

results do not reveal the mechanism for this effect; one possibility is regulation of proteolytic enzyme synthesis by N_i . However, the effect of N_i availability was transitory and had little long-term effect on mineralization or assimilation of the C contained in N_o .

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Organic Matter and Natural Carbon-13 Distribution in Forested and Cultivated Oxisols

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ABSTRACT

Soil carbon (C) distribution, natural ^{13}C abundances and their changes as a consequence of cropping were studied in three neighboring areas on an Oxisol from Brazil. One site (T_0) was under forest, while the two other sites (T_{12} and T_{50}) had been deforested, then cultivated with sugar cane for 12 and 50 yr, respectively. Soil morphological, chemical and mineralogical characteristics in all three sites were very similar. Total C content of the 0.06-m layer of T_0 was twice that of T_{12} and T_{50} , then decreased sharply with depth, to values similar to the other profiles. Delta ^{13}C had practically constant values of -25.1 , -22.8 , and -20.4% , throughout the 0 to 0.30-m layer of T_0 , T_{12} , and T_{50} respectively. These values increased in deeper layers, to about -17% , due to increased humification and possibly to deposition of organic matter from a former ^{13}C -rich vegetation. The 0.10- to 0.20-m layer was separated into particle-size fractions and alkaline extract. Carbon contents decreased from T_0 to T_{50} in the sand-size fractions and alkaline extracts, but did not change in the clay-size fractions. Delta ^{13}C values were used to estimate the proportions of C derived from forest (C_{df}) and from sugar cane (C_{dc}). Carbon derived from sugar cane represented $17.3 \pm 3.2\%$ and $40.5 \pm 2.2\%$ of total C in T_{12} and T_{50} , respectively. It reached its maximum value ($67 \pm 3.7\%$) in the coarse sand fraction of T_{12} and T_{50} and decreased with decreasing fraction size, to $13.8 \pm 9.4\%$ and $30.5 \pm 6.5\%$ in the fine clay fractions of T_{12} and T_{50} , respectively. Thus, C_{df} persisted mainly in the clay-size fraction.

STUDIES OF SOIL organic matter (SOM) are based primarily on determination of total organic carbon (C), nitrogen (N), and their distribution in a sequence of fractions separated by conventional methods. Although differing in some aspects, all fractionation methods attempt to separate SOM into classes with different degrees of decomposition (McGill et al., 1975; Turchenek and Oades, 1979; Andreux et al., 1980; Feller and Ganry, 1982; Tiessen et al., 1984). Few methods are able to relate the nature of bulk SOM or SOM fractions to their sources, however. Data on chemical structures, such as sugars (Cheshire, 1979), phenolic tracers (Hedges et al., 1982) or pyrolysis spectra (Bracewell and Robertson, 1984) do not enable cal-

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cultivation of the proportions of SOM from different plant origins.

Several investigators using isotopic methods have attempted such a determination. One of these methods is based on the natural ^{13}C abundance in SOM, which has an isotopic composition that corresponds closely to the vegetative cover which originated it (Nissenbaum and Shallinger, 1974; Deines, 1980). Carbon-13 abundance in any sample is expressed as $\delta^{13}\text{C}\text{‰}$, and is given by the relation $[(R_s - R_{st})/R_{st}] \times 1000$, where R_s is the $^{13}\text{C}/^{12}\text{C}$ ratio of the sample and R_{st} is the $^{13}\text{C}/^{12}\text{C}$ ratio of the PDB (*Pee Dee belemnite* from North Carolina) international standard. Delta ^{13}C values of higher plant species vary according to their photosynthetic cycle, from lower values in C3 plants (-22 to -33‰) to higher values in C4 plants (-9 to -16‰) (Deines, 1980). Thus, based on principles of isotopic dilution, situations in which a previous and long established vegetative cover has been replaced by another having a different ^{13}C isotopic composition may permit the study of SOM dynamics from dual origins (Barnes et al., 1983; Dzurec et al., 1985; Martinelli, 1986). However, the $\delta^{13}\text{C}$ method is restricted to situations in which the time of vegetation change is known, as pointed out by Cerri et al. (1985) and Balesdent et al. (1987). Furthermore, misinterpretations may arise from possible heterogeneity of soil layers, and the general tendency of $\delta^{13}\text{C}$ values to increase with increasing humification (Volkoff et al., 1978; Nissenbaum and Shallinger, 1974) and with soil depth (Schleser and Pohling, 1980; Volkoff et al., 1982; Becker-Heidmann and Scharpenseel, 1986).

In the Brazilian tropics, the existence of large areas of forest recently cleared for crop production has provided several situations in which relations between SOM content and soil fertility can be investigated. The present study was carried out in an area of Dark Red Latosol (Oxisol). One part of the study area was covered with native forest vegetation (C3 plants) and two other parts had been deforested and cultivated with sugar cane (C4 plant). The main objective of this paper is to show that the ^{13}C method can be used to evaluate changes in the contents of soil C from forest and sugar cane origins, but also show that more detailed data on ^{13}C distribution throughout these areas is needed if some of the errors bound to the method are to be overcome. Factors causing heterogeneity in C and ^{13}C distributions throughout the soil profile were studied with emphasis on the organic-rich 0- to 0.20-m soil layer. A particle-size fractionation method was used to illustrate trends in C distribution from both sources among soil humic constituents, and with increasing cropping time.

MATERIALS AND METHODS

Study Sites and Sampling

Samples studied were taken from three adjacent fields of a soil located on a flat area near the city of Piracicaba, ($22^\circ 43'\text{S}$; $47^\circ 38'\text{W}$), São Paulo, in southeastern Brazil. This area was previously described by Cerri et al. (1985) and Cerri (1986). The soil is a "Latossolo Vermelho Escuro" (Dark Red Latosol), according to Brazilian soil classification, and is classified as a clayey, kaolinitic, isothermic Typic Haplothox in *Soil Taxonomy*.

The first sampling site (T_0) was under natural forest vegetation. The second (T_{12}) and third (T_{50}) sites were deforested and cultivated exclusively and continuously with sugar cane (*Saccharum* spp.) for 12 and 50 yr, respectively. The T_{12} site was mechanically cleared after an accidental forest fire, and felled forest material was piled and reburned on site. The T_{50} site was manually cleared, and no burn piles were made after felling.

Production of fresh sugar cane was comparable in both cultivated areas, and was approximately $9 \times 10^4 \text{ kg ha}^{-1} \text{ yr}^{-1}$. Chemical fertilization was also the same in both areas, and consisted of 350 kg ha^{-1} of 0-13-8 (N-P-K) fertilizer in furrows at planting, 300 kg ha^{-1} of 12-0-30 (N-P-K) during initial growth, and 350 kg ha^{-1} of 12-0-30 (N-P-K) to the ratoon 3 yr following planting. No organic fertilizer was applied to any of the fields.

Soils at all three sites had a similar mineralogy and particle-size distribution in the first half-meter. Most of the differences observed in the micromorphological, physical, and chemical properties could be related to deforestation and cropping, rather than to a preexisting heterogeneity (Cerri et al., in press).

The maximum distance between sampling sites did not exceed 250 m. A composite sample of leaves and twigs from the forest live material was taken. From 1 m^2 of T_0 , the entire litter layer and litter-soil transition layer were collected separately. Aerial parts and live and decomposed roots of sugar cane were collected from T_{50} . Soil layers were sampled from the first 0.80 m from a pit within each field. Depth intervals were of 0.10 m, except in T_0 where the first three layers were 0.06-, 0.06- and 0.08-m thick. On each site, within 1 ha, the 0- to 0.20-m soil layer was collected with an auger at ten approximately equidistant spots.

Sample Processing

Litter material was manually separated into fractions differing in morphology and degree of decomposition. Root samples were picked out from the 0- to 0.20 m and soil-litter transition layers.

Plant and litter material was oven-dried at 60°C and ground in a thoroughly cleaned laboratory mill. Soil samples were homogenized, air-dried and sieved to less than $2000 \mu\text{m}$. Samples from the 0.12- to 0.20-m layer of T_0 and the 0.10- to 0.20-m layers of T_{12} and T_{50} were fractionated. Thorough disaggregation and dispersion of the soil was obtained by overnight mechanical shaking of 20 g of each sample in 200 mL of a $10 \text{ g L}^{-1} \text{ Na}_4\text{P}_2\text{O}_7$ solution adjusted to pH 11.5 with NaOH (Andreux et al., 1980). The suspension was then centrifuged at $10\,000 \times g$ for 20 min, and the separated soil residue processed once again with the $\text{Na}_4\text{P}_2\text{O}_7$ solution. The two supernatant alkaline extracts (AE) were mixed, received 20 g KCl L^{-1} of extract, and were left overnight at 5°C . The clay particles which flocculated were then separated from AE by centrifugation at $10\,000 \times g$ and added to the soil residue. The soil residue was redispersed in 200 mL of distilled water and wet-sieved with 200-, 100- and $50\text{-}\mu\text{m}$ sieves, successively. The 200- to $2000\text{-}\mu\text{m}$ (coarse sand), 100- to $200\text{-}\mu\text{m}$ (medium sand) and 50- to $100\text{-}\mu\text{m}$ (fine sand) fractions contained mainly poorly decomposed plant residues and quartz grains. These fractions were air-dried at 50°C , weighed and ground in a mechanical steel mortar.

The water suspended 0- to $50\text{-}\mu\text{m}$ fractions were acidified to pH 5.0 with dilute HCl, and centrifuged for 1 h at $10\,000 \times g$, to precipitate particles larger than approximately $0.1 \mu\text{m}$. The 0.1- to $50\text{-}\mu\text{m}$ (silt + coarse clay) and 0- to $0.1\text{-}\mu\text{m}$ (fine clay) fractions did not present recognizable organic residues. They were freeze-dried, weighed and finally ground. (The particle-size fractions defined in the above procedure and their respective names do not necessarily follow the USDA system).

Half of the AE solution was dialyzed against distilled water until no color change appeared in the renewed water. The material which remained inside or passed through the dialysis membrane was called large molecule extract (LM), and small molecule extract (SM), respectively.

Analytical Methods

Carbon contents of solid samples were determined by combustion in a C, H, N autoanalyzer. Aliquots of liquid samples were dried in porcelain vessels and combusted in a Carbon analyzer. All samples were analyzed two or three times, with a coefficient of variation less than 4%. Carbon contents of soil samples (Cs) were converted from g C g⁻¹ soil to Mg ha⁻¹ (TC), using the relation

$$TC = Cs \times L \times d \times 10^4$$

in which *L* is the thickness of the considered soil layer (in m), and *d* bulk density.

Carbon-13 composition was measured by burning samples together with Cu oxide under vacuum at 550 °C. The resulting CO₂ was then purified by trapping water vapor on dry ice, and analyzed by mass spectrometry in the isotope laboratory of CENA (Centro de Energia Nuclear na Agricultura), Piracicaba. Samples were analyzed at least twice with differences between repetitions less than 0.3‰ δ units.

Estimation of Carbon Derived from C3 and C4 Plants

Carbon derived from forest material (Cdff) and carbon derived from sugar cane crop residues (Cdffc) in any layer or SOM fraction of the cultivated soils were expressed either as percent of total C (PCdffc and PCdffc) or as g C kg⁻¹ of fraction or soil (SCdffc and SCdffc). Calculations were as follows

$$PCdffc = \frac{\delta - \delta_0}{\delta_c - \delta_0} \times 100; \quad PCdffc = 100 - PCdffc$$

Where δ = δ¹³C value of sample from cultivated soil
 δ_0 = δ¹³C value of corresponding sample from forest soil
 δ_c = δ¹³C mean value of sugar cane crop residues

SCdffc and SCdffc were obtained by multiplying PCdffc(10⁻²) and PCdffc(10⁻²) by the total C contents of the respective sample.

RESULTS AND DISCUSSION

Delta ¹³C Values of Plant Material

Total forest litter material from T₀ had a minimum δ¹³C value of -31.3 ± 0.16‰ in green material and a maximum value of -25.5 ± 0.03‰ in surface roots. Table 1 shows that the isotopic composition of the decomposing material reflected that of the initial C3 forest vegetation, but δ¹³C values of litter components increased by about 5‰ with increasing decay, from green leaves to unidentified coarse and fine materials. Sugar cane leaf material had a slightly lower δ¹³C value (-13.2‰) than root material (-12.8‰). In the calculations of Cdffc contents a mean value of -13.0‰ was used for the whole sugar cane material.

Vertical Distribution of Soil C Content and δ¹³C Values

Cumulative C content of the 0- to 0.70-m layer was higher in T₀ (126 ± 8.1 Mg ha⁻¹) than in T₁₂ (78 ± 2.9 Mg ha⁻¹) and T₅₀ (92 ± 1.2 Mg ha⁻¹). The amount

Table 1. Dry weight distribution and δ¹³C values of hand-picked forest litter components.

Litter components	Dry weight distribution	δ ¹³ C
	g kg ⁻¹ of total litter	
Green leaves and twigs	1	-31.3† ± 0.16‡
Dry, entire leaves	3	-27.8 ± 0.03
Decomposing leaves	43	-26.5 ± 0.13
Slightly decomposed twigs	45	-26.0 ± 0.03
Largely decomposed twigs	145	-25.8 ± 0.08
Surface roots	3	-25.5† ± 0.03
Unidentified coarse material	13	-25.7 ± 0.18
Unidentified fine material	646	-26.6 ± 0.53
Fecal aggregates	91	-26.1
Animal residues	10	ND
Total	1000	-26.4§
Unfractionated litter	-	-27.3 ± 0.55

† Values measured on fresh plant material.

‡ SE calculated from at least two repetitions.

§ Value recalculated from the above values (weighted mean).

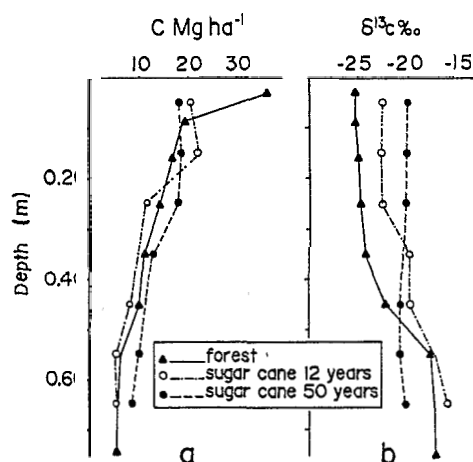


Fig. 1. Distribution of total C and δ¹³C values with depth in the three Oxisols (at a 0.05 significance level, the Tukey test's LSD between any points of the same profile or of two different profiles are 1.7 Mg ha⁻¹ and 0.59‰ for total C and δ¹³C, respectively).

of C in the upper 0- to 0.20-m layer of the forest soil (T₀) was twice that of the two other soils (Fig. 1a) as a result of the presence of decomposing forest litter. In the 0.20–0.70-m layer, differences were less pronounced; total C amounts were similar in T₀ and T₅₀ (52 ± 4.6 Mg ha⁻¹ and 56 ± 0.7 Mg ha⁻¹, respectively), but were lower in T₁₂ (40 ± 2.4 Mg ha⁻¹) than in the two other soils.

Carbon-13 abundances were rather constant in the first 0- to 0.30-m layer of each soil (Fig. 1b), with δ¹³C values of -25.1 ± 0.51‰, -22.8 ± 0.17‰, and -20.4 ± 0.19‰ in T₀, T₁₂ and T₅₀, respectively. In T₀ and T₁₂, δ¹³C values increased with depth, to -17.3 ± 0.08‰ and -16.2 ± 0.05‰ in the 0.60- to 0.70-m layer, but with a sharper pattern in T₀ than in T₁₂. In T₅₀, however, no significant change along the soil profile was noticed.

Increases in δ¹³C values with depth have been reported by several authors (Schleser and Pohling, 1980; Volkoff et al., 1982), and may result from a preferential decomposition and removal of ¹³C-impoverished components or molecules (Deines, 1980; Schleser and Pohling, 1980). Thus, δ¹³C values may

increase as a result of humification transformations. In some cases, such an increase may be emphasized by the selective migration and redeposition of clay-humic material with ^{13}C content higher than that of the whole SOM (Becker-Heidmann and Sharpenseel, 1986). However, the 8‰ increase in $\delta^{13}\text{C}$ values of SOM at 0.50- to 0.70-m was uncommon (Fig. 1b), and no enrichment of such extent was observed in any of the fractions from the upper layers. This result suggests that, together with humification processes, stable C inherited from a former C4 cycle vegetation may have been responsible for the high $\delta^{13}\text{C}$ values in depth (Cerri et al., 1985). This increase was more superficial in T_{12} than in T_0 , and did not occur in T_{50} , suggesting that the level at which this material appeared varied locally.

Spatial Variability of Surface Soil C Content and $\delta^{13}\text{C}$ Values

Mean and SDs of total C and $\delta^{13}\text{C}$ measurements on the 0- to 0.20-m layer from 10 different sampling sites of each area are presented in Table 2. The spatial variation of total soil C is in agreement with data reviewed by Campbell (1978), and temporal variations with recent observations in tropical ecosystems by

Table 2. Mean C content, $\delta^{13}\text{C}$ values and C derived from the sugar cane (SCdffc and PCdffc) in the 0- to 0.20-m layer of three soils.

	C content	$\delta^{13}\text{C}$	SCdffc	PCdffc
	g kg ⁻¹	‰	g kg ⁻¹	%
Forest soil	21 ± 1.9†	-24.99 ± 0.27	0	0
Sugar cane soil (12 yr)	22 ± 7.2	-23.66 ± 0.50	2.2 ± 0.5	11.1 ± 4.05
Sugar cane soil (50 yr)	15 ± 1.7	-20.66 ± 1.05	5.5 ± 1.6	36.1 ± 8.81
LSD*	1.4	0.87	1.0	5.92

* LSD from Tukey test for a 0.05 level of significance.

† SD calculated from 10 repetitions.

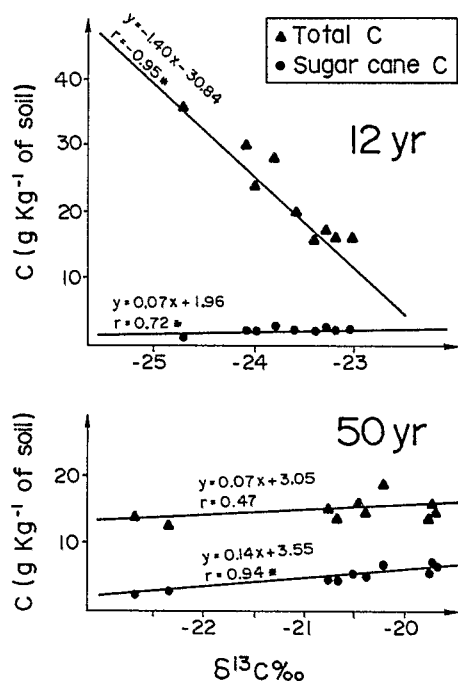


Fig. 2. Variations of total C and sugar cane C vs. $\delta^{13}\text{C}$ values in 10 surface samples of soils cultivated for 12 and 50 yr.

Sanchez et al. (1982). The main losses of SOM occurred during the first years following deforestation and cropping.

Carbon contents of T_0 and T_{12} were not statistically different, largely the result of the high SD obtained in T_{12} . In contrast, the C content of T_{50} was significantly lower than in T_0 . Mean $\delta^{13}\text{C}$ values were significantly different between the three soils, although an increase in their SD was observed from T_0 to T_{50} . Heterogeneity of C contents in the T_{12} surface soil was possibly the result of the presence of remains from burn piles of felled forest material. Total C contents varied as an inverse function of $\delta^{13}\text{C}$ values (Fig. 2). However, SCdffc was quite constant, and independent of $\delta^{13}\text{C}$ values, indicating Cdffc was almost evenly distributed in the top soil, and heterogeneity was mainly the result of Cdffc distribution. Heterogeneity was not large in the T_{50} data; the C content and SCdffc were both quite independent of $\delta^{13}\text{C}$ values. Mean values of PCdffc increased with increasing cropping time, from about 11 to 36%, as shown in Table 2. In both cases, the CV of this value was high, but the CV was higher in T_{12} than in T_{50} .

Distribution of C and ^{13}C in SOM Fractions

Particle-size fractionation following alkaline dispersion of soil samples (Table 3) yielded similar weight distribution of solid fractions in the three soils. The proportion of total C in the coarse sand-size fraction was much higher in T_0 than T_{12} and T_{50} . The two other sand-size fractions had a low contribution to total soil C, but were 50% smaller in T_{12} and T_{50} than in T_0 . As

Table 3. Distribution of C in size fractions and alkaline extracts of the 0.10- to 0.20-m layer of three soils.

Fractions	Weight recovery	C Content		Proportion of total soil C
		of fraction	of soil	
	g kg ⁻¹ soil	g C kg ⁻¹	%	
Forest soil				
Size fractions				
Coarse sand	129	24.6	3.2	17.7
Medium sand	84	7.7	0.6	3.3
Fine sand	34	13.9	0.5	2.8
Silt + coarse clay	649	10.7	6.9	38.1
Fine clay	38	18.9	0.7	3.9
Alkaline extract total	934	19.0	18.1	34.2 (22.9)†
Sugar cane soil, 12 yr				
Size fractions				
Coarse sand	109	3.6	0.4	3.1
Medium sand	84	2.1	0.2	1.6
Fine sand	28	6.3	0.2	1.6
Silt + coarse clay	647	10.5	7.0	54.7
Fine clay	47	18.0	0.8	6.2
Alkaline extract total	915	11.5	12.8	32.8 (24.2)†
Sugar can soil, 50 yr				
Size fractions				
Coarse sand	133	4.1	0.5	3.6
Medium sand	75	3.3	0.2	1.5
Fine sand	30	7.0	0.2	1.5
Silt + coarse clay	700	10.2	7.1	51.8
Fine clay	36	19.2	0.7	5.1
Alkaline extract total	973	15.7	13.7	36.5 (25.1)†

† Values in parentheses represent the amount of large molecules (LM) separated by dialysis of alkaline extract.

a consequence, the proportions of total C in the two fractions smaller than 50 μm were higher in T_{12} and T_{50} than in T_0 . More than one half of total C was concentrated in the silt + coarse clay size fractions of the two cultivated soils.

Sodium pyrophosphate ($\text{Na}_4\text{P}_2\text{O}_7$), which was used mainly as a means of dispersal, extracted larger amounts of C from T_0 , but the proportions of total C extracted were rather similar in all three soils. Only the predominance of large molecules increased, probably as a result of the substitution of organic sources.

Delta ^{13}C values of the different particle-size fractions and extracts are presented in Table 4. In T_0 , $\delta^{13}\text{C}$ values of the coarse, medium and fine sand-size fractions were lower than that of the whole soil, but were not different from each other. The values of the silt + coarse clay and fine clay-size fractions were close to each other, and slightly higher than the whole soil. The $\delta^{13}\text{C}$ value of AE was intermediate between those of the two above groups of solid fractions, but was lower than that of the total soil layer. This result is in agreement with findings by other authors (Nissenbaum and Shallinger, 1974; Volkoff et al., 1978). In T_{12} and T_{50} , the coarse sand-size fractions had $\delta^{13}\text{C}$ values higher than that of the respective whole soil. In T_{12} , $\delta^{13}\text{C}$ values decreased with size, to a minimum in the fine sand and other fine fractions, which was close to the $\delta^{13}\text{C}$ value of the whole soil. The AE fraction was the most impoverished fraction of T_{12} , however. In T_{50} , all fractions except the coarse sand-size fraction had $\delta^{13}\text{C}$ values which were similar to that of the whole soil. Values calculated for the SM fractions were only slightly lower than those obtained by analysis of the respective AE and LM fractions.

The $\delta^{13}\text{C}$ value measured in each soil layer was the result of a mixture of numerous organic compounds from both forest and crop sources. Soil alkaline dispersion and wet-sieving provided an adequate separation of SOM according to its degree of decomposition and of incorporation into fine organo-mineral particles (Andreux et al., 1980). Thus, differences ranging from 2 to 6‰ were found between fractions (Table 4). A larger contrast between the values of these fractions was noticed in the present case, at least in T_0 and T_{12} , as compared with earlier results using water dispersion (Cerri et al., 1985). This suggests that the

$\text{Na}_4\text{P}_2\text{O}_7$ solution had a higher dispersive effect over the soil particles than the water medium did.

Differential Humification of Organic Matter from Forest and Crop

Table 4 shows the estimated values of PCdfc in each particle-size fraction of the two cultivated soils. The calculation of Cdff and Cdfc contents assumed that each fraction of T_{12} and T_{50} had C isotopic composition similar to that of the corresponding fraction of T_0 at the beginning of the cultivation. Thus, the choice of the 0.10- to 0.20-m layer, rather than the upper one, was the most satisfactory for this purpose. The 0.10- to 0.20-m layer had the smallest differences in particle-size distribution and organic C and N contents between the three soils (Cerri, 1986).

The calculation also assumed that changes in $\delta^{13}\text{C}\text{‰}$ of sugar cane material due to humification are negligible within the span of time considered (50 yr). Differences in $\delta^{13}\text{C}\text{‰}$ between fractions of T_0 (Table 4) may reasonably be attributed to differences in C age. Since mean residence times for the carbon of these fractions are expected to be very different (Martel and Paul, 1974), 50 yr should not be enough to bring about large changes in ^{13}C composition. Although the humification factor is a cause of error, there is such little knowledge that further research is needed to establish the range of error.

Slightly decomposed residues of the three sand fractions represented about one-quarter of total C in T_0 , but were four-fold lower in T_{12} and T_{50} . In the two latter cases, the origin of these residues from either forest or sugar cane could not be clearly established by binocular microscope observations. Delta ^{13}C values showed that Cdfc predominated in the coarse sand-size fractions, and decreased progressively with particle size, faster in T_{12} than in T_{50} . Contrary to previous estimates (Cerri et al., 1985), PCdfc values of the coarse and medium sand-size fractions of T_{12} and T_{50} were almost equal. This result indicates the sugar cane residues decomposed rapidly, and that only a small proportion of them remained in the coarsest fractions from one year to another. Cerri (1986) previously concluded this from a "half-life time" model (Cerri, 1986). In T_{12} , C of the silt + coarse clay, fine

Table 4. Delta ^{13}C values and proportions of total C derived from the sugar cane (PCdfc) in fractions of the 0.10- to 0.20-m soil layers from three soils.

Fractions	$\delta^{13}\text{C}$			PCdfc		
	Forest soils	Sugar cane soil, 12 yr	Sugar cane soil, 50 yr	Forest soil	Sugar cane soil 12 yr	Sugar cane soil, 50 yr
	‰			%		
Whole soil	$-25.1 \pm 0.25^\dagger$	-23.0 ± 0.18	-20.2 ± 0.11	0	17.3 ± 3.2	40.5 ± 2.2
Size fractions						
Coarse sand	-28.2 ± 0.33	-18.0 ± 0.45	-18.7 ± 0.25	0	67.0 ± 3.7	62.5 ± 2.5
Medium sand	-29.0 ± 0.99	-21.3	-21.5 ± 0.91	0	47.8 ± 3.3	46.3 ± 9.0
Fine sand	-28.2 ± 0.72	-23.1	-20.8	0	33.4 ± 3.2	48.6 ± 2.5
Silt + coarse clay	-24.5 ± 0.16	-23.2 ± 0.64	-20.9 ± 0.52	0	11.2 ± 6.8	31.2 ± 5.5
Fine clay	-24.4 ± 0.27	-22.8 ± 0.84	-20.9 ± 0.56	0	13.8 ± 9.4	30.5 ± 6.5
Alkaline extract						
Total	-26.5	-24.6	-20.3	0	14.1	45.9
Large molecules	-26.3	-24.6	-19.8	0	12.8	48.9
Small molecules	-26.9^\ddagger	-24.1	-21.4^\ddagger	0	20.1	39.6

† SD are indicated only when two or more repetition were used.

‡ Values calculated by difference from the two above lines.

clay and AE fractions was still largely of forest origin. In T_{50} , about one-half of the C of the sand-size fractions, and one-third of that of the silt + coarse clay and fine clay-size fractions was of sugar cane origin. The relative increase in PCd_{fc} from T_{12} to T_{50} was about $1.5 \times$ in the fine sand-size fraction and three \times in the silt + coarse clay and fine clay-size fractions and in the alkaline extract.

In the studied soils, most of the SOM was associated with the silt + coarse clay-size fractions. This was even more pronounced in the cultivated soils, due to the high decomposition rate of sugar cane crop residues. The fine clay-size fraction, which is probably made mostly of microbial metabolites (McGill et al., 1975; Andreux et al., 1980), had the same ^{13}C isotopic composition and PCd_{fc} as the silt + coarse clay-size fraction, in spite of having higher total C content than the latter. The fact that these two clay fractions had retained larger proportions of Cd_{ff} than the coarser fractions could be explained by a protective effect of clay surfaces against degradation of organic compounds of forest origin. This would also apply in the extractable humic material (AE). However, it seems that beyond twelve years of cropping, this extractable material had been either less protected, or at least more mixed with material of sugar cane origin, than that which remained bound to the clay particles.

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