical, and biological properties of the soil.

5 Conclusions

Through a ¹⁵N tracer study in the field, we evaluated the fate of fertilizer N (urea) in a soil-maize system under local management practices in Northeast China. We showed that 52% of fertilizer N was recovered in plants, 25% was recovered in soil, and 23% was lost at harvest. Fertilizer N loss mainly occurred in the early growth stage of maize cultivation. The low contribution (37%) of fertilizer N to crop N uptake indicated that fertilizer was an important N source but not the main N source and showed that exogenous N replenishment and substitution for soil N consumption should be counted as part of the fertilization effect. Future research should clarify the relative contributions of residual fertilizer N to crop N uptake and N loss in subsequent cultivations. Our findings also reinforced the importance of straw incorporation in agricultural systems as a storage system that increases N retention and decreases N losses, although significant differences were not observed between them in maize yield and N uptake in the short term.

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