# COMMUN. SOIL SCI. PLANT ANAL., 26(11&12), 1749-1760 (1995)

# A ROUTINE METHOD TO STUDY SOIL ORGANIC MATTER BY PARTICLE-SIZE FRACTIONATION: EXAMPLES FOR TROPICAL SOILS

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<u>ABSTRACT</u>: Particle size fractionation is becoming commonly used for studying soil organic matter (OM). However, isolation of clay and silt represents a long and thus tedious step in the fractionation procedure. We propose an approach identical to the one utilized in particle size analysis with an estimation of the recoveries from aliquots ("aliquot" method) of the 0-2 and 0-20  $\mu$ m fractions and no entire isolation ("decanting" method) of clay and silt. In comparison with the "decanting" method, the fraction and carbon (C) recoveries obtained by the "aliquot" method were satisfactory, but those of nitrogen (N) being hardly interpretable because of an insufficient accuracy of the determination method. The recommended method saves time and laboratory space and could be used as a routine particle size fractionation of soil OM. Finally, this paper lists various methodological aspects of considerable significance but rarely reported in published studies.

## INTRODUCTION

More and more studies on soil organic matter (OM) rely on the particle-size fractionation of soils. The methodological aspects and field application of the different fractionation methods have been reviewed in recent years (1,2,3,4).

Like particle size analysis, the whole particle size fractionation of soil OM consists of three stages, i) a dispersion treatment, ii) a series of wet-sieving, and iii)

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a number of extractions by sedimentation and centrifugation to separate the silt and clay fractions.

Various methodological problems were associated with the first two stages and will be shortly reported in our discussion. As for a complete particle size fractionation, one of the main restraints to its routine application as a preparative measure is the long period of time needed for the separation of the clay and silt fractions. On account of the settling rates for the clay fraction and the need for six successive sedimentation cycles—at least in order to isolate this fraction—a series of soil samples are usually fractionnated within 5 to 8 days.

It may be interesting—in a non-preparative perspective to obtain fraction yields and C and N recoveries—to perform the particle size fractionation of OM according to an approach often used in particle size analysis: i.e. recoveries established from aliquots with no complete isolation of clay (0-2  $\mu$ m) or silt (2-20  $\mu$ m). The authors are not aware of any methodological study that has been published on this approach. Thus, this is the main objective of the present study which was carried out on a collection of tropical soil samples.

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#### MATERIALS AND METHODS

#### Soil Samples

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Nine ferrallitic and ferruginous soils from Burkina Faso (5), Benin (6), and Congo (7) were selected for the study. Surface (0-10 cm) horizons were sampled from paired plots with the exception of site BB 33 (Table 1) corresponding to samples having high (forest, savannah, and fallow) or low (fields under cultivation) OM contents. These soils contained predominately 1:1 type clays (kaolinite), thus providing a wide range of textures (the 0-2  $\mu$ m fraction varies from 5 to 38%) and OM contents (0.5 to 9.1%). Selected soil and site characteristics are presented in Table 1.

#### Fractionation Methods of Organic Matter

<u>Preliminary Remarks</u>: Although rarely mentioned in the literature, various methodological restraints have been taken into consideration:

a. No sonication of the whole soil (0-2 mm): In several studies using OM particle size fractionations (3,4), dispersion starts by ultrasonication of the whole soil (0-2 mm). Working under the usual conditions of ultrasound (US) application, Balesdent et al. (1) reported that an important amount (up to 50%) of C in the organic fractions (plant

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**TABLE 1- Analyses of Soils** 

Site	Vegetation	Sample.	0-2 µm(*)	2-20 μm(*)	OM	pН	CBC	EC	Ref.		
depth, cm)		n°		% of soil			cmol(+)/kg of soil				
Burkina F.	Bush fallow	311	7	3.6	0.7	6.2	3	1.7	(5)		
(0-10)	Pearl millet	331	nd	nd	0.5	5.8	nd	1.1			
	Tree fallow	221	20	14.7	2.4	6.1	2.5	1.7			
	Sorghum	211	nđ	nd	1.7	6.4	nd	1.9			
	Sorghum	8833	37.5	22.9	1.5	5.5	4.4	2.7			
Benin	Forest	DJ14	nd	nd	1.8	5.9	3.7	3.6	(6)		
(0-15)	Pałmgrove	DJ11	5.2	2.4	0,8	5.8	nd	1.3			
Congo	Savannah	SAV1	36.6	24.2	9.1	5.1	13.9	4.9	(7)		
(0-5)	Cassava	AMA1	nd	nd	5.5		nd	nd			

(\*) Results of the particle size analysis after H2O2 treatment and dispersion with sodium hexametaphosphate (HMP). CEC = cation exchange capacity at pH 7.

EC = exchangeable cation. nd = not determined

Ref. = reference

debris) associated with sand particles (>50  $\mu$ m) could be artificially transferred to the fine soil fractions (<20  $\mu$ m). Taking this into account, Feller et al. (2) proposed for soils with a medium to high aggregate stability, that dispersion be effected in two steps: (i) the soil 0-2 mm is first shaken in water containing a chemical (e.g. sodium metaphosphate, HMP) or electrochemical (cation exchange resin, % sodium saturated) dispersing agent, and (ii) after separation of sand fractions (>50  $\mu$ m) by sieving, dispersion is completed by ultrasonication of the 0-50  $\mu$ m soil suspension. In this study, HMP was preferred to a sodium-saturated resin as this dispersing agent is of a simpler use in the laboratories devoted to classical soil analyses.

b. Limitation in the duration of shaking in water for soils with coarse textures: The first step is carried out by shaking the soil suspended in water. The shaking time must be limited for soils with coarse textures in order to avoid sand particle abrasion of soil plant

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residues which can cause an artificial transfer of carbon from sand fractions to finer ones. Figure 1 illustrates such an effect for a sandy clayey soil sample that contains 60% of sand particles >50  $\mu$ m. The C content of sand fractions decreases (20 to 70%) with increasing shaking time (from 6 to 16 h). For this type of soils, the recommended shaking period is 2 h.

c. Separation of the size fractions bv wet-sieving through a succession of sieves (200  $\mu$ m, 50  $\mu$ m, and 20  $\mu$ m): The 20-50  $\mu$ m fraction is often separated after repeated sedimentation/ decantation of the 0-2  $\mu$ m and 2-20  $\mu$ m fractions instead of by sieving directly through a 20- $\mu$ m screen. Now, on the one hand, separation by sieving is less time-consuming, and on the other hand on a conceptual level, it is better to isolate fractions with recognizable plant material by size fractionation than by sedimentation. As a matter of fact, sedimentation presents the risk of mixing light organic fractions with clay and/or silt fractions the OM of which is very different (amorphous) (8).

<u>Methods of Fractionation Used</u>: The reference method, so-called "sedimentation/decanting" (abr. "dec."), must be distinguished from the test method, so-called "aliquot" (abr. "al.") method. When applying the "decanting" method, clay (0-2  $\mu$ m) and silt-size (2-20  $\mu$ m) particles were entirely extracted and separated. With the "aliquot" method, aliquots of the 0-2  $\mu$ m and 0-20  $\mu$ m fractions were withdrawn from the 0-20  $\mu$ m suspension. Carbon and N yields were obtained from C and N determined on isolated fractions ("decanting" method) or aliquots ("aliquot" method).

Fractionations were made on duplicate samples (1 for each method) of 20 g (for clays to sandy clays) or 40 g soil (for sands to clay sands):

a. Separation of the >20  $\mu$ m fractions. Soil was presoaked overnight at 4°C in 300 mL of deionized water with 0.5 g HMP (this value has been adopted after several tests made in the range 0.1-1.0 g). It was then shaken with five agate balls (diameter 10 mm) in a rotary shaker at a frequency of 50 revolutions per minute for 2 h in the case of sandy soils (no. 311, 331, DJ14, and DJ11) and six hours for the others. The soil suspension was wet-sieved through a 200- $\mu$ m and a

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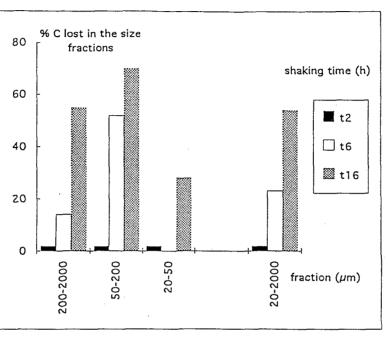


FIGURE 1.- Effect of Shaking Time on the Carbon Loss in the Different Size Fractions (200-2000, 50-200, 20-50  $\mu$ m and the whole 20-2000  $\mu$ m)

50- $\mu$ m sieve, successively. The fractions remaining on the sieves were washed with water and the washings added to the 0-50  $\mu$ m suspension. This suspension was ultrasonicated under conditions close to those described previously (2), with a probe-type ultrasound generating unit having a rated maximum power output of 600 watts. The probe (diameter 13 mm) was operated for 10 min working in 0.7:0.3 operating/interruption intervals at a setting of 9 on the intensity dial (the range being from 0 to 10).

The 0-50  $\mu$ m suspension was then sieved on a 20- $\mu$ m screen. The residual material obtained from the sieve was washed and the resulting 0-20  $\mu$ m suspension was transferred to 1-L glass cylinders. Water was added into the cylinders to bring the volumes to 1 L.

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The three fractions (>20  $\mu$ m) remaining on the sieves were dried to constant weight at 60°C and weighed. In the present study in order to avoid too many C and N determinations, these fractions were combined to give one 20-2000  $\mu$ m fraction which was finely ground.

# b. Separation of the <20 μm fractions. "Decanting" Method

The sedimentation cylinders containing the 0-20  $\mu$ m suspension were shaken by hand (30 end over end tumblings) and left to stand during the correct settling time for 0-2  $\mu$ m fractions. It is recommended to siphon off the maximum volume consistent with the size of the cylinder, e.g. the upper 30 cm every 24 h. The process was repeated until the supernatant was clear (five cycles at least). The 0-2  $\mu$ m suspension was then flocculated with a saturated solution of strontium chloride (1 mL/L suspension) and centrifuged. The pellet was washed with water, dried at 60°C, weighed, and finely ground. The solubilized fraction in the supernatant (washings) was not analysed in this study. The sediment at the bottom of the sedimentation cylinder represents the 2-20  $\mu$ m fraction. It was dried at 60°C, weighed and finely ground.

## "Aliquot" Method

The cylinders containing the 0-20  $\mu$ m suspension were shaken by hand (30 end over end tumblings) and 100 mL of the suspension were withdrawn immediately after. They constituted an aliquot of the entire 0-20  $\mu$ m fraction. After a settling time of 8 h approximately, a second aliquot was removed by siphoning the upper 10 cm of the suspension left after the first sampling. This represented an aliquot of the 0-2  $\mu$ m fraction. The two aliquot fractions were then isolated after flocculation, centrifugation, and washing as above. Once dried at 60°C, they were weighed and finely ground.

<u>Carbon and Nitrogen Determinations-Distribution of Particle Size</u> <u>Fractions:</u> Carbon and N of the particle-size fractions and the whole soils were determined using a LECO CHN-600 Elemental Analyzer.

In Tables 2a and 2b, the two methods used for the separation of the  $<20 \ \mu m$  fractions were compared with regard to fraction yield and fraction C and N contents. Results obtained after summation of the different fractions were also

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TABLE 2a - Yields, Carbon and Nitrogen Contents of Particle Size Fractions isolated by the Decanting Method (dec.) and the "Aliquot" Method (al.). Soils of Burkina Faso.

	Sample	Weight	c	N	C/N	<u>c</u>	<u>N</u>	Sample	Weight	<u> </u>	N	C/N	<u> </u>	N
(µm)	n°	% Soil NF	mg/g fra	ction		mg/g soil	NF	n°	% Soil NF	mg/g	fraction		mg/g so	a NF
20-2000	311	86.9	2.4	0.16	15.0	2.09	0.14	331	85.9	0.9	0.05	18.0	0.77	0.04
0-20 dec.		11.4	15.6	2.07	7.5	1.78	0.24		14.4	12.7	1.17	10.8	1.82	0.17
0-20 al.		11.8	16	1.80	8.9	1,88	0.21		13.6	13.4	1.20	11.2	1.83	0.16
2-20 dec.		3.3	10.4	1.00	10.4	0.34	0.03		6.4	10.2		10.2	0.65	0.06
2-20 al.		3.8	14.5	1.38	10.5	0,55	0.05		6.1	13.2	1.32	9.9	0.81	0.08
0-2 dec.		8.2	17.7	2.50	7.1	1.44	0.2		8.0	14.6		11.2	1.17	0,10
0-2 a).		8	16.7	2.00	8.4	1.33	0.16		7.5	13.6	1.10	12.4	1.02	0.08
Sum dec.		98.4			10.3	3,87	0.38		100.3			12.3	2.59	0.21
Sum al,		98.7			11.3	3,97	0.35		99.6			12.6	2.60	0.21
Soit NF(*)		100.0			8.0	4.00	0.50		100.0			9.4	2.90	0.31
Sum dec.% Si	oil NF	98.4				96.7	75.1		100.3				89.3	67.7
Sum al.% Soil	NF	98.7				99.2	70.2		99.6				89.7	67.7
20-2000	221	62.1	9.0	0.50	18.0	\$.59	0.31	211	53.3	3.9	0.20	19.5	2.08	0.11
0-20 dec.		37.7	17.2	2.07	8.3	6.48	0.78		46.1	11.9	1.23	9.7	5.48	0.57
0-20 al.		36.2	17.5	2.00	8.8	5.33	0.72		45.2	13.2		11.0	5,97	0,54
2-20 dec.		16.2	16.1	1.9	8.5	2.61	0.31		21.0	10.8	3 0.90	12.0	2.26	0.19
2-20 al.		16.6	15.7	2.00	7.9	2.61	0.33		20.6	14.2	2 0.96	14.7	2.92	0.2
0-2 dec.		21.5	18.0	2.20	8,2	3.87	0.47		25.1	12.8	3 1.50	8.5	3.21	0.38
0-2 вl.		19.6	19.0	2.00	9.5	3.72	0.39		24.6	12.4	4 1.40	8,9	3.05	0.34
Sum dec.		99,8			11.1	12.07	1.09		99.4			11.2	7.56	0.67
Sum ai.		98.3			11.5	11.92	1.03		98.5			12.4	8.05	0.65
Soil NF(*)		100.0			11.8	13.9	1.18		100.0			12.0	9.6	0.80
Sum dec.% S	oil NF	99.8				86.8	92.4		99.4				78.7	83.8
Sum al.% Soi	INF	98.3				85.8	87.3		98.5				83.6	81.3
20-2000	8833	43.1	5.1	0.30	17.0	2.20	0.13							
0-20 dec.		57.9	11.0	1.21	9.1	6.37	0.70							
0-20 al.		55.4	12	1.30	9.2	6.65	0.72							
2-20 dec.		23,9	11.0	0.80	13.8	2.63	0.19							
2-20 al.		22.2	11.85	1.45	8.2	2.63	0.32					•		
0-2 dec.		34	11.0	1.50	7.3	3.74	0.51							
0-2 al.		33.2	12.1	1.20	10.1	4.02	0,40							•
Sum dec.		101.0			10.3	8.56	0.83							
Sum al.		98.5			10.3		0.85							
Soil NF(*)		100.0			8.8	8.80	1.00							
Sum dec.% S	Soil NE	101.0				97.3	83.0							
Sum al.% So		98,5				100.5	84,9							

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TABLE 2b - Yields, Carbon and Nitrogen Contents of Particle Size Fractions isolated by the Decanting Method (dec.) and the "Aliquot" Method (al.). Soils of Benin and Congo.

Fraction	Sample	Weight	С	N	C/N	с	N	Sample	Weight	с	N	C/N	C	N
(µm)	nº	% Soil NF	mg/g	fraction		mg/g soil	NF	n°	% Soil NF	mg/g	fraction	•	mg/g sa	il NF
20.2000	DJ14	86.6	3.4	0.20	17.0	2.95	0.17	DJ11	91.1	1.9	0.10	19.0	1.73	0.09
0-20 dec.		12.8	60.8	5.53	11.0	7.77	0.71		8.6	28.2		• 11.5	2.43	0.21
0-20 al.		12.7	57.6	6.10	9.4	7.33	0.78		9.0	27.7	2.90	9.6	2.50	0.26
2-20 dec.		3.2	97.0	7.10	13.7	3.14	0.23		2.2	33.8	2.30	14.7	0.73	0.05
2-20 al.		3.4	89.3	9.04	9.9	3.03	0.31		2.5	34.1	2.90	11.8	0.87	0.07
0-2 dec.		9.6	48.5	5.00	9.7	4.63	0.48		6.5	26.3	2.50	10.5	1.70	0.16
0-2 al.		9.3	46.1	5.03	9.2	4.3	0.47		6.5	25.2	2.90	8.7	1.64	0.19
Sum dec.		99.4			12.2	10.72	0.88		99.7			13.8	4.18	0.30
Sum al.		99.4			10.8	10.27	0.95		100.2			12.0	4.24	0.35
Soil NF(*)		100.0			11.7	10.50	0.90		100.0			15.3	4.60	0.30
Sum dec.%	Soil NF	99.4				102.1	97.9		99.7				90.5	100.8
Sum al.% So	oil NF	99.4				97.8	105.5		100.2				92.1	117.8
20-2000	SAV1'	34.5	72.4	3.30	21.9	24.96	1.14	AMA1	38.2	38.3	1.80	21.3	14,63	0.69
0-20 dec.		64.8	38.7	2.73	14.2	25.07	1.77		62.8	29.9		13.8	18.75	1.36
0-20 al.		66.8	38.	3 2.30	16.7	25.60	1.54		62.1	28.8	3 2.00	14.4	17.88	1.24
2-20 dec.		24.1	53.0	3 3,30	16.3	12.94	0.79		20.8	41.3	1.70	24.3	8.59	0.35
2-20 al.		30.5	51.	1.82	28.0	15.60	0.56		21.2	41.3	3 1.81	22.9	8.77	0.38
0-2 dec.		40.7	29.	3 2.40	12.4	12.13	0.98		42.0	24.3	2 2.40	10.1	10.16	1.01
0-2 ai.		36.3	27.1	5 2.70	10.2	10.00	0.98		40.9	22.:	3 2.10	10.6	9.12	0.86
Sum dec.		99.2		2.93	17.2	50.03	2.91		101.0			16.3	33.38	2.05
Sum al.		101.3		5.60	18.9	50.56	2.67		100.3			16,9	32.51	1.93
Soil NF(*)		100.0			17.6	52,80	3.00		100.0			15,2	32.00	2.10
Sum dec.%	Soil NF	99.2				94.8	96.9		101.0				104.3	97.6
Sum al.% S	oil NF	101.3				95.8	89.2		100.3				101.6	91.9

(') NF = non fractionnated

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compared to those of the whole soils. All the results were expressed on a dry weight basis.

For the "aliquot" method, the values (weight, C, and N) corresponding to the 2-20  $\mu$ m fractions were calculated from those actually determined on the 0-2 and 0-20  $\mu$ m fractions.

According to the particle-size fractionation, the coefficients of variation concerning the fraction C contents (mg C/g of soil) accounted for 10, 5, and 5% in the 20-2000, 2-20, and 0-2  $\mu$ m fractions, respectively (2). Essentially the fraction

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yields and C recoveries will be discussed here. As a matter of fact, the less satisfactory recoveries of N reported in the present study, may be ascribed to a lower precision of the analyzer in relation to the low N contents in most of the selected soils rather than to any effect of the fractionation method used.

## **RESULTS AND DISCUSSION**

Fraction Yields, Carbon and Nitrogen Recoveries

The results of the particle size fractionations, fraction yields, C, and N recoveries are detailed in Tables 2a and 2b.

*Fraction Yields*: The cumulative fraction yield obtained by the "aliquot" method varied from 98.3 to 101.3% with a mean value of 99.4%.

**Carbon Recoveries:** The cumulative C contents of the fractions ranged from 83.8 to 101.6% of the soil C in the "aliquot" method. The lowest values, below 90 %, were obtained for soils (no. 331, 221, and 211) (Table 2a) with low C contents (2.9 - 13.9 mg C/g of soil). The mean C recoveries for the fractions isolated by the "aliquot" and the "decanting" methods were 94.0 and 93.4%, respectively, with a standard deviation of 6.5 and 8.0%, respectively. The mean 6% loss could be attributed to the solubilization of C (solubilized C not determined in the present study) during the fractionation procedure. As a matter of fact, Feller et al. (2) observed for tropical soils that this solubilized fraction represented approximately 5% of whole soil C.

<u>Nitrogen Recoveries</u>: The cumulative N contents of the fractions isolated by the "aliquot" method ranged from 67.7 to 117.8% of the soil N. These strong discrepancies with regard to the non-fractionated soil could be explained by the insufficient accuracy of the analyzer used (LECO CHN-600) as the N concentrations were very low in most of the soils studied. As a matter of fact, the apparatus commonly allows the determination of 0.3 mg N/g of sample (9) which is in good agreement with the N content found in soils (331, 311, 221, and DJ11) or their lower N concentration fractions (20-2000  $\mu$ m). The mean N recoveries were found to be for the "aliquot" and the "decanting" methods, 88.4 and 88.4%, respectively, with a standard deviation of 15.8 and 11.6%, respectively.

Comparison Between the "Aliquot" and "Decanting" Methods

The comparison related to the amounts (mg/g of fraction, Figure 2) and the contents (mg/g of soil, Figure 3) in C and N of the whole fractions  $<20 \,\mu\text{m}$ .

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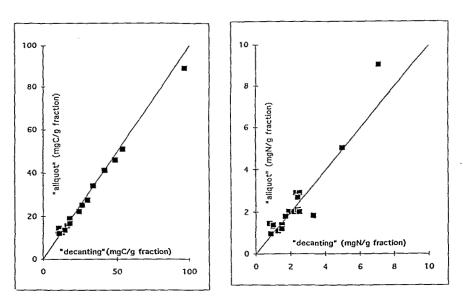


FIGURE 2.- Plots of Total Carbon and Nitrogen Amounts by "Decanting" Method Versus Total Carbon and Nitrogen Amounts by "Aliquot" Method for Fractions 2-20  $\mu$ m together with Fractions 0-2  $\mu$ m (mg/g of fraction). The line represents the theoretical values.

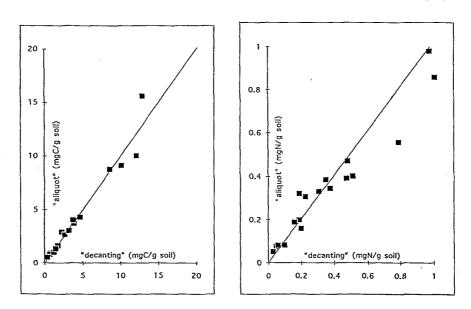
As shown in Figures 2 and 3, the "decanting" and "aliquot" methods were strongly correlated with all correlations being highly significant at the 99.9% level. However, there were poorer correlations for N ( $\mathbf{r} = 0.961$  and 0.956 for amounts and contents, respectively) than for C ( $\mathbf{r} = 0.997$  and 0.979, respectively). The relationship between the two methods is linear, passing through the origin, displaying some deviation, and more important in the case of N. This is to be put through to the variability as was discussed above concerning N.

## CONCLUSION

This paper has revealed various methodological constraints rarely taken into account in previous studies on particle size fractionation of soil OM, e.g. the influence of ultrasonic treatment and/or shaking time on the changes of plant residues associated with sand particles and on the artificial transfer of C into finer soil fractions. These methodological aspects are of importance in studies on the fate

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FIGURE 3.- Plots of Total Carbon and Nitrogen Amounts by "Decanting" Method Versus Total Carbon and Nitrogen Amounts by "Aliquot" Method for Fractions 2-20  $\mu$ m together with Fractions 0-2  $\mu$ m (mg/g of soil). The line represents the theoretical values.

of plant debris in the soil by means of isotopic techniques ( $^{14}$ C,  $^{13}$ C, or  $^{15}$ N). As a matter of fact, as there is no evaluation of these artifacts the analysis of isotopic distributions would misinterpret the observed C and N transfers, since short-term storages of OM in the finer fractions are generally explained in terms of OM that has come into solution or in terms of microbial activity (mineralization-immobilization cycle) rather than by a physical transfer of C or N from plant debris.

In other respects, it was shown that the amounts and contents in C (and probably in N) could be estimated from simple aliquots representing one-tenth of the weight of the soil fractionnated and this was done with no necessity for a complete, long, and tedious separation of fractions 2-20 and 0-2  $\mu$ m. The principal advantage of such an approach, if compared with classical ones, lies with saving time and space occupancy. The duration of the "aliquot" fractionation was estimated to be five times shorter than that of the "decanting" method, the same holds true in respect of space occupancy. However, the "aliquot" method does not allow to work

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on a preparative scale in order to isolate quantities of fractions <20  $\mu$ m, neither does beyond the whole C and N recoveries to study fraction properties, the 2-20  $\mu$ m fraction being not physically separated from the 0-2  $\mu$ m fraction.

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