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STUDY BY SIZE FRACTIONATION OF ORGANIC MATTER IN A CULTIVATED TROPICAL SOIL FERTILIZED WITH LABELLED CROP RESIDUES (^{14}C ^{15}N) AND UREA (^{15}N)*

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ABSTRACT

In Senegal, two successive millet crops were grown on a sandy soil amended with ^{14}C and ^{15}N-labelled maize straw and ^{15}N-labelled urea. After cultivation, five size fractions were separated from the top soil : size larger than 2000 µm (F2000), size 200-2000 µm (F200), size 50-200 µm (F50), size 0-50 µm (OMF) and water soluble (W) fraction. Fraction F2000 was obtained by dry-sieving, F200 and F50 by sieving under water, OMF by flocculation at pH 2.0 and W by centrifugation. Fractions F2000, F200 and F50 contained plant debris of various sizes, fraction OMF was humified organo-mineral fraction, and W was the water-soluble fraction of soil organic matter. The utilization coefficients of ^{15}N from two sources, labelled straw and labelled urea, were about 25 per cent. The ^{15}N losses were nil from straw but were about 50 per cent from urea. ^{15}N-labelled straw residues were mainly represented in F2000 F200, F50, whereas urea-derived ^{15}N was mainly present in water soluble (W) fraction and to a small extent in the OMF fraction. The main part of residual-^{14}C in soil was found in fractions larger than 50 µm. In the transformations of organic matter in soil, the size fractionnation approach combined with the use of labelled amendments allowed to study the fate of carbon and nitrogen of the added plant residues and the role of each size fraction in the humification processes.

It is well known that in intensive agriculture, the non-return of crop residues accompanies, in soils recently cleared as well as in soils under cultivation from very ancient times, a decrease in organic matter, a degradation in physical and chemical properties and a lowering in the average yield of crops (in Senegal, the works of Bouyer, 1959; Diatta, 1975; Fauck *et al.*, 1969; Feller and Milleville, 1977; Siband, 1974). In semi-arid tropics, the research on fertility of generally poor soils has shown the importance of organic matter in maintaining their fertility (Pichot, 1975). The previous studies showed that on sandy soils it was possible to increase the level of soil organic matter (Feller *et al.*, 1981) and the availability of nitrogen for plant (Guiraud *et al.*, 1980) by combining the burying of crop residues (compost) with nitrogen fertilization (urea).

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The respective effect of organic amendment and nitrogen fertilizer can only be put into evidence by labelled isotope techniques. These techniques allow to follow the fate of labelled elements (N or C) from different sources (straw and urea).

Recent studies by Amato and Ladd (1980) and Ladd *et al.* (1977) with labelled material and that of Feller (1979) and Feller *et al.* (1981), without labelled material, have shown the interest of physical methods (particle-size or density fractionation) to study the decomposition processes of crop residues in soils.

Keeping these objectives in mind, millet was cultivated in pots in a sandy soil of Senegal, with and without addition of various crop residues (straw and compost) and urea, the two being either labelled or unlabelled.

The distributions of isotopes and organic matter within soil fractions were recorded by a particle size fractionation using successive dry and water-sievings at 2000, 200 and 50 μm.

In the present paper, only the results of two treatments, which were identical agronomically but different in the nature of labelling, are presented.

MATERIAL AND METHODS

Soil. The soil used was the top horizon (0-20 cm) of a slightly leached ferrugineous tropical soil sieved through a 2 mm sieve. Its main properties are summarized in Table 1.

Labelled straw. In the absence of doubly labelled straw, we constituted a (^{14}C ^{15}N)—labelled straw for each replication of the treatment *A* from the following mixture :

—2.2 g of ^{14}C-labelled maize straw (°), source : CEA (France) (Andre *et al.*, 1978).

—1.0 g of ^{15}N-labelled maize straw (°) : cultivation of maize in presence of $Ca(^{15}NO_3)_2$ (ORSTOM, Soil Science Laboratory, Senegal).

—6.8 g of unlabelled maize straw (°), source : CEA (France).

The mixture obtained was constituted of plant debris with size more than 2 mm (0.5 to 1 cm) and presented the following characteristics :

C%=39.2, N%=2.69, C/N=14.6, E% (isotopic excess)=1.23; SRA (specific radioactivity) in kBq/gC=290

Each replication of treatment *B* received 10.0 g of unlabelled maize straw. A water extract of labelled maize straw (after 15 min agitation at 20° C and then filtration through a Durieux paper No. 111) contained 30 per cent of ^{14}C and 70 per cent of ^{15}N (thus 30% of nitrates tagged with ^{15}N) in water soluble form.

Experimental techniques. The experiment was conducted during a rainy season, in glass-house, in Senegal and consisted of two successive crops of millet (variety ICRISAT BK 560) on a sandy soil amended (in its top 20 cm) with labelled maize straw and urea. Two treatments, which differed only in the nature of labelled elements added to soil, were studied :

Treatment A

-(^{14}C ^{15}N)-labelled straw: 1.5g DM/kg soil; SRA=290 kBq/gC; E%=1.23

(°) variety INRA F7 × F2.

TABLE 1

Some physico-chemical characteristics of "Dior" soil (Senegal)

pH	Mechanical analysis* (mm)					Organic matter			Exchange properties	
	c. sand (0.2-2.0)	f. sand (0.05-0.02)	c. silt (0.05-0.02)	f. silt (0.002-0.02)	clay (0-0.002)	C (%)	N (%)	C/N	C E C meq/100 g	Base saturation (%)
5.5	24.0	64.1	4.0	3 0	4.0	2.15	0.20	10.8	1.6	50

*International method
c=coarse, f=fine

—unlabelled urea : 200 mg N/kg soil

—phosphorus (triple super) : 100 mg P_2O_5/kg soil

—potassium (KCl) : 100 mg K_2O/kg soil

Treatment B

—unlabelled straw : 1.5 g DM/kg soil

—^{15}N-labelled urea : 200 mg N/kg soil; E% = 3.86

—phosphorus (triple super) : 100 mg P_2O_5/kg soil

—potassium (KCl) : 100 mg K_2O/kg soil

For each treatment, six bottomless pots (consisting 6 replications of the top horizon) filled with about 7 kg of soil were directly placed on a tub containing 200 kg of the same soil (subsoil horizon). The assemblage was maintained at field capacity during the two successive crops (2×60 days).

Fractionation of soil. All the soil of each replication (about 7 kg) was dry-seived through a 2000 µm sieve, then an aliquot of 600 g, fraction smaller than 2000 µm, was sieved under water through 200 and 50 µm sieves. Thus, the following five size fractions were obtained by the method described by Feller (1979) :

F2000 (larger than 2000 µm) : very coarse plant debris

F200 (200-2000 µm) : coarse sands and coarse plant debris

F50 (50-200 µm) : fine sands and fine plant debris

OMF (0-50 µm) : humified organo-mineral fraction

W : Water-soluble fraction (water of fractionation).

OMF was separated from W by flocculation, at pH 2.0, and centrifugation. The fractionation was done only on the top horizon (6 replications). The subsoil horizon was analysed in mass without fractionation. In unfractionated sample, the sum of fractions represented 95±5 per cent of ^{15}N and 100±15 per cent of ^{14}C.

Measurements

Carbon. Carbon was transformed into CO_2 by combustion and then either measured by conductimetry with a Wosthoff "carmhomat" (total carbon) or trapped by means of a Packard "Oxidiser" and then subsequently determined by liquid scintillation with an Intertechnique SL 4221 (^{14}C) computer.

Nitrogen : Total nitrogen was determined by Kjeldahl digestion. The isotopic quantity of ^{15}N was obtained by mass spectrometry (Guiraud and Pacchiani, 1973) on a Varian MAT GD 150, after transformation of ammonium sulphate in molecular nitrogen by sodium hypobromite.

RESULTS AND DISCUSSION

Distribution of Tracers within the Soil-plant System after Cultivation (plant, top soil, subsoil)

The distribution of ^{15}N after cultivation appears in Table 2. The results are expressed in percentage of the quantity initially added. For the two types of additions (urea and straw) about 25 per cent of ^{15}N was found in the plant*. About 80 per cent of ^{15}N derived from straw (treatment A) was found again in soil (nil losses) but for urea (treatment B) only 28 per

cent of the added ^{15}N remained in the soil, the losses by volatilization and denitrification being about 50 per cent. This value of urea-^{15}N losses was similar to that obtained in the experiments on the same type of soil by other authors (Ganry and Guiraud, 1978; Ganry *et al.*, 1978). Thus, a low use of nitrogen by plants accompanies only losses of urea-nitrogen and not that of straw-nitrogen.

TABLE 2

^{14}C and ^{15}N distributions within the soil-plant system after cultivation (plant without roots, top soil, subsoil, losses). Results expressed as % of the total added labelled element

Treatment	Plant*		Top-soil		Subsoil		Total		Losses	
	^{14}C	^{15}N	^{14}C	^{15}N	^{14}C	^{15}N	^{14}C	^{15}N	^{14}C	^{15}N
A	0	24.8	42.1	59.7	0.5	20.9	42.6	105.4	57.4	0
B		22.5		20.1		7.8		50.5		49.5

*Without roots

Distribution of Tracers in the Top Soil after Cultivation (Table 3)

In order to facilitate the comparisons between the two elements, C and N, for the two treatments, A and B, the distribution of tracers is presented in Table 3. The results are given in percentage of the total quantity of the tracers remaining in the soil after cultivation. About 80 per cent of ^{14}C was found in fractions larger than 50 μm and only 20 per cent in organo-mineral fraction (OMF). As for ^{14}C, the main part (60 %) of ^{15}N derived from straw was found in fractions larger than 50 μm. Nevertheless ^{15}N and ^{14}C (straw) distribution was different amongst the coarse (F2000, F200) and fine fractions (F 50, OMF) : contents were higher in coarse fractions for ^{14}C and in fine fractions for ^{15}N.

TABLE 3

^{14}C and ^{15}N distributions within the different fractions of top soil after cultivation. Results expressed as % of the top soil content after cultivation

Treatment	Soil particle size fraction -									
	F2000		F200		F50		OMF		W	
	^{14}C	^{15}N	^{14}C	^{15}N	^{14}C	^{15}N	^{14}C	^{15}N	^{14}C	^{15}N
A	13.1	3.9	53.4	33.3	11.9	23.3	19.2	29.8	2.4	9.7
B		3.0		6.4		7.9		21.8		60.9

*Without roots

The distribution of urea-^{15}N differed very much from that of straw-^{15}N, as more than half of the remaining urea-^{15}N was still in water-soluble form (W) at the end of the experiment.

In conclusion, if incorporation of nitrogen in different organic fractions of soil is more rapid than that of carbon, however, the incorporation process is still far from completion after a season of cultivation and the nitrogen distribution varies widely with the type of addition.

Characteristics of Labelled Fractions

The study of the isotopic excesses of ^{15}N and the specific radioactivities of ^{14}C (Table 4) allows to specify in a better way the humification pathways of carbon and nitrogen according to the nature of addition, organic (straw) or soluble (urea). For treatment A, the values of specific radioactivities *R* indicated that respectively half and one third of carbon in fractions F2000 and F200 derived from the added straw. On the other hand, the low labelling of finer fractions, F50, OMF and W, is an index of their low renewal by the recently added straw. The isotopic excess for the OMF fraction was very low, whereas it was about 10 for the water soluble fraction (W).

It is of interest to compare the values of ratio $r=^{14}C/^{15}N$ of different fractions with that of initial plant material ($r=340$). The value of this ratio rises to 800 for the fraction F2000 and then decreases with the size of fractions to 150 for OMF, thereby indicating a different distribution of ^{14}C and ^{15}N.

Let us point out in the end, that r, equal to 800 in F2000 fraction, is higher than that of initial straw (340). The

TABLE 4

Isotope characteristics of plants and different particle-size fractions of the top soil after cultivation

Treatment	Plant or soil fraction	C/N	*R***	*E***	$r=^{14}C/^{15}N$***
A	Plant*	15	0	12	0
	F2000	22	50	32	802
	F200	15	34	31	385
	F50	10	7	13	123
	OMF	9	4	5	154
	W	nd	nd	10	59
B	Plant*	15		45	
	F2000	nd		34	
	F200	nd		15	
	F50	nd		9	
	OMF	nd		7	
	W	nd		67	

nd : not determined.

*without roots.

**R=Specific radioactivity expressed as% of the SRA of initial labelled straw.

E=^{15}N isotopic excess expressed as % of isotopic excess (E) of initial addition (straw, urea).

***=Ratio $r=^{14}C/^{15}N$ is expressed in KBq per mg of ^{15}N. The ratio for the initial straw is 340.

labelled residual straw in this fraction has, thus, lost a part of its nitrogen (an increase in r). The analysis of initial mixture, in fact, indicates that 70 per cent of the initial total ^{15}N was in water-soluble form (probably cell contents), whereas only 30 per cent of ^{14}C was water-soluble. Therefore, one can calculate the ratio r (r=800) of the insoluble part of the initial plant mixture (probably cell walls) which is thus identical with that found for F2000 fraction. Thus, the F2000 fraction is mainly composed of cell-wall.

In conclusion, in a season of cultivation the first phases of transformations of added plant material concern, on priority, the redistribution of carbon and nitrogen of cell-contents.

For treatment B, two results are worth noting : the higher value of the isotopic excess of water-soluble fraction W and the lower values of the isotopic excess of finer fractions. The former is an index of a low incorporation of initial urea nitrogen and the latter indicates a low rate of renewal of nitrogen within these fractions.

CONCLUSIONS

This experiment of labelling of an annual addition of maize straw and its transformations in soil during four months of cultivation of millet has given the following results :

—the humification of half of the carbon remaining in the soil is just beginning (fractions larger than 200 μm).

—the utilization coefficients of straw-N and urea-N are almost similar but the losses are nil for straw-N and 50 per cent for urea-N. The incorporation of urea-N in soil is low.

—in short-term experiments the distribution pathways of C and N derived from the same plant addition differ and are function of the initial composition of the plant material.

These results illustrate the interest of the size fractionation approach for the study of soil organic matter in general and the transformations of crop residues in particular. The use of isotope techniques combined with the size fractionation approach permits to follow :

—the fate of the amendments, depending upon its nature (straw or urea) or upon the element studied (C, N).

—the participation of each fraction in diverse processes of humification.

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