Nitrogen utilization and transformation in red soil fertilized with urea and ryegrass*

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Abstract The influence of fertilization with urea and ryegrass on nitrogen utilization and transformation in red soil has been studied by using ^{15}N tracer method. When urea and ryegrass were applied alone or in combination, the percentage of N uptaken by ryegrass from labelled urea was 3 and 1.7 times that from labelled ryegrass for the application rate of 200 mgN·kg^{-1} and 100 mgN·kg^{-1}, respectively; combining application of ryegrass and urea reduced uptake of urea N and increased uptake of ryegrass N by ryegrass plant, but the percentage of N residued in soil increased for urea and decreased for ryegrass; when urea and ryegrass were applied alone, the percentage of N residued in soil from labelled ryegrass was more than 69% while that from labelled urea was less than 25%, and much more ryegrass N was incorporated into humus than urea N.

Keywords Nitrogen, Utilization, Transformation, Red soil, Ryegrass

1 Introduction

The area of red soils in China covering about more than 2 million km^2 accounts for 22.7% of the whole country's area.[1] Most of the red soils are deficient in nutrient elements which are indispensable to plant production; moreover, red soils are readily to be eroded and the physical characteristics are not suitable for arable practice and crop production. For these reasons, effective measures must be taken to ameliorate the fertility and productivity of red soils. One of the commonest methods used is to enhance the input of organic matters. Ryegrass plants have strongly vigorous fibrous roots, it can not only prevent the red soils from being eroded, but also be used as a source of animal forage. Cultivating ryegrass in red soils and application of ryegrass as a green manure to the soils have been demonstrated to be feasible for the amelioration of the soils.[1] Direct green manure application to soil has been reported to increase soil organic matter, available N and other nutrients in the plow layer.[2] The potential of green manure to maintain or even improve soil properties and productivity is less well documented than its actual potential to substitute for inorganic N.[3]

To develop practices that increase fertilizer use efficiency and to provide sustainable red soil fertility and productivity, the present study was carried out with a view to determine the effects of fertilization with ryegrass and urea on utilization and transformation of nitrogen in red soil.

2 Materials and methods

2.1 Treatment of soil and ryegrass plantation

The red clayey soil was taken from surface layer of fallow land in Longyou county of Zhejiang Province. The properties of the soil were presented in Table 1 of Ref.[4]. The soil sample was air-dried and ground to pass through a 2 mm sieve. 250 g of each air-dried soil sample was put into 200 ml polyethylene pots and added with pulverized ryegrass or/and urea as presented in Table 1, and then mixed thoroughly. To obtain uniformly labelled ryegrass, ^{15}N labeled $(NH_4)_2SO_4$ (10.5% abundance) was applied to soil and mixed well, then ryegrass was sown in the soil one month later. The properties of the added ryegrass were shown in Table

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2. Each treatment was triplicated. The mixtures as well as the control soil were adjusted to 40% of the maximum water holding capacity (WHC). The pots were pre-incubated indoors under room temperature for 20 d. Each pot was weighed everyday and the weight loss was compensated by the addition of distilled water to keep the moisture content constant. After 20 d of incubation, 130 ryegrass seeds were sown in each pot, and then weighed in order to adjust the soil moisture as mentioned above.

Table 1 Adopted treatments and labelled fertilizer abundance in the experiment

Treatment No	Application rate of fertilizer (mg N/kg air-dried soil)					
	Urea	Ryegrass	Labelled-urea		Labelled-ryegrass	
			Rate	^{15}N abundance	Rate	^{15}N abundance
1	0	0	0	–	0	–
2	0	0	200	2.793	0	–
3	0	0	0	–	200	5.209
4	0	100	100	4.346	0	–
5	100	0	0	–	100	5.209
6	0	0	100	2.793	0	–
7	0	0	0	–	100	5.209

Table 2 The properties of the applied ryegrass

Ryegrass type	C weight fraction/%	N weight fraction/%	C/N ratio
^{15}N-ryegrass	47.36	3.52	13.45
Unlabelled ryegrass	49.39	3.33	14.83

2.2 Sampling procedure of soil and ryegrass

At the time of 20 d incubation, fresh soil sample from each pot corresponding to 5 g air-dried soil was taken for the determination of mineral N, microbial biomass N. After ryegrass seeds germinated, the pots were managed as the conventional management of ryegrass production and the soil in pots was kept in field water content during ryegrass growth period. The first harvest of ryegrass was carried out when the ryegrass grew into 17 d-old seedlings. The aboveground ryegrass was clipped. After harvest, pots were kept being managed as before. The second harvest of ryegrass was carried out after 41 d later. The whole ryegrass plants were separated from the moist soil and washed free of adhering soil under tap water. All the ryegrass plants were dried at 60°C until a constant mass. Then, the ryegrass plants were pulverized and kept in a desiccator until the determination of total N and ^{15}N abundance. After the second harvest, the fresh soil in each pot was mixed thoroughly, and soil sample corresponding to 5 g air-dried soil from each pot was taken for the determination of soil total N, microbial biomass N, mineral N, humus N and ^{15}N abundance.

2.3 Soil and ryegrass analysis

Analyses performed included total soil N and ^{15}N abundance, soil mineral N and ^{15}N abundance, soil humus N and ^{15}N abundance, soil microbial biomass N, ryegrass plant total N and ^{15}N abundance, and ryegrass dry matter mass.

Total N in the soil and ryegrass samples was determined by Kjeldahl digestion method with a mixed catalyst (mK_2SO_4:$mCuSO_4$:mSe = 100:10:1).[5] Mineral N in soil samples was extracted with 20% (w/v) NaCl and the extracts were analyzed by distillation with 10 mol/L NaOH and Zn-$FeSO_4$ (mZn:$mFeSO_4$=1:5) agent.[5,6] The microbial biomass N in soil samples was determined using a modified chloroform fumigation-direct extraction procedure.[7] Soil samples were fumigated with chloroform for 24 h in a vacuum desiccator and extracted with 30 ml 0.5 mol/L K_2SO_4 for 30 min at 25°C following the removal of chloroform. The other homogeneous soil samples (unfumigated) were extracted in the same manner when the fumigation commenced. Extracts were subjected to Kjeldahl digestion for total N determination. The difference in K_2SO_4 extractable N between the fumigated and unfumigated soil samples represented the chloroform-labile N fraction of the soils, and a correction factor (Kn) of 0.54 was applied to estimate the microbial biomass N.

For extraction and analysis of humus N, the fumigated soil samples following 0.5 mol/L K_2SO_4 extraction were extracted with 0.1 mol/L NaOH solution, the extracts were concentrated and subjected to Kjeldahl digestion and the N in extracts was measured with distillation method.[8] ^{15}N abundance of all the samples was analysed with mass spectrometer.

With the total N content and ^{15}N abundance, the amount of N in various forms from labelled source can be calculated as the following:

$$N_{dff}\ (\text{N}\ derived\ from\ fertilizer\ or\ ryegrass) = \frac{^{15}\text{N}\ atom\ percent\ excess\ of\ sample}{^{15}\text{N}\ atom\ percent\ excess\ of\ labelled\ source}$$

$$Amount\ of\ N\ in\ samples\ from\ labelled\ source = amount\ of\ total\ \text{N} \times N_{dff}$$

3 Results and discussion

3.1 Effect of application of urea and ryegrass on microbial biomass N and mineral N in soil before cultivating ryegrass

As can be seen from Fig.1, at the time of 20 d incubation, the amount of mineral N in treatment 2 was the highest among the treatments, the amount of mineral N in treatment 3 and treatment 7 was the lowest. Paul *et al.* had suggested that N immobilization occurred during the flush of microbial activity.[9] In our experiment, when ryegrass was applied alone, the amount of mineral N in soil was much less than that in the control. The reason for the decrease of mineral N may be attributed to immobilization of inorganic N in soil by microorganisms with ryegrass application. When urea was applied in combination with ryegrass (treatment 4 and treatment 5) or 200 mg ryegrass N·kg^{-1} was applied alone (treatment 3), the microbial biomass N was much higher than those in the other treatments; it indicated that addition of ryegrass stimulated the microbial activity in soil.

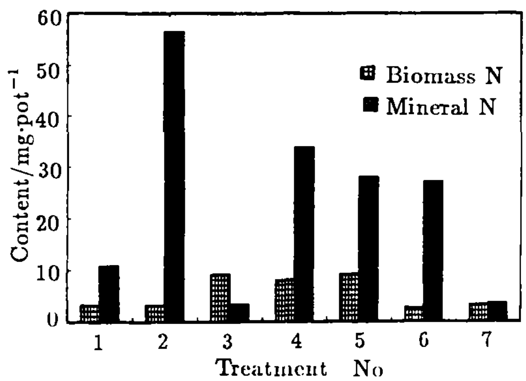

Fig.1 Microbial biomass N and mineral N in different treatments before cultivating ryegrass

3.2 Effect of different treatments on ryegrass plant dry mass and N accumulation

Dry mass of ryegrass plant and N accumulation were influenced significantly by the fertilization way (Table 3). The largest yield of dry matter was obtained when urea and ryegrass were applied in combination. The two treatments also resulted in higher accumulation of N in ryegrass plant; however, when 200 mg urea N·kg^{-1} was applied alone, the amount of N accumulated in ryegrass plant was higher than that when urea and ryegrass were applied in combination. The amount of both dry matter and N accumulation at second harvest in treatment 6 and treatment 7 was significantly less than that in the other treatments. As can be seen from Fig.1 and Table 3, the dry matter and N accumulation in ryegrass plant were related to the content of mineral N in soil to some extent. The results indicated that ryegrass growth and N accumulation in ryegrass plant mainly depended on the amount of available N in soil and that addition of organic matter to soil was also favorable to ryegrass growth and uptake of N by ryegrass.

3.3 Effect of different treatments on N transformation in red soil

As shown in Table 4, when ryegrass was applied alone or in combination with urea, the content of microbial biomass N was increased compared with that when urea was applied alone. It can be seen that application of ryegrass increased accumulation of humus N significantly. The amount of humus N with 200 mg ryegrass N·kg^{-1} application in soil was significantly higher than that with 100 mg ryegrass N·kg^{-1}. When ryegrass was applied in combination with urea, the amount of humus N was a little higher than that when 100 mg ryegrass N·kg^{-1} was applied alone (treatment 7). The increase in humus N in soil may be attributed to urea, because when urea was applied alone (treatment 2, treatment 5), the amount of humus N in soil was higher than that in the control. It can also be seen from Table 5 that when ryegrass was applied alone or in combination with urea, more ryegrass N was incorporated into red soil humus N, and more N derived from ryegrass or urea was residued in red soil than those when urea was applied alone. Among all the treatments, when urea was applied alone, the mineral N in red soil reached the highest.

Table 3 Effects of different treatments on ryegrass plant dry mass and N accumulation (per pot)

Treatment No	Dry mass of the first harvest/g	N accumulated /mg	Dry mass of the second harvest/g	N accumulated /mg
1	0.135	5.7	0.375	4.6
2	0.153	9.0	1.023	30.6
3	0.160	6.7	1.173	16.9
4	0.183	9.0	1.623	25.5
5	0.187	9.7	1.457	24.2
6	0.153	8.6	1.223	18.4
7	0.163	6.6	0.928	11.2
$LSD_{0.05}$	0.153	1.0	0.341	3.7
$LSD_{0.01}$	0.212	1.5	0.473	5.1

Table 4 The content of N in different forms in soil after ryegrass harvest (per pot)

Treatment No	Biomass N/mg	Humus N/mg	Mineral N/mg
1	0	17.8	3.1
2	1.6	19.7	5.9
3	3.9	29.3	3.6
4	2.7	25.6	2.3
5	4.1	24.9	3.5
6	3.9	19.6	3.3
7	5.1	23.5	2.8
$LSD_{0.05}$	2.4	3.6	1.0
$LSD_{0.01}$	3.4	5.0	1.3

Table 5 N derived from labelled fertilizer (N_{dff}) in soil after ryegrass harvest (per pot)

Treatment No	Humus N N_{dff}	Content /mg	Mineral N N_{dff}	Content /mg	Residual N N_{dff}	Content /mg
2	0.1294	2.6	0.3487	2.1	0.1529	15.5
3	0.3265	9.6	0.1216	0.4	0.3108	42.1
4	0.1010	2.6	0.1035	0.2	0.1053	12.5
5	0.1326	3.3	0.1192	0.4	0.1316	15.3
6	0.0717	1.4	0.3137	1.0	0.066	6.1
7	0.2074	4.9	0.1231	0.3	0.1605	18.2
$LSD_{0.05}$		0.7		0.04		1.8
$LSD_{0.01}$		1.0		0.05		2.5

3.4 Effect of different treatments on the balance of fertilizer N

It can be seen from Table 6 when urea and ryegrass were applied alone, the percentage of N uptaken by ryegrass from urea was 3 times that from ryegrass, the percentage of N transformed to humus N from ryegrass was significantly higher than that from urea, but the percentage of mineral N from ryegrass was significantly less than that from urea. Combining application of urea and ryegrass reduced the difference in N uptaken by ryegrass, recovery of humus N and mineral N from urea and ryegrass. When ryegrass and urea were applied alone, the N residued in soil from ryegrass was also significantly higher than that from urea. Combining application of urea and ryegrass could increase the residual N from urea but reduce the residual N from ryegrass in soil. There was about 60~70% of ryegrass N residued in soil, perhaps most of the ryegrass N was retained in the undecomposed forms because transformed humus N only accounted for less than 20% of the ryegrass N, furthermore, the mineral N and microbial biomass N also accounted for a very little percent of the ryegrass N. When ryegrass was applied alone, the utilization of ryegrass N by growing ryegrass was much lower than that when urea was applied alone or in combination with ryegrass, but the residual N in soil was the highest among all the treatments; furthermore, when ryegrass was applied to red soil alone, the loss of ryegrass N was reduced significantly compared with that in the other treatments. It can also be seen from Table 6 that when urea was applied alone, the utilization of urea N was the highest, but the residual N in red soil was lowest. When urea was applied in combination with ryegrass, the utilization of urea N was reduced, while the utilization of ryegrass N was significantly increased. With combined application of urea and ryegrass, the residual urea N in soil was increased significantly but the residual ryegrass N in soil was decreased significantly. Also, when urea was applied in combination with ryegrass, the loss of N was greatly reduced.

Table 6 N uptaken by ryegrass and N recoveries from labelled N after ryegrass harvest (%)

Treatment No	N uptaken by ryegrass	Recovery of humus N	Recovery of mineral N	Residual N in soil	Total recovery of N	Loss of N
2	58.6	5.2	4.2	25.0	83.6	16.4
3	19.2	19.2	0.8	70.6	89.8	10.2
4	50.0	10.4	0.8	40.8	90.8	9.2
5	28.4	13.2	1.6	60.2	88.6	11.4
6	58.8	5.6	4.0	24.4	83.2	16.8
7	19.2	19.6	1.2	69.6	88.8	11.2
$LSD_{0.05}$	5.1	1.9	1.0	7.8	5.6	–
$LSD_{0.01}$	7.1	2.6	1.4	10.9	7.8	–

From above results, it can be concluded that urea N was much more favourable to be uptaken by growing ryegrass than ryegrass N, but urea N was much more easily to be lost from soil than ryegrass N during ryegrass growth. Combining application of urea and ryegrass reduced uptake and loss of urea N and increased the urea N residued in soil compared with those when urea was applied alone, so it can be assumed that application of organic matter to soil could improve soil organic matter and reduce the loss of nutrients. The above results were identical to other reports.[10~12] From our experiment, it could be suggested that combining application of urea and ryegrass (both at the application rate of $100\,mg{\cdot}kg^{-1}$ soil) was appropriate for the growth of ryegrass plant in red soil and the improvement of red soil fertility.

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