

## The mineralization and transformation of both added organic nitrogen and native soil N in red soils from four different ecological conditions\*

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**Abstract** The  $\text{NH}_4^+$ -N, microbial biomass-N, humus-N, and extractable organic N derived from the added  $^{15}\text{N}$ -labelled ryegrass and soil indigenous pool were measured separately with  $^{15}\text{N}$  tracing techniques. Based on the recovery of  $\text{NH}_4^+$ - $^{15}\text{N}$  and lost- $^{15}\text{N}$  (mainly as  $\text{NH}_3$ ), more than 30% of the added ryegrass  $^{15}\text{N}$  was mineralized in 15 d. The amount of mineralized N increased with time up to 90 d for all soils except for the upland soil in which it decreased slightly. The mineralization of ryegrass N and incorporation of ryegrass- $^{15}\text{N}$  into microbial biomass was greatest in upland soil. The transformation of ryegrass  $^{15}\text{N}$  into humus  $^{15}\text{N}$  occurred rapidly in 15 d, with higher humus  $^{15}\text{N}$  occurring in the upland or tea-garden soil than the paddy and unarable soil. The addition of ryegrass caused additional mineralization of soil indigenous organic N and enhanced the turnover of both microbial biomass N and stable organic N in soils.

**Keywords** Agro-ecosystems, Microbial biomass, Ryegrass, Nitrogen transformations

### 1 Introduction

Organic materials have beneficial effects on biological, chemical and physical properties of soils in the tropical and subtropical regions.<sup>[1,2]</sup> Added organic materials can stimulate microbial activities (because of increased source of energy and nutrients for microorganisms), and can enhance the decomposition of passive organic matter in soil due to the increased microbial growth. Nutrients released from the mineralization of organic matter may be readily available to plants, or may be incorporated into microbial biomass as a fraction of potentially-available plant nutrients.<sup>[3]</sup> The decomposition of added organic materials could accelerate the transformation of nutrients and the formation of new soil organic matter. Therefore, increasing the input of organic materials is an useful approach for improvement of soil fertility.<sup>[4~6]</sup>

Red soils, which are highly-weathered and similar to the soil Taxonomic Order Ultisol in properties, and widely distributed in southern China. Most of the red soils in China have low

nutrient content, high acidity, and poor physical properties. This is because of being subjected to severe soil erosion as a result of deforestation. Great efforts have been made in the last decade to improve red soils for sustainable crop production. Organic materials have been proven to be the most effective amendment for the red soils.<sup>[1,7,8]</sup> However, many factors may affect the decomposition and transformation of organic materials in soils. An understanding of the fate of organic components in red soils from different agro-ecosystems is essential for the successful management of red soil fertility and structure in agriculture. Our previous study on the transformation of organic N in two red soils using  $^{15}\text{N}$ -labelled ryegrass demonstrated that the transformation rates of organic N to mineral N and microbial biomass N were significantly different between the sandy and the clayey soils.<sup>[9,10]</sup> The decomposition and turnover of organic C and N in soil could also be affected by other factors such as plant cover, rotation and cultivation.<sup>[11~13]</sup> In this

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study, the transformation of both added ryegrass  $^{15}\text{N}$  and indigenous soil organic N was investigated.  $^{15}\text{N}$ -labelled ryegrass was used so that mineralized N, microbial biomass N and humus N (from both the added ryegrass and soil indigenous pool) could be traced and examined separately.

## 2 Materials and methods

### 2.1 Soils

Four red acid soil samples were collected from the surface soil layer (0~15 cm) in Longyou County, Zhejiang Province, China. All four soils were derived from a quaternary red earth and all had similar clay mineral composition. However, biological properties and fertility of the soils varied because of each having

been under different vegetation conditions for ten years. The four soils were collected from paddy, upland, non-arable, eroded bareland and tea-garden, respectively. The samples were air-dried and ground to pass through 1-mm sieve before use. The major properties are listed in Table 1. Soil pH was measured in deionized water at a soil water ratio of 1:1. Organic C was determined by dichromate digestion, and total N was determined by a wet digestion-distillation method. Cation exchange capacity (CEC) was measured by the ammonium-acetate-displacement method.<sup>[14]</sup> Labile N was determined with an alkaline-hydrolysis method, labile P was extracted by the Bray-Pl procedure, and labile K was extracted by the N ammonium-acetate extraction procedure<sup>[15]</sup>

Table 1 Properties of the red soils

Soil No	Vegetational state	pH (H <sub>2</sub> O)	Clay g·kg <sup>-1</sup>	Organic C g·kg <sup>-1</sup>	CEC /cmol·kg <sup>-1</sup>	Total N g·kg <sup>-1</sup>	Labile nutrients/mg·kg <sup>-1</sup>		
							N	P	K
1	Paddy	6.2	502	9.41	9.55	1.1	153.4	5.3	165
2	Upland	4.8	287	8.55	5.64	0.9	99.1	11.8	80
3	Non-arable	5.1	354	0.87	6.86	0.3	35.8	5.3	80
4	Tea-garden	4.6	505	6.51	11.41	1.1	113.2	7.4	190

### 2.2 Preparation of uniformly $^{15}\text{N}$ -labelled ryegrass

$^{15}\text{N}$ -( $\text{NH}_4$ )<sub>2</sub>SO<sub>4</sub> with a 11.38 atom percent excess was used to label the soil in a pot which was placed in field and glucose was added to the  $^{15}\text{N}$ -( $\text{NH}_4$ )<sub>2</sub>SO<sub>4</sub> amended soil to promote  $^{15}\text{N}$  immobilization. The soil moisture was kept at field-holding capacity under natural conditions. After three months, appropriate amounts of P (50 mg·kg<sup>-1</sup> as CaHPO<sub>4</sub>), K (100 mg·kg<sup>-1</sup>), and micronutrient fertilizers were applied to the  $^{15}\text{N}$ -labelled soil, and then ryegrass seeds were sown. No fertilizer was applied during the growth period. The ryegrass was harvested after two-month growth, dried at 45°C, and ground to pass through 0.5-mm sieve. Total N of the labelled ryegrass was 2.7% of dry matter with a 4.207 atom percent excess of  $^{15}\text{N}$ .

### 2.3 Incubation of soil with ryegrass

30 g of each soil sample (oven-dry basis) in three replication was placed into a 100-ml glass beaker with soil moisture adjusted to 50% of water-holding capacity (WHC), and then pre-incubated for 10 d at 25°C. After pre-

incubation, the soils were each mixed with 0.9 g of labelled ryegrass (a rate of 30 g·kg<sup>-1</sup> with 0.821 mg ryegrass N·kg<sup>-1</sup> soil). The soil was incubated at 25°C, whose moisture was adjusted and maintained at 50% WHC by weighing.

### 2.4 Sampling and analysis

Subsoil samples were taken to analyze for the amounts and  $^{15}\text{N}$  abundance of total N, microbial biomass N, mineral N, and humus N at intervals of 15, 30 and 90 d after incubation. Total N was determined by Kjeldahl-digestion method.<sup>[14]</sup> Mineral N was extracted with 3.5 mol/L NaCl and the extract was analyzed for  $\text{NH}_4^+$ -N with the NaOH-distillation procedure. The amounts of  $\text{NO}_3^-$ -N and  $\text{NO}_2^-$ -N as detected by Zn-FeSO<sub>4</sub> were negligible in both soil and ryegrass, and thus only  $\text{NH}_4^+$ -N was determined for all samples. A fumigation-extraction method was used to measure soil microbial biomass N.<sup>[16]</sup> The CHCl<sub>3</sub>-fumigated with 0.5 mol/L K<sub>2</sub>SO<sub>4</sub> immediately following the removal of CHCl<sub>3</sub> by vacuum pumping. Un-fumigated soils were extracted under the same conditions at the time fumigation started. Total N in the K<sub>2</sub>SO<sub>4</sub> extract was determined

by Kjeldahl-digestion method. The flush ( $F$ ) i.e. the difference in extractable N between the fumigated and unfumigated samples was used to calculate microbial biomass N ( $B_n$ ) by applying a  $k_{EN}$  value of 0.54.<sup>[16]</sup> Humus N was extracted for 16 h with 0.1 mol/L NaOH at soil/solution ratio of 1:20 following 0.5 mol/L  $K_2SO_4$  extraction of the fumigated soils. The extract was concentrated by evaporation at low

temperature and analyzed for total N with Kjeldahl digestion-distillation method.  $^{15}N$  abundance of all the samples was measured with a mass spectrometer. Based on total N and  $^{15}N$  abundance, the amounts of total N, microbial biomass N, mineral N, and humus N (from both the added ryegrass and soil indigenous pool) were calculated separately with the following equation:

$$N \text{ amount from ryegrass} = \frac{\text{total N} \times {}^{15}N \text{ atom percent excess of sample}}{{}^{15}N \text{ atom percent excess of ryegrass}}$$

The amount of N derived from soils was the difference between total N and the calculated  $^{15}N$ -N derived from the ryegrass. All soil samples and analyses were in triplication and the results were expressed as percent of applied  $^{15}N$ -N or initial soil-indigenous N.

### 3 Results

#### 3.1 Transformation dynamics of ryegrass $^{15}N$ in soils

Ryegrass straw was rapidly decomposed in the soils with a net release of  $NH_4^+-N$  (Fig.1a). Extractable  $NH_4^+-N$  (in percent of applied ryegrass- $^{15}N$ ) in the soils ranged from 5.9 to 17.4%, with the highest percentage in upland

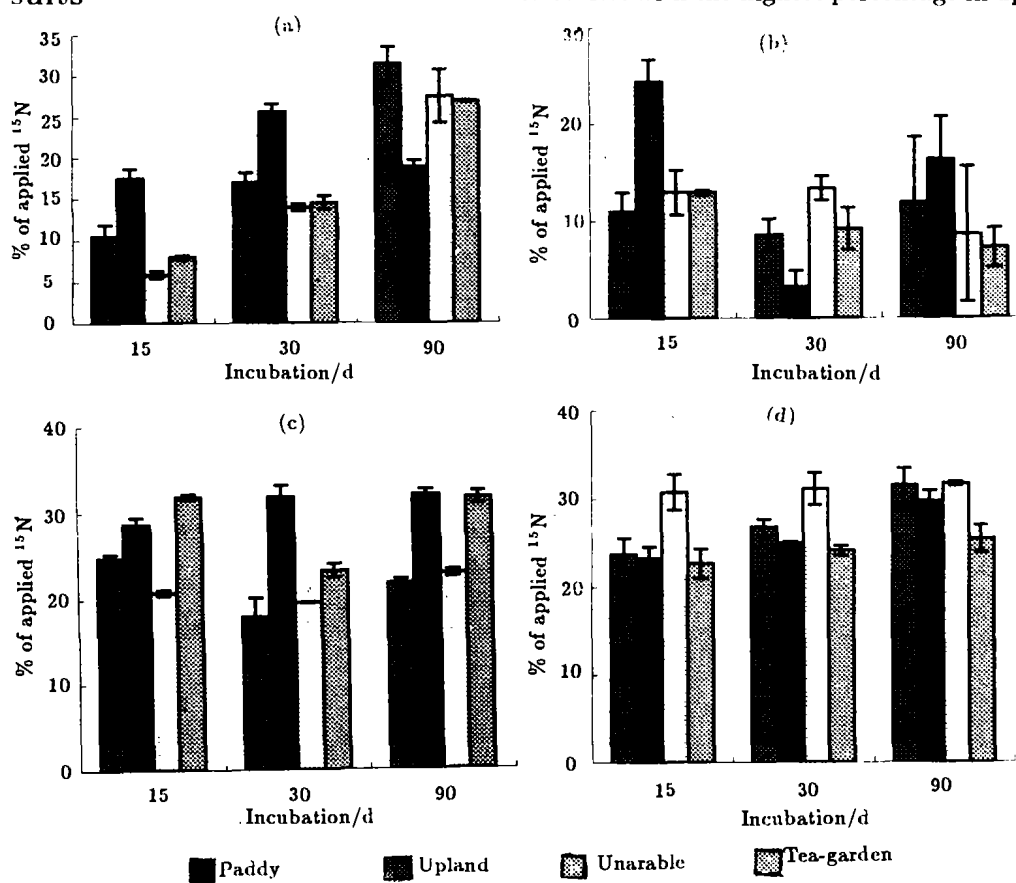


Fig.1 Amount of  $NH_4^+-^{15}N$  (a), microbial biomass- $^{15}N$  (b), humus- $^{15}N$  (c) and lost- $^{15}N$  (d) derived from  $^{15}N$ -ryegrass in four red soils: paddy, upland, unarable and tea-garden

soil and the lowest in non-arable soil at the day 15 of incubation. The extractable  $\text{NH}_4^+-^{15}\text{N}$  values were raised to 13.91 to 25.61% of applied  $^{15}\text{N}$  with the same tendency among soils at the day 30 of incubation. By the end of incubation (90 d), the extractable  $\text{NH}_4^+-^{15}\text{N}$  in soils increased to 31.31, 27.37 and 26.81%, respectively, for paddy soil, non-arable soil, and tea-garden soil. On the contrary, the corresponding values for upland soil decreased to 18.74%. These results indicated that some of the ryegrass-N was in a plant available form, however, the mineralization rate of ryegrass-N varied among the soils from different vegetation conditions.

Approximately 13 to 24% of ryegrass  $^{15}\text{N}$  was incorporated into soil microbial biomass (Fig.1b). Microbial biomass  $^{15}\text{N}$  reached maximum in 15 d for the upland and tea-garden soil, in 30 d for non-arable soil, and in 90 d for the paddy soil. Maximum microbial biomass  $^{15}\text{N}$  in the upland soil nearly doubled that in any other soils at the day 15 of incubation. The mineralization of ryegrass N in soils appeared to be related to the microbial activities. The high extractable  $\text{NH}_4^+-\text{N}$  in the upland soil was in good agreement with its high microbial biomass- $^{15}\text{N}$ .

Humus  $^{15}\text{N}$  accounted for 20.72 to 31.79%

of the added ryegrass- $^{15}\text{N}$  at the 15 d of incubation, with the highest value in tea-garden soil followed by upland soil (Fig.1c). Humus  $^{15}\text{N}$  increased in the upland soil but declined in the others at the day 30 of incubation. Humus  $^{15}\text{N}$  again increased in all soils by the end of incubation (90 d), with the highest values in the upland and tea-garden soil. It is evident that the humification of ryegrass occurred at an early stage of ryegrass decomposition, and that a considerable part of ryegrass N was transformed into humic fractions in soils within a short term.

The difference between total  $^{15}\text{N}$  and the sum of  $\text{NH}_4^+-^{15}\text{N}$ , microbial biomass  $^{15}\text{N}$  and humus  $^{15}\text{N}$  was termed as residual  $^{15}\text{N}$ . This difference may include non-extractable soil organic and inorganic  $^{15}\text{N}$  and undecomposed ryegrass  $^{15}\text{N}$ . The residual  $^{15}\text{N}$  was about 25 to 30% of the added ryegrass  $^{15}\text{N}$  in 15 d, but decreased to 0.5 to 6.3% by the end of incubation for all soils. Residual  $^{15}\text{N}$  in upland soil was small at the day 15 but slightly increased at the day 30, and then again declined to a very small percent of total ryegrass  $^{15}\text{N}$  at the day 90 of incubation (Table 2). It appeared that fixation of  $^{15}\text{N}$  derived from ryegrass was small in the soils (seen from Tables 1,2).

**Table 2** Dynamics of extractable organic  $^{15}\text{N}$  and residual  $^{15}\text{N}$  derived from  $^{15}\text{N}$ -labelled ryegrass in red soils

N forms	Incubation time /d	Fractions of applied N			
		Soil type			
		Paddy/%	Upland/%	Non-arable/%	Tea-garden/%
Extractable organic $^{15}\text{N}$	15	0.66±0.11	1.40±0.12	1.40±0.23	0.21±0.04
	30	0.76±0.13	8.97±1.49	2.10±0.26	3.73±0.27
	90	0.94±0.16	0.89±0.23	8.99±0.18	2.66±0.48
Residual $^{15}\text{N}$	15	30.00±2.46	4.99±0.30	28.47±2.28	24.67±0.37
	30	29.27±3.40	7.06±0.66	20.02±0.73	25.33±2.33
	90	2.03±0.29	2.29±0.21	0.50±0.09	6.26±0.25

The difference between the added ryegrass  $^{15}\text{N}$  and the total  $^{15}\text{N}$  in the soil (determined at the end of incubation) was considered as lost  $^{15}\text{N}$ . The lost  $^{15}\text{N}$  at most could account for 23 to 31% of the added ryegrass  $^{15}\text{N}$  with a lower value in the tea-garden soil (Fig.1d). There was no significant difference among the other three soils (Fig.1d). Most of  $^{15}\text{N}$  loss occurred in the first 15 d of incubation, and afterwards, the accumulated  $^{15}\text{N}$  loss slowly increased with time. The major form of lost N was likely

$\text{NH}_3$  because little  $\text{NO}_3^--\text{N}$  or  $\text{NO}_2^--\text{N}$  was detected in either soil or ryegrass.<sup>[9]</sup> These results showed that  $\text{NH}_3$  volatility during decomposition of ryegrass at early stage was likely responsible for the loss of the added ryegrass  $^{15}\text{N}$ .

Kjeldahl N in 0.5 mol/L  $\text{K}_2\text{SO}_4$  extracts of unfumigated soils consisted of  $\text{NH}_4^+-\text{N}$  and extractable organic N (EON). The EON could be calculated by subtracting  $\text{NH}_4^+-\text{N}$  from total Kjeldahl N in the extract. The amount of EON was small for most of the tested soil samples and

was closely related to the microbial biomass  $^{15}\text{N}$  (Table 2 and Fig.1b).

### 3.2 Effect of ryegrass addition on the transformation of native soil N

The amount of  $\text{NH}_4^+-\text{N}$  in the ryegrass-amended soil tended to increase with incubation time for all soils except the upland soil for which  $\text{NH}_4^+-\text{N}$  reached maximum in 15 d and then slightly decreased with incubation time (Fig.2a). The humus N from native soil pool increased with incubation time (Fig.2c). The percentages of soil N in form of EON were higher at the end of incubation than that in early period of incubation (Fig.2b). Humus N from soil in native pool was less than that from ryegrass in 15 d, but higher than that from ryegrass in 30 d and 90 d.

grass after 30 d of incubation (Fig.2c). By comparing humus N in the control soils with that in ryegrass-amended soils, it was shown that the mineralization of indigenous soil humus N was increased by the addition of ryegrass (Table 4). The decrease in the amount of humus N in the ryegrass-amended soils was accompanied by the increase in the sum of  $\text{NH}_4^+-\text{N}$ , microbial biomass N, EON and lost N. The residual N from the control soils was much higher than that from ryegrass, and decreased with incubation time (seen from Table 3). It was shown clearly from above results that addition of ryegrass promoted the transformation of soil organic N and increased the turn over rate of stable soil organic matter.

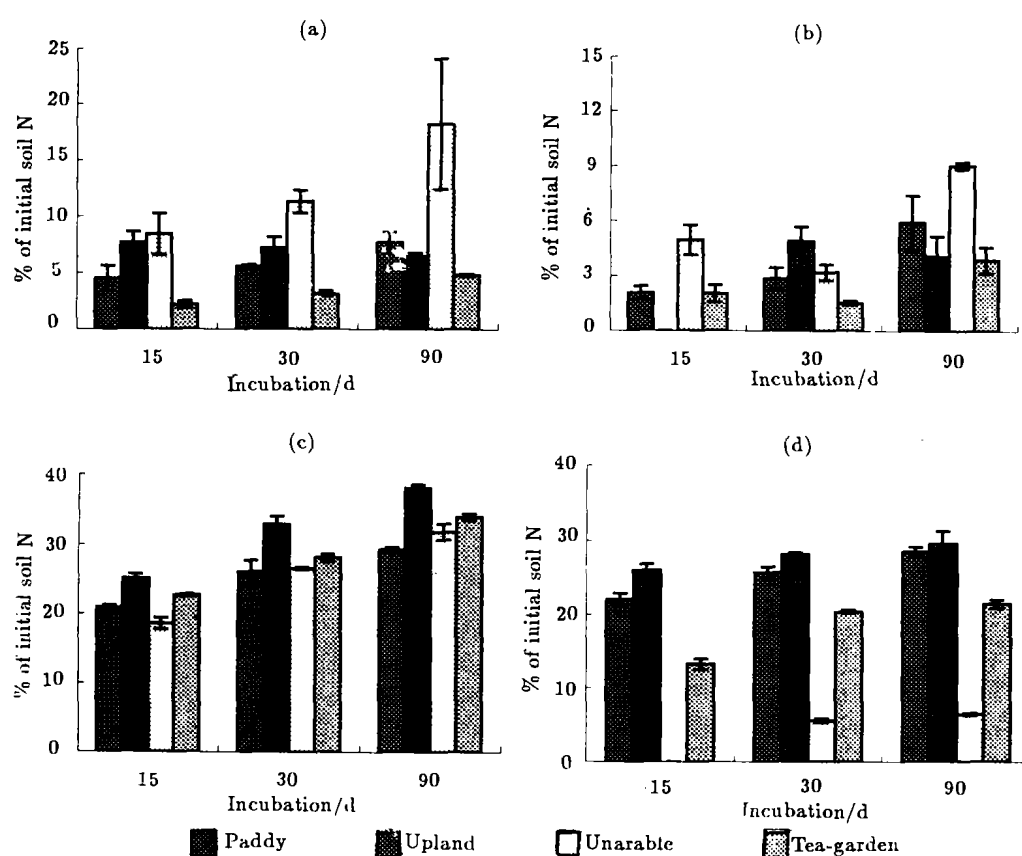


Fig.2 Amount of  $\text{NH}_4^+-\text{N}$  (a), extractable organic-N (b), humus-N (c) and lost-N (d) from native soil N in four soils: paddy, upland, unarable and tea-garden

**Table 3** Dynamics of N transformation from indigenous organic N in red soils

N forms	Incubation time/d	Fractions of native soil N			
		Soil type			
		Paddy/%	Upland/%	Non-arable/%	Tea-garden/%
Residual N	15	46.59	35.44	65.44	57.46
	30	39.87	26.91	49.78	46.03
	90	28.84	23.61	38.60	35.83

Because of ryegrass addition the loss of indigenous soil N was considerable for all soils except for non-arable soil, but the proportion of lost N in the total was less for soil organic N (Fig.2d) than ryegrass- $^{15}\text{N}$  (Fig.2d). For the

un-arable soil only a small amount of native soil N was lost from the soil after 30 d incubation, showing that organic N in the non-arable soil was more stable and less bioavailable than in the arable soils.

**Table 4** Humus N in soils with and without ryegrass amendment at the day 15 of incubation

Soils	Amount of humus N in the soils/ $\text{mg}\cdot\text{kg}^{-1}$		Soil humus N decrease due to amendment /%	Other N increase in amended soils/%
	With	Without		
Paddy	1.134	1.426	20.48	32.48
Upland	1.183	1.588	25.50	39.46
Non-arable	0.293	0.339	13.57	19.27
Tea-garden	1.261	1.484	15.03	19.85

## 4 Discussion

The mineralization of ryegrass N was rapid in red soils. A large part of mineralized N remained in soil in the form of  $\text{NH}_4^+\text{-N}$ , which is readily available to plants. It is interesting that even in the acid soils  $\text{NH}_3$  was still produced and lost into the atmosphere through volatilization. This occurred during the decomposition of ryegrass straw although  $\text{NH}_3$  only accounted for a small percent of the total N. The amounts of  $\text{NH}_4^+\text{-}^{15}\text{N}$  plus the  $\text{NH}_3\text{-}^{15}\text{N}$  constituted a major part of the mineralized  $^{15}\text{N}$  and could be used to roughly estimate the mineralization rate of ryegrass  $^{15}\text{N}$  in soils. The net N mineralization rate of ryegrass- $^{15}\text{N}$  decreased among the soils with the following sequence: upland > non-arable > paddy > tea-garden. The mineralization rate increased with time at initial stage and declined gradually after passing its peak as a result of a decrease in decomposable substrate. The upland soil that had the highest mineralization rate of ryegrass N at initial stage (15 d) had the lowest mineralization rate of ryegrass N at the late stage of incubation (90 d). The mineralization rate of ryegrass N was related to clay content of the soils. Soils with less clay content generally had a higher mineralization rate of added ryegrass  $^{15}\text{N}$ .

The transformation rate of ryegrass  $^{15}\text{N}$  was calculated as the difference between the added ryegrass  $^{15}\text{N}$  and the percentage of residual  $^{15}\text{N}$  in samples. This was because all of the mineral  $^{15}\text{N}$ , extractable  $^{15}\text{N}$ , humus  $^{15}\text{N}$ , and lost  $^{15}\text{N}$  were transformed from the added ryegrass  $^{15}\text{N}$ . Transformation rates of ryegrass N for the four soils ranged from 70 to 95%, with the highest values in the upland soil at the day 15 and the day 30 of incubation. By the end of incubation, the transformation rates were more than 90% for all soils with the lowest value in the tea-garden soil. Apparently, the decomposition of the ryegrass straw was nearly completed in 90 d, and plants could benefit from the mineralization of added ryegrass straw within a growing season.

In addition to providing nutrients to the soil and increasing soil N storage, the application of ryegrass also enhanced the turnover of soil organic matter. Compared with the control, humus N in the ryegrass-amended soil decreased by 13 to 25%. Meanwhile, 19 to 39% more of total soil N from the ryegrass-amended soils was transferred to mineral N, microbial biomass N, and extractable organic N compared with that from the controls (Table 5). Obviously, the addition of ryegrass straw accelerated the turnover of soil organic matter and increased the avail-

ability of soil N.

**Table 5** The sum of  $\text{NH}_4^+ -^{15}\text{N}$  plus lost  $^{15}\text{N}$  and transformation rate of ryegrass- $^{15}\text{N}$  in red soils

N transformation	Incubation time/d	Transformation of ryegrass $^{15}\text{N}$ (of applied $^{15}\text{N}$ )			
		Paddy/%	Upland/%	Unarable/%	Tea-garden/%
$\text{NH}_4^+ -^{15}\text{N}$ + lost $^{15}\text{N}$	15	34.27	40.74	36.62	30.70
	30	43.71	50.65	45.13	38.70
	90	62.85	48.60	59.15	52.41
Transformation rate	15	70.00	95.01	71.53	75.33
	30	71.73	92.94	79.98	74.67
	90	97.69	97.71	99.50	93.74

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