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Effects of N fertilization and maize straw on the dynamics of soil organic N and amino acid N derived from fertilizer N as indicated by ^{15}N labeling

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ABSTRACT

The accumulation and availability of soil organic nitrogen (N), especially amino acid N, is highly important for N retention in the soil and N supply for crop growth. However, it remains obscure how the conversion of fertilizer N to both soil organic N and amino acid N is affected by management practices such as fertilization and residue amendment. In present study, we conducted an outdoor ^{15}N labeling pot experiment in Mollisols of Northeast China, where plants were consecutively cropped after each of three cultivations (spring wheat-buckwheat-spring wheat). We aimed to evaluate the effects of maize straw applications on the dynamics of organic N and amino acid N derived from fertilizer N. Six treatments were set: no N fertilization (CK), application of only maize straw (M), low N levels (N_1), N_1 plus maize straw (N_1 + M), high N levels (N_2), and N_2 plus maize straw (N_2 + M). The results showed that organic N and amino acid N dominated the available N derived from fertilizer N, but their contents decreased significantly as crop cultivation continued. Compared with N_2, N_2 + M, which involved the application of maize straw, significantly increased the content of organic N derived from fertilizer N by an average of 20.1% during the first crop cultivation. Compared with N_1 and N_2, N_1 + M and N_2 + M promoted the content of fertilizer-derived amino acid N by an average of 12.5% across five sampling dates. During the first crop cultivation, straw amendment also significantly increased both plant uptake and soil residues of fertilizer N in N_1 + M and N_2 + M. Our results demonstrated that substrate amendments such as crop residues may constitute a practical agricultural management approach for promoting the transformation of fertilizer N to soil organic N, thereby increasing both fertilizer N use efficiency and retention in our study crop-soil systems.

1. Introduction

Nitrogen (N) is the primary limiting nutrient for crop production in terrestrial ecosystems (Smil, 1999). The productivity of agricultural crops strongly depends on high levels of fertilizer N applied to the soil (Alam et al., 2006; Raun and Johnson, 1999). However, excessive N application can cause significant ecological and environmental problems, such as increased production of N_2O, which is both a greenhouse gas and an ozone-depleting substance, and nitrate leaching, which can reduce water quality (de Klein et al., 2001; Di and Cameron, 2002).

Although soil inorganic N is available for crop growth and is a common source of various N losses, such as NH_3 volatilization, nitrification-denitrification processes, and N leaching (Davies and Williams, 1995; Magalhães et al., 2005), soil inorganic N constitutes only a small proportion of the total soil N, < 10% (Haynes, 2005). Under certain conditions, soil organic N can account for 90% of the total soil N; thus, the transformation of soil organic N plays an important role in the N supply for crop growth and the minimization of N loss (Murphy et al., 2000; Schulten and Schnitzer, 1998; Stevenson, 1982). Soil organic N comprises various N compounds ranging from high-molecular-weight polyphenol-bound N to low-molecular-weight amino acids (Antia et al., 1991; Warren, 2014), and soil amino acid N accounts for a large proportion (Hou et al., 2009; Senwo and Tabatabai, 1998; Stevenson, 1982). Given their higher turnover rate than that of other more complex substrates, soil amino acids can be rapidly immobilized and mineralized by soil microorganisms (Finzi and Berthrong, 2005; Jones, 1999; Jones and Kielland, 2002; Jones et al., 2005b; Tate, 2000). Amino acid N is an important storage pool for immobilized N (Amelung and Zhang, 2001; Lü et al., 2013) and a dominant transitional available N form for plants and soil microorganisms (Henry and Jefferies, 2003; Näsholm et al., 1998; Werdin-Pfisterer et al., 2009).

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Table 1
Soil physical and chemical properties.

Chemical properties							
Total C	Total N	Total P	Total K	Avail. N	Avail. P	Avail. K	Fixed NH_4^+
g kg − 1				mg kg − 1			
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.

The immobilization and mineralization of soil organic N and amino acid N are biological processes mediated by soil microorganisms. Thus, energy and nutrient availability for soil microbial growth may be critical for N retention in the soil and subsequent N utilization by crops, similar to the importance of regulating the accumulation and transformation of soil organic N and amino acid N (Hu et al., 2016; Zhang et al., 2015). Zhang et al. (2015) reported that the turnover of amino acids is closely associated with microbial metabolism and that glucose addition significantly decreased the mineralization of both newly synthesized and originally present amino acids; however, an abundance of inorganic N accelerated the rapid decomposition of the newly synthesized amino acids and inhibited the mineralization of the originally present amino acids. Hu et al. (2016) reported that both microbial activity stimulated by available substrates and C and N bio-availability govern the dynamics and maintenance of soil proteinaceous amino acids. These results showed that addition of readily degradable C and inorganic N controls the dynamics and availability of soil organic N and amino acid N.

After being applied to the soil, fertilizer N is transformed into different forms, such as inorganic N (i.e., exchangeable NH_4^+-N and NO_3^--N), or organic N or clay-mineral-fixed NH_4^+ (Lu et al., 2010a; Schneiders and Scherer, 1998; Stewart et al., 1963). To enhance fertilizer N use efficiency and reduce N losses, appropriate fertilization practices should be developed to immobilize excess fertilizer N into the soil organic N pool. In addition, the slow release of newly synthesized organic N to the plant-available pool possibly constitutes another mechanism for enhancing N retention in crop-soil systems (Haynes, 2005). Amending the soil by adding crop residues is a common agricultural practice that can significantly contribute to enhance soil fertility and C sequestration (Bierke et al., 2008; Fuentes et al., 2009; Gangwar et al., 2006). At the same time, crop residues with available energy and nutrients strongly influence soil microorganism activity as well as the transformation of fertilizer N to soil organic N and its availability for crop growth. Therefore, information on the effects of soil amendments with crop residues that have high C/N ratios on the transformation of fertilizer N to soil organic N and N retention in crop-soil systems is urgently needed.

In China, more than one-quarter of N fertilizers that are produced worldwide and have generally low use efficiencies but high loss rates are applied (Jing et al., 2007; Li et al., 2007; Zhu and Chen, 2002). Therefore, regarding the sustainable development of agriculture in China, understanding the effects of fertilization practices on the transformation of fertilizer N in the soil is important. Thus far, the effects of high-C/N crop residue applications on the transformation of applied N to crop-available N, especially organic N and amino acid N still remains in question. In this study, we examined the effects of crop residue application on the conversion of N derived from fertilizers to soil organic N, especially amino acid N and N uptake by crops in a Mollisol in Northeast China for three crop cultivations using ^{15}N-labeled tracer techniques. We hypothesized that applying crop straw would enhance the transformation of fertilizer N to soil organic N, especially amino acid N, and therefore increase fertilizer N uptake by crops and its N residue in soil.

2. Materials and methods

2.1. Study site

An outdoor pot experiment, 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, 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; the 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, and the total solar radiation ranges from 5410 to 5600 kJ cm^{-2}. The duration of the frost-free season is 147–168 days. The test soil 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

For this experiment, 6.5 kg of homogenized fresh field soil was mixed together with phosphorus (P) and potassium (K) fertilizer and/or maize straw, after which the mixture added to plastic pots that had an outer diameter of 25 cm and a height of 15 cm. A total of 15 spring wheat seeds (or 8 buckwheat seeds) were sown in each pot.

Six treatments were established in the study: no N fertilization (CK); the application of only maize straw at 2.31 g dry matter kg^{-1} soil (M), which is equal to 5000 kg dry matter ha^{-1} and represents the amount routinely applied to local fields; a low-application level of fertilizer N at 44.64 mg N kg^{-1} soil (N_1), which is equal to 100 kg N ha^{-1} and represents the lowest N application level among local fertilization practices; a low-application level of fertilizer N with maize straw (N_1 + M); a high-application level of fertilizer N at 89.28 mg N kg^{-1} soil (N_2), which is equal to 200 kg N ha^{-1} and represents the highest N application level among local fertilization practices; and a high-application level of fertilizer N with maize straw (N_2 + 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_1, N_1 + M, N_2 and N_2 + M). Each treatment had 20 pots available to sample five times with four replicates, resulting in a total of 120 plots.

In all the treatments, concentrated super-phosphate and potassium sulfate were also applied via basal applications at 27.95 mg P kg^{-1} soil (equal to 60 kg P ha^{-1}) and 46.59 mg K kg^{-1} soil (equal to 100 kg K ha^{-1}), respectively. Maize straw with 428.23 g C kg^{-1} and 7.14 g N kg^{-1} was added to half of the pots. The N are supplied as topdressing at the three tillering stages of the crops, i.e., on 9 May 2006, 25 July 2006, and 4 May 2007. Labeled $(^{15}NH_4)_2SO_4$ with a ^{15}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. Soil and plant sampling

All soil and plant samples from the 6 treatments and 4 replicates were collected by destructive sampling at five sampling dates: 19 May (tillering), 5 June (flowering), 3 July 2006 (ripening of spring wheat during the first cultivation), 1 October 2006 (ripening of buckwheat during the second cultivation), and 29 June 2007 (ripening of spring wheat during the third cultivation). All collected soil samples in each pot were ground and sieved through a 2-mm mesh and homogenized. A fresh soil subsample of approximately 150 g was used to determine exchangeable NH_4^+-N, NO_3^--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, amino acid N, fixed NH_4^+, and ^{15}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 ^{15}N abundance.

2.4. Analysis of soil properties, inorganic N, fixed NH_4^+, total N and ^{15}N enrichment

The results of the analysis of the soil physical and chemical properties were reported previously (Lu et al., 2010a, 2010b). The exchangeable NH_4^+-N and NO_3^--N was measured using 2 mol L^{-1} KCl extractions, MgO and Devarda's alloy distillation method (Keeney and Nelson, 1982), and fixed NH_4^+ was determined by the KOBr-KOH method (Silva and Bremner, 1966). The plant N was determined by the regular Kjeldahl method (Bremner, 1960; Bremner and Mulvaney, 1982), and the soil N was determined by hydrofluoric acid modification of the Kjeldahl method to include total fixed ammonium (Bremner and Mulvaney, 1982; Keeney and Bremner, 1967). The regular Kjeldahl method is suitable for the analysis of soils containing typically low levels of NO_3^- and NO_2^-, and such method does not recover total fixed ammonium and significant amounts of NO_3^--N or NO_2^--N (Bremner and Mulvaney, 1982). Soil N can be determined by salicylic acid-thiosulfate modification of the Kjeldahl method, resulting in the inclusion of NO_3^--N and NO_2^--N (Bremner and Mulvaney, 1982). Thus, the present hydrofluoric acid-modified method of determining soil N includes organic N, NH_4^+-N and fixed NH_4^+ but excludes NO_3^--N and NO_2^--N. The atom% ^{15}N of exchangeable NH_4^+-N, NO_3^--N, fixed NH_4^+ and soil N in an acidified aqueous distillate was measured using a Finnigan Mat model 251 isotope ratio mass sepectrometer (USA). All operation procedures were carried out in a sequence from lower to higher atom% ^{15}N to avoid cross-contamination.

The contents of soil N (N_s in mg N kg^{-1} soil), NH_4^+-N (N_{NH4} in mg N kg^{-1} soil), NO_3^--N (N_{NO3} in mg N kg^{-1} soil), and fixed NH_4^+ (N_f in mg N kg^{-1} soil) derived from applied fertilizer N were calculated using the following formula:

$$N_s,\ N_{NH4},\ N_{NO3},\ \text{or}\ N_f = N_x \times \frac{(b-c)}{a} \tag{1}$$

where N_x represents the contents of soil N, NH_4^+-N, NO_3^--N or fixed NH_4^+ (mg N kg^{-1} soil); a represents the APE of the applied labeled fertilizer N, which is the difference between the ^{15}N abundance of $(^{15}NH_4)_2SO_4$ (50.12%) and the natural ^{15}N abundance (0.366%); b represents the ^{15}N abundance of N, NH_4^+-N, NO_3^--N or fixed NH_4^+ in the soils treated with labeled fertilizer N; and c represents the ^{15}N abundance of N, NH_4^+-N, NO_3^--N or fixed NH_4^+ in the soils that were not treated with labeled fertilizer N ("c" in the CK treatment was used for the N_1 and N_2 treatments, and 'c' in the M treatment was used for the N_1 + M and N_2 + M treatments).

The content of organic N (N_o in mg N kg^{-1} soil) derived from applied fertilizer N was calculated as the difference between the soil N derived from applied fertilizer N and the sum of exchangeable NH_4^+-N and fixed NH_4^+ derived from applied fertilizer N, as soil N determined by the hydrofluoric acid-modified Kjeldahl method did not include NO_3^--N and NO_2^--N (Bremner and Mulvaney, 1982; Keeney and Bremner, 1967):

$$N_o = N_s - (N_{NH4} + N_f) \tag{2}$$

The percentages of organic N or amino acid N derived from applied fertilizer N out of applied fertilizer N (N_oP or $N_{aa}P$) at five different sampling dates and their change rates (CR, %) for the three different crop cultivations were calculated using the following formula (Westerman et al., 1972):

$$N_oP \text{ or } N_{aa}P = \frac{N_j}{N_{fer}} \times 100 \tag{3}$$

Where N_j (j = 1, 2, 3, 4, and 5) is the content of organic N (mg N kg^{-1} soil) or amino acid N (mg N kg^{-1} soil) derived from applied fertilizer N at five sampling dates and where N_{fer} is the amount of applied labeled fertilizer N during the first crop cultivation (mg N kg^{-1} soil).

$$CR_1(\%) = \frac{N_1 - N_3}{N_1} \times 100 \quad (4)$$

$$CR_2(\%) = \frac{N_3 - N_4}{N_1} \times 100 \quad (5)$$

$$CR_3(\%) = \frac{N_4 - N_5}{N_1} \times 100 \quad (6)$$

where N_1, N_3, N_4, and N_5 represent the content of organic N (mg N kg^{-1} soil) or amino acid N (mg N kg^{-1} soil) derived from applied fertilizer N at the sampling dates of 19 May 2006, 3 July 2006, 1 October 2006 and 29 June 2007, respectively, and where CR_1, CR_2 and CR_3 represent the change rate of organic N or amino acid N derived from applied fertilizer N during the first, second, and third crop cultivations, respectively.

2.5. Determination of soil amino acids and ^{15}N enrichment

Soil amino acids were determined by 6 mol L^{-1} HCl hydrolyzation-purification-derivation and LC-MS method (Amelung and Zhang, 2001; He et al., 2011; Hou et al., 2009). Specifically, a specific amount of air-dried soil sample was hydrolyzed with 6 mol L^{-1} HCl for 12 h at 105 °C. After 80 μL of 2.5 mmol L^{-1} L-2-aminobutyric acid was added as internal standard, the soil hydrolysates was filtered and dried under vacuum in a rotary evaporator. The residue was then redissolved in 0.05 mol L^{-1} HCl and transferred to a resin-filled polypropylene column filled with 3 g of Dowex 50WX8 cation exchange resin for sample purification. After three successive rinses with 0.1 mol L^{-1} oxalic acid, 0.01 mol L^{-1} HCl and deionized water, the amino acids were eluted with 2.5 mol L^{-1} $NH_3{\cdot}H_2O$. The eluted solution was then dried under vacuum, after which the amino acids were redissolved in 0.1 mol L^{-1} HCl for centrifugation. The supernatant was then freeze-dried, and the amino acids were dissolved in 0.02 mol L^{-1} HCl and subjected to 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (AQC) precolumn derivatization in accordance with the method modified by Hou et al. (2009). Derivatization of an amino acid standard was also performed; L-2-aminobutyric acid served as an internal standard for each analysis.

Fifteen individual amino acids and the ^{15}N incorporated into individual amino acids were analyzed using a Waters 2695 HPLC-MS system equipped with an XTerraR MS C18 column and an electrospray ionization source (He et al., 2011; Hou et al., 2009). The mobile phase consisted of three solvents, 1.5 m mol L^{-1} ammonium formate, acetonitrile, and water, at a flow rate of 0.2 mL min^{-1}. The mass spectrometer was set up as follows: capillary voltage, 3000 V; cone voltage, 25 V; extractor and RF lens voltage, 4 and 0.4 V, respectively; ion source temperature, 105 °C; desolvation temperature, 120 °C; desolvation gas flow (N_2), 2.5 L min^{-1}; and cone gas flow (N_2), 1.7 L min^{-1}. The intensities of the quasimolecular peaks in AQC-amino acids as well as the adjacent intensities within the zones were measured under selective ion monitoring (SIM) mode. The HPLC-MS data were analyzed using Waters MassLynx 3.5 software. The isotope-amended and the original soil samples were determined in the same assay.

The ^{15}N enrichment in each amino acid was expressed as the APE and calculated as follows (He et al., 2011):

$$APE_i = \frac{(R_e - R_c)}{[1 + (R_e - R_c)]} \times 100 \quad (7)$$

where R_e represents the isotopic ratio of the AQC-amino acid in soil samples to which labeled fertilizer N was applied and where R_c represents the corresponding ratio in the absence of applied fertilizer N.

The individual and total contents of N derived from applied fertilizer N of fifteen amino acids are calculated as follows (He et al., 2011):

$$N_{ai} = C_{ti} \times N\%_i \times \frac{APE_i}{a} \quad (8)$$

$$N_{aa} = \sum_{i=1}^{n} Nai \quad (n = 1, 2....., 15) \quad (9)$$

where C_{ti} represents the concentration of a specific amino acid (mg kg^{-1} soil); $N\%_i$ represents the N percentage of the specific amino acid; a represents the APE of applied labeled fertilizer N, which is difference between the ^{15}N abundance of $(^{15}NH_4)_2SO_4$ (50.12%) and the natural ^{15}N abundance (0.366%); N_{ai} represents the content of the specific amino acid N derived from applied fertilizer N; and N_{aa} represents the total N contents of fifteen amino acids.

2.6. Statistical analysis

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 ($P < 0.05$) plant uptake and soil residues of applied fertilizer N as well as the dynamics and change rate of organic N and amino acid N derived from applied fertilizer N.

3. Results

3.1. Plant N uptake, soil residue and total losses from applied fertilizer N under different N rates and straw application conditions

Both treatment and cultivation significantly affected the plant uptake of soil N and applied fertilizer N, and significant interactions were observed ($P < 0.001$, Fig. 1A, B). For the first crop cultivation, compared to CK and M treatments, N fertilization significantly reduced plant uptake from soil N ($P < 0.05$, Fig. 1A). For the second and third crop cultivations, higher N inputs enhanced plant uptake of soil N than lower N inputs ($P < 0.05$, Fig. 1A). During the first and third crop cultivations, compared with the N_1 treatment, the N_1 + M treatment with its straw amendment significantly reduced the plant uptake of soil N by an average of 8.0% ($P < 0.05$, Fig. 1A). For all three crop cultivations, higher N inputs significantly increased plant uptake of fertilizer N compared to lower N rates ($P < 0.05$, Fig. 1B). During the first crop cultivation, compared with the N_1 and N_2 treatments, both the N_1 + M and N_2 + M treatments (with straw amendment) also significantly increased the plant uptake of fertilizer N by an average of 31.5% ($P < 0.05$, Fig. 1B). During the third crop cultivation, the plant uptake of fertilizer N in the N_2 + M treatment was still 9.4% greater than that in the N_2 treatment ($P < 0.05$, Fig. 1B).

Both treatment and cultivation significantly affected soil residues and the total losses of applied fertilizer N, and significant interactions were observed ($P < 0.001$, Fig. 2A, B). Compared with the N_1 and N_2 treatments, the N_1 + M and N_2 + M treatments (with straw amendment) significantly increased the soil residues of fertilizer N by an average of 9.4% during the first crop cultivation ($P < 0.05$, Fig. 2A). Similarly, compared with the N_2 treatment, the N_2 + M treatment (with straw amendment) significantly increased soil residues of fertilizer N by an average of 9.7% during the second crop cultivation ($P < 0.05$, Fig. 2A). Furthermore, compared with the N_2 treatment, the N_2 + M treatment (with straw amendment) also significantly reduced fertilizer N loss by an average of 24.3% during the first crop cultivation ($P < 0.05$, Fig. 2B).

3.2. N transformation from fertilizer to soil organic N and amino acid N under different N rates and straw application conditions

The contents of organic N (N_o) and amino acid N (N_{aa}) derived from applied fertilizer N were significantly affected by both treatment and date, and significant interactions between these two factors were observed ($P < 0.001$, Fig. 3A, B). The contents of organic N and amino

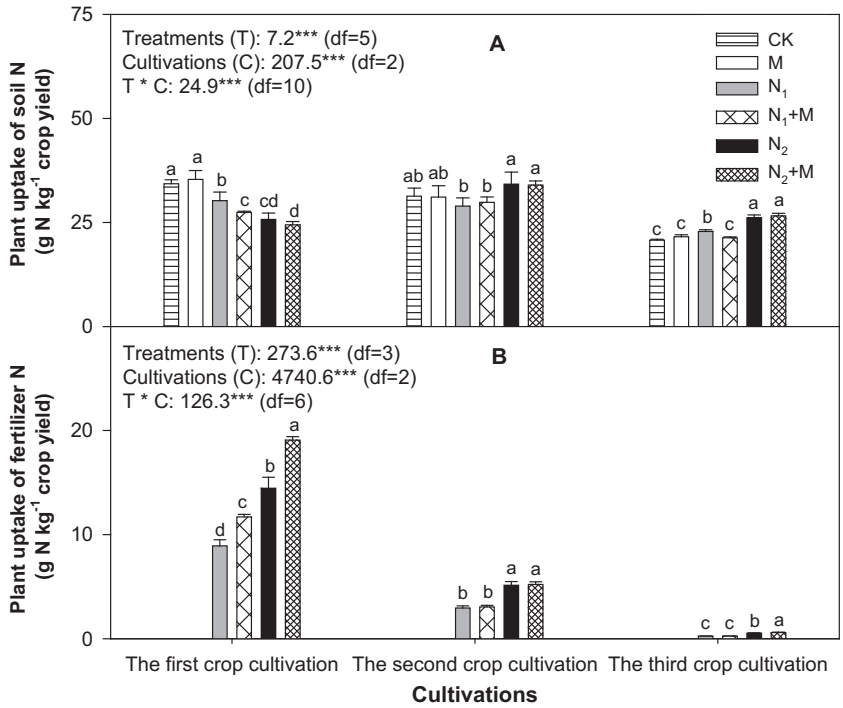

Fig. 1. The effects of N application rates and maize straw amendment on plant uptake of soil N (A) and applied fertilizer N (B) among three different crop cultivations. Different letters within each sampling date indicate significant differences at α = 0.05. Error bars indicate the standard deviations. 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.

acid N decreased with consecutive crop cultivation for all treatments (Fig. 3A, B). Across all five sampling dates, the fertilizer-derived organic N and amino acid N in the N_2 and N_2 + M treatments, which averaged 19.8 and 8.0 mg N kg^{-1} soil, respectively, were significantly greater than those in the N_1 and N_1 + M treatments, which averaged 11.6 and 6.7 mg N kg^{-1} soil, respectively ($P < 0.05$, Fig. 3A, B). Compared with the N_2 treatment, the N_2 + M treatment (with straw amendment) significantly increased the content of organic N from fertilizer N by an average of 20.1% during the first crop cultivation ($P < 0.05$, Fig. 3A). Moreover, maize straw also significantly promoted fertilizer-derived amino acid N by an average of 12.5% for the N_1 + M and N_2 + M treatments at the five sampling dates ($P < 0.05$, Fig. 3B).

The percentages of organic N (N_oP) and amino acid N ($N_{aa}P$) to applied fertilizer N were also significantly affected by treatment and date, and significant interaction effects were observed ($P < 0.05$, Table 2). On average, 34.4% and 21.0% of applied labeled fertilizer N were converted to organic N and amino acid N, respectively, after 10 days of fertilization (Table 2). The percentages of organic N and amino acid N from fertilizer N inputs decreased significantly as crop cultivations continued (Table 2). Straw application significantly

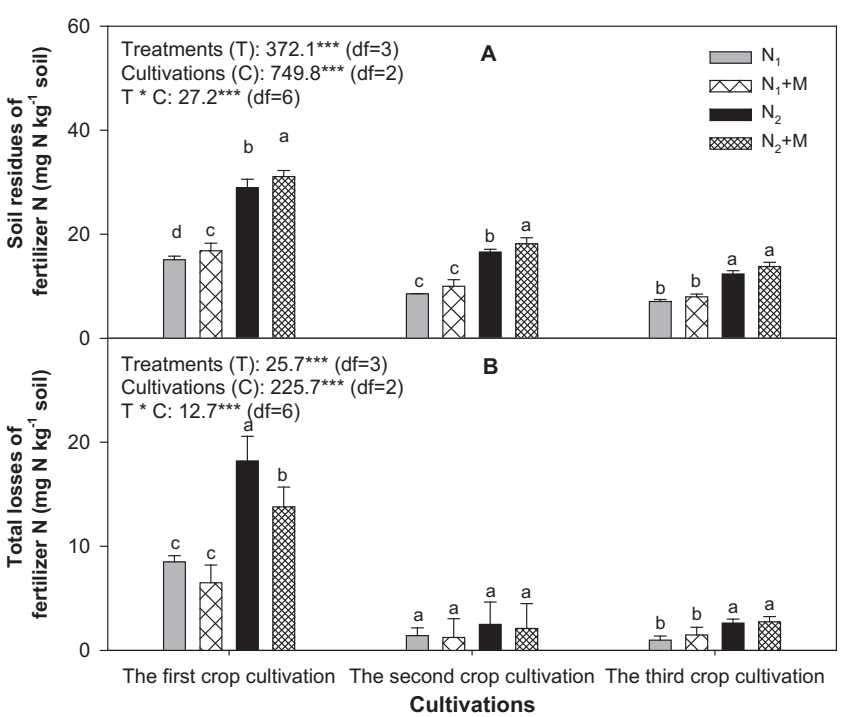

Fig. 2. The effects of N application rates and maize straw amendment on the soil residues (A) and total losses (B) of applied fertilizer N among three different crop cultivations. Different letters within each sampling date indicate significant differences at α = 0.05. Error bars indicate the standard 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.

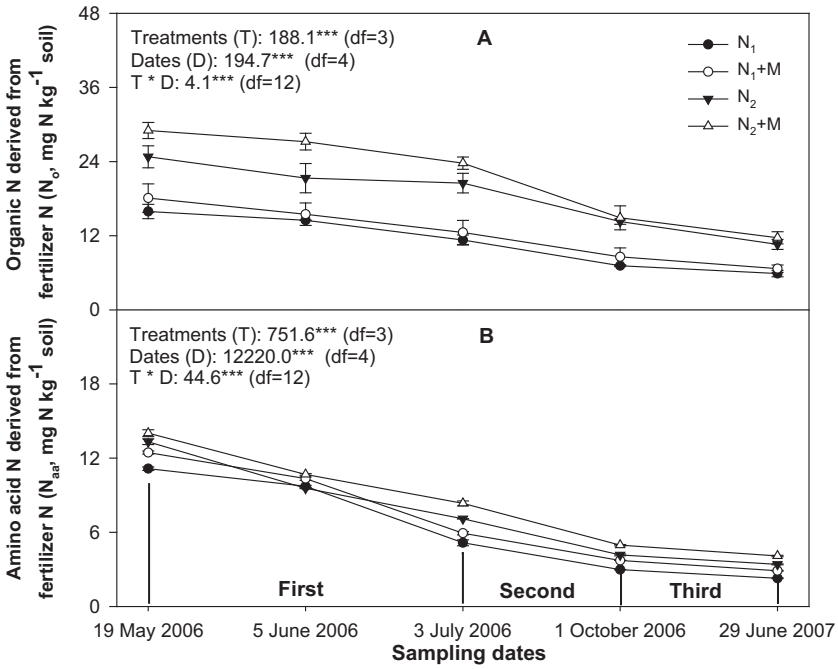

Fig. 3. The effects of N application rates and maize straw amendment on the contents of organic N (N_o) (A) and amino acid N (N_{aa}) (B) derived from fertilizer N at five sampling dates. Different letters within each sampling date indicate significant differences at $\alpha = 0.05$. Error bars indicate 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.

Table 2
The percentages of organic N and amino acid N derived from fertilizer N out of applied fertilizer N as well as the proportion of amino acid N to organic N at five sampling dates (mean ± SD).

Dates	Treatments	Organic N from fertilizer out of applied fertilizer N (N_oP, %)	Amino acid N from fertilizer out of applied fertilizer N ($N_{aa}P$, %)	Amino acid N to organic N (%)
19 May 2006	N1	36.0 ± 2.6 b	25.2 ± 0.3 b	70.3 ± 5.9 a
	N1 + M	40.7 ± 5.2 a	28.0 ± 0.3 a	69.6 ± 9.6 a
	N2	28.0 ± 2.0 c	15.1 ± 0.3 d	54.0 ± 4.9 b
	N2 + M	32.8 ± 1.5 b	15.9 ± 0.3 c	48.3 ± 1.8 b
5 June 2006	N1	32.8 ± 0.1 ab	22.0 ± 0.2 b	67.1 ± 0.4 a
	N1 + M	34.9 ± 4.1 a	23.3 ± 0.3 a	67.4 ± 7.2 a
	N2	24.0 ± 2.7 c	10.8 ± 0.0 d	45.2 ± 4.8 b
	N2 + M	30.7 ± 1.5 b	12.0 ± 0.1 c	39.3 ± 1.9 b
3 July 2006	N1	25.6 ± 1.9 ab	11.6 ± 0.5 b	45.6 ± 2.1 a
	N1 + M	28.1 ± 4.8 a	13.3 ± 0.3 a	48.1 ± 8.1 a
	N2	23.2 ± 1.8 b	8.0 ± 0.0 d	34.7 ± 2.7 b
	N2 + M	26.6 ± 1.1 ab	9.4 ± 0.2 c	35.2 ± 2.2 b
1 October 2006	N1	16.2 ± 0.4 a	6.7 ± 0.0 b	41.7 ± 0.8 a
	N1 + M	19.3 ± 3.2 a	8.4 ± 0.0 a	44.3 ± 7.6 a
	N2	16.2 ± 0.5 a	4.7 ± 0.1 d	29.3 ± 1.2 b
	N2 + M	16.7 ± 2.2 a	5.6 ± 0.1 c	33.7 ± 4.8 b
29 June 2007	N1	13.3 ± 1.2 a	5.2 ± 0.1 b	39.1 ± 2.9 ab
	N1 + M	15.0 ± 1.3 a	6.5 ± 0.1 a	43.6 ± 4.4 a
	N2	12.0 ± 0.9 a	3.9 ± 0.1 d	32.3 ± 2.9 b
	N2 + M	13.1 ± 1.1 a	4.6 ± 0.1 c	35.2 ± 3.5 b
Effects	Treatment (T)	21.3*** (df = 3)	4068.2*** (df = 3)	50.8*** (df = 3)
	Dates (D)	166.6*** (df = 4)	12,029.8*** (df = 4)	62.5*** (df = 4)
	T × D	2.4* (df = 12)	487.8*** (df = 12)	2.8** (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. Different letters within each column indicate significant differences at $\alpha = 0.05$.

increased the percentages of organic N to N inputs in the $N_1 + M$ and $N_2 + M$ treatments on 19 May 2006 but only in the $N_2 + M$ treatment on 5 June 2006 ($P < 0.05$, Table 2). At the five sampling dates, straw amendment significantly increased the percentages of amino acid N out of fertilizer N, which averaged 12.0% in the $N_1 + M$ and $N_2 + M$ treatments ($P < 0.05$, Table 2).

3.3. Change rates of organic N and amino acid N derived from applied fertilizer N

Based on fertilizer-derived organic N and amino acid N on 19 May, 1 July, and 1 October 2006 as well as 29 June 2007, the change rates of fertilizer-derived organic N and amino acid N during the three crop cultivations were calculated (Fig. 4A, B). Straw amendment and N inputs did not significantly affect the change rates of organic N derived from fertilizer N ($P > 0.05$, Fig. 4A), but the change rates of fertilizer-derived amino acid N were significantly affected by treatment and cultivation ($P < 0.05$, Fig. 4B). Compared with the N_2 treatment, the $N_2 + M$ treatment (with straw amendment) significantly reduced the change rates of amino acid N during the first cultivation ($P < 0.05$, Fig. 4B). Cultivation significantly influenced the change rates of organic N and amino acid N derived from fertilizer N ($P < 0.01$, Fig. 4A, B). Regarding organic N, the change rates during the first and second crop cultivations were significantly greater than those during the third crop cultivation ($P < 0.05$, Fig. 4A). For amino acid N, the overall change rates decreased with consecutive crop cultivations (Fig. 4B).

4. Discussion

4.1. N transformation from fertilizer to soil organic N and amino acid N

Our results showed that approximately 23–41% of fertilizer N applied to soils could be recovered as organic N during the first crop cultivation (Table 2) and that fertilizer-derived organic N accounted for 37–86% of the total residual labeled fertilizer N (Fig. 2A and Fig. 3A). These results are consistent with reports of organic N being a major N pool in agricultural soils (Jones et al., 2004; Murphy et al., 2000), and immobilization of chemical fertilizer N in the soil is a major mechanism of N storage for subsequent crop use (De Neve et al., 2004; Macdonald et al., 2002). The dissolved organic N pool is mainly composed of free amino acid and hydrolyzable petides or proteins (Yu et al., 2002; Warren, 2014). Jones et al. (2004) hypothesized that the first pool of dissolved organic N, mainly free amino acids and proteins, can be turned over rapidly by soil microbes, which does not build up in soil; however, the second dissolved organic N is of a high-molecular-weight,

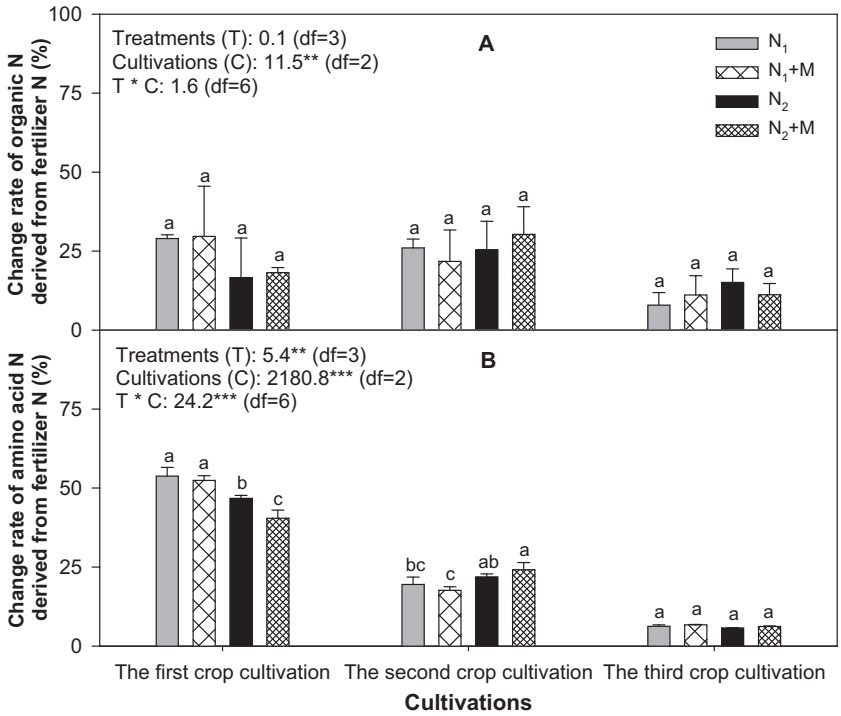

Fig. 4. The effects of N application rates and maize straw amendment on the change rates of organic N (A) and amino acid N (B) derived from fertilizer N among three different crop cultivations. Different letters within each sampling date indicate significant differences at $\alpha = 0.05$. Error bars 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.

which dominates dissolved organic N potential to N loss due to slow turnover rates. These studies suggest a dynamic role of the soil organic N pool, especially for amino acid N with respect to the transformation of fertilizer N to crop-soil N cycling (Jones et al., 2005a).

Soil hydrolyzed amino acid N accounted for 4–28% of applied fertilizer N and > 30% of recovered soil organic N among the three crop cultivations (Table 2). These results confirmed that soil amino acid N, mainly polypeptide, i.e., protein and/or peptide, is a major fraction of soil organic N and dominates both organic N turnover and N flux in the soil (Muruganandam et al., 2009; Jones and Kielland, 2002; O'Dowd et al., 1999; Stevenson, 1982). Lü et al. (2013) reported that basal fertilizer N is initially and significantly converted to amino acids by biological processes, which could constitute a transitional pool of N transformation from the newly formed amino acids N to other different fractions (such as amino sugar, hydrolyzable ammonium N, hydrolyzable unknown N and acid insoluble N) in soil organic N pool available for crop growth in a maize growing season (Bremner, 1960; He et al., 2011).

When maize straw was not applied, 32.0% and 20.1% of fertilizer N applied to the soil were quickly transformed to organic N and amino acid N after ten days of $(^{15}NH_4)_2SO_4$ top dressing (Table 2). Applying maize straw significantly increased the percentages of fertilizer-derived organic N and amino acid N by 13.1% and 11.3%, respectively, in the N_1 + M treatment and by 17.2% and 5.3%, respectively, in the N_2 + M treatment (Table 2). In our study, higher N rates increased the stimulating effects of straw on fertilizer-derived organic N proportion, but not the percentage of fertilizer-derived amino acid N, suggesting that due to C limitation for microbial activity after higher N inputs, instead of amino acid N, more N has been converted to hydrolyzable ammonium N, such as acid-labile organic constituents, exchangeable and clay-fixed ammonium (Lu et al., 2010b; Lü et al., 2013). We previously reported that the combined application of fertilizer N and maize straw significantly reduced the accumulation of inorganic N, especially NH_4^+-N in the soil (Lu et al., 2010a), and reduced fertilizer N losses (Fig. 2B). Corbeels et al. (2000) reported that applying wheat straw rather than sunflower stalks increased fertilizer N immobilization in the soil organic fraction. Ros et al. (2010) reported that crop residue amendment greatly increased dissolved and extractable soil organic N, mainly because soluble compounds in the amendments could be rapidly decomposed and used by the soil microbial community to form an organic N pool. In present study, the transformation of fertilized N to the soil organic N pool, especially amino acid N, was stimulated by adding crop residues to the soil in conjunction with fertilizer N. These results supported our working hypothesis in which combined maize straw-fertilizer practices promoted the transformation of fertilizer N to organic N, especially amino acid N, which is the most dynamic and bioavailable fraction and the primary source of mineralizable N (Haynes, 2005). Consequently, amending maize straw in conjunction with fertilizer N enhanced the retention of applied fertilizer N in the soil (Fig. 2A) and minimized N losses (Fig. 2B). Steiner et al. (2008) also reported that organic amendments significantly increase the retention of applied fertilizer N in the soil.

4.2. Change rates of organic N and amino acid N derived from applied fertilizer N

On average, the change rates of organic N and amino acid N were 60.6% and 75.4%, respectively, among the three crop cultivations, suggesting a relatively high activity of newly synthesized organic N and the amino acid N pool derived from fertilizer N occurred (Fig. 4A, B). Hansen et al. (2005) reported that organic N mineralizes easier if formed more recently. The higher change rate of amino acid N than that of organic N among the three crop cultivations confirmed that soil amino acid N is a more labile fraction of the soil organic N pool. Our results agreed with the findings in which the turnover rates of amino acid N are greater than the turnover rates of other organic N fractions (Jones and Kielland, 2002; Jones et al., 2005b; Tate, 2000). This phenomenon occurs presumably because amino acids can be taken up directly by microbial cells by specific transporters, while other fractions, such as proteins, must be degraded into peptides or amino acids before being transported into those cells (Jones, 1999; Vinolas et al., 2001).

Using an aerobic 30-week intermittent leaching experiment with ^{15}N isotope tracing, Zhang et al. (2015) reported that the mineralization rate of newly synthesized soil amino acids was faster than that of

originally present amino acids, and the simulated turnover times of the newly synthesized and originally present amino acids were approximately 13 years and 25 years, respectively, suggesting that more rapid N cycling of the newly synthesized amino acid pool from fertilizer N occurs and that a potentially rapid transitional pool for N supply exists. Glucose addition inhibits mineralization of newly synthesized amino acids (Zhang et al., 2015); our results agreed with those of this report, as maize straw application reduced the change rate of fertilizer-derived amino acid N in the soil that received high N fertilization during the first crop cultivation. These results indicated that the addition of available C (such as crop residue return) should be the key agricultural management approach for regulating the accumulation, mineralization and availability of fertilizer-derived amino acid N (Fig. 3B and Fig. 4B).

The change rates of organic N were 23.4%, 25.9% and 11.3% during the first, second and third crop cultivations, and the corresponding percentages of amino acid N were 48.4%, 20.8% and 6.2%, respectively. The decrease in change rates of organic N and amino acid N during the crop cultivation implied that the release and availability of newly formed organic N and amino acid N could persist for a relatively long time, as rapid releases during the first and second crop cultivations but a slow release during the last crop cultivation were recorded in our study. Zhang et al. (2015) reported that the decomposition of newly synthesized amino acids could be divided into a rapid phase and a relatively slow phase. Springob and Kirchmann (2003) also reported that new amino acids can gradually stabilize over time via humification. This slow release during the last crop cultivation might be due to the chemical and physical protection of protein or peptides (Schulten and Schnitzer, 1998); for example, the retention of soil organic molecules, especially amino acids in soil microsized pores, considerably reduces N availability, microbial accessibility and pore water exchange (McCarthy et al., 2008; Zhuang et al., 2008). Also, Lü et al. (2013) reported that residual fertilizer N was stabilized in the soil mainly in the microbially associated fractions at the end of the maize growing season and that proteinaceous amino acids play a mediating role in both the retention and translocation of fertilizer N; thus, the accessibility of these amino acids to microorganisms and depolymerization control the process of fertilizer N cycling in crop-soil systems (Nannipieri and Eldor, 2009) as well as the N supply for the next season or long-term N storage.

5. Conclusions

Transformation of fertilizer N to soil organic N available to plants in crop-soil systems is critical for meeting the goal of increasing N use efficiency while minimizing N losses for sustainable agriculture. Our study suggests that organic N is a major N pool in agricultural soil and that immobilization of fertilizer N in the soil is a major mechanism of N storage for subsequent crop use. This finding confirms that soil amino acid N is a major labile fraction of soil organic N, constitutes a transitional pool for the internal transformation of fertilizer N and dominates both the organic N turnover and N supply for crop growth. The decrease in change rates of organic N and amino acid N with the continuation of crop cultivation implies that the release and availability of newly formed organic N and amino acid N is a relatively long process, which is generally fast during the first and second crop cultivations but slow during the third crop cultivation. More importantly, applying maize straw that has a wide range of C/N ratios greatly enhanced the accumulation of fertilizer-derived organic N and amino acid N during the first crop cultivation. Throughout the three crop cultivations, straw amendment also promoted fertilizer N retention in the crop-soil systems. This study provides important insights into the role of maize straw applications to soil in conjunction with N fertilization in controlling both the accumulation and transformation of organic N and amino acid N from fertilizer N for plant uptake of N nutrients.

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