Study of organic N transformation in red soils by ^{15}N tracer method*

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Abstract Uniformly ^{15}N -labelled ryegrass was used to investigate NH_4^+ -production, microbial transformation and humification of organic N in two types of red soils by incubating the soils amended with labelled material. The results showed that there was little significant difference in biomass N transformation in the tested soils between ^{15}N tracer method and conventional method, but the amount of NH_4^+ -N from ryegrass measured with ^{15}N method was significantly higher than that measured with conventional method. The results also indicated that there was more NH_4^+ -N released from the ryegrass in the clayey soil than in the sandy soil at all sampling time. By 120 d of incubation, humified N was less than 10% of the amount of the applied N in two types of red soils and the amount of residual N in the clayey red soil was obviously higher than that in the sandy red soil.
Keywords ^{15}N tracer method, Organic N turnover, Red soil, Ryegrass

1 Introduction

Nutrient cycling in soils is closely related to organic matter turnover. The organisms in saprophytic soil which act as a driving force in nutrient cycling rely on available organic compounds as a source of energy and carbon. Consequently, knowledge about the stabilization and decomposability of organic matter in soils is indispensable for understanding the fate of plant nutrients in organic materials added to soils. The decomposability of organic matter in soil was often studied by measuring the amount of the releasing CO_2 after adding organic materials to soils.[1] Isotope technique is widely applied to study of organic matter in soils, but mostly focused on studying decomposition of organic matters in soils with ^{14}C labelled materials[2~5]; moreover, some researchers have studied the behaviour of organic N in soils using ^{15}N -labelled materials. The research subject mainly includes the availability of N in organic matter to crops, mineralization, immobilization and microbial turnover related to mineralization of organic N in various types of soils, and utilization efficiency of straw and animal manure by crops under different conditions.[6~10]

To understand the fate of organic N in red soils, the experiment was conducted under controlled conditions using ^{15}N -labelled material. The following researches were carried out: the values of NH_4^+ -N and biomass N measured by ^{15}N method were compared with those measured by conventional method; mineralization, microbial transformation and humification of ryegrass -N were investigated.

2 Materials and methods

2.1 Soils

Two types of classic red soils (sandy and clayey soils) were selected in the study. The soil samples were taken from the surface layer of fallow land in Longyou County of Zhejiang Province. The air-dried soils were passed through a sieve of 2 mm in diameter. The basic properties of the soils were listed in Table 1.

Table 1 Properties of the soils

Item	pH (H_2O)	Olsen P/ mg·kg^{-1}	Org./ g·kg^{-1}	N/ mg·kg^{-1}	Changeability /cmol·kg^{-1}				
					K^+	Na^+	Ca^{++}	Mg^{++}	CEC
Red clayey	4.16	0.31	8.4	100	0.19	0.02	0.69	0.30	6.62
Red sandy	4.55	0.24	6.5	110	0.06	0.02	0.38	0.11	4.53

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2.2 Preparation of ^{15}N-labelled plant material

Ryegrass shoots were uniformly labelled with ^{15}N by growing them in a nutrient solution -glass granule cultural system with the presence of ^{15}N -$(NH_4)_2SO_4$. The shoots were harvested at the time of 4 weeks after germination, dried under 50°C and ground (<40 mesh). The total N content of the material was 5.01% with 0.07979 atom fraction excess of ^{15}N. N in the form of NO_3^- and NO_2^- could not be detected in the material.

2.3 Incubation of samples

5 g of each air -dried soil (<20 mesh) was put into 50 ml flasks, adjusted to 40% of soil water holding capacity (WHC), pre -incubated at 25°C for 10 d. Then each pre -incubated soil sample was mixed with 200 mg of ^{15}N -ryegrass, finally they were adjusted to 60% of WHC and incubated at 25°C. During the course of incubation, water content was kept constant by adding distilled water every day.

2.4 Sampling and analysis

The samples were taken to analyse NH_4^+ -N and biomass N on incubation of 30, 60 and 120 d, respectively. At the end of incubation (120 d), the humified N and residual N in soils were also analysed.

NH_4^+-N was extracted with 0.20 g/ml NaCl solution and determined by distillation with NaOH. The fumigation -extraction method was used to measure the biomass N.[11] After the removal of $CHCl_3$, the $CHCl_3$-fumigated soil samples were extracted immediately with 0.5 mol/L K_2SO_4 for 30 min at 25°C, the unfumigated samples were extracted at the moment when fumigation commenced. Total N in the 0.5 mol/L K_2SO_4 extracts was analysed with Kjeldahl digestion method.

The distilled solutions of NH_4^+-N biomass N were acidified, concentrated and analysed with mass spectrometer for ^{15}N abundance.

For extraction and analysis of humified N, the soil sample following 0.5 mol/L K_2SO_4 extraction were extracted with 0.1 mol/L NaOH. The extracts were adjusted to pH 1~1.5 with 0.5 volume fraction HCl solution to agglutinate the humic acid and the agglutinative substance was separated from solution by centrifugation. The fulvic acid remained in the solution.[12] The precipitates and the solution were subjected to Kjeldahl digestion to analyse total N in the form of humic acid and fulvic acid, respectively. The residue following 0.1 mol/L NaOH extraction was digested with Kjeldahl method to analyse residual N. All analyses were triplicated and the results have been expressed as % of the applied N.

NH_4^+-N extracted from samples included NH_4^+-N from both the added ryegrass and the soil. With ^{15}N tracer method, the control samples were not necessary for measuring the NH_4^+-N released from ryegrass, because the fraction of NH_4^+-N from the ^{15}N -labelled ryegrass (expressed as $NDFG$) could be calculated by measuring the ^{15}N abundance of the samples and ryegrass:

$$NDFG = \frac{^{15}\text{N atom fraction excess of sample}}{^{15}\text{N atom fraction excess of ryegrass}}$$

then the amount of NH_4^+-N from ryegrass equals $NDFG$ multiplied by the total NH_4^+-N of the sample. The value which total NH_4^+-N minus this part is equal to the amount of NH_4^+-N from soil. But with conventional method (the amount of NH_4^+-N from ryegrass is equal to the difference of the amount of NH_4^+-N between soil amended with ryegrass and control soil), whether the N was from ryegrass or from soil could not be distinguished, so control soil sample (without adding ryegrass) incubated under the same conditions should be used to measure the soil NH_4^+-N.

The flushes F_N of extractable N which are the difference between 0.5 mol/L K_2SO_4 extractable N in the fumigated samples and in the unfumigated control samples include those from ryegrass and soil. F_N from ryegrass can be calculated by measuring the ^{15}N abundance of 0.5 mol/L K_2SO_4 extractable N for both fumigated and unfumigated samples:

$F_{^{15}N}$=extractable N in fumigated sample × $NDFG(F)$ -extractable N in unfumigated sample × $NDFG(NF)$

where,

$$NDFG(F) = \frac{^{15}\text{N atom fraction excess of fumigated sample}}{^{15}\text{N atom fraction excess of ryegrass}};$$

$$NDFG(NF) = \frac{^{15}\text{N atom fraction excess of unfumigated sample}}{^{15}\text{N atom fraction excess of ryegrass}}$$

3 Results and discussion

3.1 Mineralization of organic N in red soils

By comparing the results of two methods, we found that the amount of NH_4^+-N from ryegrass measured with ^{15}N method at all sampling time was 4.4~5.7% higher than that measured with conventional method for the sandy soil and 3.6~3.8% for the clayey soil (see Table 2). It can also be found from Table 3 that the amount of NH_4^+-N from soil amended with ^{15}N-labelled ryegrass which was calculated with ^{15}N method was less than that from the control soil measured with conventional method. According to above results, it was concluded that ^{15}N tracer method may be more reliable than the conventional one for measuring mineralization of organic N.

Table 2 Amount of NH_4^+-N from ryegrass in incubated soil samples measured with ^{15}N method and conventional method

Incubation days	Sandy				Clayey			
	^{15}N		Conventional		^{15}N		Conventional	
	mg	%	mg	%	mg	%	mg	%
30	3.558	35.51	3.377	33.70	4.826	48.17	4.652	46.42
60	3.546	35.39	3.354	33.47	4.408	43.90	4.245	42.37
120	2.846	28.41	2.730	27.24	3.484	39.75	3.840	38.32

Table 3 The amount of NH_4^+-N from soil in incubated soil sample measured with ^{15}N method and conventional method

Incubation days	Sandy/mg		Clayey/mg	
	^{15}N	Con.	^{15}N	Con.
30	0.201	0.382	0.215	0.389
60	0.253	0.445	0.279	0.442
120	0.308	0.424	0.285	0.428

As can be seen from Table 2, substantial parts of organic N added to soils were mineralized to NH_4^+-N in the red soils on 30 d of incubation. The amount of NH_4^+-N didn't change on 60 d of incubation and decreased on 120 d of incubation in the sandy soil, while that in the clayey soil decreased during whole incubation. The amount of NH_4^+-N remained in the soils on 120 d of incubation was about 0.80 of that in the samples on 30 d of incubation. It can be seen from the results that the NH_4^+-N released may be utilized by microorganisms or be converted to new soil organic matter. There was more NH_4^+-N remained in the clayey soil than in the sandy soil among all the samples on 30, 60 and 120 d of incubation. One of the reasons may be that more NH_4^+-N was lost in the form of NH_3 in the sandy soil than in the clayey soil according to the other experiments (to be published). Only a little amount of NO_3^--N, NO_2^--N was detected in all samples, so it can be assumed that NO_3^--N, NO_2^--N were minor constituents involving in mineralization of organic N in the red soils.

3.2 Transformation of organic N into biomass N

As can be seen from Table 4, no significant difference was found between $F_{^{15}N}$ from ryegrass and total F_N in incubated soil samples, so it indicated that F_N from soil biomass could be neglected.

Table 4 F_N measured with two methods (mg N/sample)

Incubation days	30		120	
Measure methods	^{15}N	Con.	^{15}N	Con.
Sandy	0.851	0.814	0.541	0.566
Clayey	0.560	0.593	0.372	0.395

Table 5 Biomass N in soils amended with ^{15}N-labelled ryegrass

Incubation days	sandy		clayey	
	mg	% of app.N	mg	% of app.N
30	1.577	15.75	1.037	10.36
60	1.896	18.94	–	–
120	1.001	10.60	0.689	6.88

The biomass N from ryegrass could be calculated by applying a F_N value of 0.54 according to Brookes's *s* suggestion.[11]. The results in

Table 5 showed that 15.75% and 10.35% of organic N on 30 d of incubation was converted into biomass N in the sandy soil and in the clayey soil, respectively. The biomass N increased significantly on 60 d of incubation in the sandy soils. By the end of incubation, biomass N both in the sandy and clayey soils decreased by more than 30% compared with that in the sample incubated for 30 d.

3.3 Humification of organic N in the red soils

At the end of incubation (120 d), after the samples were extracted with 0.5 mol/L K_2SO_4 to exclude the extractable N, the residues were extracted with 0.1 mol/L NaOH. The extracts were treated with HCl in order to separate humic acid and fulvic acid, the N in humic acid and fulvic acid then was analysed. The black soil samples were also treated with the same procedure. The increased amount of N in humic acid (hn) and fulvic (fn) by adding ryegrass was considered as the amount of N humified from ryegrass.

The results in Table 6 showed that the extractable humic N in the clayey soil samples was a little higher than that in the sandy soil samples, but it didn't exceed 10% of the applied organic N, hn was a little higher than fn in the sandy soils, but it was opposite in the clayey soils. The residues after extracting with 0.1 mol/L NaOH thoroughly were digested and analysed for residual N in soils. The residual N includes inextractable humic N, NH_4^+-N immobilized by soil particles and undecomposed organic N. The amount of residual N in soils accounted for 0.0682 and 0.2042 of the applied organic N in the sandy and clayey soils, respectively.

Table 6 Amount of humic N in soil samples incubated with ryegrass for 120 d

Item	Humic acid		Fulvic acid		sum	
	mg	% of app.N	mg	% of app.N	mg	% of app.N
Sandy	0.427	4.27	0.359	3.58	0.786	7.84
Clayey	0.461	4.60	0.536	5.34	0.997	9.95

Table 7 Recoveries (%) of various N from samples

Item	NH_4^+-N	Bio. N	Hum. N	Res. N	Sum
Sandy	28.41	10.01	7.84	6.82	53.08
Clayey	39.75	6.88	9.95	20.42	77.00

From the results in Table 7, we can see that added organic N has different fates in the sandy and clayey soils. More mineralized N and residual N, which may be mainly in undecomposed form, remained in the clayey soils than in the sandy soils, but more N in the form of NH_3 was left in the sandy soils than in the clayey soils according to the other experiments (to be published). The results also showed that microorganisms were more active in aerated sandy soils than in clayey soils.

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