

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

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Summary. The fate of N when incorporated in a ferrallitic soil was investigated during a 3-month incubation, using either ¹⁵N-labelled urea or ¹⁵N-labelled crop residues (sugarcane roots and leaves). The organic matter was characterized by particle-size fractionation. The urea-derived organic ¹⁵N was mainly found in the clay-sized fractions and was ascribed to biological activity. The plant-derived ¹⁵N 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 ¹⁵N recovered in the various clay fractions (coarse, fine, and very fine) were similar, irrespective of the nature of the added ¹⁵N. The very fine clay fraction ($F < 0.05 \mu\text{m}$) 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 N₂ 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; Chabaliér 1985; Chotte et al. 1990). Therefore, a fairly good understanding of the

cadés, 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. 1977 a, 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. ¹⁵N-labelled urea and plant fragments were used as N sources in a laboratory ¹⁵N-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

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 $\mu\text{g g}^{-1}$ 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 fertilizers were then added as above. The corresponding

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 (F0–0.2 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:

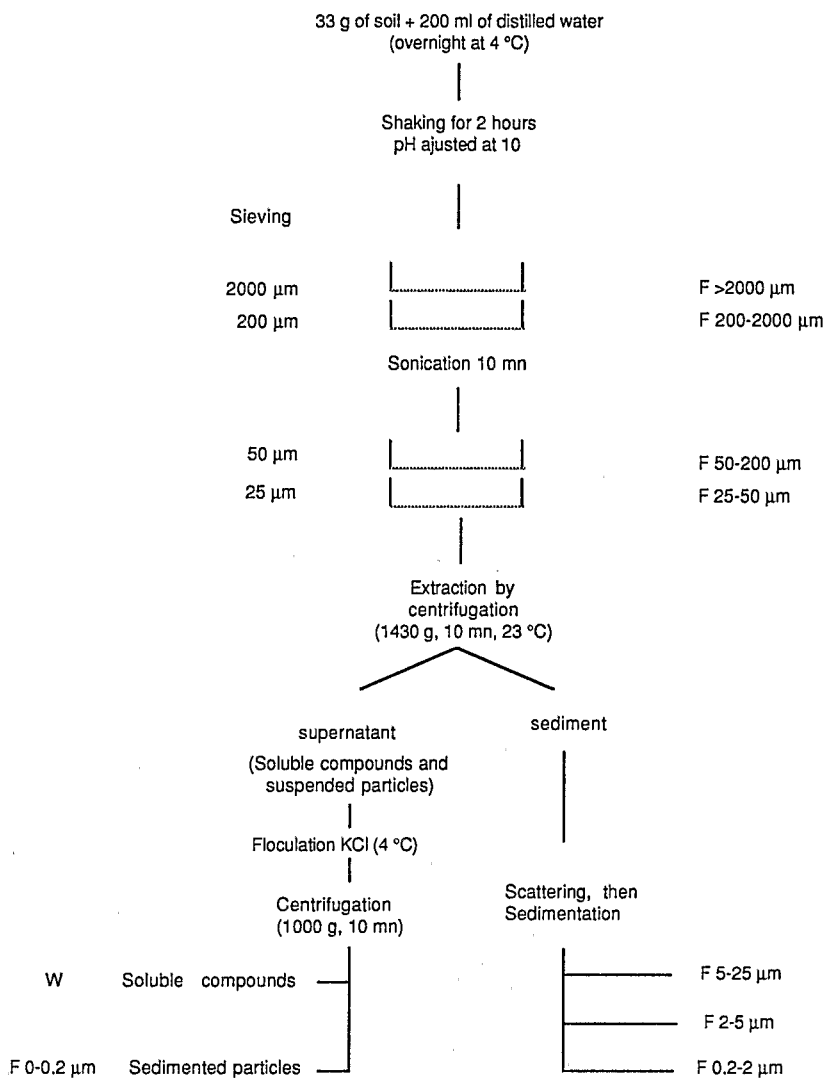


Fig. 1. Major procedure used for particle-size fractionation of soil organic matter

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 ($>50\ \mu\text{m}$) and coarse silt-sized ($25\text{--}50\ \mu\text{m}$) fractions consisted predominantly of more or less decaying plant fragments. This organic matter contained about $24.9\pm 3.7\%$ of soil C and $13.1\pm 2.1\%$ of the soil organic N. The average C:N ratio varied from 33 for the $>2000\ \mu\text{m}$ fraction to 16 for the $25\text{--}50\ \mu\text{m}$ fraction.

The finer silt-sized fractions ($5\text{--}25\ \mu\text{m}$ and $2\text{--}5\ \mu\text{m}$) 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 $20\pm 2\%$ of the soil organic C and $16.9\pm 2\%$ of the soil organic N. The average C:N ratio varied from 14.4 ($5\text{--}25\ \mu\text{m}$) to 11.8 ($2\text{--}5\ \mu\text{m}$).

The organic matter of the coarse, fine, and very fine clay-sized fractions ($0.2\text{--}2\ \mu\text{m}$, $0.05\text{--}0.2$, and

$0\text{--}0.05\ \mu\text{m}$) was predominantly amorphous, mixed with very scarce plant and microbial fragments. These fractions contained about $46\pm 2.3\%$ of the total soil C and $58\pm 2.3\%$ 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

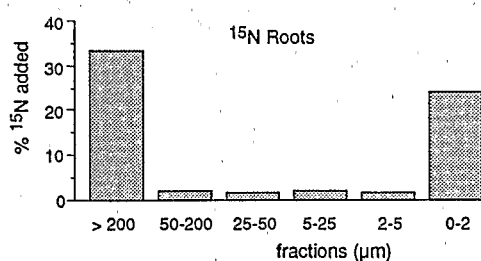
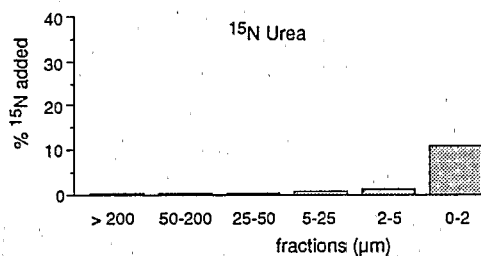
Table 3. Recovery of added ^{15}N determined after 3 months of incubation as a percentage of ^{15}N added

Experiment	Recovered ^{15}N (%)				Unrecovered ^{15}N (%)
	Total ^{15}N	Exchangeable inorganic ^{15}N (only $\text{NO}_3^- - \text{N}$)	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								Sum
	$F > 2000 \mu\text{m}$	$F 200 - 2000 \mu\text{m}$	$F 50 - 200 \mu\text{m}$	$F 25 - 50 \mu\text{m}$	$F 5 - 25 \mu\text{m}$	$F 2 - 5 \mu\text{m}$	$F 0.2 - 2 \mu\text{m}$	$F 0 - 0.2 \mu\text{m}$	
^{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	
$^{15}\text{N}_{\text{org}}$ (% added ^{15}N)	0.05	0.6	0.3	0.5	0.7	1.3	4.9	6.0	14.4
$^{15}\text{N}_{\text{org}}$ (% 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	
$^{15}\text{N}_{\text{org}}$ (% added ^{15}N)	6.7	26.5	2	1.7	2	1.9	9.7	14.4	64.9
$^{15}\text{N}_{\text{org}}$ (% 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	
$^{15}\text{N}_{\text{org}}$ (% added ^{15}N)	9.3	20.4	1.4	2.6	3.1	3.5	10.1	12.4	62.8
$^{15}\text{N}_{\text{org}}$ (% recovered ^{15}N)	14.8	32.5	2.2	4.1	4.9	5.6	16.1	19.7	100

 ^{15}N Leaves

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

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

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 frac-

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 ($18 \text{ mEq } 100 \text{ g}^{-1}$), 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 ($> 200 \mu\text{m}$ material), about 50% and in the finest fractions ($< 2 \mu\text{m}$ material; 35–37%). Conversely, in the ^{15}N urea-enriched samples only 5% of the organic ^{15}N was included in the $> 200 \mu\text{m}$ material whereas 76% occurred within the $< 2 \mu\text{m}$ 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 $> 200 \mu\text{m}$ 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 $< 2 \mu\text{m}$ 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 $> 200 \mu\text{m}$ 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

soils therefore support previous findings made in temperate soils on the nature and dynamics of soil organic N.

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