Biol Fertil Soils (1991) 12:182–188

Biology and Fertility of Soils

Immobilization of nitrogen from urea and plant residues in a ferrallitic soil: Laboratory experiments and study by size-fractionation

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Received October 15, 1990

Summary. The fate of N when incorporated in a ferrallitic soil was investigated during a 3-month incubation, using either ^{15}N-labelled urea or ^{15}N-labelled crop residues (sugarcane roots and leaves). The organic matter was characterized by particle-size fractionation. The urea-derived organic ^{15}N was mainly found in the clay-sized fractions and was ascribed to biological activity. The plant-derived ^{15}N was observed both in the sand-sized and in the clay-sized fractions; the former pool was ascribed to the persistence of crops residues, the latter to biological immobilization. The relative proportions of organic ^{15}N recovered in the various clay fractions (coarse, fine, and very fine) were similar, irrespective of the nature of the added ^{15}N. The very fine clay fraction ($F < 0.05$ µm) showed the highest isotopic excess, and thus gave rise to the highest turnover rate.

Key words: Nitrogen – urea – crop residues – particle-size fractionation – ferrallitic soil

In tropical agricultural soils lacking N_2 fixation, about 50% of the N uptake by plants is known to be derived from chemical fertilizers, whereas the other part is provided by organic amendments, crop residues, and soil organic matter (Guiraud et al. 1979; Chabalier 1985; Chotte et al. 1990). Therefore, a fairly good understanding of the nature and dynamics of soil organic N is required before improvements in soil management and crop yield can be obtained.

In the past, soil organic matter and soil organic N were studied mainly by chemical fractionation (acid hydrolysis and alkaline extraction). The resulting chemical compartments, however, were poor representatives of the functional ones, and this traditional approach provided very little insight into soil N turnover, especially in cultivated soils (Tiessen et al. 1984). During the last two decades, physical fractionation methods (involving size-fractionation procedures and densimetry) have provided more representative compartments. These procedures have given rise easily to the isolation of two very different entities, viz., a pool of a poorly transformed organic material, associated with sand-sized and coarse silt-sized fractions, and a pool of humified, amorphous, organic matter, associated with fine-silt-sized and clay-sized fractions (Oades and Turchenek 1978; Adams 1982; Feller et al. 1983; Tiessen and Stewart 1983; Balesdent et al. 1987, 1988).

This type of approach has been used in the past to investigate N mineralization and immobilization processes in temperate soils (McGill et al. 1975; Ladd et al. 1977a, b, but only a few of the data refer to tropical soils. The aim of the present work was to study N immobilization in a clayey ferrallitic soil cultivated with sugarcane. ^{15}N-labelled urea and plant fragments were used as N sources in a laboratory ^{15}N-tracer experiment, and a particle-size fractionation method was implemented.

Materials and methods

Soil used

The soil samples were taken from the 0–20 cm layer of a ferrallitic soil (Clayey Eutropept) under sugarcane (*Saccharum officinarum* L.) in Martinique. The field was part of the Galion farm, in an area subjected to about 1800 mm of annual rainfall. A composite sample was obtained by mixing 30 subsamples taken randomly within a 400-m^2 surface. Table 1 shows the main physicochemical features.

Production of crop residues

^{15}N-labelled roots and leaves were obtained from a previous greenhouse pot experiment. Sugarcane (cultivar B64 277) was cultivated on the selected soil in 20-kg jars with ^{15}N-labelled urea plus K_2HPO_4 fertilizer (64 µg P and 166 µg K g^{-1} soil). After 9 months of growth, the sugarcane roots were removed by hand, carefully washed with water, and dried at 50 °C. The main veins of the leaves were separated from the limb with scissors. Roots, foliar veins, and limbs were cut into fragments. Only fragments ranging from 2 to 10 mm and 0.2 to 2 mm for

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roots, and from 2 to 10 mm for foliar veins and limbs, were weighed and set aside for the experiment.

Incubation experiments

Urea fertilizer and the root and leaf fragments were used in the following three experiments (four replicates for each treatment): (1) ^{15}N-urea fertilizer: A solution of ^{15}N-labelled urea (isotopic excess 30.09‰) plus K_2HPO_4 was added to 143 g dry soil in 200-cm^3 pots, so that field capacity was obtained (determined at pF 2.5). N, P, and K were added at 172, 38, and 96 μg g^{-1} soil, respectively. (2) Addition of ^{15}N-labelled roots: 0.37 g of 0.2–2 mm ^{15}N-labelled root fragments plus 1.01 g of 2–10 mm fragments were mixed with 143 g dry soil. Unlabelled urea plus K_2HPO_4 fertilizers were then added as in the previous experiment. (3) Addition of ^{15}N-labelled leaves: 0.58 g leaf-limb fragments plus 0.8 g vein fragments were mixed with 143 g dry soil. Unlabelled urea plus K_2HPO_4 fertilizer were then added as above. The corresponding additions of C and N, and their isotopic excess for both root and leaf fragments, are shown in Table 2.

The incubation experiments were performed in the dark for 3 months at 27–30 °C. The jars were alternatively covered and opened for 2 days and their moisture content was adjusted to the initial value every 4 days by weighing them and adding water as required. Changes in the water content reached 10% of the initial moisture level. No leaching of water with ^{15}N occurred.

Table 1. Main features of the soil studied

Analysis	Content
X-ray diffraction of clay minerals (<2 μm)	K (+++) H (+++) Qz (+) Cr (+)
Clay + fine silt content (% soil)	63.9
C (% soil)	2.37
N (‰ soil)	2.2
pH_{H_2O}	5.7
pH_{KCl}	4.8
Total P_2O_5 (‰ soil)	1.23
Available P_2O_5 (‰ soil)	0.05
Exchange capacity (mEq 100 g^{-1})	18.3
Exchangeable bases (mEq 100 g^{-1})	8.7
Bulk density (g cm^{-3})	1.1
H_2O content at pF 2.5 (% soil)	34.5
H_2O content at pF 4.2 (% soil)	25.9

K, kaolinite; H, halloysite; Qz, quartz; Cr, cristobalite; +, not abundant; +++, very abundant

Particle-size fractionation of soil organic matter

Before and at the end of the incubation period, a particle-size fractionation of the organic matter was performed according to Fig. 1. (François 1988). Briefly, 33 g dry soil was suspended overnight in distilled water at 4 °C, and stirred at room temperature (23 °C) for 2 h on a rotary shaker (50 min^{-1}) in the presence of three glass beads of 1.5 cm diameter. The pH was maintained close to 10 by adding the necessary volume of 0.1 *M* NaOH every 15 min. The soil was sieved at 2000 and at 200 μm, sonicated (20 kHz, 8 A, 10 min), and sieved again to pass 50 and 25 μm. The particles <25 μm were centrifuged at 1430 g for 10 min at 23 °C, up to the quantitative extraction of a dispersed fraction (*F* 0–0.2 μm). This suspended material was flocculated with a saturated KCl solution, washed with 1 *M* KCl, centrifuged at 10000 g and washed three times with distilled water. Both a solid fraction (0–0.2 μm) and a pool of soluble compounds ("W") were obtained. The previously sedimented particles, ranging from 0.1 to 25 μm, were scattered and allowed to settle in order to obtain the following three fractions: 0.2–2 μm, 2–5 μm, and 5–25 μm. All the fractions were dried at 50 °C.

The reproductibility of this procedure was tested for the weight of each fraction obtained and the C and N content of each fraction. The recovery rate of the total soil reached 99.1±1.1% on a weight basis. Recovery of C reached 90.9±6.8% and of N, 92.7±4.1%.

In addition, with the same fractionation steps, taken just after the 25-μm sieving step, a very fine clay fraction (0–0.05 μm) was isolated from the 0–25 μm material as follows. The 0–25 μm suspension was centrifuged at 10000 g for 20 min, and then the suspended material (0–0.05 μm) was flocculated with 1 *M* KCl and recovered by centrifugation (10000 g, 10 min).

Morphological characterization of the fractions

In order to test the reliability of the separation, the various fractions were examined with a binocular lens and both light and transmission electron microscopes.

Analytical determinations

Organic C was measured in the soil, fractions, and plant fragments with a Carlo Erba analyzer CHN 1106. A total N analysis was performed on the same material, according to Guiraud and Fardeau (1977) in order to include NO_3^--N. Exchangeable inorganic N was extracted from 38 g dry soil with 1 *M* KCl (1:5 w:v). Analysis of NH_4^+, NO_2^-, and NO_3^- was performed by steam distillation (Bremner 1965): A fixed N analysis was performed according to Silva and Bremner (1966). Organic N was calculated as the difference between total N and inorganic exchangeable plus fixed N. Gaseous losses of labelled N were asusmed to be equal to the unrecovered ^{15}N determined at the end of the experiment. The ^{15}N abundance in the various fractions was obtained with a mass spectrophotometer (VG Micromass 622). The uncertainties affecting both N analysis and ^{15}N determinations, tested on a finely ground soil sample (10 replicates), reached 16 μg N g^{-1} and 0.5 μg ^{15}N g^{-1}, respectively.

The specific surface area of the various clay fractions was measured by N_2 adsorption. The N_2 gas adsorption isotherms were obtained at liquid N temperature (77 K) according to Feller et al. (1991).

Table 2. Analytical features of buried crop residues (sugarcane roots and leaves)

		Buried ovendry material (mg g^{-1} soil)	Buried C (mg g^{-1} soil)	Buried N (mg g^{-1} soil)	C:N	Isotopic excess
Root fragments	Total	9.65	4.19	0.061	63	12.63
	>2000 μm	7.06	3.07	0.045	68	12.71
	200–2000 μm	2.59	1.12	0.016	52	12.46
Leave fragments	Total	9.65	4.54	0.098	47	16.3
	Limbs	4.06	1.91	0.061	32	16.53
	Veins	5.59	2.63	0.037	71	16.16

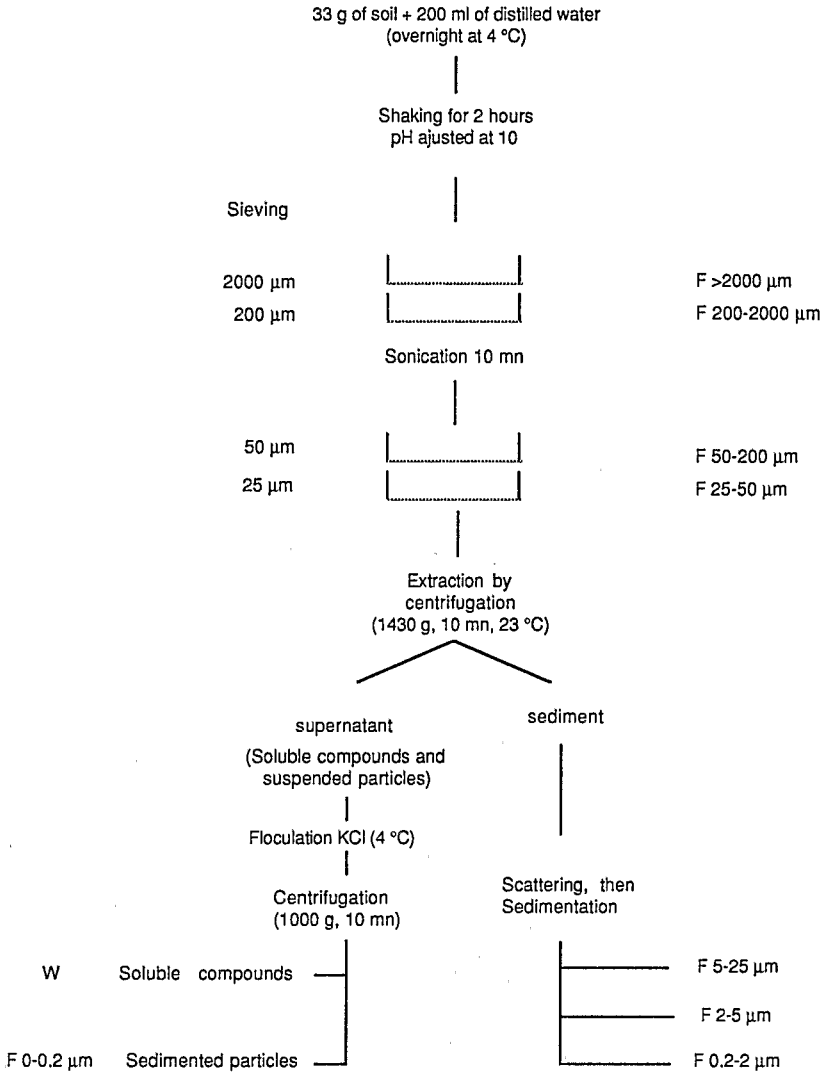

Fig. 1. Major procedure used for particle-size fractionation of soil organic matter

Results

Morphological and chemical features of the various fractions

Morphological and chemical features of the various fractions have been described elsewhere (François 1988); only a summary is presented here.

The organic matter included within the sand- (>50 µm) and coarse silt-sized (25–50 µm) fractions consisted predominantly of more or less decaying plant fragments. This organic matter contained about 24.9±3.7% of soil C and 13.1±2.1% of the soil organic N. The average C:N ratio varied from 33 for the >2000 µm fraction to 16 for the 25–50 µm fraction.

The finer silt-sized fractions (5–25 µm and 2–5 µm) contained a mixture of both isolated mineral particles and microaggregates, the latter comprising agglomerates of silt, clays, bacteria, fungi, and plant fragments. These fractions contained about 20±2% of the soil organic C and 16.9±2% of the soil organic N. The average C:N ratio varied from 14.4 (5–25 µm) to 11.8 (2–5 µm).

The organic matter of the coarse, fine, and very fine clay-sized fractions (0.2–2 µm, 0.05–0.2, and 0–0.05 µm) was predominantly amorphous, mixed with very scarce plant and microbial fragments. These fractions contained about 46±2.3% of the total soil C and 58±2.3% of the total soil N. The average C:N ratio varied from about 8 to 10.

Recovery rate of added ^{15}N at the end of 3 months of incubation

The recovery rate of the added ^{15}N, determined after 3 months of incubation for each treatment, is shown in Table 3. Unrecovered ^{15}N (i.e., probable gaseous losses) reached 9.4% for the urea-treated samples, 7.3% for the sugarcane roots (enriched samples) and 11.4% for the sugarcane leaves (enriched samples). Inorganic C derived from the added ^{15}N reached about 67% in the urea-treated samples, but only 18–19% in the enriched plant fragments, and was always exclusively in the NO_3^- form. Samples enriched with ^{15}N-labelled plant fragments contained a much greater quantity of organic ^{15}N (70–75% of the ^{15}N added) than those amended with urea (23.5%), presumably indicating that some of the added labelled plant fragments were rather persistent. Fixed ^{15}N

Table 3. Recovery of added ^{15}N determined after 3 months of incubation as a percentage of ^{15}N added

Experiment	Recovered ^{15}N (%)				Unrecovered ^{15}N (%)
	Total ^{15}N	Exchangeable inorganic ^{15}N (only NO_3^--N)	Organic ^{15}N	Fixed ^{15}N	
^{15}N urea	90.6 ± 1.8	66.9 ± 5.1	23.5 ± 6.2	< 0.1	9.4
^{15}N roots	92.7 ± 12.7	18.0 ± 1.5	74.7 ± 12.9	< 0.1	7.3
^{15}N leaves	88.6 ± 6.7	19.0 ± 3	69.6 ± 9	< 0.1	11.4

Means of four replicates ± SE

Table 4. Distribution of organic N (N_{org}) and ^{15}N in various fractions after 3 months of incubation

Experiment	Fractions								
	$F > 2000$ µm	$F 200-2000$ µm	$F 50-200$ µm	$F 25-50$ µm	$F 5-25$ µm	$F 2-5$ µm	$F 0.2-2$ µm	$F 0-0.2$ µm	Sum
^{15}N urea N_{org} (mg g^{-1} soil)	0.008	0.049	0.058	0.097	0.135	0.181	0.565	0.627	
Isotopic excess	0.291	0.610	0.246	0.287	0.266	0.363	0.443	0.500	
$^{15}N_{org}$ (% added ^{15}N)	0.05	0.6	0.3	0.5	0.7	1.3	4.9	6.0	14.4
$^{15}N_{org}$ (% recovered ^{15}N)	0.3	4.2	2.1	3.5	4.9	9.1	34.1	41.8	100
^{15}N roots N_{org} (mg g^{-1} soil)	0.009	0.091	0.069	0.085	0.151	0.133	0.557	0.575	
Isotopic excess	5.740	2.360	0.233	0.162	0.11	0.113	0.141	0.203	
$^{15}N_{org}$ (% added ^{15}N)	6.7	26.5	2	1.7	2	1.9	9.7	14.4	64.9
$^{15}N_{org}$ (% recovered ^{15}N)	10.3	40.8	3.1	2.6	3.1	2.9	14.9	22.2	100
^{15}N leaves N_{org} (mg g^{-1} soil)	0.021	0.1	0.07	0.103	0.157	0.163	0.584	0.530	
Isotopic excess	6.82	3.22	0.322	0.403	0.308	0.344	0.272	0.368	
$^{15}N_{org}$ (% added ^{15}N)	9.3	20.4	1.4	2.6	3.1	3.5	10.1	12.4	62.8
$^{15}N_{org}$ (% recovered ^{15}N)	14.8	32.5	2.2	4.1	4.9	5.6	16.1	19.7	100

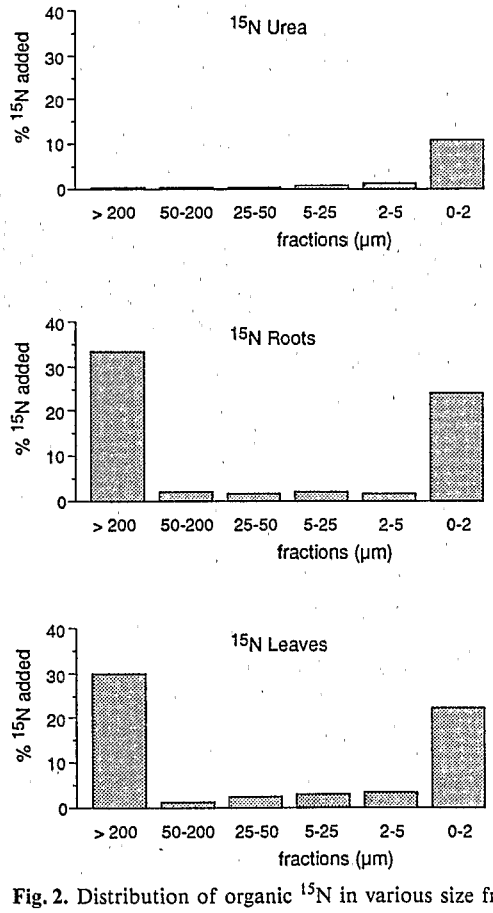

Fig. 2. Distribution of organic ^{15}N in various size fractions at the end of the 3 months of incubation

was always less than 0.1% of the initial ^{15}N added, and is not considered further. This was due to the mineralogical features of the soil, where kaolinite and halloysite constituted the major clay minerals (Table 1).

Distribution of organic ^{15}N among the various fractions of the soil

The distribution of the organic ^{15}N among the various fractions of the soil is shown in table 4 and figure 2. The organic ^{15}N in the W fraction (soluble compounds) was always less than 0.1% of the initially added ^{15}N, and therefore is not considered further.

About 9.1, 9.8, and 6.8% of the added ^{15}N was lost during the fractionation procedure in samples amended with urea, sugarcane roots, and sugarcane leaves, respectively. Consequently, the ^{15}N recovery rate fell from 90.4 to 81.3%, from 92.7 to 82.9%, and from 88.6 to 81.8%, respectively, in these samples.

On the whole, the distribution of organic ^{15}N within the various fractions in the urea-amended samples was different to that of the samples enriched with plant fragments. In the former, about 11% of the initially added ^{15}N (i.e., 76% of the organic ^{15}N recovered) was recovered in the clay-size fractions (0.2–2 µm and 0–0.2 µm), whereas the rest of the soil contained less than 3.5% of this initial ^{15}N (i.e., less than 24% of the organic ^{15}N recovered). In contrast, in the samples amended with plant fragments, 22–24% of the initially added ^{15}N (i.e., 35–37% of the organic ^{15}N recovered) was similarly recovered in the corresponding clay-sized fractions, but a

second large pool of organic ^{15}N occurred in the two coarsest fractions (30–33% of the added ^{15}N, i.e., 47–51% of the organic ^{15}N recovered). A more detailed examination of the different fractions showed that the proportion of organic ^{15}N contained within the >2000 µm and 200–2000 µm fractions changed dramatically during the 3-month incubation period (Table 5). In the samples amended with root fragments, the organic ^{15}N present in the >2000 µm fraction decreased from 68.8 to 6.7% of the added ^{15}N whereas the proportion in the 200–2000 µm fraction did not change appreciably. In the leaf enriched samples, all the added ^{15}N was initially contained in the >2000 µm fraction. At the end of the incubation period, however, this fraction contained only 9.3% of the added ^{15}N, whereas 20.4% had been transferred to the 200–2000 µm fraction. Thus, the added plant residues were rapidly fragmented when mixed in with the soil. Surprisingly, the medium-sized fractions did not appear to be significantly enriched with ^{15}N as a consequence of these fragmentation processes (Fig. 2).

Among the three treatments, the 0–0.2 µm fraction was more enriched with organic ^{15}N than the 0.2–2 µm fraction (Table 4). This was attributed mainly to the very fine clay fraction (0–0.05 µm) (Table 6), in which the isotopic excess was always greater than that in the two other clay fractions. This 0–0.05 µm fraction also had the highest total C and N contents. The organic ^{15}N distribution among the three clay fractions (expressed as a percentage of the total organic ^{15}N in the 0–2 µm material) was very similar for all three treatments, and appeared to bear some relationship to the specific surface area (Table 6).

Table 5. Variation in added ^{15}N-labelled plant fragments within the two coarsest fractions during 3 months of incubation

Experiment	Fractions	Before incubation	After incubation
Roots	*F*>2000 µm	68.8	6.7
	*F*200–2000 µm	31.2	26.5
Leaves	*F*>2000 µm	100	9.3
	*F*200–2000 µm	0	20.4

Values show ^{15}N as a percentage of ^{15}N added to samples of root and of leaf fragments at the beginning of the experiment

Discussion

Both the morphological and the chemical features of the various fractions supported the reliability of the particle-size fractionation scheme that was used.

On a morphological basis, the isolation of three fractions, <2 µm, 2–25 µm, and F>25 µm, was technically easy and gave a fairly good separation between the poorly transformed plant residues (predominantly in the >25 µm fractions) and the amorphous, humified, organic compounds (predominantly in the <2 µm fractions). The medium-sized fractions, however, remained a mixture, and the microbial biomass was obviously distributed among fractions containing "non-humified" as well as "humified" organic matter. The fungal biomass, which was found predominantly within the medium-sized fractions (2–25 µm), is known to contain humic acid-like polymers (Linhares and Martin 1978) and thus is likely to constitute a mixture of unseparable (on a physical basis at least) fine fragments and amorphous, humic-like, compounds. The proposed fractionation procedure does not provide a good separation of this mixture. However, as the microbial biomass contains barely more than 5% of the total soil organic C and 10% of the total soil N (Adams and Laughlin 1981; Anderson and Domsch 1989), this should not constitute a real problem.

On a chemical basis, the C:N ratio of the various fractions gradually decreased from the coarsest (C:N = 16–33), to the medium (C:N = 11–14), and the finest fractions (C:N = 8–10), reflecting the progressive increase in the degree of humification in their respective organic matter contents. This confirmed, from a chemical point of view, the large difference seen between the fractions >25 µm and those <2 µm.

Under our experimental conditions, the ^{15}N that remained unrecovered at the end of the 3-month incubation period probably represents gaseous losses. These losses were quite low, however, and did not exceed 12% of the added ^{15}N. For the samples enriched with plant fragments, these values agree very well with data by Kanazawa and Yoneyama (1980a, b), Ladd et al. (1981), and Feller et al. (1982). Gaseous losses following the application of urea fertilizer are known to vary considerably, depending on experimental conditions. In the pre-

Table 6. Chemical, isotopic, and physical features of the coarse (0.2–2 µm), fine (0.05–0.2 µm), and very fine (0–0.05 µm) clay fractions

Experiment	Fraction (µm)	C (mg g^{-1})	N (mg g^{-1})	E %	$^{15}N_{org}$ (% total recovered)	$^{15}N_{org}$ (% in clay material)	SSA (% of total SSA of 0–2 µm fraction)
Urea	*F*0.2–2	17.2	2.1	0.443	34.1	44.9	40.2
	*F*0.05–0.2	19.5	2.3	0.411	27.2	35.8	49.3
	*F*0–0.05	38.0	4.3	0.827	14.6	19.2	10.5
Roots	*F*0.2–2	16.8	2.0	0.141	14.9	46.6	39.9
	*F*0.05–0.2	17.4	2.2	0.185	12.0	37.5	51.9
	*F*0–0.05	35.1	3.5	0.305	5.1	15.9	8.3
Leaves	*F*0.2–2	17.5	2.1	0.272	16.1	51.1	41.4
	*F*0.05–0.2	17.0	2.2	0.338	11.1	35.2	50.5
	*F*0–0.05	33.1	3.3	0.546	4.3	13.7	8.1

E %, atom % ^{15}N excess; $^{15}N_{org}$, organic ^{15}N; SSA, BET-N_2 specific surface areas of 40, 63.4, and 91.3 m^2 g^{-1} for the 0.2–2 µm, 0.05–0.2 µm, and 0–0.05 µm fractions, respectively

sent work, the low ^{15}N-urea losses can be attributed to the following factors: (1) The urea was added in solution and therefore mixed rapidly with the soil; (2) the soil pH remained in the acidic range during the early days of the experiments (data not shown), which is likely to have promoted a rapid conversion of NH_3 to NH_4^+; and (3) the soil had a fairly high cationic exchange capacity (18 mEq $100\,g^{-1}$), which is likely to have favoured the retention of NH_4^+.

The particle-size fractionation procedure that was used allowed us to individualize several pools of organic N after the 3-month incubation period. There were considerable differences between samples enriched with plant fragments and those enriched with ^{15}N urea. In the former, the organic ^{15}N occurred mainly in the two coarsest fractions (>200 µm material), about 50% and in the finest fractions (<2 µm material; 35–37%). Conversely, in the ^{15}N urea-enriched samples only 5% of the organic ^{15}N was included in the >200 µm material whereas 76% occurred within the <2 µm fractions. Since there was no possibility of residually adsorbed urea, owing to the fractionation procedure used, this latter ^{15}N pool must be ascribed either to biological or chemical immobilization processes. Chemical immobilization (mainly by NH_3 fixation on organic matter) was considered negligible, due to the acidic pH of the soil. Therefore these data suggest strongly that the major part of the organic ^{15}N in the >200 µm material from the samples enriched with leaf and root fragments was mainly inherited from the initially added ^{15}N-labelled debris. Conversely, the organic ^{15}N located in the <2 µm fractions comprised the main proportion of the biologically immobilized ^{15}N, which favoured organic N accumulation in the clay fractions rather than in the coarsest ones, despite the high C:N ratio in the coarsest fractions.

The change affecting the organic ^{15}N distribution within the >200 µm material during the 3-month incubation period revealed an important fractionation process. However, there was no appreciable transfer of ^{15}N-labelled plant debris to the medium-sized fractions (2–200 µm). This suggests that the fragmentation of the plant debris strongly favoured rapid mineralization.

As a proportion of the total organic ^{15}N included in the 0–2 µm material, the coarse, fine, and very fine clay fractions contained 45–50%, 35–40%, and 14–19%, respectively, whatever the nature of the initially added material (Table 6). This distribution seems to be more or less related to the specific surface area of the corresponding clay fractions, which represented 40, 50, and 10% of the total specific surface area of the clay-sized material (Table 6). This suggests some relationship between the specific surface area and the storage of immobilized organic ^{15}N. Further, since the finest of these three clay fractions exhibited the highest isotopic excess, it appeared to be the most active one in terms of biological immobilization of N and, presumably, was enriched with microbial metabolites. This assumption agrees with other findings (^{14}C, ^{15}N, and $\delta^{13}C$ studies) related to C and N turnover in fine and/or very fine clay fractions in temperate soils (McGill et al. 1975; Ladd et al. 1977a, b; Amato and Ladd 1980; Balesdent et al. 1987). Our results in tropical soils therefore support previous findings made in temperate soils on the nature and dynamics of soil organic N.

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