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ORIGINAL PAPER

Influence of ^{15}N-labeled ammonium sulfate and straw on nitrogen retention and supply in different fertility soils

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Abstract Microbial immobilization/mineralization and mineral fixation/release of ammonium are important for N retention and supply. However, the rates of such processes vary among different fertility soils and fertilization management practices. Three long-term different fertilized soils were used to simulate a range in soil fertility level and incubated with different N amendments for 144 days. The dynamics of ^{15}N derived from ammonium sulfate (AS) or straw in different soil N pools and the ammonium sulfate-N or straw-N retention and supply were studied. In the absence of straw, the amount of ammonium sulfate-N present as fixed ammonium was 1.1–3.5-fold higher than that present as soil microbial biomass N (SMBN), although ammonium sulfate-derived SMBN and its mineralization increased by increasing soil fertility level. Straw addition significantly ($P < 0.05$) enhanced the relative importance of the SMBN pool on ammonium sulfate-N retention and supply compared with the fixed ammonium-N pool, and the former exceeded the latter in higher fertility soils. Regardless of soil fertility levels, straw addition significantly blocked the release of ammonium sulfate-N from the fixed ammonium-N pool. The SMBN pool was more important in straw-N retention and supply than the fixed ammonium-N pool, confirming that straw-N cycling depended more on biotic processes. The percentage of mineralized ammonium sulfate-N or straw-N from SMBN was higher than that released from fixed ammonium, indicating the higher availability of SMBN. Generally, the mineral fixation/release of ammonium was the main process for mineral fertilizer N retention and supply in the low fertility soil with or without straw addition, whereas microbial immobilization/mineralization became the main process in the high fertility soil with straw addition. Our results gave insights on the ammonium sulfate-N or straw-N retention and supply in different fertility soils, providing suggestions for optimizing straw management and synchronizing N supply with crop demand.

Keywords Ammonium sulfate-N · Straw-N · Soil fertility · Soil microbial biomass N pool · Fixed ammonium-N pool · N retention and supply

Introduction

Application of mineral fertilizers, especially N fertilizer, compensates the increase in grain demand of the growing population. Nevertheless, numerous issues have emerged with increasing application rate of N fertilizers; such issues include reduced N use efficiency and aggravated fertilizer losses through ammonium volatilization, denitrification, leaching, and runoff, which may generate environmental risks (Richter and Roelcke 2000; Zhu and Chen 2002). The time-space discrepancy between N supply and crop demand becomes the main limitation for improving N use efficiency (Choi et al. 2004). Therefore, mineral fertilizer N supply must be synchronized with crop demand (Cassman et al. 2002; Bindraban et al. 2015), and this requires further research (Nannipieri and Paul 2009; Miao et al. 2011).

Agricultural organic wastes, especially straw, are considered an important N source in agricultural production. Burning straw for managing excessive crop residues is a common practice that has resulted in greenhouse gas emissions and organic N losses (Graham et al. 2002; Mandal et al. 2004; Singh et al. 2005), which

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can be addressed by returning straw to farmland (Dominguez-Escriba and Porcar 2010). The combined application of straw with mineral fertilizer N reduces the superfluous accumulation of mineral N in soil and its losses (Dong et al. 2014; Wang et al. 2015) due to the biotic processes (Bengtsson and Bergwall 2000), particularly the drastic increase in microbial immobilization of fertilizer N (Bird et al. 2001). Microbial immobilization of applied N can effectively prevent N losses during the early growth stages when the demand for N remains low, whereas remineralization may enhance soil N supply when plant N demand increases (Herai et al. 2006; Sugihara et al. 2010). Consequently, soil microbial biomass N (SMBN) may serve both as a sink and a source of N. Furthermore, straw-N is a major source of recently formed SMBN and should not be neglected (Ocio et al. 1991).

Abiotic processes in soil also play an important role in N retention and supply (Davidson et al. 2003; Moritsuka et al. 2004). Johnson et al. (2000) reported that biotic N immobilization proportionally decreased in soils by increasing N saturation, whereas abiotic immobilization proportionally increased. Among these abiotic processes, ammonium fixation and its release can be particularly important in regulating soil N supply and improving fertilizer N use efficiency (Scherer and Mengel 1986). However, competition for N exists between these biotic and abiotic processes (Raju and Mukhopadhyay 1974; Breitenbeck and Paramasivam 1995; Tahovská et al. 2013), and their contributions to N retention and supply also vary with N fertilizer types (Fitzhugh et al. 2003). Few studies investigated the relationship between these two processes and their responses to different N amendments particularly in soils with different fertility levels (Miyajima 2015).

Long-term different fertilization can alter soil physical, chemical, and microbial properties at varying degrees (Zhang et al. 2009; Liu et al. 2010). Across different fertilized soils, the rates of N immobilization and remineralization vary widely because of the diverse composition and activity of different microbial communities (Vinten et al. 2002; Liang et al. 2013; Yu et al. 2013). The capacity for ammonium fixation and release also differs because of changes in the contents of soil original fixed ammonium, potassium, and organic matter after long-term different fertilization (Hinman 1966; Graham et al. 2002). Thus far, limited information is available concerning the effects of long-term fertilization on these biotic and abiotic processes with different N amendments. This information is critical for regulating soil N supply and optimizing fertilization management practices according to different soil fertility levels.

In this study, a 144-day incubation experiment was conducted using ^{15}N cross-labeling. Three soils under 25-year different fertilization were used to simulate a range in soil fertility level. Ammonium sulfate (AS) alone or in combination with straw was applied to each fertility level. The objectives of this study were to investigate (1) the fate of AS-N or straw-N in soils with different fertility levels and (2) the AS-N or straw-N retention and supply in different fertility soils.

Materials and methods

Site description and soil sampling

Soil samples (0–15 cm) were collected from a 25-year-old field trial initiated in 1990 and located at the Shenyang Experimental Station of the Institute of Applied Ecology, Liaoning province, China (41° 32′ N, 123° 23′ E). Samples were collected from three long-term fertilized soils: (1) no fertilizer (NF); (2) inorganic N, P, and K fertilizers (NPK); and (3) pig manure combined with NPK fertilizers (NPK + M). For NPK and NPK + M treatments, the annual application rates of N, P, and K in the forms of urea, superphosphate, and potassium chloride were 150, 25, and 60 kg ha^{-1}, respectively. Pig manure was applied as composted manure (80% of the harvest grain of corn [*Zea mays* L.] from this treatment was used to feed pig, and 50% of stalk was ground and used as litter). After the feed was consumed, all the excretion and litters were composted and returned back to the original treatment in the following spring). Soil sampling was conducted in April before corn planting. After removal of bigger roots and vegetations, soils were air dried and sieved (<2 mm). The physicochemical properties of soils after 25 years of different fertilization are shown in Table 1.

Experimental design

A 2-week pre-incubation was conducted in a constant-temperature incubator at 25 °C in the dark, with the soil water content adjusted to 40% of the water-holding capacity (WHC, 42.51%). There were four treatments for each soil in the incubation experiment: (1) control, without N addition (CK); (2) ^{15}N-labeled ammonium sulfate (^{15}AS, atom% ^{15}N, 10.08); (3) ^{15}N labeled ammonium sulfate plus unlabeled wheat straw (^{15}AS + S); and (4) unlabeled ammonium sulfate plus ^{15}N-labeled wheat straw (AS + ^{15}S, atom% ^{15}N, 4.94). The total amount of applied N (equivalent to 107.14 mg N kg^{-1} dry soil, i.e., 180 kg N ha^{-1}) was equivalent among all N-amended treatments, with AS-N accounting for 75% and straw-N accounting for 25%. After fully mixed with N amendments, soils were weighed (equivalent to 130 g oven-dry weight) and placed in 500-ml glass bottles (a total of 216 bottles). The experimental setup (three soils × four treatments × three replicates) was repeated six times to allow for destructive sampling after 0.5, 3, 12, 36, 72, and 144 days of incubation. The C/N ratios of the labeled and unlabeled wheat straw were 80.49 and 78.53 g kg^{-1}, respectively and the corresponding N contents were 4.76 and 4.79 g kg^{-1}. During the 144-day incubation, the soil moisture was maintained at 50% of the WHC. Loss of moisture was replenished every 3 days by adding distilled water based on constant weight.

Table 1 Physicochemical properties of soils after 25 years of different fertilization

Soil	Chemical properties						Physical properties (mechanical composition)		
	Total C ($g\ kg^{-1}$)	Total N ($g\ kg^{-1}$)	Available P ($mg\ kg^{-1}$)	Available K ($mg\ kg^{-1}$)	pH	Fixed NH_4^+ ($mg\ kg^{-1}$)	Sand ($g\ kg^{-1}$)	Silt ($g\ kg^{-1}$)	Clay ($g\ kg^{-1}$)
NF	8.79b	0.95b	3.90c	54.37c	6.23a	124.13c	163.78a	585.83a	212.26a
NPK	9.21b	0.97b	13.50b	86.81b	5.35b	145.60b	163.23a	585.65a	213.84a
NPK + M	11.10a	1.20a	23.00a	125.16a	5.51b	162.53a	161.63a	584.97a	215.38a

Long-term fertilized soils: *NF* no fertilizer applied, *NPK* inorganic N, P, and K fertilizers, *NPK + M* pig manure + inorganic N, P, and K fertilizers. Different letters within columns indicate significant difference ($P < 0.05$) between the soils. Values are means ($n = 3$)

Analysis of soil chemical properties

Soil microbial biomass N was determined by chloroform fumigation extraction (Brookes et al. 1985; Vance et al. 1987). Thirty grams of the fresh soil was fumigated with chloroform at 25 °C for 24 h and another 30 g was not fumigated. After removing the chloroform, all subsamples were extracted with 100 ml 0.5 M K_2SO_4, filtered, and freeze-stored before steam distillation.

Soil samples were extracted with 2 M KCl for determination of exchangeable NH_4^+-N and NO_3^--N contents. The filtrate was analyzed by steam distillation with MgO and Devarda's alloy (Keeney and Nelson 1982). Soil residues were washed with 0.1 M KCl three times, oven dried at 50 °C, and sieved (0.15 mm) prior the determination of NH_4^+-N content fixed by clay minerals (fixed ammonium) as already described (Silva and Bremner 1966; Shen et al. 1984). Two grams of the soil sample was treated with 40 ml KOBr, digested with concentrated sulfuric acid, and steam distilled.

Samples for analysis of ^{15}N atom % were prepared by steam distillation, where the liberated NH_3 was trapped with 0.003 mol L^{-1} H_2SO_4 solution. The trapped N was evaporated to dryness in an oven at 65 °C and analyzed by an isotope ratio mass spectrometer (Delta plus XP) (Shen et al. 1984).

Calculations and statistical analysis

SMBN was determined by the following equation

$$\mathrm{SMBN} = \left(\mathrm{N_{fumigated}}-\mathrm{N_{non\text{-}fumigated}}\right) \Big/ \mathrm{K_{EN}} \tag{1}$$

where $N_{fumigated}$ and $N_{non\text{-}fumigated}$ correspond to N extracted by 0.5 M K_2SO_4 from fumigated and non-fumigated soils, respectively, and K_{EN} is the conversion ratio of SMBN (equivalent to 0.45) (Jenkinson 1988).

The amount of fertilizer-derived ^{15}N in a certain N pool was calculated as follows

$$\mathrm{N_{dff}}(\mathrm{mg/kg}) = \mathrm{N\ content_{soil\ N\ pool}} \times {}^{15}\mathrm{N\ atom\%\ excess_{soil\ N\ pool}} / {}^{15}\mathrm{N\ atom\%\ excess_{fertilizer}} \tag{2}$$

^{15}N recovery in a specific N pool was calculated as follows

$$\mathrm{Recovery}\ (\%) = 100 \times \mathrm{N_{dff}}/\mathrm{content\ of\ N\ applied} \tag{3}$$

All statistical analyses were performed with SPSS 17.0 and Excel. Duncan method at the significance level of 0.05 was used to assess the effects of treatments, with soil fertility level as the main plot treatment and N amendment as the subplot treatment. Data were presented as means ($n = 3$).

Results

Dynamics of four N pools

Addition of external N significantly ($P < 0.05$) increased the soil exchangeable NH_4^+-N compared with CK, with the increase higher in the AS treatment than that in the AS + S treatment (Fig. 1a). The exchangeable NH_4^+-N concentration in the NF soil was significantly lower than that in the NPK and NPK + M soils. Soil exchangeable NH_4^+-N concentration decreased rapidly when incubation was extended. By day 36, no significant difference existed among the soils and treatments.

Soil NO_3^--N showed an opposite trend compared to that of exchangeable NH_4^+-N during incubation and reached the maximum on day 144 (Fig. 1b). The concentration of NO_3^--N generally ranked as NF < NPK < NPK + M. Compared with CK, the AS treatment significantly increased soil NO_3^--N content and the effect on increment decreased by increasing soil fertility level. The increasing effect was less pronounced in the AS + S treatment than in the AS treatment, especially in NF soil, where the effect became negative after day 36.

Soil microbial biomass N significantly increased in the AS + S treatment compared with that in CK and AS treatments

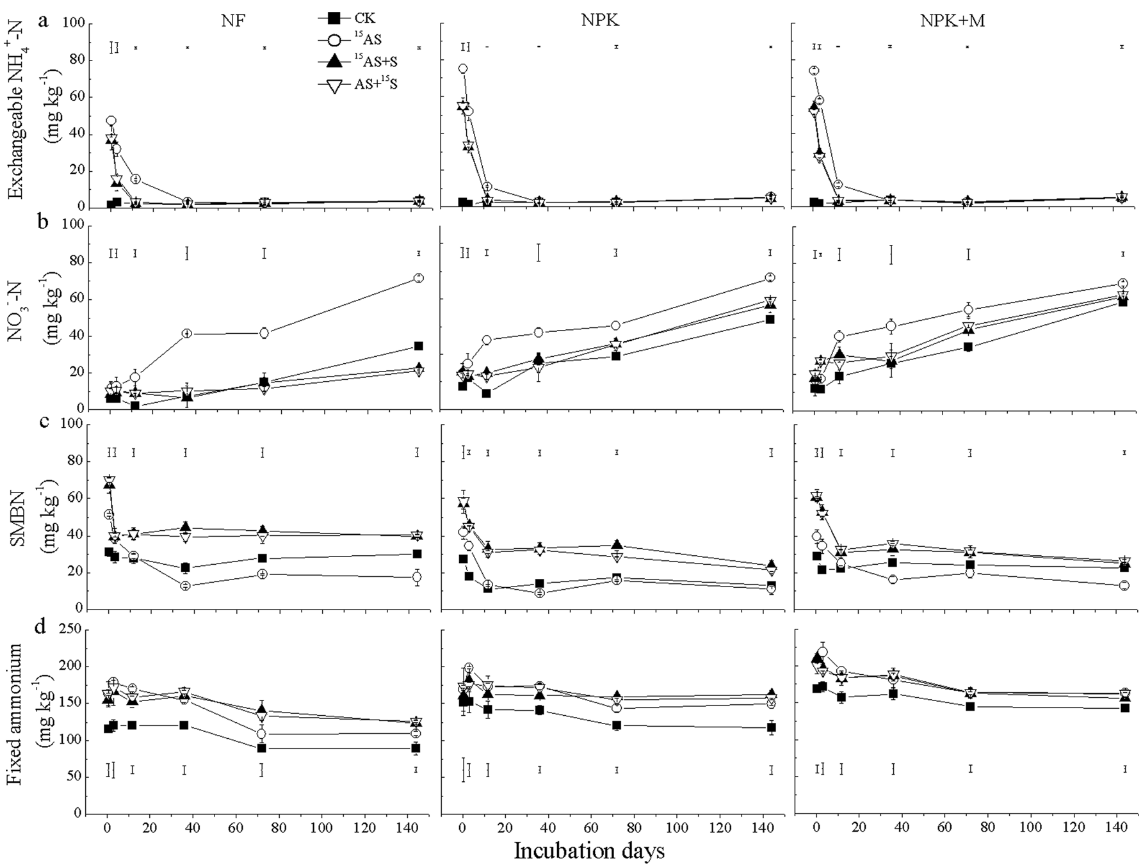

Fig. 1 Dynamics of exchangeable NH_4^+-N (**a**), NO_3^--N (**b**), SMBN (**c**), and fixed ammonium (**d**) during the 144-day incubation in three different long-term fertilized soils. Long-term fertilized soils: *NF* no fertilizer applied, *NPK* inorganic N, P, and K fertilizers, *NPK + M* pig manure + inorganic N, P, and K fertilizers; treatments: *CK* without N addition, *^{15}AS* ^{15}N-labeled ammonium sulfate, *^{15}AS + S* ^{15}N-labeled ammonium sulfate + unlabeled wheat straw, *AS + ^{15}S* unlabeled ammonium sulfate + ^{15}N-labeled wheat straw; *SMBN* soil microbial biomass N. *Vertical bars* represent standard deviations of the mean ($n = 3$). *Separate bars* indicate the range of least significant difference (*LSD*) ($t = 0.05$) for different treatments

(Fig. 1c). Addition of ammonium sulfate significantly increased SMBN before day 12, after which the SMBN was lower than that in CK in all soils.

Soil fixed ammonium ranked as NF < NPK < NPK + M (Fig. 1d). The amount of fixed ammonium reached the maximum by day 3, after which the value declined. External N addition markedly increased the content of fixed ammonium compared with CK, with the increases higher in the NF soil than in the NPK and NPK + M soils.

Partitioning of labeled N in four N pools

The trend of AS-N present as exchangeable NH_4^+-N was similar with or without straw (Fig. 2a). The straw addition significantly decreased the amount of AS-N present as NO_3^--N in soils, and the decreasing effect was the strongest in the NF soil (Fig. 2b). Straw-derived N in these two N pools remained low during incubation, indicating that AS-N was the main contributor to total variations in the AS + S treatment.

The amount of AS-N present as SMBN peaked on day 0.5 in NF and NPK soils and on day 3 for the NPK + M soil (Fig. 2c). Compared with the AS treatment, straw addition drastically increased the amount and percentage of AS-N present as SMBN (Table 2), which ranked as NF < NPK < NPK + M. The amount of AS-N present as fixed ammonium ranked as NF > NPK > NPK + M (Fig. 2d) and was significantly higher in the AS treatment than in the AS + S treatment.

The partitioning of external N in the SMBN and fixed ammonium-N pools varied among different soils and external N types (Tables 2 and 4). In the treatments without straw, though both the amount and the percentage of AS-N present as fixed ammonium were higher than that present as SMBN, the differences between these two N pools decreased with increasing soil fertility. Straw addition significantly increased the amount and percentage of AS-N present as SMBN, which exceeded that present as fixed ammonium in the NPK and NPK + M soils. For straw-N, the amount present as SMBN or fixed ammonium remained low during the incubation, but larger amounts

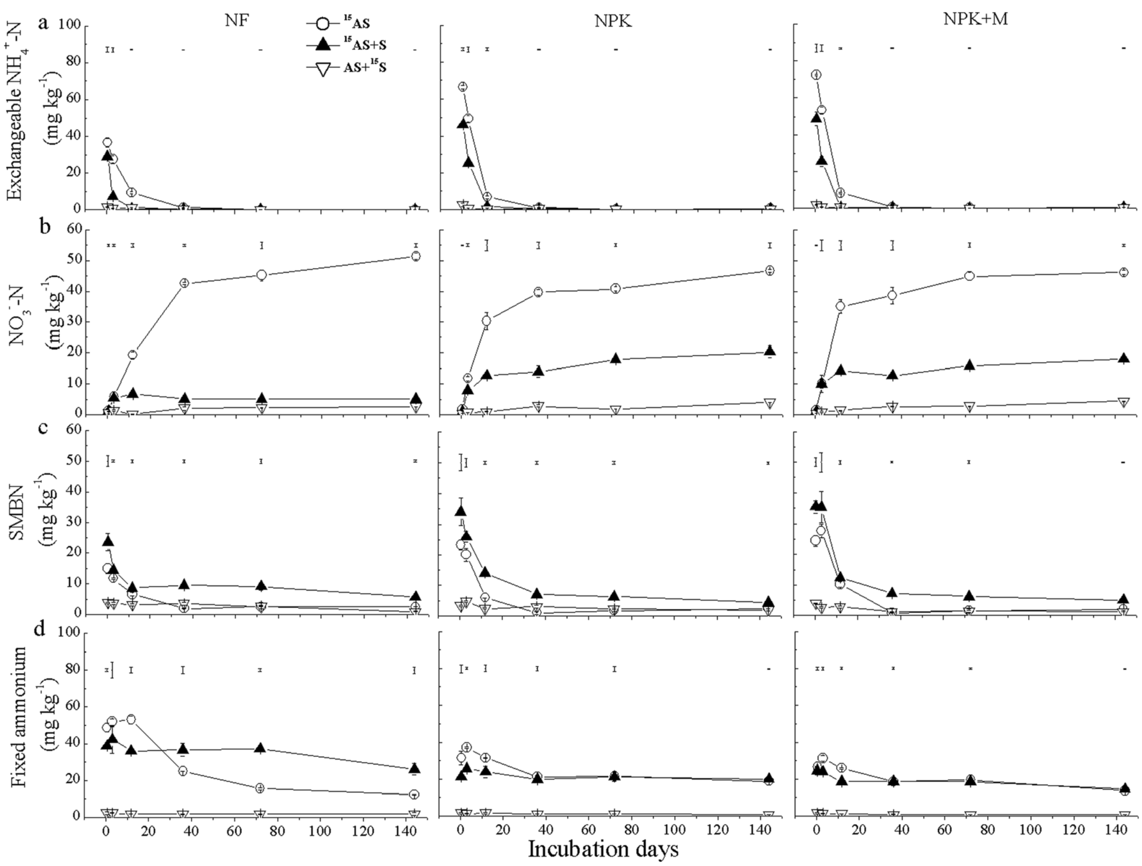

Fig. 2 Exchangeable NH_4^+-N (**a**), NO_3^--N (**b**), SMBN (**c**), and fixed ammonium (**d**) derived from the ^{15}N-labeled external N during the 144-day incubation in three different long-term fertilized soils. Long-term fertilized soils: *NF* no fertilizer applied, *NPK* inorganic N, P, and K fertilizers, *NPK + M* pig manure + inorganic N, P, and K fertilizers; N added treatments: *^{15}AS* ^{15}N-labeled ammonium sulfate, *^{15}AS + S* ^{15}N-labeled ammonium sulfate + unlabeled wheat straw, *AS + ^{15}S* unlabeled ammonium sulfate + ^{15}N-labeled wheat straw; *SMBN* soil microbial biomass N. *Vertical bars* represent standard deviations of the mean (n = 3). *Separate bars* indicate the range of least significant difference (*LSD*) (t = 0.05) for different treatments

were generally retained in the SMBN pool than in the fixed ammonium-N pool.

Mineralization and release of labeled N from SMBN and fixed ammonium

Mineralization of labeled N from SMBN or release of labeled N from fixed ammonium differed among soils with different fertility levels and treatments (Tables 3 and 4). In the AS treatment, the mineralized amount and percentage of AS-N from SMBN ranked as NF < NPK < NPK + M; the trend was the opposite for the released AS-N from fixed ammonium (Table 3). Although the mineralized amount of AS-N from SMBN significantly increased with straw addition, the mineralized percentage declined. Straw addition significantly decreased both the released amount and percentage of AS-N from fixed ammonium compared with the AS treatment. The amount and percentage of mineralized straw-N from SMBN were higher than those released from fixed ammonium, regardless of soil fertility level.

^{15}N recovery in soils

The recovery of AS-N in soil NO_3^--N pool was the highest in the AS treatment, followed by fixed ammonium-N pool (Table 5). Straw addition significantly reduced the recovery of AS-N in the soil NO_3^--N pool and increased that in the fixed ammonium-N, SMBN, and total N pools. The recovery of AS-N in soil, which ranked as NF > NPK > NPK + M, was significantly higher than that of straw-N. The recovery of straw-N in the mineral N (NH_4^+-N plus NO_3^--N) pool ranked as NF < NPK < NPK + M.

Discussion

Retention of labeled N in soils

The NF soil had a significantly higher capacity for ammonium fixation than the NPK and NPK + M soils (Table 2), due to the lack of N and K fertilizers for a long term. Indeed, the capacity of ammonium fixation can be reduced by long-term

Table 2 Amount and percentage of labeled N present as fixed ammonium and SMBN

Soil	Treatment	Fixation/Immobilization of labeled N			
		Amount (mg kg^{-1})		Percentage (%)	
		Fixed NH_4^+	SMBN	Fixed NH_4^+	SMBN
NF	^{15}AS	52.99a	15.03b	49.46b	14.03b
	^{15}AS + S	45.15b	23.62a	56.20a	29.39a
	AS + ^{15}S	2.42c	3.78c	9.02c	14.12b
NPK	^{15}AS	36.95a	23.24b	34.49a	21.69b
	^{15}AS + S	25.58b	33.98a	31.83a	42.28a
	AS + ^{15}S	1.34c	4.70c	4.99b	17.56b
NPK + M	^{15}AS	31.17a	27.60b	29.09a	25.76b
	^{15}AS + S	23.69b	35.50a	29.49a	44.18a
	AS + ^{15}S	1.26c	3.96c	4.69b	14.80c

Long-term fertilized soils: *NF* no fertilizer applied, *NPK* inorganic N, P, and K fertilizers, *NPK + M* pig manure + inorganic N, P, and K fertilizers; N added treatments: *^{15}AS* ^{15}N-labeled ammonium sulfate, *^{15}AS + S* ^{15}N-labeled ammonium sulfate + unlabeled wheat straw, *AS + ^{15}S* unlabeled ammonium sulfate + ^{15}N-labeled wheat straw; *SMBN* soil microbial biomass N. Different letters within columns indicate significant difference ($P < 0.05$) between treatments in the same soil. Values are means ($n = 3$)

application of N or K fertilizer because of the saturation of the interlayer space (Liu et al. 1997). This suggested that few sites were probably available for ammonium fixation in the NPK and NPK + M soils (Liang et al. 2012). The hindering effect of the organic molecule might also contribute to lower AS-N fixation in these two soils (Porter and Stewart 1970). Therefore, the ammonium fixation of the AS-N by clay minerals was probably the main factor explaining the low exchangeable NH_4^+ content in the NF soil. Consequently, the nitrification rate (reflected by the slope of the AS-derived NO_3^--N curve) was low, particularly before day 12 due to reduction in the reaction substrate for nitrification (Drury and Beauchamp 1991). After day 36, the amount of AS-N present as NO_3^--N in the NF soil was higher than that in the NPK and NPK + M soils, coupling with the significant release of fixed ammonium (Fig. 2).

Though the amount of AS-N present as fixed ammonium decreased with straw addition, the percentage did not decrease significantly, which even increased remarkably in the NF soil. Considering that less AS-N was applied in the AS + S treatment than in the AS treatment, this phenomenon was probably due to the fact that the percentage of NH_4^+ fixed by clay minerals generally increases by decreasing the amount of added NH_4^+ (Nommik and Vahtras 1982). The fixation of AS-N in soils was probably not influenced by K^+ of straw as also suggested by Scherer et al. (2014) when NH_4^+ and straw were added simultaneously. However, Ma et al. (2015) showed that both the amount and the percentage of fertilizer N fixed by clay minerals decreased after the addition of glucose. This discrepancy may be attributed to the C source that was more easily used by soil microorganisms with a rapid microbial N immobilization (Blagodatskaya et al. 2007), and less fertilizer N was available for ammonium fixation.

The amount of AS-N present as SMBN ranked as NF < NPK < NPK + M, confirming what reported by Pu et al. (2002). It is well established that long-term combined application of N, P, and K fertilizers and manure increases both soil

Table 3 Amount and percentage of released or mineralized labeled N from fixed ammonium or SMBN

Soil	Treatment	Release/mineralization of labeled N from fixed ammonium/SMBN			
		Amount (mg kg^{-1})		Percentage (%)	
		Fixed NH_4^+	SMBN	Fixed NH_4^+	SMBN
NF	^{15}AS	40.76a	12.49b	76.90a	82.92a
	^{15}AS + S	18.93b	18.05a	41.36b	76.28b
	AS + ^{15}S	0.99c	2.84c	41.15b	75.02b
NPK	^{15}AS	18.19a	20.73b	49.23a	89.15a
	^{15}AS + S	5.97b	29.57a	21.44b	86.74a
	AS + ^{15}S	0.64c	2.76c	44.16a	58.54b
NPK + M	^{15}AS	17.71a	25.69b	56.77a	93.10a
	^{15}AS + S	9.47b	30.48a	39.94b	85.81b
	AS + ^{15}S	0.71c	2.78c	55.39a	70.17c

Long-term fertilized soils: *NF* no fertilizer applied, *NPK* inorganic N, P, and K fertilizers, *NPK + M* pig manure + inorganic N, P, and K fertilizers; N added treatments: *^{15}AS* ^{15}N-labeled ammonium sulfate, *^{15}AS + S* ^{15}N-labeled ammonium sulfate + unlabeled wheat straw, *AS + ^{15}S* unlabeled ammonium sulfate + ^{15}N-labeled wheat straw; *SMBN* soil microbial biomass N. Different letters within columns indicate significant difference ($p < 0.05$) between treatments in the same soil. Values are means ($n = 3$)

Table 4 Main effects of soils and treatments on the amount of labeled N present as fixed ammonium or SMBN and the relative release or mineralization

Factor		Main effects			
		Fixation/immobilization (mg kg^{-1})		Release/mineralization (mg kg^{-1})	
		Fixed NH_4^+	SMBN	Fixed NH_4^+	SMBN
Soil	NF	33.52a	14.14b	20.10a	11.12b
	NPK	21.29b	20.64a	8.11b	17.69a
	NPK + M	18.71b	22.36a	9.30b	19.65a
Treatment	AS	40.37a	21.95b	25.55a	19.63b
	AS + S	33.15b	35.18a	11.96b	28.82a
Soil		0.000**	0.004**	0.002**	0.005**
Treatment		0.000**	0.000**	0.000**	0.000**
Soil × treatment		0.000**	0.002**	0.000**	0.001**

Long-term fertilized soils: *NF* no fertilizer applied, *NPK* inorganic N, P, and K fertilizers, *NPK + M* pig manure + inorganic N, P, and K fertilizers; treatments: *AS* ^{15}N-labeled ammonium sulfate, *AS + S* ammonium sulfate + wheat straw; *SMBN* soil microbial biomass N. Values of *AS + S* were the sum of ^{15}N-labeled ammonium sulfate content in the labeled ammonium sulfate + wheat straw treatment (*^{15}AS + S*) and ^{15}N-labeled wheat straw in the ammonium sulfate + labeled wheat straw treatment (*AS + ^{15}S*). Different letters within columns indicate significant difference ($P < 0.05$) between soils or treatments

**Statistical significance at $P < 0.01$

microbial biomass and enzymatic activity (Nannipieri et al. 2003; Chakraborty et al. 2011), generating the maximum contribution of the SMBN pool to AS-N retention in NPK + M soil. Straw addition significantly increased the amount and percentage of AS-N present as SMBN and correspondingly decreased those of AS-N present as NO_3^--N, reducing the risk of nitrate leaching (Gentile et al. 2009). Probably, the addition of organic C increased microbial N immobilization (Shindo and Nishio 2005; Said-Pullicino et al. 2014), resulting in intensified competition between heterotrophic microorganisms and nitrifiers for AS-N and decreased nitrification (Bengtsson et al. 2003; Zhao et al. 2014).

The amount of straw-N present as SMBN and fixed ammonium remained low during the entire incubation (Fig. 2), with the former pool being more important than the latter pool, regardless of soil fertility levels (Table 2). This could be attributed to the straw-N present as fixed ammonium was related to NH_4^+-N originated from microbial mineralization (Nieder et al. 1996; Shindo and Nishio 2005). The presence of straw-N as fixed ammonium confirmed that organic N can be the source of fixed ammonium-N pool after its mineralization, explaining the increase in soil fixed ammonium content after crop harvest. The increase may be attributed to the fixation of NH_4^+ derived from the mineralization of crop residues,

Table 5 Recovery of labeled N (%) in various soil N pools at the end of the incubation

Soil	Treatment	NH_4^+-N	NO_3^--N	Fixed NH_4^+	SMBN	Total N
NF	^{15}AS	0.03b	47.98a	11.41b	2.38c	64.61c
	^{15}AS + S	0.08b	6.39c	32.65a	6.92a	73.17b
	AS + ^{15}S	0.52a	9.89b	6.73c	3.54b	84.77a
NPK	^{15}AS	0.33a	43.47a	17.51b	2.35c	64.44c
	^{15}AS + S	0.19a	25.37b	24.99a	5.48b	68.90b
	AS + ^{15}S	0.21a	15.08c	2.60c	7.25a	74.43a
NPK + M	^{15}AS	0.24b	43.11a	12.56b	1.78c	58.39c
	^{15}AS + S	0.28b	22.60b	17.71a	6.25a	66.00b
	AS + ^{15}S	1.57a	16.27c	2.02c	4.41b	76.70a

Long-term fertilized soils: *NF* no fertilizer applied, *NPK* inorganic N, P, and K fertilizers, *NPK + M* pig manure + inorganic N, P, and K fertilizers; N added treatments: *^{15}AS* ^{15}N-labeled ammonium sulfate, *^{15}AS + S* ^{15}N-labeled ammonium sulfate + unlabeled wheat straw, *AS + ^{15}S* unlabeled ammonium sulfate + ^{15}N-labeled wheat straw; *SMBN* soil microbial biomass N. Different letters within columns indicate significant difference ($P < 0.05$) among treatments in the same soil such as roots, leaves, and root exudates (Matsuoka and Moritsuka 2011). With the increase in soil fertility level, the amount of straw-N present as fixed ammonium decreased, while that present as SMBN increased. There might be two reasons for this. Firstly, a large amount of straw-N was immobilized or nitrified (as confirmed by the straw-N recovery; Table 5) because of the high decomposition rate of straw mediated by high microbial biomass value in high-fertility soils (An et al. 2015). Secondly, few location sites were available for ammonium fixation in high-fertility soils. Therefore, it can be concluded that the external N retention was affected by the different fertility levels.

Supply of labeled N

The mineralization of SMBN and the release of fixed ammonium are important for the N supply to plant (Hayatsu et al. 2008). The AS-N supply significantly differed among different treated soils. In the AS treatment, both the amount and the percentage of released AS-N from fixed ammonium in the NF soil were higher than those in the NPK and NPK + M soils (Table 3), where the original fixed ammonium and potassium were higher. Consequently, the release of AS-N from fixed ammonium was the main process for AS-N supply. However, the amount and percentage of mineralized AS-N from SMBN were enhanced by the increasing soil fertility and exceeded that released from fixed ammonium in the NPK and NPK + M soils, indicating that the role of SMBN pool in N supply was enhanced with the increase in soil fertility level. The discrepancy in the N supply depended on the different capacities of ammonium fixation/release and the various microbial activities in different fertility soils (Qiu et al. 2012).

Straw addition further increased the effect of SMBN on AS-N supply, correspondingly decreased the effect of fixed ammonium-N pool. The decreasing effect of straw addition on fixed NH_4^+ release was stronger in the NF than in NPK and NPK + M soils. It had been observed that the increase in microbial N immobilization stimulated by organic C addition can favor the release of fixed ammonium (Scherer and Werner 1996; Tang et al. 2008). In the present study, however, the blocking effect of K^+ derived from straw on the release of fixed ammonium was stronger than the promoting effect by microbial N immobilization with straw addition, especially in the NF soil. Thereby, fertilizer N cycling in soil was remarkably influenced by types, compositions, and amounts of added organic C (Trinsoutrot et al. 2000).

The mineralization percentage of AS-N from SMBN during incubation was higher than that released from fixed ammonium in all cases, although straw addition decreased the mineralization percentage of AS-N from SMBN. Ma et al. (2015) found similar release percentage from fixed ammonium in a soil similar to NF after 96 days of incubation. However, it has been found the mineralization percentage of labeled SMBN was lower than that of our study after 144 days of incubation. This demonstrated that the mineralization of fertilizer-N from SMBN lasted longer than its release from fixed ammonium. Therefore, the SMBN pool maybe a more continuous N supply source and more synchronized to satisfying the plant N demand than the fixed ammonium-N pool (Hayatsu et al. 2008; Sugihara et al. 2012).

Notably, compared with the AS treatment, the treatment with straw and AS in NF soil increased the external N (labeled AS-N plus straw-N) retention and decreased its subsequent supply. This favored soil fertility improvement, increasing N storage in the SMBN and fixed ammonium-N pools (Table 5). By contrast, though the straw addition to NPK and NPK + M soils also significantly increased external N retention by soil microorganisms and clay minerals, the total amount of external N mineralized and released was not considerably influenced. This indicated that in low-fertility soil, the combined addition of straw and AS benefits the soil fertility improvement from both biotic and abiotic processes. While in high-fertility soil, the partial substitution of AS with straw can decrease AS-N losses, with total N supply through these two processes unaffected. However, further studies must be conducted to explore the optimal amount of straw addition, as well as the ratio of straw and fertilizer N.

Recovery of labeled N

The N retention and N supply processes differed among soils and responded differently to straw addition, thereby significantly influencing the residual forms and the recovery rate of added N (Yadvinder-Singh et al. 2009; Azeez and Van Averbeke 2010). In the AS treatment, the recovery rate was ranked as NF > NPK > NPK + M (Table 5), and the differences were mainly due to NO_3^--N and the transformation of AS-N into soil microbial necromass N (SMNN, including microbial metabolites and residues), which were supposed to be higher in the NF soil due to its lower microbial activity and N cycling rate than the other two soils (Arnebrant et al. 1996).

Straw addition significantly increased the recovery of AS-N, confirming what was already reported (Wu et al. 2010; Yang et al. 2015). This was probably attributed to the higher AS-N recovery as fixed ammonium-N and organic N (including SMBN and SMNN), which was in accordance with the decreased percentage of AS-N released from fixed ammonium and that mineralized from SMBN after straw addition (Table 3). The recovery of straw-N in soil was higher than that of AS-N, which was probably due to (1) large amounts of straw had not been decomposed by the end of the incubation, and (2) the mineralized percentage of straw-N from SMBN pool was significantly lower than that of AS-N (Table 3), resulting in higher recovery of straw-N in SMBN or SMNN pool. With increasing soil fertility, the recovery of straw-N in the mineral N pool increased, indicating that more straw-N was mineralized due to increased microbial activities (Tu et al. 2006; Zhang et al. 2015).

In general, the recoveries of added N in soil ranked as NF > NPK > NPK + M. Considering the absence of leaching N losses in the incubation experiment, the losses were probably occurring as NH_3 volatilization or as N_2 and N_xO due to the nitrification-denitrification. Since the NH_3 volatilization was directly influenced by ammonium fixation (San Francisco et al. 2011), the AS-N losses through NH_3 volatilization were probably the lowest in NF soil compared with the other soils considering that the amount of AS-N present as fixed ammonium was the highest. Consequently, amounts of the substrate for nitrification were the least in the NF soil, decreasing the risk of N losses through nitrification-denitrification (Abbasi and Adams 2000). The N losses were also influenced by the application of organic wastes. In the present study, straw addition significantly decreased the recovery of AS-N as NO_3^--N in soil, reducing the possibility of gaseous losses from AS-N. This was contrary to what reported by Zhai et al. (2011), who suggested larger amounts of N_2O emissions when organic manure was applied. The discrepancy may depend on (1) the increased N_2O can be derived from soil organic N mineralized from the priming effect after the organic manure application (Kuzyakov 2010); (2) the difference in the C/N ratios of applied organic manure may have also contributed to the discrepancy (Cheng et al. 2015). Generally, organic amendments with higher C/N ratios were more beneficial to decreasing the risk of gaseous N losses through nitrification-denitrification due to the microbial immobilization of N (Fisk et al. 2015), while Trinsoutrot et al. (2000) pointed that biochemical characteristics of the organic amendments should also be considered because of their various C or N availabilities to soil microorganisms.

Therefore, more mineral fertilizer N should be applied when straw is returned to the low- than high-fertility soils. For the high-fertility soils, straw application favored AS-N retention without visibly affecting the total amount of external N (labeled AS-N plus straw-N) supply. However, straw returning probably prevented the release of fixed ammonium in the application season and resulted in considerable amounts of N losses during the fallow season (Jayasundara et al. 2010; Chantigny et al. 2014). Thus, the period of straw application under field conditions should be carefully considered to avoid the drawbacks due to the simultaneous application of straw and N fertilizer. Straw returning in autumn instead of in spring would be a more effective approach for the N management in the study region. In addition, the amount of added straw to minimize N losses and to synchronize the relative N supply with the crop demand should be optimized (Han et al. 2004).

Conclusion

The relative importance of SMBN and fixed ammonium-N pools in the retention of AS-N or straw-N and the N supply differed among soils subjected to different long-term fertilization (causing different soil fertility levels) and N amendments. In the NF soil, fixed ammonium-N pool was more important in AS-N retention and supply than SMBN pool with or without straw addition. With the increase in soil fertility level, the role of SMBN pool in AS-N retention and supply enhanced; straw addition further increased the role of SMBN pool, which exceeded that of fixed ammonium-N pool in the NPK and NPK + M soils. However, straw addition decreased the released amount and percentage of AS-N from fixed ammonium, especially in the NF soil. The mineralized percentage of AS-N from SMBN also declined with straw addition, though the mineralized amount increased. The SMBN pool was more important than the fixed ammonium-N pool in straw-N retention and supply, suggesting that straw-N cycling depended more on the biotic processes than the fixed ammonium turnover. In all cases, the mineralized percentage of AS-N or straw-N from SMBN was higher than that released from fixed ammonium, indicating the higher availability of SMBN. Our results provide information for optimizing straw management measures and synchronizing soil N supply with the crop demand in soils with different fertility levels.

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