ORIGINAL PAPER

Effects of applied urea and straw on various nitrogen fractions in two Chinese paddy soils with differing clay mineralogy

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Abstract Combined application of synthetic nitrogen (N) fertilizers and organic materials can enhance soil quality, but little is known about the distribution of fertilizer N among different soil fractions after crop harvest. A pot experiment using ^{15}N tracer was employed to address this question with three treatments, i.e., labeled urea-only (^{15}NU), labeled urea + rice straw (^{15}NU-S) and labeled rice straw + urea (^{15}NS-U) applied to a Ferallic Cambisol (1:1 type soil clay mineral) and a Calcaric Fluvisol (2:1 clay mineral). Soil microbial biomass N, fixed ammonium (fixed NH_4^+), exchangeable ammonium and soil organic N fractions by hydrolysis (6 N HCl) and their isotope abundance were determined after the rice harvest. Soil newly formed N in urea + straw (U-S) treatments (^{15}NU-S, ^{15}NS-U) was the sum of labeled urea-N in ^{15}NU-S and labeled straw-N in ^{15}NS-U. Compared with ^{15}NU, U-S significantly ($P<0.05$) increased the content and percentage of newly formed total soil N, acid insoluble N, amino acid N, and hydrolysable unknown N in both soils. In U-S treatment, straw amendment significantly ($P<0.05$) reduced the content and percentage of newly formed fixed-NH_4^+-N in Fluvisol as compared with ^{15}NU treatments. Soil microbes contributed to the larger percentage of newly formed amino acid N ($P<0.01$) in Cambisol as compared with Fluvisol. Fertilizer N in various soil fractions was therefore strongly affected by clay mineral type and microbes after the combined application of organic materials and synthetic N fertilizer.

Keywords Soil microbial biomass nitrogen · Clay mineral type · Acid-hydrolysable organic nitrogen fractions · ^{15}N tracer · Paddy soils

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Introduction

Nitrogen (N) is the major essential nutrient for increasing and maintaining agricultural production worldwide. However, there is increasing concern about the effects of synthetic N fertilizers on transformations of soil N and on soil quality. In

China, the use of mineral fertilizers has increased greatly over the past 30 years (Ju et al. 2007). The N uptake efficiency of crops was reported to be only 28–41% (Zhu 1997), and most of the remaining N was lost or remained in the soil. The combination of organic matter (e.g., straw) and synthetic N fertilizer was shown to improve the chemical and biological properties of soil (Nardi et al. 2004) and to decrease losses of synthetic fertilizer N. This improvement is mainly through the accumulation of soil organic N which is derived from straw-N and from straw-induced fertilizer N immobilization (Luxhøi et al. 2007). While there have been some studies on N during the decomposition of organic residues (Blumfield et al. 2004) and on the bioavailability of residue N (Halvorson et al. 2005), little is known about the quantity and quality of residue N in different soil fractions.

Soil microorganisms turn over soil organic matter and soil microbial biomass is both a source and sink of nutrients (Burger and Jackson 2003). Microbes can utilize the energy provided by carbon (C) sources to increase N immobilization, even though soil microbial biomass C and N account for only a small part (1–5%) of the total C and N pools, respectively (Jenkinson and Ladd 1981; Smith and Paul 1990; Sparling 1985). Ammonium (NH_4^+) is the preferred form of N for microbial assimilation (Burger and Jackson 2003; Recous et al. 1990), and this form can also be fixed by clay minerals, especially 2:1 clay minerals. The rate of NH_4^+ fixation by clay minerals is rapid, with the proportion of fixed-NH_4^+-N to total soil N reaching 20% in some typical 2:1 cultivated soils (Nieder et al. 2011; Wen et al. 1995). Inorganic N losses can therefore be reduced by N immobilization in soil microbial biomass N and fixed-NH_4^+-N pools. However, the effects of synthetic N fertilizers and organic materials on the soil microbial biomass N and fixed-NH_4^+-N are inconsistent. Several studies have reported that soil microbial biomass N was increased by synthetic N fertilizers (Goyal et al. 1992; Sarathchandra et al. 2001) and organic materials (Zaman et al. 1999). In the case of fixed-NH_4^+-N, ammonium can be captured by the interlayers of clay minerals after application of N fertilizer (Azam et al. 1994; Nieder et al. 2011). After addition of organic materials, however, the diffusion of NH_4^+ into the interlayers of clay minerals can be blocked by low-molecular-weight material from the decomposing organic matter (Nieder et al. 2011). Ultimately, this can result in contraction of the interlayers (Porter and Stewart 1970). On the other hand, the N immobilized by microbes and NH_4^+ fixed by clay minerals can release N into the soil solution and thus both the N pools can become available to plants when the microbes die or the clay interlayers expand (Nieder et al. 2011).

The N partly immobilized in soil organic matter is usually released very slowly, and this slow release can increase soil fertility and reduces N losses. Hence, to better understand the fate of N in soil and its role in soil fertility, it is useful to determine the quantity and quality of fertilizer N in soil organic fractions. Using the acid hydrolysis method described by Bremner (1965), soil organic N can be divided into total hydrolysable N and acid insoluble N, with total hydrolysable N comprising amino acid N, ammonia N, amino sugar N and hydrolysable unknown N. It has previously been reported that long-term applications of manures and synthetic N fertilizers increase amino acid N and amino sugar N but seldom form hydrolysable unknown N (Johnsson et al. 1999; Xu et al. 2003). Nannipieri and Paul (2009) reported that hydrolysable unknown N consisted of stable amino compounds in soil organic N. Moreover, amino sugar N was mainly microbially derived N (Wander et al. 2007). However, there have been very few studies in which ^{15}N labeling techniques have been used to study the effects of combinations of organic residues and fertilizer N on soil organic N fractions.

Double-season rice cropping is currently practiced on the Dongting Lake floodplain in South China, and the planted area accounted for 26% of Hunan province's crop in 2007. Correspondingly, the rate of synthetic fertilizer N reached 330 kg ha^{-1} per season to achieve high rice yields (Hunan Statistics Notebook 2008). This has the potential to adversely affect the water quality of Dongting Lake, and N immobilization in soil would be a good strategy to decrease N losses. In view of the complex effects of straw and synthetic N fertilizers on the different soil fractions, it is necessary to clarify how much available N and how much immobilized N are present in the various N pools after applications of straw and/or synthetic N fertilizers. In addition, we need to understand the distribution and fate of N from synthetic fertilizers and organic materials (straw) in different N fractions of intensively managed soils. The aims of the present study were therefore to: (1) quantify urea N and straw N in different soil fractions such as exchangeable NH_4^+, soil microbial biomass N, fixed-NH_4^+-N and different N fractions by 6 N HCl hydrolysis; (2) compare the effects of synthetic fertilizer N with or without straw on these soil fractions; and (3) elucidate the distributions of urea N and straw N in soil fractions in response to soil clay mineral type. We therefore conducted a pot experiment in which two paddy soils from Dongting Lake Plain that differ in their dominant clay minerals were treated with ^{15}N-labeled urea and ^{15}N-labeled rice straw. One soil is dominated by a 1:1 (kaolinite) and the other by a 2:1 (montmorillonite) clay mineral.

Materials and methods

Sites and soils

Two typical soils of Dongting Lake Plain, a Ferallic Cambisol clay paddy soil and a Calcaric Fluvisol clay

paddy soil (FAO 2002; Zhang et al. 2003), were collected to a depth of 20 cm at Ningxiang village (28°16′N, 112°33′E) and Datong village (28°50′N, 112°22′E) at the end of October 2004. In this region a typical subtropical mainland monsoon climate is prevalent and the annual precipitation ranges from 1,250 mm to 1,700 mm, with more than 55% occurring from April to August. Annual average temperature is about 17°C and current agricultural practice is an intensive double rice cropping system. The parent material of Cambisol is quaternary red clay and the main clay mineral is 1:1 kaolinite. Fluvisol is derived from river and lake deposits and the dominant clay mineral is 2:1 montmorillonite. The soil clay mineral composition, pH, content of soil organic C, total N, P and K, exchangeable NH_4^+ and fixed-NH_4^+ of both soils were determined before the experiment and are shown in Table 1. Both soils were air-dried and sieved through a 1-cm mesh before the pot experiment began.

Experimental procedures and design

An equivalent N amendment pot experiment was carried out in a net house with a glass roof from April 28 to July 19 2005. Soil type was the main plot factor and urea with or without straw was the subplot factor in a split-plot design with four replicates. The treatments were applied as follows: (1) control (CK); (2) labeled urea-only (^{15}NU); 3) urea + rice straw (U-S); and the U-S treatment was divided into two sub-treatments: (i) labeled urea + rice straw (^{15}NU-S) and (ii) urea + labeled rice straw (^{15}NS-U). In the U-S treatment, the proportion of urea N to straw N was 3:1. Each 20-cm-diameter pot contained 3.5 kg air-dried soil uniformly treated with 0.8 g N, 0.4 g P_2O_5, and

0.6 g K_2O in the fertilizer treatments. The same amounts of P and K fertilizers were added to the control soil. The contents of N, P, and K in rice straw were included in the total amount of fertilizer so that the N, P, and K rates were equivalent in each pot. At the start of the pot experiment the soil was flooded for 3 days and then six 20-day-old rice seedlings were transplanted into each pot (two seedlings per pocket). The soil in these pots was flooded every day. The synthetic fertilizers added to the soil were $(NH_2)_2CO$, NaH_2PO_4, and KCl. The rice cultivar used was Xiangyou Hybridized 520S/86-1. The ^{15}N abundance of the urea was 10.21%.

Before the pot experiment the ^{15}N labeled rice straw was produced by keeping rice plants in a water-tight chamber (75×50×15 cm) with a low fertility soil for an 85-day growing season from the beginning of May to the end of July 2004. The soil was enriched with 30.22% $(^{15}NH_4)_2SO_4$ as a basal and top-dressed fertilizer. The relatively high N isotope abundance in straw was obtained by cutting the rice spikes at the grain filling stage. The plants were protected from rain by a plastic film positioned 2 m above the chamber. At the end of the experiment the straw was oven-dried at 60°C and pulverized in a mill at 25,000 rpm. The cultivar used for both labeled and unlabeled rice straw was "Hybridization 770" and the unlabeled rice was harvested from the field. The labeled rice straw had an organic C content of 427.2±0.05 g kg^{-1}, total N content of 11.5±0.05 g kg^{-1}, total P content of 2.5± 0.004 g kg^{-1}, total K content of 15.6±0.3 g kg^{-1} and ^{15}N abundance of 13.8±0.07%. In the unlabeled rice straw, the organic C, total N, total P, total K contents were 406.7± 0.07, 10.5±0.04, 1.2±0.02, and 17.6±0.4 g kg^{-1}, respectively, and the ^{15}N abundance was 0.36±0.001%.

Sample analysis and data calculation

After the rice was harvested and the roots immediately removed, exchangeable ammonium (NH_4^+-N) in fresh soil was extracted with 2 M KCl (1:5 w/v, oven-dry basis) and then recovered by steam distillation with MgO. Nitrate-N (NO_3^--N) was ignored because the paddy soils were flooded during rice growth. Similarly, soil microbial biomass N was measured by the anaerobic $CHCl_3$ fumigation-extraction method as described by Inubushi et al. (1991) after the fresh soil above was air-dried for up to 1 h to allow free water to drain. The N content of fumigated and unfumigated samples extracted with 0.5 M K_2SO_4 (1:4 w/v, oven-dry basis) was determined by the Kjeldahl method. Soil microbial biomass N was estimated using the equation SMBN=E_N/K_{EN}, where E_N was calculated as N extracted by K_2SO_4 from fumigated soil minus N extracted by K_2SO_4 from unfumigated soil, and the K_{EN} value used was 0.45 (Jenkinson 1988).

The remaining fresh soil with roots removed was air-dried and passed through a 0.15-mm sieve and then fixed-

Table 1 Properties of the Feralic Cambisol and Calcaric Fluvisol

Soil property	Feralic Cambisol	Calcaric Fluvisol
Organic C (g kg^{-1})	19.4	29.4
Total soil N (g kg^{-1})	2.2	3.4
Total P(g kg^{-1})	0.7	1.3
Total K (g kg^{-1})	17.3	26.3
NH_4^+-N (mg kg^{-1})	58.8	110.6
Fixed-NH_4^+-N (mg kg^{-1})	142.8	303.4
pH	5.4	7.8
Total clay mineral (%)	48.8	59.6
Silicon (%)	50.9	25.3
Orthoclase (%)	0.3	1.3
Plagioclase (%)	–	4.5
Calcite (%)	–	3.6
Dolomite (%)	–	3.5
Pyrite (%)	–	1.3
Siderite (%)	–	0.9

"–" not detected

NH_4^+ was determined according to the method of Silva and Bremner (1966) with the modifications described by Scherer and Mengel (1979) as described in detail by Lin et al. (2004). Different soil organic N fractions were determined according to Stevenson (1996). Total soil N was determined by the Kjeldahl method (Bremner 1996). Soil total P and K were determined as reported by Kuo (1996) and Helmke and Sparks (1996), respectively. The quantity and type of soil clay minerals were determined with an X-ray diffractometer (Rigaku D/MAX 2500, Japan). Soil pH was measured in a 1:2.5 soil/water mixture with 2-mm sieved air-dried soil.

All distilled solutions were quantified by titration and then acidified and concentrated to 3–5 ml in an oven at 60 °C for N isotope analysis by mass spectrometry (Finnigan, MAT251, Germany) according to Hauck et al. (1996). The fertilizer or straw N in different soil fractions was calculated using the following equations:

$$Ndfx_p = \left[content_p \times ape_p(\%) \right] / ape_x(\%)$$

where Ndfx is N derived from fertilizer or straw, p is the soil N pool, ape is ^{15}N atom percent excess, and x is the applied urea N or straw N.

The fertilizer or straw N microbially assimilated was calculated using a similar equation:

$$[content_f \times ape_f(\%) - content_u \times ape_u(\%)]/0.45/ape_x(\%),$$

where f and u are fumigated N and unfumigated N, respectively.

In this study, the recovery rate in the fractions is the percentage of labeled fertilizer N present in the target fraction at the end of the experiment. The amounts of applied labeled N at the beginning of the experiment were 228.6 mg N kg^{-1} in ^{15}NU, 171.4 mg N kg^{-1} in $^{15}NU\text{-}S$, and 57.1 mg N kg^{-1} in $^{15}NS\text{-}U$. Labeled N percentage in soil fractions is the total N of each soil fraction present as urea N or straw N. Thus, in the U-S treatment, the percentage of newly formed N in the soil fractions is the sum of labeled urea N percentage in $^{15}NU\text{-}S$ and labeled straw N percentage in $^{15}NS\text{-}U$ in the target fraction. Correspondingly, the content of newly formed N is the sum of labeled urea N in $^{15}NU\text{-}S$ and labeled straw N in $^{15}NS\text{-}U$.

Statistical analysis

Data were calculated on oven-dried weight basis and were analyzed using analysis of variance with soil type as the main plot treatment and N source as the subplot treatment in a split-plot design. Statistical analysis of all variables was carried out using the SAS 8.0 software package and mean values were compared using least significant difference (LSD) at the 5% level.

Results

In comparison with the control, addition of urea with or without straw increased soil microbial biomass N content in Fluvisol ($P<0.05$, Table 2), and the urea + straw treatment increased soil microbial biomass N content in Cambisol and amino acid N content in Fluvisol ($P<0.05$). Except for amino sugar N, the total N content of different soil fractions and total soil N in Fluvisol were significantly higher than in Cambisol ($P<0.01$). The amino sugar N contents in both soils were ~0.1 mg kg^{-1}.

Compared with the urea-only treatment, the urea + straw treatment increased newly formed soil microbial biomass N content in Cambisol ($P<0.05$, Fig. 1a), decreased newly formed fixed-NH_4^+-N content and percentage in Fluvisol ($P<0.05$, Fig. 1b, e), and decreased newly formed exchangeable NH_4^+-N percentage in both soils ($P<0.05$, Fig. 1f). Conversely, the urea + straw treatment did not significantly ($P>0.05$) affect newly formed soil microbial biomass N content or percentage in Fluvisol (Fig. 1a, d), newly formed fixed-NH_4^+-N content or percentage in Cambisol (Fig. 1b, e), or newly formed exchangeable NH_4^+-N content in either soil. Soil characteristics themselves significantly affected the content and percentage of newly formed fixed-NH_4^+-N and exchangeable NH_4^+-N ($P<0.05$ or $P<0.01$).

The urea + straw treatment increased the content and percentage of newly formed total soil N and acid insoluble N in both soils ($P<0.05$; Fig. 2a, c, d, f), and those of newly formed total hydrolysable N in Cambisol ($P<0.05$; Fig. 2b, e) in comparison with the urea-only treatment. Addition of urea with or without straw made greater contributions to the percentage of newly formed total soil N, total hydrolysable N, and acid insoluble N in Cambisol than in Fluvisol (Fig. 2c, d, e), and this may attributed to the significant difference in soil characteristics ($P<0.01$). In both soils rice straw N made greater contributions to the content and percentage of newly formed acid insoluble N (Fig. 2c, f) than those of newly formed total soil N, total hydrolysable N (Fig. 2a, b, d, e), and the percentages of newly formed total soil N, total hydrolysable N and acid insoluble N were less than 3%.

Compared with the urea-only treatment, the urea + straw treatment increased the content and percentage of newly formed amino acid N and hydrolysable unknown N ($P<0.05$; Fig. 3a, d, e, h) in both soils and increased the content of newly formed amino sugar N in Cambisol (Fig. 3c, g). In contrast, the urea + straw treatment exerted no significant effect on the content or percentage of newly formed ammonia N in either soil in comparison with the urea-only treatment ($P>0.05$; Fig. 3b). In the same treatment, the percentages of newly formed amino acid N and hydrolysable unknown N were significant greater in Cambisol than in Fluvisol ($P<0.01$ or $P<0.05$; Fig. 3e, h). The

Table 2 Total N content of different soil fractions and total soil N (TSN) in the Ferallic Cambisol and Calcaric Fluvisol (in g N kg^{-1})

Treatment	SMBN ($\times 10^{-3}$)	Fixed-NH$_4^+$-N ($\times 10^{-3}$)	NH$_4^+$-N ($\times 10^{-3}$)	TSN	N fraction by 6 N HCl hydrolysis					
					THN	AIN	AAN	AN	ASN	HUN
Cambisol										
CK	70.0±4.8b	162.7±10.9a	8.1±0.3b	2.0±0.01a	1.7±0.05a	0.3±0.04a	0.6±0.01a	0.5±0.01a	0.1±0.01a	0.6±0.05a
U	74.3±2.0ab	158.8±6.1a	8.7±1.1b	2.1±0.02a	1.7±0.05a	0.4±0.03a	0.6±0.03a	0.5±0.02a	0.1±0.02a	0.6±0.03a
U-S	81.5±4.8a	161.4±9.8a	10.9±0.9a	2.0±0.02a	1.7±0.07a	0.4±0.07a	0.6±0.01a	0.4±0.02a	0.1±0.01a	0.6±0.07a
Fluvisol										
CK	84.5±2.0b	350.0±2.3a	10.8±2.2b	3.3±0.00a	2.6±0.07a	0.7±0.08a	0.9±0.01b	0.7±0.01a	0.1±0.01a	0.8±0.04a
U	107.2±3.6a	354.5±7.0a	12.8±1.4ab	3.2±0.08a	2.5±0.08a	0.7±0.05a	0.9±0.02b	0.7±0.00a	0.1±0.02a	0.8±0.04ab
U-S	113.7±6.1a	363.8±11.1a	15.5±4.7a	3.3±0.05a	2.6±0.10a	0.7±0.06a	1.0±0.06a	0.7±0.03a	0.1±0.01a	0.7±0.04b
ANOVA										
Treatment	**	n.s.	**	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Soil	**	**	**	**	**	**	**	**	n.s.	**
Soil × Treatment	**	n.s.	n.s.	n.s.	n.s.	n.s.	*	n.s.	n.s.	n.s.

Values are mean ± standard deviation of four replicates except for U-S. Value of U-S is mean of four replicates of labeled urea + straw and four replicates of urea + labeled straw

SMBN soil microbial biomass N, *THN* total hydrolyzed N, *AIN* acid insoluble N, *AAN* amino acid N, *AN* ammonia N, *ASN* amino sugar N, *HUN* hydrolysable unknown N, *CK* control, no fertilizer applied, *U* labeled urea-only, *U-S* urea + straw

n.s. not significant at *P*>0.05; *significant at *P*<0.05; **significant at *P*<0.01. Lower case letters within columns denote significant difference (*P*<0.05) in the same soil
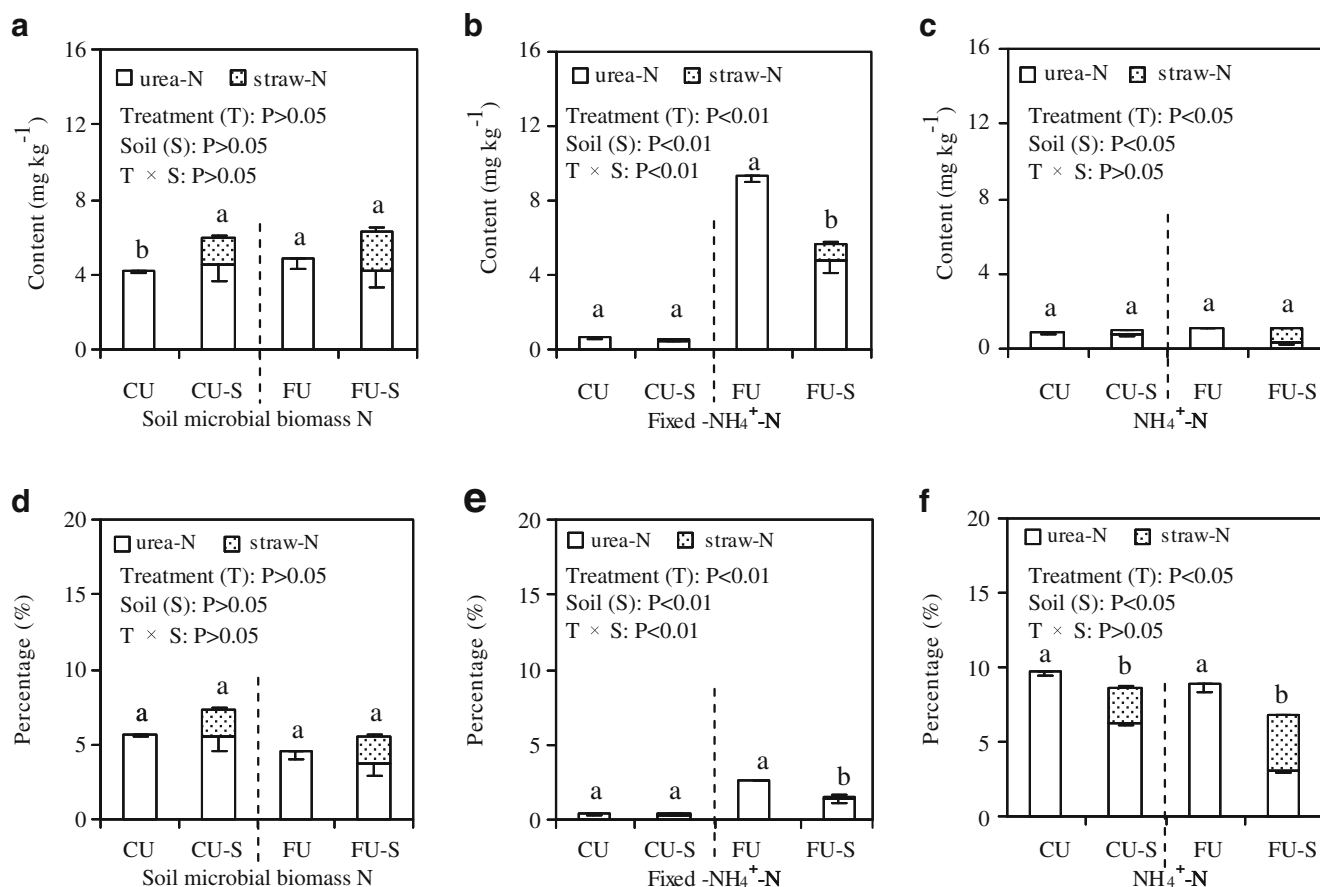
Fig. 1 Content and percentage of newly formed N in soil microbial biomass N, fixed ammonium (fixed-NH_4^+-N) and NH_4^+-N in Cambisol (C) and Fluvisol (F). U urea-only treatment, U-S urea and straw combination treatment. Data shown are means ± standard deviation of four replicates; different lower letters denote significant difference ($P<0.05$) in the same soil. In each soil fraction of U-S, newly formed N content (or percentage) is the sum of labeled urea N content (or percentage) in labeled urea + straw treatment (^{15}NU-S) and labeled straw N content (or percentage) in urea + labeled straw treatment (^{15}NS-U) in the target fraction

percentage of newly formed N in all total hydrolysable N fractions was less than 3%, except for amino acid N in urea + straw treatment in Cambisol (Fig. 3e, f, g, h). Again, soil characteristics themselves significantly impacted the percentage of newly formed amino acid N, the content of newly formed ammonia N, and the content and percentage of newly formed hydrolysable unknown N ($P<0.01$ or $P<0.05$).

For total soil N, total hydrolysable N, acid insoluble N, amino acid N, and hydrolysable unknown N, the recovery rate of straw N was much greater than that of urea N ($P<0.05$, Table 3). Compared with the urea-only treatment, the urea + straw treatment increased the recovery rate of urea N or straw N in soil microbial biomass N in Cambisol ($P<0.05$) and increased the recovery rate of straw N in soil microbial biomass N in Fluvisol ($P<0.05$), but did not significantly increased the recovery rate of urea N in soil microbial biomass N in Fluvisol ($P>0.05$). Conversely, the urea +straw treatment decreased the recovery rate of urea N in fixed-NH_4^+ in Fluvisol ($P<0.05$). In both soils, for total hydrolysable N fractions, addition of urea with or without

straw did not significantly affect the recovery rate of urea N ($P>0.05$) except for the larger urea-derived amino acid N in Cambisol ($P<0.05$), therefore the urea + straw treatment increased the recovery rate of urea because of the straw N/ urea N ratio of 1:3 with equivalent N amendment in the experiment.

Discussion

Soil microbial biomass N, fixed-NH_4^+-N, and NH_4^+-N

Both soil microbial biomass N and fixed-NH_4^+-N are potential sources of available N for crops and they can regulate N status in the soil and decrease N losses. Our results indicate that straw can provide energy for microorganisms, resulting in the largest total N content of soil microbial biomass in the urea + straw treatment (Table 2). In Cambisol the content of newly formed soil microbial biomass N in the urea+straw treatment was significantly
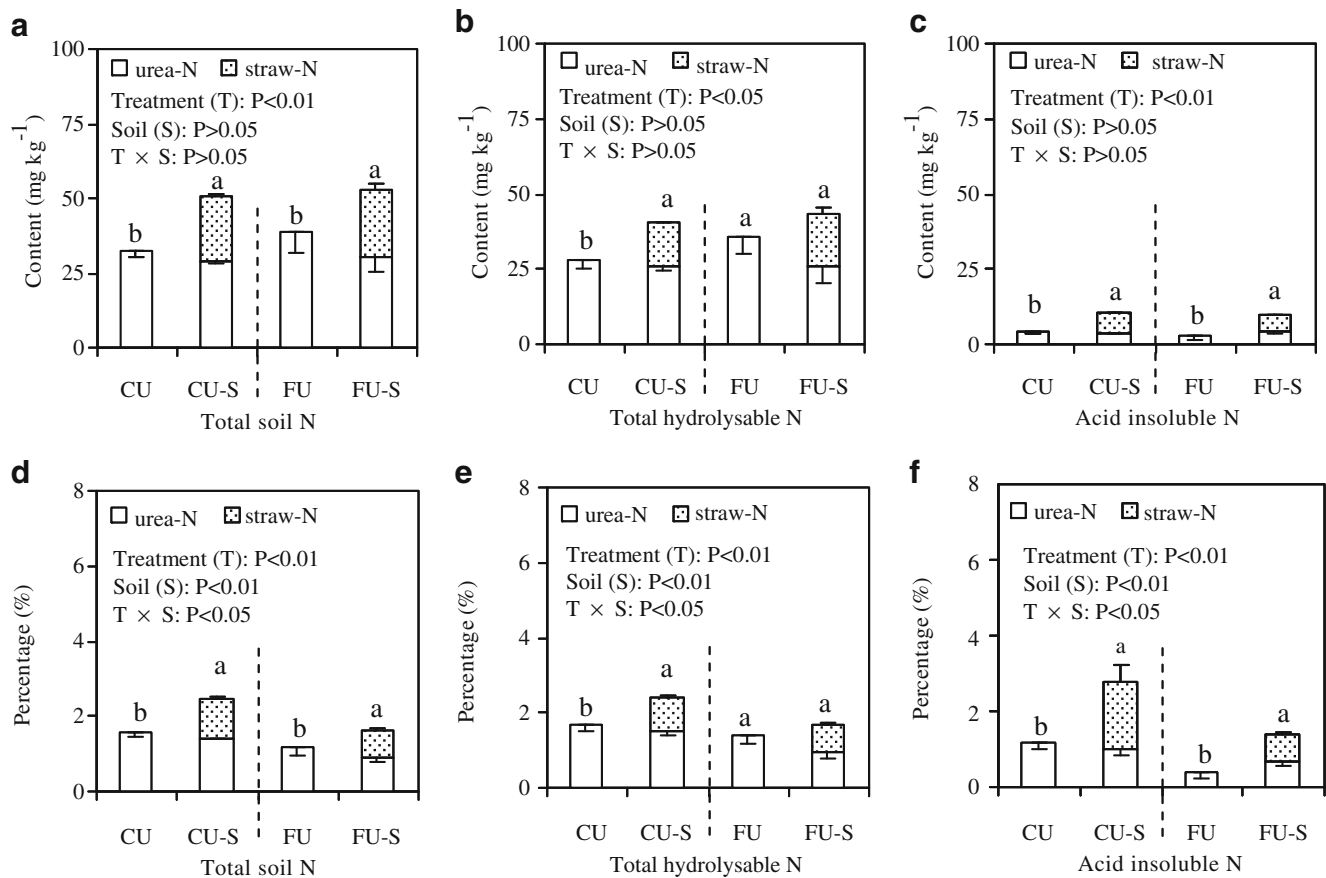
Fig. 2 Content and percentage of newly formed N in total soil N, total hydrolysable N and acid insoluble N in Cambisol (*C*) and Fluvisol (*F*). *U* urea-only treatment, *U-S* urea and straw combination treatment. Data shown are means ± standard deviation of four replicates; different lower case letters denote significant difference (*P*<0.05) in the same soil. In each soil fraction of U-S, newly formed N content (or percentage) is the sum of labeled urea N content (or percentage) in labeled urea + straw treatment (^{15}NU-*S*) and labeled straw N content (or percentage) in urea + labeled straw treatment (^{15}NS-*U*) in the target fraction

higher ($P<0.05$) than in the urea-only treatment (Fig. 1a), but there was no statistically significant difference in Fluvisol ($P>0.05$). Moreover, within the same treatment the percentage of newly formed soil microbial biomass N in Cambisol was larger than in Fluvisol. This may be related to the difference in fertility of the two soils. The lower fertility Cambisol (Table 1) had lower levels of available C and N sources for microbes in comparison with Fluvisol, and therefore the decomposition rate of straw in Cambisol may have increased and more fertilizer N may have been immobilized in the soil microbial biomass. In contrast, more available C and N in the higher fertility Fluvisol may have been released to supply microbial metabolism due to the priming effect of added fertilizer N (Blagodatskaya and Kuzyakov 2008; Kuzyakov et al. 2000). The N uptake efficiency of labeled fertilizer N by the aboveground parts of rice also supports this explanation. The efficiencies in the ^{15}NU, ^{15}NU-S, and ^{15}NS-U treatments were 55.5%, 56.5%, and 42.2%, respectively, in Cambisol and 51.0%, 55.2%, and 40.1%, respectively, in Fluvisol (Peng et al. 2011).

Soil mineralogical type, organic substrate amendment and pH impact on the content of fixed-NH_4^+. As shown in Table 2 and Fig. 1, Fluvisol (2:1-type soil) contained higher fixed-NH_4^+ content and newly formed fixed-NH_4^+-N than Cambisol (1:1-type soil). The wedge zone of 2:1-type clay minerals has three adsorption positions, the inside position (i-P), the edge position (e-P), and the surface plane position (p-P), while the 1:1 type clay mineral soil lacks the i-P. The fixed-NH_4^+-N in e-P and p-P is available to crops and microbes and the N fixed in these sites is readily exchanged with other cations including K^+. This phenomenon may also be induced by pH. In Cambisol the main acidifying factors contributing to the low pH were Al^{3+} and H^+. Thus, in this soil type the protons will displace some specifically adsorbed cations (Sparks and Liebhard 1982) or neutralize negative charges resulting in decreased adsorption positions of NH_4^+. The urea + straw treatment decreased the amount of fixed-NH_4^+-N in Fluvisol and this may be attributed mainly to the low molecular weight organic matter blocking diffusion of NH_4^+ into the
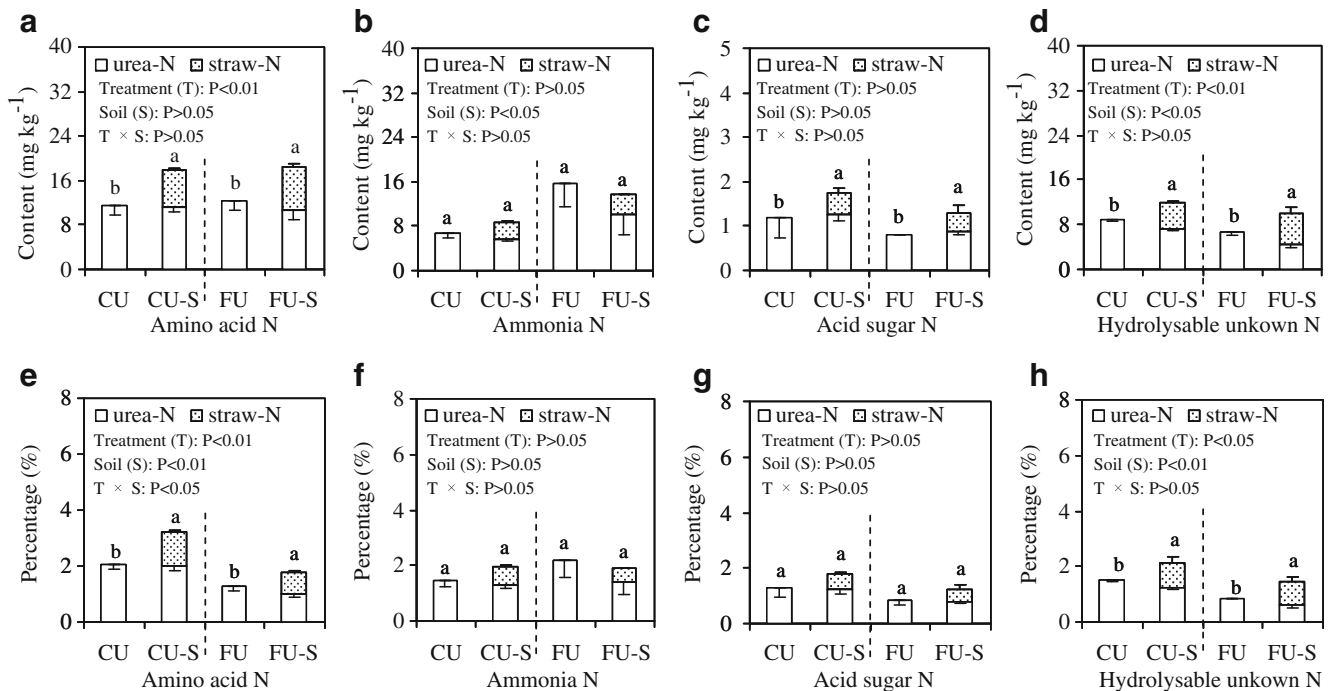
Fig. 3 Content and percentage of newly formed N in soil different hydrolysable N fractions in Cambisol (*C*) and Fluvisol (*F*). *U* urea-only treatment, *U-S* urea and straw combination treatment. Data shown are means ± standard deviation of four replicates; different lower letters denote significant difference (*P*<0.05) in the same soil.

In each soil fraction of U-S, newly formed N content (or percentage) is the sum of labeled urea N content (or percentage) in labeled urea + straw treatment (^{15}NU-S) and labeled straw N content (or percentage) in urea + labeled straw treatment (^{15}NS-U) in the target fraction

interlayers of clay minerals (Porter and Stewart 1970). These low molecular weight organic materials may have been derived from the microbial decomposition of the straw. The phenomenon above is strongly supported by the difference in fixed-NH_4^+-N recovery rate between urea N and straw N among ^{15}NU, ^{15}NU-S, and ^{15}NS-U treatments in both soils (Table 3) and fixed-NH_4^+-N in the interaction between treatment and soil type (Table 3; Fig. 1b, e). Ammonium fixation is also strongly affected by soil Eh (Schneiders and Scherer 1996, 1998; Zhang and Scherer 2000); that is, decreasing Eh increases the negative charges. Moreover, the Eh value decreases with addition of organic material to flooded soils (Schneiders and Scherer 1996), but this is not consistent with the findings of Porter and Stewart (1970). We deduce that oxidation from O_2 secreted from rice roots might counteract the decrease in Eh, especially in pot experiments in which there is a large biomass of rice roots per unit volume of soil. In addition, microbial N immobilization induced by the C source (Burger and Jackson 2003) can compete for NH_4^+ with clay minerals and decrease NH_4^+ fixation. However, the urea + straw treatment did not significantly (*P*>0.05) affect the total fixed-NH_4^+-N content in either soil (Table 2) or the content and percentage of newly formed fixed-NH_4^+-N in Cambisol (Fig. 1b, e) in comparison with

the urea-only treatment. Therefore, in Fluvisol N immobilization did not have any discernible effect on the decline in content or percentage of newly formed fixed-NH_4^+-N in the urea + straw treatment (Fig. 1b, e).

Total soil N, acid insoluble N, and Total hydrolysable N Fractions

Ju et al. (2006) reported that total soil N, acid insoluble N and total hydrolysable N increased after long-term fertilizer application and a similar result was found for the content and percentage of newly formed total soil N, acid insoluble N and total hydrolysable N in the present study; especially in the urea + straw treatment (Fig. 2), the urea + straw treatment increasing the content of newly formed total soil N by 56.0% in Cambisol and by 37.5% in Fluvisol in comparison with the urea-only treatment. Straw N was the dominant component in acid insoluble N (Fig. 2c, g), and the recovery rate of straw N was higher than that of urea N in both soils (*P*< 0.05, Table 3), and this may be due to the lower availability of straw N to microbes compared with urea N. This further suggests that the availability of acid insoluble N to crops was lower than that of total hydrolysable N fractions. In fact, acid insoluble N has also been reported to be biodegradable (Schulten and Schnitzer 1998)

Table 3 Recovery rate of labeled fertilizer N in different soil fractions and total soil N (TSN) in the two soils (%)

Treatment	$SMBN_S/N_S$	$Fixed\text{-}NH_4^+\text{-}N_S/N_S$	$NH_4^+\text{-}N_S/N_S$	TSN_S/N_S	N fraction by 6 N HCl hydrolysis					
					THN_S/N_S	AIN_S/N_S	AAN_S/N_S	AN_S/N_S	ASN_S/N_S	HUN_S/N_S
Cambisol										
^{15}NU	1.8±0.04b	0.3±0.06a	0.4±0.05a	14.2±1.0c	12.3±1.4c	1.9±0.4b	5.0±0.7c	2.9±0.4b	0.5±0.2a	3.9±0.2b
$^{15}NU\text{-}S$	2.7±0.5a	0.3±0.04a	0.4±0.02a	17.0±0.6b	14.9±0.6b	2.1±0.2b	6.6±0.6b	3.3±0.2b	0.7±0.1a	4.3±0.3b
$^{15}NS\text{-}U$	2.5±0.1a	0.2±0.02a	0.4±0.02a	37.3±1.4a	25.9±0.9a	11.4±0.7a	11.7±0.3a	5.1±0.7a	0.9±0.2a	8.3±0.3a
Fluvisol										
^{15}NU	2.1±0.3b	4.1±0.1a	0.5±0.03b	16.8±2.8b	15.6±2.4b	1.2±0.4b	5.4±0.7b	6.9±1.9a	0.4±0.01a	3.0±0.3b
$^{15}NU\text{-}S$	2.5±0.5b	2.8±0.4b	0.2±0.04c	17.6±2.7b	15.1±3.1b	2.5±0.5b	6.1±0.8b	5.8±2.1a	0.5±0.04a	2.6±0.3b
$^{15}NS\text{-}U$	3.6±0.4a	1.7±0.2c	1.3±0.1a	39.9±3.5a	30.8±2.9a	9.1±0.7a	13.9±1.1a	6.2±0.1a	0.7±0.3a	10.0±1.8a
ANOVA										
Treatment	n.s.	**	**	**	**	**	**	n.s.	*	**
Soil	n.s.	**	**	n.s.	n.s.	**	n.s.	**	n.s.	n.s.
Soil × Treatment	n.s.	**	**	n.s.	n.s.	**	n.s.	n.s.	n.s.	n.s.

Values are mean ± standard deviation of four replicates

SMBN soil microbial biomass N, *THN* total hydrolyzed N, *AIN* acid insoluble N, *AAN* amino acid N, *AN* ammonia-N, *ASN* amino sugar N, *HUN* hydrolysable unknown N, ^{15}NU labeled urea-only, $^{15}NU\text{-}S$ labeled urea + straw, $^{15}NS\text{-}U$ urea + labeled straw

Subscript "S" indicates labeled urea in ^{15}NU or $^{15}NU\text{-}S$ treatments and labeled straw in $^{15}NS\text{-}U$ treatment

n.s. not significant at $P>0.05$; *significant at $P<0.05$; **significant at $P<0.01$; lower case letters within columns denote significant difference ($P<0.05$) in the same soil

and derived from amino-N bonded with organic materials (Leinweber and Schulten 2000).

Among the total hydrolysable N fractions, amino acid N is an important N source for plants and microbes, and microbes play a key role in synthesizing amino acid N. Application of urea + straw increased amino acid N (Fig. 3a), possibly because the straw provided a C source for microbes. The relative high percentage of fertilizer N assimilated microbially (Fig. 1d) in the lower fertility Cambisol may have contributed, at least in part, to the higher content and percentage of newly formed amino sugar N (Fig. 3c, g) as discussed for soil microbial biomass N in the previous paragraph. Ammonia N is derived from the breakdown of amides, hydroxyamino acids, and some other amino acids and amino sugars, from the deamination of purines and pyrimidines, and from the release of partly fixed-NH_4^+ (Xu et al. 2003). Thus, in the present study ammonia N could have been derived from the breakdown of organic N, mineral N (NH_4^+-N) and released from soil clay minerals. The release of fixed-NH_4^+-N resulted to some extent in more newly formed ammonia-N in Fluvisol (2:1-type clay) than in Cambisol (1:1-type clay). Because the low-molecular-weight organic material from decomposed rice straw can block the diffusion of NH_4^+ into the interlayers of clay minerals (Porter and Stewart 1970), the urea + straw treatment decreased the content and percentage of newly formed ammonia N (Fig. 3b, f) in Fluvisol, but the difference was not significant compared with the urea-only treatment (P >0.05). Hydrolysable unknown N is derived partly from microbial nucleic acids and is regarded as stable soil compounds (Nannipieri and Paul 2009), but to date Hydrolysable unknown N has not been characterized completely because of its complexity (Schulten and Schnitzer 1998). In our study the urea + straw treatment increased the hydrolysable unknown N fraction in both soils ($P<0.05$; Fig. 3d, h). In another study, however, the hydrolysable unknown N content was not significantly different between fertilizer and control treatments in a long-term experiment (Xu et al. 2003). In all of the total hydrolysable N fractions except ammonia N,

the percentage of newly formed N was greater in Cambisol than in Fluvisol (Fig. 3e, g, h). This was mainly because of the differences between the soils in fertility and clay type (Table 1).

The newly formed acid insoluble N and hydrolysable unknown N in Cambisol occurred as 63.9% and 39.2% straw N and in Fluvisol as 54.7% and 56.3% straw N (Figs. 2c and 3d), and the percentage was much larger than the straw N added at the beginning of the experiment (25%). Because acid insoluble N and hydrolysable unknown N are more resistant to degradation than amino acid N, ammonia N, and amino sugar N (Mishra et al. 2005; Schulten and Schnitzer 1998; Nannipieri and Paul 2009), we divided the soil organic N fractions into easily degradable (amino acid N, ammonia N, and amino sugar N) and degradation-resistant (acid insoluble N and hydrolysable unknown N) parts (Table 4) to calculate the amount of available N to subsequent crops. Although the urea + straw treatment increased the contents of newly formed easily degradable and degradation-resistant parts, the percentage of the easily degradable part in total newly formed N decreased and correspondingly that of the degradation-resistant part increased. In addition, there were larger amounts of the newly formed easily degradable part in Fluvisol (high fertility) than in Cambisol (low fertility). Even so, most of the newly formed N in the soils was available because the newly formed easily degradable part accounted for at least 56.0% of N in both soils.

In addition, the effect of urea + straw on newly formed N was greater in the low fertility Cambisol than in the high fertility Fluvisol. The newly formed N was generally more available to crops than the "native" soil organic N (Table 4). Thus, if the availability of newly formed N to the next crop is deducted, the actual percentage of newly formed N in the total N of the different soil fractions should be less than 3% according to the percentage figure of newly formed N (Figs. 1d, e, f; 2d, e, f; and 3e, f, g, h). This further indicates that long-term application of organic materials (e.g., straw) is required to increase soil fertility.

Table 4 Easily degraded and degradation-resistant parts of newly formed N in both soils

Easily degradable part: amino acid N + ammonia N + amino sugar N; degradation-resistant part: hydrolysable unknown N + acid insoluble N;

U urea-only treatment, *U-S* urea and straw combination treatment

Treatment	Content of constituent parts (mg kg^{-1})		Percentage of constituent parts in soil total newly formed N (%)	
	Easily degraded	Degradation-resistant	Easily degraded	Degradation-resistant
Cambisol				
U	19.3	13.1	59.4	40.6
U-S	28.3	22.2	56.0	44.0
Fluvisol				
U	29.0	9.5	75.3	24.7
U-S	33.3	19.6	63.0	37.0

Conclusions

In most cases, in different soil fractions the combination of synthetic N fertilizer and organic material (compared with synthetic fertilizer application alone) significantly increased the content of the newly formed N from fertilizer N (the sum of urea N plus straw N), and the percentage of newly formed N in the low fertility soil (Cambisol<Fluvisol) ($P<$ 0.05), except that the fractions were affected by the predominant soil clay mineral type. For example, Fluvisol accumulated more fixed-NH_4^+-N and ammonia N than Cambisol but the urea + straw treatment decreased newly formed fixed-NH_4^+-N and ammonia N in Fluvisol. The urea + straw treatment significantly ($P<0.05$) increased the content and percentage of newly formed amino acid N or the content of newly formed amino sugar N as a result of microbial synthesis. In addition, the actual percentage of newly formed N in different soil fractions was less than 3% if the availability of newly formed N was considered. Therefore, both an increase in soil fertility and the distribution of N in soils of different clay type are affected after the combined application of synthetic N fertilizer and organic material.

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