

## Effects of N fertilization and maize straw on the transformation and fate of labeled ( $^{15}\text{NH}_4$ ) $_2\text{SO}_4$ among three continuous crop cultivations

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### ABSTRACT

The transformation and fate of fertilizer N applied to soil is particularly important for increasing N retention in soil-crop systems and minimizing N loss from agroecosystems. However, it is obscure how the transformation and fate of fertilizer N will be affected by N input rates and crop residue amendment. In present study, an outdoor experiment was conducted in Mollisols of Northeast China throughout three crop cultivations using  $^{15}\text{N}$ -labeled tracer techniques to evaluate the effects of N fertilization levels and maize straw application on the conversion of applied fertilizer N to newly synthesized organic N, recently fixed  $\text{NH}_4^+$ , and mineral N in soil-crop systems. Generally, six treatments were set up: no N fertilization (CK), application of only maize straw (M, 16.49 mg N kg<sup>-1</sup> soil), low N input levels (N<sub>1</sub>, 44.64 mg N kg<sup>-1</sup> soil), N<sub>1</sub> plus maize straw (N<sub>1</sub> + M, 61.13 mg N kg<sup>-1</sup> soil), high N input levels (N<sub>2</sub>, 89.28 mg N kg<sup>-1</sup> soil), and N<sub>2</sub> plus maize straw (N<sub>2</sub> + M, 105.77 mg N kg<sup>-1</sup> soil). The results showed that the majority of applied fertilizer N was rapidly transformed into newly synthesized organic N, followed by recently fixed  $\text{NH}_4^+$ . Compared with the N<sub>1</sub> and/or N<sub>2</sub> treatments, maize straw amendment in the N<sub>1</sub> + M and N<sub>2</sub> + M treatments significantly increased the residual amount of applied fertilizer N in soil by accelerating the conversion of fertilizer N to newly synthesized organic N or recently fixed  $\text{NH}_4^+$ . Among three crop cultivations, the percentages of recovery of applied fertilizer N in crops (PR-N<sub>crop</sub>) and in soil (PR-N<sub>soil</sub>) were averagely 61.1% and 15.8%, respectively, while the percentage of N loss (PL-N) was 23.1%. Applying maize straw significantly elevated the PR-N<sub>crop</sub> and PR-N<sub>soil</sub> but decreased the PL-N compared to those without maize straw. These results suggested that the application of maize straw with high C/N ratio is important to retain applied fertilizer N in soil-crop systems. Balancing carbon and nitrogen inputs in soil through proper combination of fertilizer N and maize straw will be a potentially practical management to increase fertilizer N recovery in crop and soil, and therefore reduce fertilizer N loss from crop-soil systems.

### 1. Introduction

Nitrogen (N) is one of the primarily limiting nutrient elements in agriculture production, and the crop productivity strongly depends on the application rate of fertilizer N (Malhia et al., 2001; Alam et al., 2006). However, excessive N application may cause severe ecological and environmental problems, including nitrate contamination of groundwater, eutrophication of surface waters, and greenhouse effects, etc. (Abril et al., 2007; Dunn et al., 2005; Islam et al., 2007; Vallejo et al., 2006).

Fertilizer N applied to soil can be converted into different N forms by multiple soil physical/chemical processes, including mineral N (i.e., extractable  $\text{NH}_4^+$ -N and  $\text{NO}_3^-$ -N), newly synthesized organic N, or

recently clay-mineral-fixed  $\text{NH}_4^+$  (Liang and MacKenzie, 1994; Lu et al., 2010a; Lü et al., 2013; Schneiders and Scherer, 1998). The N uptakes and yields of crops are closely related to the availability of inorganic and organic N forms (Amanullah, 2016; Zhang et al., 2016). Soil mineral N is not only the available N source for crop growth, but also the common source of various N losses (Zhu, 2000). The superfluous mineral N in excess of crop N demand could be lost by  $\text{NH}_3$  volatilization, nitrification-denitrification processes, and N leaching (Cassman et al., 2002; Lin et al., 2007; Zhu and Chen, 2002). Whereas the immobilization and fixation of fertilizer N in soil organic N and fixed  $\text{NH}_4^+$  pools, respectively, are critical processes of N transformation in soil, which play an important role in regulating soil N-supply potential (Elmaci et al., 2002; Reddy et al., 2003). It was reported that

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**Table 1**  
Soil physical and chemical properties.

Chemical properties	Total C g kg <sup>-1</sup>	Total N	Total P	Total K	Avail. N mg kg <sup>-1</sup>	Avail. P	Avail. K	Fixed NH <sub>4</sub> <sup>+</sup>
	15.56	1.47	0.47	26.81	30.8	15.8	244.5	172.4
Physical properties	Texture (%)			Clay Minerals (< 2 μm, %)				
	Sand	Silt	Clay	Quartz	Chlorite	Illite	Kaolinite	Smectite
	38.9	35.3	25.8	11.0	14.0	35.0	27.0	13.0

Sand: 2–0.02 mm; Silt: 0.02–0.002 mm; Clay: < 0.002 mm.

remarkable quantities of fixed NH<sub>4</sub><sup>+</sup> could be released during a growing season in the luvisol (Van Praag et al., 1980) and Chicot soil (Liang and MacKenzie, 1994; Lu et al., 2010b). Lu et al. (2018) also found high conversion rates of newly synthesized organic N, especially amino acid N from fertilizer N for the period of three crop cultivations. And thus, how to reduce the superfluous accumulation of mineral N derived from fertilizer in soil, and more importantly accelerate the conversion of fertilizer N to newly synthesized organic N and/or recently fixed NH<sub>4</sub><sup>+</sup> are crucial to increase the recovery of fertilizer N in crop and soil, and decrease fertilizer N loss (Zhu, 2000; Haynes, 2005).

Understanding the controlling factors of transformation and fate of fertilizer N is of considerable importance in developing proper N management practice for maximizing N retention while minimizing its losses in crop-soil systems (Quiñones et al., 2007; Takahashi et al., 2004). Specifically, the transformation and fate of fertilizer N are affected by many factors, such as the growing environment of crop (i.e. soil type and climate), N management practices (fertilizer type, rate, timing, and methods of N application), and crop development period (Angás et al., 2006; Lü et al., 2013; Raghuram et al., 2006). Application of crop residues back to soil is a common but advantageous agricultural practice, which has drawn more and more attention for its significant benefits to soil fertility and carbon sequestration (Bierke et al., 2008; Fuentes et al., 2009; Gangwar et al., 2006). Crop residues with sufficient energy and nutrient sources strongly affect the microbial immobilization of mineral N, and consequently influence the conversion of fertilizer N to organic N or clay-mineral fixed NH<sub>4</sub><sup>+</sup> and N availability for crop growth (Dawson et al., 2008; Lu et al., 2010b, 2018; Rodrigues et al., 2006; Semenov et al., 2007; Singh et al., 2007). Studies showed that soil receiving crop residues with C/N ratios over 30 would lead to a high N immobilize rate, and thus decrease the accumulation of mineral N in soil, and reduce the environmental pollution risk caused by N loss (Khalil et al., 2005; Mungai and Motavalli, 2006). It is critical to accurately evaluate the effects of N fertilization and crop residue amendment on the transformation and fate of applied fertilizer N to different soil N pools in crop-soil systems, because these processes affect the N use efficiency, crop productivity, agriculture sustainability, and environment pollution risk (De Neve et al., 2004; Gentile et al., 2008, 2009; Kumar and Goh, 2002; Macdonald et al., 2002).

However, the conversion of applied fertilizer N to soil mineral N, organic N or clay-mineral-fixed NH<sub>4</sub><sup>+</sup>, as three key processes involved in fertilizer N fate in agricultural ecosystems, still remain obscure in Mollisols of Northeast China. By evaluating these transformation processes using <sup>15</sup>N-labeled tracer techniques, we can clearly understand how much N in each soil N pool is derived from fertilizer N as well as their availability for subsequent crop growth. In present study, we evaluated the effects of N input rates and maize straw application on the conversion of applied fertilizer N to three soil N pools, i.e. soil mineral N, organic N and clay-mineral-fixed NH<sub>4</sub><sup>+</sup>, and the fate in crop-soil systems in a Mollisol in Northeast China for three crop cultivations with the help of <sup>15</sup>N tracer method. We hypothesized that applying maize straw would reduce the accumulation of fertilizer derived-mineral N and promote the conversion of fertilizer N to newly synthesized

organic N and/or recently fixed NH<sub>4</sub><sup>+</sup>, and therefore increase the fertilizer N retention in crop and soil, but accordingly decrease the N loss from agro-ecosystems.

## 2. Materials and methods

### 2.1. Study site

An outdoor pot experiment was conducted at the National Field Observation and Research Station of Agro-ecosystems in Shenyang (41°31'N, 123°24'E), located on the lower Liao River plain, in which Liaochun-9 spring wheat (*Triticum aestivum* L., from 8 April, 2006 to 3 July, 2006), Liaoqiao-2 buckwheat (*Fagopyrum esculentum* M., from 10 July, 2006 to 1 October, 2006), and Liaochun-9 spring wheat (from 3 April, 2007 to 29 June, 2007) were consecutively planted. This region consists of a humid and semi-humid continental monsoon climate in a warm-temperate zone. The mean annual temperature is 7–8 °C, and the minimum and maximum monthly mean temperatures are –13 °C in January and 24 °C in July, respectively. The mean annual precipitation is approximately 700 mm. The mean active accumulated temperature (≥ 10 °C) is 3300–3400 °C, and the total solar radiation ranges from 5410 to 5600 kJ cm<sup>-2</sup>. The duration of the frost-free season is 147–168 days. The test soil with pH 6.1 is classified as a Mollisol in accordance with U.S. soil taxonomy. Soil samples at a depth of 0–20 cm were collected from the Jilin Institute of Soil and Fertilizer, sieved to pass through a 5-mm mesh, and homogenized thoroughly. The essential physical and chemical properties of the test soil are provided in Table 1.

### 2.2. Experimental design

Six treatments were established in the study: no N fertilization (CK); the application of only maize straw at 2.31 g dry matter kg<sup>-1</sup> soil (M), which is equal to 5000 kg dry matter ha<sup>-1</sup> and represents the amount routinely applied to local fields; a low-application level of fertilizer N at 44.64 mg N kg<sup>-1</sup> soil (N<sub>1</sub>), which is equal to 100 kg N ha<sup>-1</sup> and represents the lowest N application level among local fertilization practices; a low-application level of fertilizer N with maize straw (N<sub>1</sub> + M); a high-application level of fertilizer N at 89.28 mg N kg<sup>-1</sup> soil (N<sub>2</sub>), which is equal to 200 kg N ha<sup>-1</sup> and represents the highest N application level among local fertilization practices; and a high-application level of fertilizer N with maize straw (N<sub>2</sub> + M). The CK and M treatments were used to calculate the atom percent excess (APE), i.e., the isotopic enrichments of N fertilization and maize straw application treatments (N<sub>1</sub>, N<sub>1</sub> + M, N<sub>2</sub> and N<sub>2</sub> + M). Each treatment had 20 pots available to destructively sample five times with four replicates, resulting in a total of 120 plots.

Specifically, 6.5 kg of homogenized fresh soil was mixed together with phosphorus (P) and potassium (K) fertilizer and/or maize straw based on experimental treatments, before added to a plastic pot of a 25-cm outer diameter with a 15-cm height. In all the treatments, concentrated super-phosphate and potassium sulfate were applied via basal applications at 27.95 mg P kg<sup>-1</sup> soil (equal to 60 kg P ha<sup>-1</sup>) and

46.59 mg K kg<sup>-1</sup> soil (equal to 100 kg K ha<sup>-1</sup>), respectively. Maize straw with 428.23 g C kg<sup>-1</sup> and 7.14 g N kg<sup>-1</sup> was added to half of the pots according to treatments. A total of 15 spring wheat seeds (or 8 buckwheat seeds) were sown in each pot. Fertilizer N was applied as topdressing at the three tillering stages of the crops, i.e., on 9 May, 2006, 25 July, 2006, and 4 May, 2007. Labeled (<sup>15</sup>NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> with a <sup>15</sup>N abundance of 50.12% (Shanghai Research Institute of Chemical Industry, Shanghai, China) was dissolved in deionized water and applied during the first crop cultivation, and non-labeled fertilizer N was applied during next two crop cultivations. During the experiment, soil moisture content was adjusted daily with deionized water to 60% of the maximum water-holding capacity (WHC).

### 2.3. Sampling and analytical methods

All soil and plant samples from the 6 treatments and 4 replicates were collected by destructive sampling method at five sampling dates: 19 May (tillering stage), 5 June (flowering stage), and 3 July, 2006 (ripening stage of spring wheat during the first cultivation); 1 October, 2006 (ripening stage of buckwheat during the second cultivation); and 29 June, 2007 (ripening stage of spring wheat during the third cultivation). All collected soil samples in each pot were sieved through a 2-mm mesh and homogenized. A soil subsample of approximately 150 g was used to determine mineral N, i.e., exchangeable NH<sub>4</sub><sup>+</sup>-N and NO<sub>3</sub><sup>-</sup>-N, and moisture content. A subsample of approximately 200 g of fresh soil was air-dried and sieved through a 150-μm mesh for measuring soil N, fixed NH<sub>4</sub><sup>+</sup>, and <sup>15</sup>N abundance. The sampling soil process was based on the coning and quartering method to obtain a representative soil subsample. All plant samples were collected and dried at 65 °C until constant weight before being ground and sieved through a 250-μm mesh for the analysis of total N and <sup>15</sup>N abundance.

The analysis methods of soil physical and chemical properties were reported previously (Lu et al., 2010a, 2010b). The exchangeable NH<sub>4</sub><sup>+</sup>-N and NO<sub>3</sub><sup>-</sup>-N were measured using 2 mol L<sup>-1</sup> KCl extractions, MgO and Devarda's alloy distillation method (Keeney and Nelson, 1982), and fixed NH<sub>4</sub><sup>+</sup> was determined by the KOBBr-KOH method (Silva and Bremner, 1966). The plant N was determined by the regular Kjeldahl method (Bremner, 1960; Bremner and Mulvaney, 1982). The soil N was determined by hydrofluoric acid modification of the Kjeldahl method which includes organic N, NH<sub>4</sub><sup>+</sup>-N and total fixed ammonium, but excludes NO<sub>3</sub><sup>-</sup>-N and NO<sub>2</sub><sup>-</sup>-N (Bremner and Mulvaney, 1982; Keeney and Bremner, 1967; Lu et al., 2018). The <sup>15</sup>N abundance of exchangeable NH<sub>4</sub><sup>+</sup>-N, NO<sub>3</sub><sup>-</sup>-N, fixed NH<sub>4</sub><sup>+</sup> and soil N in an acidified aqueous distillate was measured using a Finnigan Mat model 251 isotope ratio mass spectrometer (USA). All operation procedures were carried out in a sequence from lower to higher atom% <sup>15</sup>N to avoid cross-contamination.

### 2.4. Methods of calculation

The content of soil N (N<sub>s</sub>, mg N pot<sup>-1</sup>), extractable NH<sub>4</sub><sup>+</sup>-N (N<sub>NH4</sub>, mg N pot<sup>-1</sup>), extractable NO<sub>3</sub><sup>-</sup>-N (N<sub>NO3</sub>, mg N pot<sup>-1</sup>), or recently fixed NH<sub>4</sub><sup>+</sup> (N<sub>f</sub>, mg N pot<sup>-1</sup>) derived from applied fertilizer N at five sampling dates was calculated using the following formula:

$$N_s, N_{NH_4}, N_{NO_3}, \text{ or } N_f = N_x \times \frac{(b-c)}{a} \quad (1)$$

where N<sub>x</sub> represents the content of soil N, NH<sub>4</sub><sup>+</sup>-N, NO<sub>3</sub><sup>-</sup>-N or fixed NH<sub>4</sub><sup>+</sup> (mg N pot<sup>-1</sup>); a represents the APE of the applied labeled fertilizer N, which is the difference between the <sup>15</sup>N abundance of (<sup>15</sup>NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> (50.12%) and the natural <sup>15</sup>N abundance (0.366%); b represents the <sup>15</sup>N abundance of soil N, NH<sub>4</sub><sup>+</sup>-N, NO<sub>3</sub><sup>-</sup>-N or fixed NH<sub>4</sub><sup>+</sup> in the soils treated with labeled fertilizer N; and c represents the <sup>15</sup>N abundance of soil N, NH<sub>4</sub><sup>+</sup>-N, NO<sub>3</sub><sup>-</sup>-N or fixed NH<sub>4</sub><sup>+</sup> in the soils that were not treated with labeled fertilizer N ("c" in the CK treatment was used for the N<sub>1</sub> and N<sub>2</sub> treatments, and "c" in the M treatment was used

for the N<sub>1</sub> + M and N<sub>2</sub> + M treatments).

The content of fertilizer derived-mineral N (N<sub>m</sub>, mg N pot<sup>-1</sup>) at five sampling dates was calculated as the sum of extractable NH<sub>4</sub><sup>+</sup>-N and NO<sub>3</sub><sup>-</sup>-N derived from applied fertilizer N:

$$N_m = N_{NH_4} + N_{NO_3} \quad (2)$$

The content of newly synthesized organic N (N<sub>o</sub>, mg N pot<sup>-1</sup>) derived from applied fertilizer N at five sampling dates was calculated as the difference between the soil N derived from applied fertilizer N and the sum of exchangeable NH<sub>4</sub><sup>+</sup>-N and recently fixed NH<sub>4</sub><sup>+</sup> derived from applied fertilizer N, as soil N determined by the hydrofluoric acid-modified Kjeldahl method did not include NO<sub>3</sub><sup>-</sup>-N and NO<sub>2</sub><sup>-</sup>-N:

$$N_o = N_s - (N_{NH_4} + N_f) \quad (3)$$

The residual amount of applied fertilizer N in soil (N<sub>r</sub>, mg N pot<sup>-1</sup>) at five sampling dates was calculated as the sum of newly synthesized organic N, recently fixed NH<sub>4</sub><sup>+</sup> and fertilizer derived-mineral N:

$$N_r = N_o + N_f + N_m \quad (4)$$

The percentage of newly synthesized organic N (N<sub>o-P</sub>, %), recently fixed NH<sub>4</sub><sup>+</sup> (N<sub>f-P</sub>, %), or fertilizer derived-mineral N (N<sub>m-P</sub>, %) out of residual fertilizer N at five sampling dates was calculated using the following formula:

$$N_{o-P}, N_{f-P}, \text{ or } N_{m-P} = \frac{N_j}{N_r} * 100 \quad (5)$$

where N<sub>j</sub> represents the content of newly synthesized organic N (mg N pot<sup>-1</sup>), recently fixed NH<sub>4</sub><sup>+</sup> (mg N pot<sup>-1</sup>), or mineral N (mg N pot<sup>-1</sup>) derived from applied fertilizer N at five sampling dates; and N<sub>r</sub> represents the residual amount of applied fertilizer N in soil (mg N pot<sup>-1</sup>).

The percentage of recovery of applied fertilizer N in crop (PR-N<sub>crop</sub>, %) or soil (PR-N<sub>soil</sub>, %) was calculated according to the following formulas:

$$PR-N_{crop} = \frac{N_{total} * (d - c)}{N_{fertilizer} * a} * 100 \quad (6)$$

$$PR-N_{soil} = \frac{N_r}{N_{fertilizer}} * 100 \quad (7)$$

where N<sub>total</sub> is the content of total N in the crop (mg N pot<sup>-1</sup>); N<sub>fertilizer</sub> is the content of applied labeled fertilizer N during the first crop cultivation (mg N pot<sup>-1</sup>); N<sub>r</sub> is the residual amount of applied fertilizer N in soil at five sampling dates (mg N pot<sup>-1</sup>); a represents the APE of the applied labeled fertilizer N, which is the difference between the <sup>15</sup>N abundance of (<sup>15</sup>NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> (50.12%) and the natural <sup>15</sup>N abundance (0.366%); d represents the <sup>15</sup>N abundance of total N in the crop treated with labeled fertilizer N; and c represents the <sup>15</sup>N abundance of total N in the crop that were not treated with labeled fertilizer N ("c" in the CK treatment was used for the N<sub>1</sub> and N<sub>2</sub> treatments, and "c" in the M treatment was used for the N<sub>1</sub> + M and N<sub>2</sub> + M treatments).

The percentage of applied fertilizer N loss (PL-N, %) was calculated according to the following formulas:

$$PL-N = 100\% - PR-N_{crop} - PR-N_{soil} \quad (8)$$

### 2.5. Statistical analysis of data

All the data are presented as the means ± standard deviations. Two-way ANOVA (mixed model, SAS 9.3, SAS institute Inc.) was used to evaluate whether fertilization practice and sampling date significantly affect (P < 0.05) the transformation of applied fertilizer N to three different soil N pools and the fate of fertilizer N in crop-soil systems.

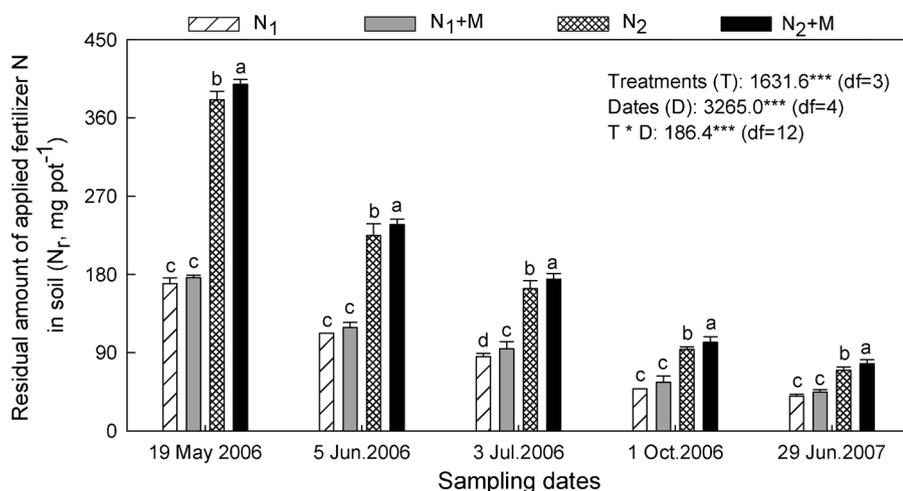


Fig. 1. The effects of N application rates and maize straw amendment on the residual amount of applied fertilizer N in soil at five sampling dates. Different letters within each sampling date indicate significant differences at  $\alpha = 0.05$ . Error bars indicate the standard deviations. F-value, P-value (\* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ ), and degree of freedom of ANOVA were also provided for main factors of treatment and date as well as interaction.

### 3. Results

#### 3.1. Transformation of applied fertilizer N to three different soil N pools among three crop cultivations

Both treatment and date significantly affected the residual amount of applied fertilizer N in soil, with significant interactions observed ( $P < 0.001$ , Fig. 1). The residual amount of applied fertilizer N in soil decreased significantly with consecutive crop cultivation for all treatments ( $P < 0.001$ , Fig. 1). The residual amount of applied fertilizer N in soil was enhanced significantly by an average of 94.9% in the N<sub>2</sub> and N<sub>2</sub>+M treatments compared with the N<sub>1</sub> and N<sub>1</sub>+M treatments at five sampling dates ( $P < 0.05$ , Fig. 1). Compared with the N<sub>1</sub> treatment, the N<sub>1</sub>+M treatment (with straw amendment) significantly increased the residual amount of applied fertilizer N by an average of 10.6% at the ripening stage of the first crop cultivation (3 Jul. 2006) ( $P < 0.05$ , Fig. 1). Similarly, compared with the N<sub>2</sub> treatment, the N<sub>2</sub>+M treatment (with straw amendment) significantly increased the residual amount of applied fertilizer N in soil by an average of 5.6% during the first crop cultivation, and 8.9% and 10.7% at the ripening stages of the second and third crop cultivations, respectively ( $P < 0.05$ , Fig. 1).

The transformation of applied fertilizer N to three soil N pools was affected significantly by both treatments and date, with significant interaction between these two factors observed (Table 2). The contents of fertilizer derived-mineral N (N<sub>m</sub>), recently fixed NH<sub>4</sub><sup>+</sup> (N<sub>f</sub>), and newly synthesized organic N (N<sub>o</sub>) decreased significantly with consecutive crop cultivation for all treatments ( $P < 0.001$ , Fig. 2). Across all five sampling dates, the contents of N<sub>m</sub>, N<sub>f</sub> and N<sub>o</sub> in the N<sub>2</sub> and N<sub>2</sub>+M treatments were greater significantly than those in the N<sub>1</sub> and N<sub>1</sub>+M treatments, and straw amendment in the N<sub>2</sub>+M treatment significantly decreased the content of N<sub>m</sub> compared with N<sub>2</sub> treatment and significantly increased the content of N<sub>o</sub> in the N<sub>1</sub>+M and N<sub>2</sub>+M

Table 2

Results (F and p values) of two-way ANOVAs on the contents of newly synthesized organic N, recently fixed ammonium, and fertilizer derived-mineral N.

	Newly synthesized organic N (N <sub>o</sub> ) (mg pot <sup>-1</sup> )	Recently fixed ammonium (N <sub>f</sub> ) (mg pot <sup>-1</sup> )	Fertilizer derived-mineral N (N <sub>m</sub> ) (mg pot <sup>-1</sup> )
Treatments (T)	187.8*** (df = 3)	1142.1*** (df = 3)	111.6*** (df = 3)
Dates (D)	196.4*** (df = 4)	4339.6*** (df = 4)	297.0*** (df = 4)
T*D	4.2*** (df = 12)	419.3*** (df = 12)	31.2*** (df = 12)

F-value, P-value (\* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ ), and degree of freedom of ANOVA were also provided for main factors of treatment and date as well as interaction.

treatments compared with N<sub>1</sub> and N<sub>2</sub> treatments ( $P < 0.05$ , Fig. 2).

The N<sub>o</sub> was higher significantly than the N<sub>f</sub> and N<sub>m</sub> in the N<sub>1</sub> and N<sub>1</sub>+M treatments at five sampling dates ( $P < 0.05$ , Fig. 2A, B). The same trend was found in the N<sub>2</sub> and N<sub>2</sub>+M treatments at other four sampling dates except for the tillering stage of the first crop cultivation (19 May, 2006) ( $P < 0.05$ , Fig. 2C, D). The N<sub>f</sub> was higher significantly than the N<sub>m</sub> in the N<sub>1</sub> and N<sub>2</sub> treatments at the tillering stage of the first crop cultivation (19 May, 2006) and at the ripening stages of the second and third crop cultivations (1 Oct. 2006 and 29 Jun. 2007) ( $P < 0.05$ , Fig. 2A, C). The same trend was found in the N<sub>1</sub>+M and N<sub>2</sub>+M treatments at the tillering and flowering stages of the first crop cultivation (19 May, 2006 and 5 Jun. 2006), and at the ripening stage of the third crop cultivation (29 Jun. 2007) ( $P < 0.05$ , Fig. 2B, D). The percentage of fertilizer derived-mineral N (N<sub>m</sub>-P) to residual fertilizer N varied from 1.3% to 24.4% in all treatments. The corresponding percentages of recently fixed NH<sub>4</sub><sup>+</sup> (N<sub>f</sub>-P) and newly synthesized organic N (N<sub>o</sub>-P) were 10.4–38.9% and 36.8–85.3%, respectively (Fig. 3). The N<sub>m</sub>-P and N<sub>f</sub>-P decreased significantly as crop cultivations continued (Fig. 3). On the contrary, the N<sub>o</sub>-P increased significantly as crop cultivations continued (Fig. 3).

#### 3.2. Fate of applied fertilizer N in soil-crop systems among three crop cultivations

Both treatments and date significantly affected the percentage of recovery of applied fertilizer N in crop and soil ( $P < 0.001$ , Fig. 4A, B), and the percentage of applied fertilizer N loss ( $P < 0.01$ , Fig. 4C). The percentage of recovery of applied fertilizer N in crop (PR-N<sub>crop</sub>) increased significantly with the extending of growth stages among the first crop cultivation ( $P < 0.05$ , Fig. 4A), and then declined significantly with the second and third consecutive crop cultivations ( $P < 0.05$ , Fig. 4A). Compared with higher N inputs (N<sub>2</sub> and N<sub>2</sub>+M), lower N rates (N<sub>1</sub> and N<sub>1</sub>+M) significantly increased the PR-N<sub>crop</sub> by an average 118.4% at the tillering stage of the first crop cultivation (19 May, 2006), but the trend was opposite at the ripening stage of the third crop cultivation (29 Jun. 2007), with an average decline of 34.5% ( $P < 0.05$ , Fig. 4A). Straw amendment in the N<sub>1</sub>+M and N<sub>2</sub>+M treatments significantly reduced the PR-N<sub>crop</sub> by an average of 6.3% compared to N<sub>1</sub> and N<sub>2</sub> treatments at the tillering stage of the first crop cultivation (19 May, 2006) ( $P < 0.05$ , Fig. 4A). Compared with the other three treatments, the N<sub>2</sub>+M treatment significantly promoted the PR-N<sub>crop</sub> by an average of 5.7% at the ripening stage of the first crop cultivation (3 Jul. 2006) ( $P < 0.05$ , Fig. 4A). Straw amendment in the N<sub>1</sub>+M and N<sub>2</sub>+M treatments significantly increased the PR-N<sub>crop</sub> by an average of 8.5% compared with N<sub>1</sub> and N<sub>2</sub> treatments at the ripening stage of the second crop cultivation (1 Oct. 2006) ( $P < 0.05$ , Fig. 4A).

The percentage of recovery of applied fertilizer N in soil (PR-N<sub>soil</sub>)

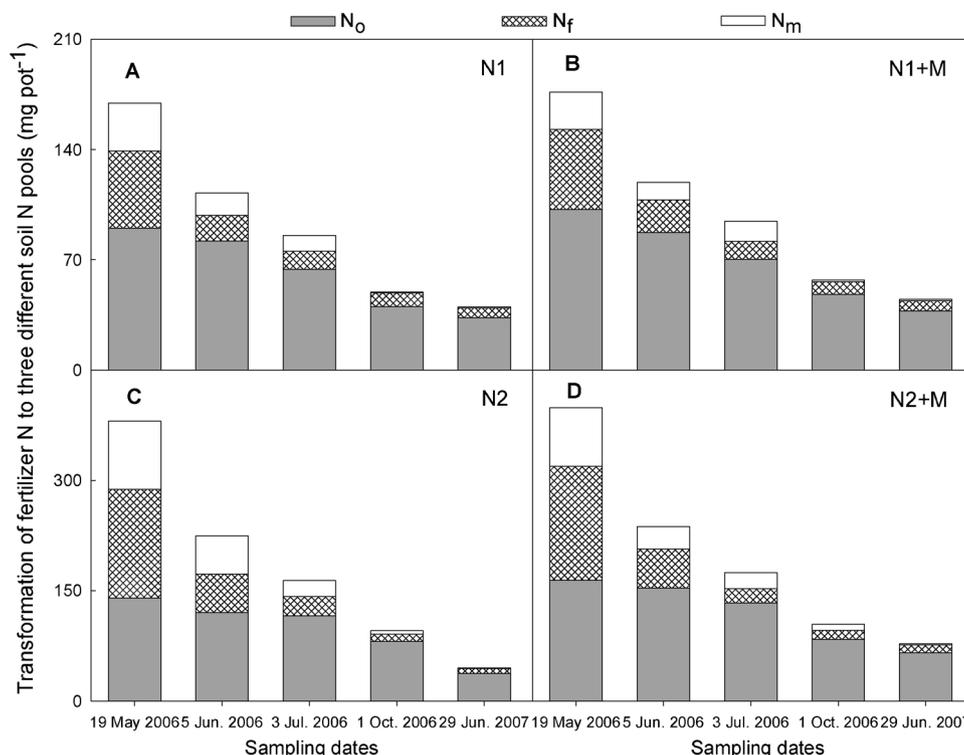


Fig. 2. The effects of N application rates and maize straw amendment on the transformation of applied fertilizer to three different soil N pools, newly synthesized organic N ( $N_o$ ), clay mineral-fixed  $NH_4^+$  ( $N_f$ ), and fertilizer derived-mineral N ( $N_m$ ), at five sampling dates.

decreased significantly with consecutive crop cultivation ( $P < 0.001$ , Fig. 4B). Compared with  $N_1$  treatment, straw amendment in the  $N_1 + M$  treatment significantly increased the  $PR-N_{soil}$  by 10.6%, 15.9%, and 11.7% at the ripening stages of the first (3 Jul. 2006), second (1 Oct. 2006) and third crop cultivations (29 Jun. 2007) (Fig. 4B). Compared with  $N_2$  treatment, straw amendment in the  $N_2 + M$  treatment

significantly increased the  $PR-N_{soil}$  by 4.7% at the tillering stage of the first crop cultivation (19 May, 2006) and by 10.7% at the ripening stage of the third crop cultivation (29 Jun. 2007) (Fig. 4B).

The percentage of applied fertilizer N loss (PL-N) increased gradually with extending of growth stages among the first crop cultivation, and then decreased significantly at the ripening stages of the second

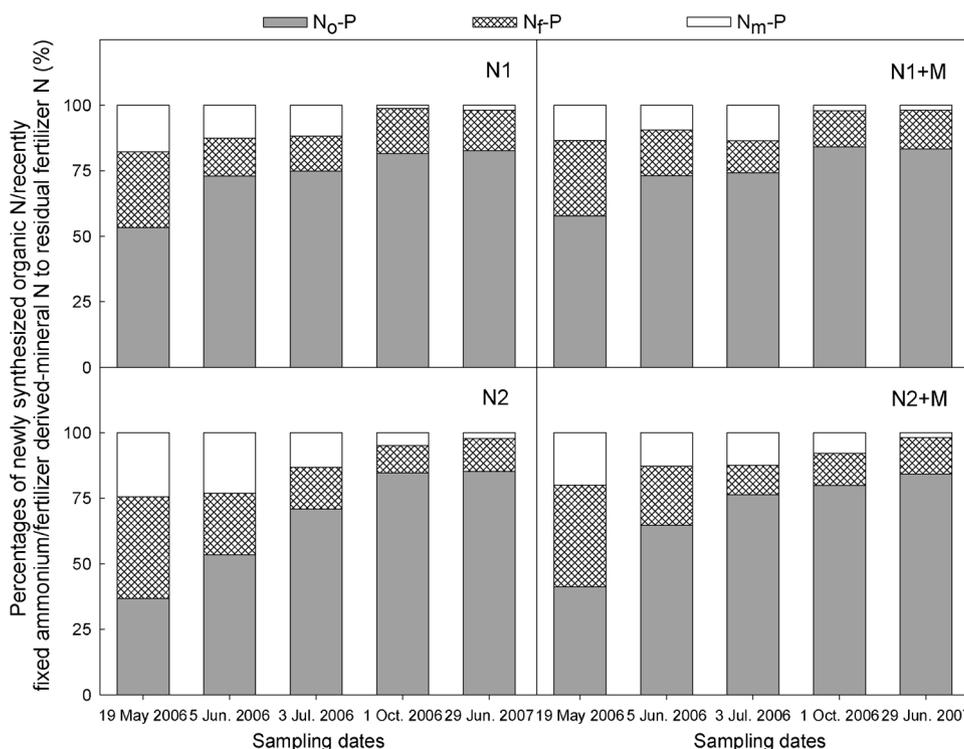
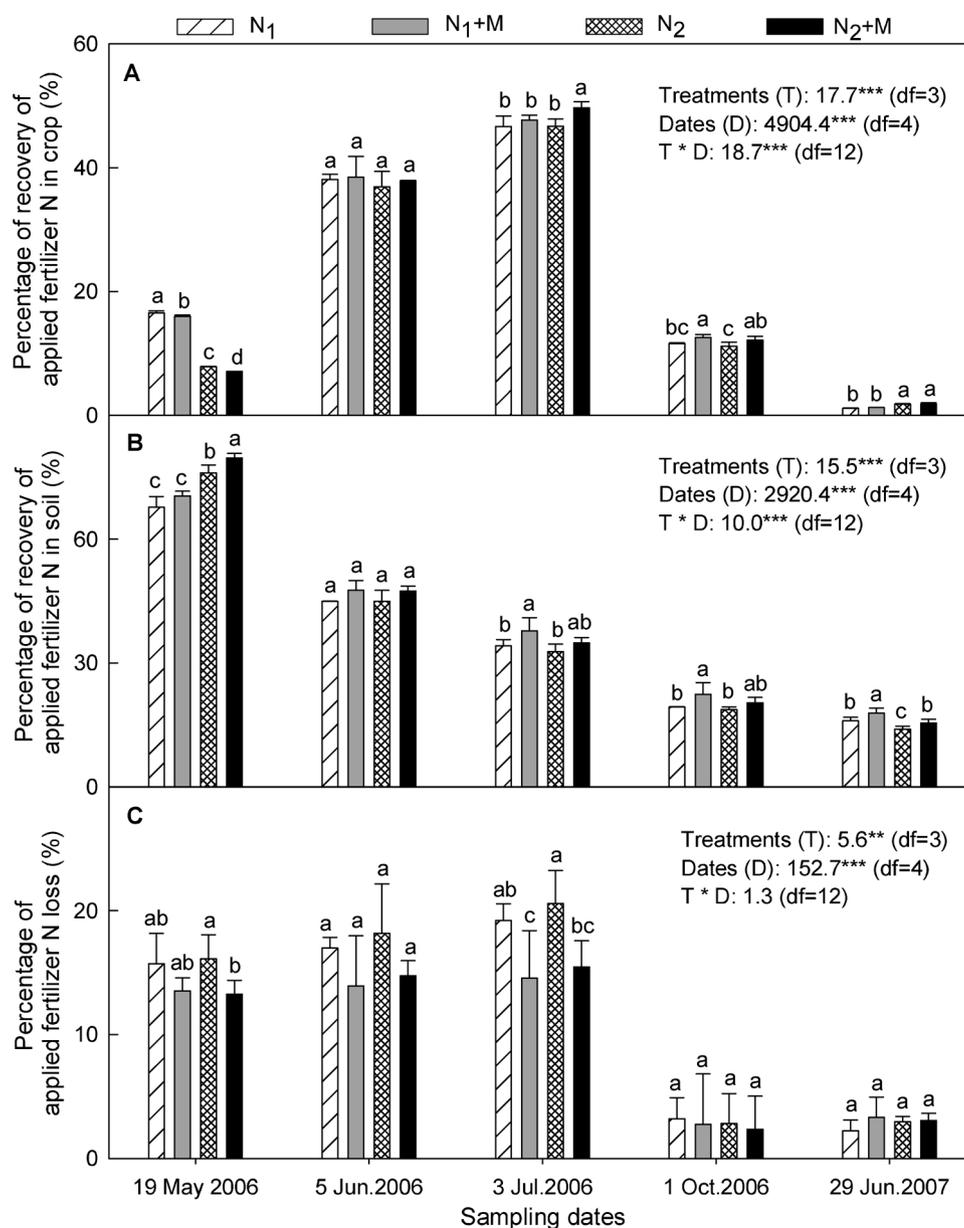


Fig. 3. The percentage of three different soil N pools derived from applied fertilizer N out of the residual fertilizer N at five sampling dates.



**Fig. 4.** The effects of N application rates and maize straw amendment on the percentage of recovery of applied fertilizer N in crop (A) and soil (B), and the percentage of applied fertilizer N loss (C) at five sampling dates. Different letters within each sampling date indicate significant differences at  $\alpha = 0.05$ . Error bars indicate the standard deviations. F-value, P-value (\* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ ), and degree of freedom of ANOVA were also provided for main factors of treatment and date as well as interaction.

and third crop cultivations (Fig. 4C). Compared with  $N_1$  treatment, straw amendment in the  $N_1 + M$  treatment significantly reduced the PL-N by 24.2% at the ripening stage of the first crop cultivation (3 Jul. 2006). Compared with  $N_2$  treatment, straw amendment in the  $N_2 + M$  treatment significantly decreased the PL-N by 17.7% and 24.9% at the tillering (19 May, 2006) and ripening stages of the first crop cultivation (3 Jul. 2006) ( $P < 0.05$ , Fig. 4C).

#### 4. Discussions

##### 4.1. Transformation of applied fertilizer N to three different soil N pools among three crop cultivations

Fertilizer N applied to soil is converted into different N forms, such as fertilizer-derived mineral N (i.e., fertilizer-derived exchangeable  $NH_4^+$ -N and  $NO_3^-$ -N), fertilizer-derived new synthesized organic N, or fertilizer-derived recently clay-mineral-fixed  $NH_4^+$  (Lu et al., 2010a; Lü

et al., 2013; Schneiders and Scherer, 1998). Our results from present pot experiment showed that both treatment and date significantly affected the residual amount of applied fertilizer N in soil and its transformation to different soil N pools (Figs. 1 and 2). Newly synthesized organic N accounted for the majority of applied fertilizer N irrespective of N-fertilization treatment or sampling dates, followed by recently clay-mineral-fixed  $NH_4^+$  and fertilizer derived-mineral N (Fig. 3), suggesting that fertilizer N applied to soil was mainly transformed into newly synthesized organic N by N immobilization of soil microbes. These results also indicated that newly synthesized organic N and recently fixed  $NH_4^+$  play an important role in retaining and enhancing the N-supplying capacity of soil, which is consistent with previous reports that immobilization of fertilizer N in soil may be regarded as a means of storing N for subsequent crops (Burger and Jackson, 2003; De Neve et al., 2004; Glendinning et al., 1997, 2001; Haynes, 1999; Kumar and Goh, 2002).

Our previous results showed that straw amendment at two N levels

significantly degraded the conversion of applied fertilizer N to mineral N pool (Lu et al., 2010a), but promoted fertilizer N conversion to newly synthesized organic N and recently fixed  $\text{NH}_4^+$  pools, compared with straw-free control (Lu et al., 2010b, 2018). Xu et al. (2005) reported that the combined application of chemical fertilizer and rice straw decreased the level of soil mineral N compared to that of chemical fertilizer alone. Chaves et al. (2006, 2008) found that application of cereal straw with a 105 C/N ratio significantly increased microbial biomass N but decreased the amount of mineral N. Mubarak et al. (2001) reported that N immobilization shortly occurred after adding crop residue with a high C/N ratio to soil. Cheng et al. (2017) found from this meta-analysis that the quantity and quality of exogenous organic C input affected the  $\text{NO}_3^-$  immobilization of soil microorganisms, and plant residues addition increased microbial  $\text{NO}_3^-$  immobilization and reduced soil  $\text{NO}_3^-$  accumulation.

Accelerating the transformation of fertilizer N to soil organic N and fixed  $\text{NH}_4^+$  pool could minimize the N loss of applied fertilizer. At the same time, the re-mineralization and release of the newly synthesized organic N and recently fixed  $\text{NH}_4^+$  would decide the N availability and N-synchronous supply of applied fertilizer to subsequent crops. The fact that the contents of newly synthesized organic N and recently fixed  $\text{NH}_4^+$  decreased significantly with consecutive crop cultivation for all treatments showed that newly synthesized organic N and recently fixed  $\text{NH}_4^+$  were quite active, and thus could be rapidly converted and released to be available for subsequent crops (Fig. 2). Our results further supported that the microbial immobilization of applied fertilizer N is an important mechanism of N storage for subsequent crop N utilization, in which newly synthesized organic N was an important N pool derived from applied fertilizer (Lu et al., 2018). Our results also confirmed that the recently fixed  $\text{NH}_4^+$  derived from applied fertilizer was an important available N source for crops in season and next season (Lu et al., 2010b). Our previous studies have shown that straw amendment had less effect on the release rates of recently fixed  $\text{NH}_4^+$  and the change rates of newly synthesized organic N (Lu et al., 2010b, 2018), which suggested a relatively high activity of recently fixed  $\text{NH}_4^+$  and the newly synthesized organic N derived from applied fertilizer N irrespective of N levels or straw amendment. The higher release percentages of recently fixed  $\text{NH}_4^+$  than the change rates of newly synthesized organic N confirmed that recently clay-mineral-fixed  $\text{NH}_4^+$  is a more labile N fraction of soil N pools derived from applied fertilizer N. In contrast, the N release of newly synthesized organic N was a relatively long-term process. Our results that the  $\text{N}_r\text{-P}$  decreased significantly with consecutive crop cultivation but the  $\text{N}_c\text{-P}$  increased significantly with consecutive crop cultivation, further verified the above-mentioned results (Fig. 3).

#### 4.2. Fate of applied fertilizer N in soil-crop systems among three crop cultivations

Our results from present pot experiment showed that the  $\text{PR-N}_{\text{crop}}$  for all treatments was averagely 47.7% during the first crop cultivation. The N uptakes of crop for applied fertilizer mainly occurred between the tillering stage and the flowering stage (19 May–5 Jun. 2016), which accounted for the 54.5% of the  $\text{PR-N}_{\text{crop}}$  from the first crop cultivation. The  $\text{PR-N}_{\text{crop}}$  for all treatments declined significantly during the second crop cultivation, amounting to 11.9%, with the  $\text{PR-N}_{\text{crop}}$  for all treatments, and was even lower than 1.5% during the third crop cultivation. These results suggested that the N availability of applied fertilizer to crops mainly occurred among the first and second crop cultivations. These results were consistent with the high release potential of recently fixed  $\text{NH}_4^+$  and newly synthesized organic N, indicating that temporary immobilization/fixation and subsequent release of applied fertilizer N improved N synchrony between N availability and crop requirement (Lu et al., 2018; Vanlauwe et al., 2001a, 2001b, Vanlauwe et al., 2002). Compared to the  $\text{N}_1$  and  $\text{N}_1 + \text{M}$  treatments with low N application rate, the  $\text{N}_2$  and  $\text{N}_2 + \text{M}$  treatments with high N application rate significantly

reduced the  $\text{PR-N}_{\text{crop}}$  on May 19, 2006, but this effect was not observed for the consecutive crop cultivations (Fig. 4A). A major reason was that low N application rate could meet the low N demand of crop at the tillering stage of the first crop cultivation, and thus, different N application rate resulted in the significant differences in the  $\text{PR-N}_{\text{crop}}$  in spite of nearly the same amount of applied fertilizer N was absorbed by crop biomass for all treatments. Compared with  $\text{N}_1$  and  $\text{N}_2$  treatments, straw amendment in the  $\text{N}_1 + \text{M}$  and  $\text{N}_2 + \text{M}$  treatments increased the  $\text{PR-N}_{\text{crop}}$  among three crop cultivations except for the tillering stage of the first crop cultivation (19 May, 2006), with the significant differences at the ripening stage of the first and second crop cultivations (Fig. 4A). These results suggested that straw amendment had positive effects on the N use efficiency of applied fertilizer N.

The  $\text{PR-N}_{\text{soil}}$  decreased significantly with consecutive crop cultivation. Compared with  $\text{N}_1$  and  $\text{N}_2$  treatments, straw amendment in the  $\text{N}_1 + \text{M}$  and  $\text{N}_2 + \text{M}$  treatments increased the  $\text{PR-N}_{\text{soil}}$  among three crop cultivations, with the significant differences at the tillering stage of the first crop cultivation (19 May, 2006) and at the ripening stage of three crop cultivations, indicating that the combination application of chemical fertilizer and crop residues was beneficial for fertilizer N storage in soil and subsequent crop use (Glendinning et al., 1997, 2001). Although the  $\text{PR-N}_{\text{soil}}$  amounted to 20.2% at the ripening stage of the second crop cultivation, the  $\text{PR-N}_{\text{crop}}$  for all treatments was only averagely 1.5% during the third crop cultivation. The low availability of residual fertilizer N during the third crop cultivation could be due to the chemical and physical protection of newly synthesized organic N which considerably reduces N availability, microbial accessibility, and pore water exchange (Schulten and Schnitzer, 1997; McCarthy et al., 2008; Zhuang et al., 2008; Lu et al., 2018).

The  $\text{PL-N}$  for all treatments was averagely 17.4% during the first crop cultivation, in which 84.0% of N loss from applied fertilizer in the first crop cultivation occurred within 10 days after fertilization, suggesting that soil nitrogen nutrient management to accelerate the transformation of applied fertilizer N to soil organic N and fixed  $\text{NH}_4^+$  pools was vital for minimizing fertilizer N loss within short time following fertilization. The  $\text{PL-N}$  for all treatments was similar during the second and third crop cultivations, averagely 2.8%. Compared with  $\text{N}_1$  and  $\text{N}_2$  treatments, straw amendment in the  $\text{N}_1 + \text{M}$  and  $\text{N}_2 + \text{M}$  treatments reduced the  $\text{PL-N}$  during the first and second crop cultivations, with the significant differences at the ripening stage of the first crop cultivation (3 Jul. 2006), indicating that loss risk of applied fertilizer N may also be reduced through combined application of N fertilizer and maize straw (Gentile et al., 2008, 2009). Xu et al. (2005) also reported that the combined application of chemical fertilizer and rice straw significantly increased the recovery of chemical fertilizer N compared to the application of chemical fertilizer alone. Kimani et al. (2003) pointed out that integrated soil fertility management (ISFM) is currently promoted as a management approach, and supported that the combined application of mineral fertilizer and crop residues benefit crop N synchrony and N loss reduction through interactive effects between both types of inputs.

## 5. Conclusions

The challenge in managing soil organic matter pools is how to balance C and N inputs to synchronize the supply of available N with plant uptake during peak periods of crop N demand, and thus minimize N loss of fertilizer. Our study indicated that maize straw amendment with a high C/N ratio is an important regulator of transformation and fate of applied fertilizer N. The temporary immobilization and fixation of applied fertilizer N are two important mechanisms of N storage and N synchrony supply for subsequent crops. Application of maize straw increased the soil residue of applied fertilizer N and accelerated the conversion of residual fertilizer N to newly synthesized organic N and recently fixed  $\text{NH}_4^+$  without affecting the N availability of the two N pools. After being applied to soil, fertilizer N was mainly converted into

newly synthesized organic N, followed by recently clay-mineral-fixed  $\text{NH}_4^+$ . Maize straw amendment also elevated the  $\text{PR-N}_{\text{crop}}$  and  $\text{PR-N}_{\text{soil}}$  in crop and soil, but degraded the PL-N. Utilization and loss of applied fertilizer N mainly occurs in the first crop cultivation, especially within ten days after fertilization for the N loss. Thus, accommodating the application proportion of carbon source and nitrogen nutrition in soil through properly combined application of chemical fertilizer and maize straw may be a potential means for regulating the transformation and fate of applied fertilizer N, increasing fertilizer N retention, and minimizing fertilizer N loss in crop-soil systems.

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