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EFFECT OF NITROGEN FERTILIZER AND MAIZE STRAW INCORPORATION ON NH_4^+-^{15}N AND NO_3^--^{15}N ACCUMULATION IN BLACK SOIL OF NORTHEAST CHINA AMONG THREE CONSECUTIVE CROPPING CYCLES

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ABSTRACT

A pot experiment was conducted to evaluate the effect of nitrogen (N) fertilizer and maize straw incorporation on the accumulation of NH_4^+-^{15}N and NO_3^--^{15}N in soil inorganic N pool among three consecutive cropping cycles, aimed to search for an effective N management practice to decrease superfluous accumulation of soil inorganic N and fertilizer N losses. The results showed that the amounts of soil NH_4^+-^{15}N, NO_3^--^{15}N and inorganic ^{15}N, and their percent to applied ^{15}N-labeled fertilizer declined significantly with sampling time ($p \leq 0.001$). Compared to low N application rate (44.64 mg N kg^{-1} soil), high N application rate (89.28 mg N kg^{-1} soil) enhanced significantly the amounts of soil NH_4^+-^{15}N, NO_3^--^{15}N and inorganic ^{15}N by 238.6%, 132.9% and 197.3%, respectively ($p \leq 0.001$). In contrast, maize straw addition declined significantly the amounts of soil NH_4^+-^{15}N and inorganic ^{15}N by 21.4% and 16.1% compared to without maize straw ($p \leq 0.001$). The results suggested that a combined application of chemical fertilizer and maize straw with a wide C/N ratio is an important means for reducing the superfluous accumulation of fertilizer N as soil inorganic N to subsequently lower its loss.

Keywords: N fertilizer, maize straw, NH_4^+-^{15}N and NO_3^--^{15}N accumulation, Black soil

INTRODUCTION

Nitrogen (N) is one of the critical nutrients for crop production and is generally applied in large quantities in form of fertilizer to soils (Malhia *et al*., 2001; Murshedul *et al*., 2006; Singh *et al*., 2007; Kong *et al*., 2008). However, most plants only utilize less than one-half of fertilizer N applied, and the loss of fertilizer N was high (Zhu, 2000; Zhu and Chen, 2002). Nitrogen management in agro-ecosystems has been extensively studied due to its importance in improving crop yield and quality, and in mitigating the negative effects of fertilizer N losses such as nitrate contamination of groundwater, eutrophication of surface water, and greenhouse effect (Hillin and Hudak, 2003; De Paz and Ramos, 2004; Alam *et al*., 2006; Dambreville *et al*., 2008).

Soil exchangeable inorganic N is the common source of various N losses (Zhu, 2000), whereas the immobilization and release of fertilizer N in soil organic N and fixed NH_4^+ pools are important processes regulating fertilizer N transformation in soil, and play an important role in controlling soil N-potential supply (Mubarak *et al*., 2001; Macdonald *et al*., 2002; Elmaci *et al*., 2002; Lu *et al*., 2010). Therefore, a key challenge in minimizing loss of chemical fertilizer N is how to decrease the superfluous accumulation of soil exchangeable inorganic N, accelerate its transformation to other N forms (such as organic N and fixed NH_4^+), and synchronize the supply of available N with plant uptake during peak periods of crop N demand (Zhu, 2000; Lin *et al*., 2007). Understanding the accumulation of fertilizer N in soil inorganic N pool under different fertilization practices is of considerable importance in developing proper fertilization practice for minimizing fertilizer N loss while maximizing its use efficiency (Angás *et al*., 2006; Lu *et al*., 2008).

China consumes more than one quarter of total fertilizer N of the world (Li *et al*., 2007; Zhu *et al*., 2008). However, loss of fertilizer N in China is high (Zhu and Chen, 2002; Luo *et al*., 2006; Jing *et al*., 2007). Especially for Northeast China, which is famous for commercial crop production in China, decreasing fertilizer N loss and increasing its utilization efficiency is very important for sustainable development of agriculture in this region (Hu *et al*., 2007; Ma *et al*., 2007; Peng *et al*., 2007). Black soil (Hapli-Udic Isohumosols) is the main agricultural soil in Northeast China, but until now, researches focusing on the effects of different fertilization practices on the accumulation of soil inorganic N are still lacking. In this study, a pot experiment was conducted to examine the effect of N fertilizer and maize straw incorporation on the accumulation of NH_4^+-^{15}N and NO_3^--^{15}N in the inorganic N pool of the black soil among three consecutive cropping cycles, aimed to search for an effective N management practice to decrease superfluous accumulation of soil inorganic N and fertilizer N loss.

MATERIALS AND METHODS

Study site

The study was conducted at the National Field Observation and Research Station of Shenyang Agro-ecosystems, a member of Chinese Ecosystem Research Network (CERN) established in 1987. This Station locates on the Lower Liao River Plain, with a humid and semi-humid continental monsoon climate of warm-temperate zone. Mean annual temperature is 7-8°C, with minimum and maximum mean monthly temperature in January (-13°C) and July (24°C), respectively. Mean active accumulated temperature (≥10°C) is 3300-3400°C. The total solar radiation is 5410-5600 kJ cm^{-2}. The duration of frost-free season is 147-168 d. Mean annual precipitation is about 700 mm.

Experimental design

Black soil samples of 0-20 cm (Hapli-Udic Isohumosols) were collected from the Jilin Institute of Soil and Fertilizer, sieved through 5-mm, and adequately homogenized.

An outdoor pot experiment consecutively cropped with Chinese spring wheat (*Triticum aestivum* L. cv. Liaochun 9, from 8 April 2006 to 3 July 2006), buckwheat (*Fagopyrum eaculentum* moench. cv. Liaoqiao-2, from 10 July 2006 to 1 Oct. 2006), and spring wheat (*Triticum aestivum* L. cv. Liaochun 9, from 3 April 2007 to 29 June 2007) was conducted. 6.5 kg of the soil was put into each pot with an outer diameter of 25 cm and a height of 15 cm, and each pot was sown with 15 spring wheat seeds or 8 buckwheat seeds. Six Treatments were set up, and each treatment had 20 pots and sampled for five times with four replicates at each sampling date.

During the experiment, soil moisture content was adjusted daily with deionized water to about 60% water

Table 1. Physical and chemical characters of the tested soil

Chemical characters	TC	T.N	T.P	T.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 characters	Mechanical composition (%)			Clay mineral composition (<2um, %)				
	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 varies from 2 to 0.02mm, Silt from 0.02 to 0.002 mm, and Clay is < 0.002 mm.

Table 2. Fertilization treatments of the outdoor pot experiment

Treatments	**Amount of fertilizer application**		
	N fertilizer	**Maize straw**	**P, K fertilizer**
CK	No N fertilization	No Maize straw	27.95 mg P kg^{-1} soil (60 kg P ha^{-1}), 46.59 mg K kg^{-1} soil (100 kg K ha^{-1})
N_1	44.64 mg N kg^{-1} soil (100 kg N ha^{-1}, low N application rate)	No Maize straw	27.95 mg P kg^{-1} soil (60 kg P ha^{-1}), 46.59 mg K kg^{-1} soil (100 kg K ha^{-1})
N_2	89.28 mg N kg^{-1} soil (200 kg N ha^{-1}, high N application rate)	No Maize straw	27.95 mg P kg^{-1} soil (60 kg P ha^{-1}), 46.59 mg K kg^{-1} soil (100 kg K ha^{-1})
M	No N fertilization	2.31 g dry matter kg^{-1} soil (5000 kg ha^{-1})	27.95 mg P kg^{-1} soil (60 kg P ha^{-1}), 46.59 mg K kg^{-1} soil (100 kg K ha^{-1})
N_1 + M	44.64 mg N kg^{-1} soil (100 kg N ha^{-1}, low N application rate)	2.31 g dry matter kg^{-1} soil (5000 kg ha^{-1})	27.95 mg P kg^{-1} soil (60 kg P ha^{-1}), 46.59 mg K kg^{-1} soil (100 kg K ha^{-1})
N_2 + M	89.28 mg N kg^{-1} soil (200 kg N ha^{-1}, high N application rate)	2.31 g dry matter kg^{-1} soil (5000 kg ha^{-1})	27.95 mg P kg^{-1} soil (60 kg P ha^{-1}), 46.59 mg K kg^{-1} soil (100 kg K ha^{-1})

holding capacity (WHC). The basic properties of the tested soil and fertilization treatments of outdoor pot experiment were given in Table 1 and Table 2.

Maize straw, concentrated super-phosphate and potassium sulfate were applied as basal. Chemical N fertilizer was dissolved using de-ionized water and applied as top-dressing at the three tillering stage of the crops, i.e., on 9 May 2006, 25 July 2006, and 4 May 2007, respectively. Labeled $(^{15}NH_4)_2SO_4$ (Shanghai Research Institute of Chemical Industry) with 50.12 atom% ^{15}N was applied in the first cropping cycle, and non-labeled urea-N fertilizer was applied in the following two cropping cycles.

Sampling and analytical methods

Soil samples from the pots were collected on 19 May, 5 June, and 3 July 2006 (tillering anaphase, flowering, and ripening stage of spring wheat in the first cropping cycle), on 1 October 2006 (ripening stage of buckwheat in the second cropping cycle), and on 29 June 2007 (ripening stage of spring wheat in the third cropping cycle) by destructive sampling method. All fresh soil samples were sieved (<2 mm), and mixed homogeneously. About 100 g fresh subsamples were used to determine moisture content, soil NH_4^+-N and NO_3^--N, and their atom% ^{15}N.

Total carbon was measured using TOC-5000A automatic analyzer (Shimadzu Corporation, Japan). Total P and K were measured by sodium carbonate fusion and molybdenum antimony-ascorbic acid colorimetric method (Olsen and Sommers, 1982). Available P and K were determined by extraction method with sodium bicarbonate (Olsen *et al*., 1954) and ammonium acetate (Pratt, 1965), respectively. Soil mechanical composition and clay mineral composition was measured by pipette method and X-ray diffraction analysis, respectively (Whitting, 1965).

Total N was determined by the Kjeldahl method (Bremner and Mulvaney, 1982), inorganic N was measured by 2M KCl extraction-MgO-Devarda alloy distillation method (Keeney and Nelson, 1982), and fixed NH_4^+ was determined by KOBr-KOH method (Silva and Bremner, 1966). Subsequently, atom% ^{15}N in the acidified aqueous distillate was measured using a Finnigan Mat model 251 Isotope Ratio Mass Sepectrometer (USA). All the operation procedure was carried out from lower to higher atom% ^{15}N to avoid cross-contamination.

Methods of calculation

The amount (mg N kg^{-1} soil) of soil NH_4^+-^{15}N (C_{NH4}), NO_3^--^{15}N (C_{NO3}) and inorganic ^{15}N (C_i), and percent of soil NH_4^+-^{15}N, NO_3^--^{15}N and inorganic ^{15}N to applied ^{15}N-labeled fertilizer (P_{NH4}, P_{NO3} and P_i) was calculated according to the following formulas:

$$C_{NH4,\ NO3} = C * \frac{(b-c)}{(a-c)} \qquad (1)$$

$$C_i = C_{NH4} + C_{NO3} \qquad (2)$$

Where C is the amount (mg N kg^{-1} soil) of soil NH_4^+-N or NO_3^--N and a is the atom% ^{15}N of ^{15}N-labeled fertilizer, b is the atom% ^{15}N of treated soil NH_4^+-N or NO_3^--N, c is the atom% ^{15}N of background soil NH_4^+-N or NO_3^--N, respectively.

$$P_{NH4}\,(\%) = \frac{C_{NH4}}{C_f} * 100 \qquad (3)$$

$$P_{NO3}\,(\%) = \frac{C_{NO3}}{C_f} * 100 \qquad (4)$$

$$P_i\,(\%) = P_{NH4} + P_{NO3} \qquad (5)$$

where C_f is the amount of ^{15}N-labeled fertilizer applied (mg N kg^{-1} soil), C_{NH4} and C_{NO3} is the amount (mg N kg^{-1} soil) of soil NH_4^+-^{15}N and NO_3^--^{15}N.

Statistical analysis of data

Three-way Analysis of Variance (ANOVA) with SPSS 13.0 statistical package was conducted to detect effect of N fertilizer and maize straw incorporation on amount and distribution of NH_4^+-^{15}N and NO_3^--^{15}N in soil inorganic N pool. Differences with a probability level of $p \leq 0.05$ were considered significant.

RESULTS

Amounts of soil NH_4^+-^{15}N and NO_3^--^{15}N

^{15}N-labeled fertilizer existed in form of soil NH_4^+-^{15}N and NO_3^--^{15}N in soil inorganic ^{15}N pool. The amount of soil NH_4^+-^{15}N declined significantly with sampling time, and was significantly different between the treatments with the low and high N application rates ($p \leq 0.001$, Table 3, Figure 1). Compared to the low N application rate, the high N application rate significantly increased the

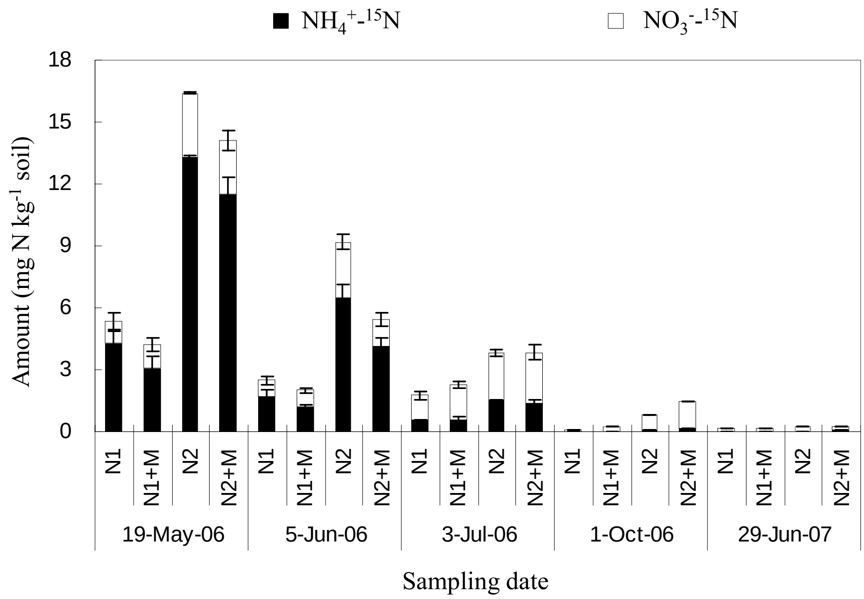

Figure 1. Amounts of soil NH_4^+-^{15}N, NO_3^--^{15}N and inorganic ^{15}N across three cropping cycles (Bars indicate the standard errors)

amount of soil NH_4^+-^{15}N by 238.6% across three consecutive cropping cycles, and this effect was the largest in the flowering stage and then weakened with time ($p \leq 0.001$, Table 3, Figure 1). In contrast, the application of maize straw significantly decreased the amount of soil NH_4^+-^{15}N by 21.4% among the first cropping cycle, and this effect was the largest in the flowering stage and then weakened with time ($p \leq 0.001$, Table 3, Figure 1).

Table 3. Results of three-way Analysis of Variance on the amount of soil NH_4^+-^{15}N, NO_3^--^{15}N and inorganic ^{15}N

	NH_4^+-^{15}N		**NO_3^--^{15}N**		**Inorganic ^{15}N**	
	F value	*P* value	*F* value	*P* value	*F* value	*P* value
Time	622.11	<0.001	108.98	<0.001	592.20	<0.001
Nitrogen	506.76	<0.001	198.60	<0.001	639.47	<0.001
Maize straw	24.97	<0.001	0.59	0.445	20.24	<0.001
T*N	188.23	<0.001	14.93	<0.001	167.88	<0.001
T*M	9.26	<0.001	7.32	<0.001	13.57	<0.001
N*M	4.43	0.040	7.37	0.009	9.10	0.004
T*N*M	2.22	0.078	2.46	0.061	5.02	0.001

The amount of soil NO_3^--^{15}N also significantly declined with sampling time ($p \leq 0.001$, Table 3, Figure 1). Compared to the low N application rate, the high N application rate significantly increased the amount of soil NO_3^--^{15}N by 132.9% across three consecutive cropping cycles, and this increasing pattern weakened with time ($p \leq 0.001$, Table 3, Figure 1). However, applying maize straw had no effects on the amount of soil NO_3^--^{15}N ($p > 0.05$, Table 3, Figure 1). The change trend of soil inorganic ^{15}N was the same with that of soil NH_4^+-^{15}N (Table 3, Figure 1).

In soil inorganic ^{15}N pool, the proportion of soil NH_4^+-^{15}N accounting for inorganic ^{15}N obviously decreased with sampling time, however, soil NO_3^--^{15}N accounting for inorganic ^{15}N increased with sampling time (Figure 2). For example, the proportion of soil NH_4^+-^{15}N accounting for inorganic ^{15}N across four fertilization treatments was 78.54%, 68.77%, 33.48%, 11.08% and 12.46% on average at the five consecutive sampling dates, respectively, and that of soil NO_3^--^{15}N accounting for inorganic ^{15}N was averagely 21.46%, 31.23%, 66.52%, 88.92% and 87.54%, respectively (Figure 2). The proportion of soil NH_4^+-^{15}N was obviously higher than that of soil NO_3^--^{15}N at the tillering anaphase and flowering stage of the first cropping cycle, and vice versa in the ripening stage of three cropping cycles (Figure 2).

Distribution of ^{15}N-labeled fertilizer in soil inorganic N pool

The percent of soil NH_4^+-^{15}N to applied ^{15}N-labeled fertilizer (P_{NH4}) decreased significantly with sampling time ($p \leq 0.001$, Table 4, Table 5). On 19 May 2006, the P_{NH4} was averagely 11.12% across four fertilization treatments, and the corresponding proportions were 1.45%, 0.07% and 0.03% on 3 July, 1 October 2006 and 29 June 2007, respectively (Table 4). Compared to the low N application rate, the high N application rate remarkably elevated the P_{NH4} by 69.1% across three consecutive cropping cycles, and this effect was the highest at the flowering stage and then

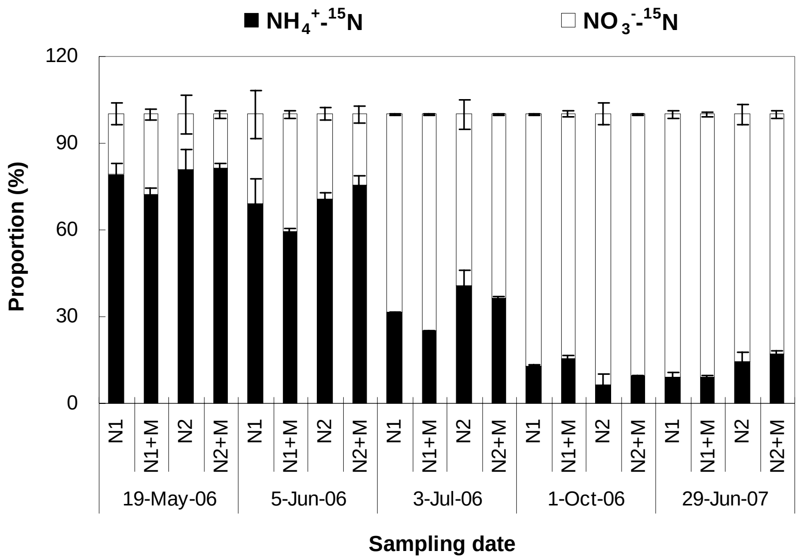

Figure 2. Proportions of NH_4^+-^{15}N and NO_3^--^{15}N in soil inorganic N pool across three cropping cycles (Bars indicate the standard errors).

weakened with time ($p \leq 0.001$, Table 4, Table 5). In contrast, maize straw addition significantly lowered the P_{NH4} by 19.6% in the tillering anaphase of the first cropping cycle, and this effect disappeared with sampling time, compared to those without maize straw addition ($p \leq 0.01$, Table 4, Table 5).

The percent of soil NO_3^--^{15}N to applied ^{15}N-labeled fertilizer (P_{NO3}) significantly decreased with sampling time ($p \leq 0.001$, Table 4, Table 5). On 19 May 2006, the P_{NO3} was averagely 2.90% across four fertilization treatments, and the corresponding proportions were 2.86%, 0.66% and 0.21% on 3 July, 1 October 2006 and 29 June 2007, respectively (Table 4). The high N application rate significantly increased the P_{NO3} by 13.0% across three consecutive cropping cycles, compared to the low N application rate, and this increasing pattern weakened with time ($p \leq 0.05$, Table 4, Table 5). However, no significant effect from maize straw addition was observed in the P_{NO3} ($p > 0.05$, Table 4, Table 5).

The percent of soil inorganic ^{15}N to applied ^{15}N-labeled fertilizer (P_i) significantly decreased with sampling time ($p \leq 0.001$, Table 4, Table 5). The highest P_i was 14.01% in the tillering anaphase across four fertilization treatments. The change trend of P_i was the same with P_{NH4} in different fertilization treatments (Table 4, Table 5).

DISCUSSION

Chemical N fertilizer existed in form of inorganic N. Thus it was expected that application of chemical N fertilizer enhanced the size of soil inorganic N pool. Our observation supported the above speculation that high N application

Table 4. Percent of soil NH_4^+-^{15}N, NO_3^--^{15}N and inorganic ^{15}N to applied ^{15}N-labeled fertilizer (P_{NH4}, P_{NO3} and P_i, respectively) across three cropping cycles (mean ± S.E).

Sampling date	Treatments	P_{NH4}	P_{NO3}	P_i
19-May-06	N_1	9.63±1.41	2.50±0.40	12.13±1.81
	N_1+M	6.82±1.06	2.60±0.49	9.42±1.56
	N_2	15.01±0.02	3.55±0.15	18.56±0.13
	N_2+M	13.00±2.53	2.93±0.95	15.93±3.49
5-June-06	N_1	3.92±0.62	1.75±0.52	5.67±1.15
	N_1+M	2.66±0.35	1.81±0.35	4.47±0.70
	N_2	7.36±1.88	3.03±0.27	10.39±1.61
	N_2+M	4.6±0.45	1.49±0.20	6.09±0.65
3-July-06	N_1	1.26±0.05	2.74±0.03	4.00±0.08
	N_1+M	1.28±0.43	3.81±1.23	5.09±1.65
	N_2	1.76±0.01	2.55±0.39	4.31±0.40
	N_2+M	1.58±0.09	2.74±0.20	4.31±0.29
1-October-06	N_1	0.03±0.00	0.19±0.03	0.22±0.04
	N_1+M	0.06±0.01	0.35±0.01	0.42±0.01
	N_2	0.05±0.01	0.78±0.09	0.83±0.08
	N_2+M	0.14±0.02	1.30±0.05	1.44±0.04
29-June-07	N_1	0.02±0.00	0.20±0.03	0.22±0.03
	N_2	0.03±0.01	0.20±0.04	0.23±0.04
	N_1+M	0.02±0.00	0.23±0.03	0.25±0.03
	N_2+M	0.04±0.01	0.19±0.03	0.23±0.04

rate significantly enhanced the amount of NH_4^+-^{15}N and NO_3^--^{15}N in soil inorganic N pool ($p \leq 0.001$, Table 2, Figure 1), and subsequently increased the loss of ^{15}N-labeled fertilizer by 4.4% across three consecutive cropping cycles (Lu, unpublished). Angás *et al*. (2006) also found that soil mineral N increased with the increase of N fertilization rates, and applying more N than the crop needed elevated the superfluous accumulation of inorganic N and its loss.

In contrast, applying maize straw significantly declined the amount of soil NH_4^+-^{15}N and inorganic ^{15}N by 16.2% and 17.3%, and degraded the loss of ^{15}N-labeled fertilizer by 12.4% in the tillering anaphase of the first cropping cycle among four fertilization treatments compared to those without maize straw

Table 5. Results of three-way Analysis of Variance on Percent of soil NH_4^+-^{15}N, NO_3^--^{15}N and inorganic ^{15}N to applied ^{15}N-labeled fertilizer (P_{NH4}, P_{NO3} and P_i, respectively).

	P_{NH4}		**P_{NO3}**		**P_i**	
	F value	*P* value	*F* value	*P* value	*F* value	*P* value
Time	364.09	<0.001	122.06	<0.001	320.08	<0.001
Nitrogen	63.00	<0.001	4.55	0.037	48.75	<0.001
Maize straw	9.62	0.003	0.01	0.911	5.69	0.020
T*N	25.85	<0.001	8.75	<0.001	20.89	<0.001
T*M	4.63	0.003	6.03	<0.001	5.27	0.001
N*M	0.03	0.862	3.68	0.062	1.26	0.266
T*N*M	0.43	0.788	1.39	0.254	0.94	0.445

addition. The results also showed that 14.8% loss of ^{15}N-labeled fertilizer in the tillering anaphase accounted for 64.3% of the overall loss across three consecutive cropping cycles, which indicated that the loss of ^{15}N-labeled fertilizer occurred mainly within 10 days after fertilization. A major reason for this was that low N application rate could meet the low N demand of spring wheat at the tillering stage, while high N application rate resulted in excessive N loss at the tillering stage. However, maize straw with a wide C/N ratio could provide plentiful carbon and energy sources to stimulate soil microbial activity, and accelerate the transformation of soil inorganic ^{15}N into organic ^{15}N (increasing the amount of soil organic ^{15}N by 13.9%, and then decrease the loss percent of ^{15}N-labeled fertilizer (Lu, unpublished, Gentile *et al*., 2009; Nayak *et al*., 2007; Chaves *et al*., 2006).

The amount of soil NH_4^+-^{15}N was higher than that of soil NO_3^--^{15}N at the tillering anaphase and flowering stage, and then the trend was reversed at the ripening stages of three cropping cycles (Figure 1, 2), which suggested that the nitrification of soil NH_4^+-^{15}N was low within 27 days after fertilization, and strengthened thereafter. The proportion of soil NH_4^+-^{15}N accounting for inorganic ^{15}N decreased with time, and that of soil NO_3^--^{15}N accounting for inorganic ^{15}N increased with time, which further confirmed the above-mentioned results (Figure 2).

CONCLUSIONS

Our study indicated that high N application rate significantly enhanced the amount of soil NH_4^+-^{15}N, NO_3^--^{15}N and inorganic ^{15}N, compared to low N application rate. In contrast, maize straw with a wide C/N ratio is important in regulating the accumulation of NH_4^+-^{15}N and NO_3^--^{15}N in soil inorganic N pool. Maize straw addition lowered the amounts of soil NH_4^+-^{15}N, inorganic ^{15}N, and their percent to applied ^{15}N-labeled fertilizer, and then decreased the percent loss of ^{15}N-labeled fertilizer. Thus, a combined application of chemical fertilizer and maize straw with a wide C/N ratio is an important means for reducing the superfluous accumulation of N fertilizer as soil inorganic N to subsequently lower its loss.

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