Soil Organic Matter Dynamics and Sustainability of Tropical Agriculture
Edited by K. Mulongoy and R. Merckx

A Wiley-Sayce Co-Publication

1.2

Soil microhabitats and the importance of the fractionation method

J.L. Chotte, L. Jocteur Monrozier, G. Villemin and A. Albrecht

Summary

The microhabitats of a Vertisol were studied as part of a research programme on organic matter in tropical soils. This paper presents the results of a comparative study of the effect of soil disruptive energy (method A < method B) on: the weight of size fractions; the morphological characteristics of size fractions isolated through particle size fractionation; and the distribution of microorganisms in the different size fractions. An increase in disruptive energy significantly modified the characteristics of fractions. The > 250 µm size fraction, isolated by method A, was composed of organic residues and coarse sand coated with a clay matrix. These macro-aggregates were colonized by numerous microorganisms (one third of the total amount of soil microbial biomass carbon). The increase in disruptive energy (method B) destroyed the macro-aggregates, leading to the release of organic residues and sand. The organo-mineral matrix and associated microorganisms were transfered to other fine fractions. The increase in dispruptive energy also led to a rise in the weight and microbial biomass C content of the 0-2 µm size fraction.

Soil microorganisms are actively involved in the transformation of organic matter and the recycling of nutrients needed for plant growth. For these microorganisms, the soil represents a complex and heterogeneous environment, characterized by three phases: a dominant solid phase, in which particles of varying sizes and characteristics occur; an aqueous phase; and a gaseous phase. The main factors controlling the distribution of soil microorganisms are soil structure, defined by the size, shape and organization of the particles (simple or complex) and associated voids (Brewer, 1964) and the concentration in assimilable carbon (Gray and Williams, 1971). Elliott and Coleman (1988) proposed a distribution pattern of soil microorganisms based on pore size. Hattori (1988) showed that protozoa (> 97%) and fungi (90%) tended to occur on the surfaces of the larger pores of macro-aggregates, whereas bacteria (90%) occurred within the pores. These findings indicate that, because of the low mobility of non-filamentous organisms and bacteria, soil is a patchwork of heterogeneous sites colonized by different groups of microorganisms (Kanazawa and Filip, 1986; Gupta and Germida, 1988). Several workers have found that the physicochemical conditions of these microhabitats control microbial activity (Seifert, 1964; Adu and Oades, 1978; Elliott, 1986).

Many studies have been conducted to characterize soil microhabitats. In most of them, a physical fractionation method was applied to a soil which had been dispersed (Ahmed and Oades, 1984; Kanazawa and Filip, 1986; Gupta and Germida, 1988; Jocteur Monrozier et al., 1991). Fractions were isolated by successive sievings, sedimentation and/or centrifugation. The energy used during the dispersion process or particle size fractionation modified the characteristics of isolated size fractions. Ahmed and Oades (1984) found that the distribution of C and N in fine size fractions differed from that in coarse size fractions. Elliott (1986) reported a higher N mineralization rate in disrupted macro-aggregates (>300 µm) than in intact macro-aggregates.

The characterization of soil microhabitats was part of a study intitiated by the Institut Français de Recherche Scientifique pour le Développement en Coopération (ORSTOM) in Martinique on soil organic matter dynamics in tropical pasture systems. The work described here focused on a Vertisol. Its objective was to measure the disruptive energy effect of two fractionation methods on: weight of isolated size fractions; morphological characteristics of these size fractions; and distribution of microorganisms in the size fractions. Particular emphasis was placed on the significance of macro-aggregates (> 250 µm) as microbiological sites.

MATERIALS AND METHODS

The top layer (0-10 cm) of a Vertisol in Martinique which had been under pasture (*Digitaria decumbens)* for 7 years (total organic C 3.30 %) was sampled and air-dried. The soil was carefully sieved (2 mm) to remove coarse fragments of plant roots, put in a plastic flask (80 g) and incubated with urea (160 mg N/kg soil) and glucose (2.5 mg C/kg soil) for 3 days at 28 ± 1°C, with the soil moistened at 100% of water-holding capacity.

Two methods were used to isolate the size fractions (*see* Figure 1):

Method A After suspension in water (250 ml), the soil sample was dispersed by rolling for 2 hours. It was then fractionated by wet sieving, sedimentation and centrifugation in order to separate the size fractions into these categories: > 250 µm, 50-250 µm, 20-50 µm, 2-20 µm and 0-2µm.

Method B A replicate of the soil sample used in method A was dispersed more energetically by rolling the soil suspension in water with five agate marbles (1 cm diameter) for 16 hours (Bruckert, 1979). The dispersed soil was then fractionated following the same procedure as in method A.

A fumigation-incubation extraction method was used to determine the microbial biomass of the unfractionated soil and the size fractions, calculated thus: biomass C = 21 x (ninhydrin-reactive N after 10 days fumigation with chloroform minus ninhydrin-reactive N prior to fumigation) (Amato and Ladd, 1988). The soil fractions (wet samples) were observed with a stereo microscope. Subsamples for transmission electron microscopy (TEM) were fixed in buffered 1% OsO_4 for 16 hours (Villemin and Toutain, 1987). After the buffer was washed off, the samples were enclosed in 2% agar, dehydrated by solvent exchange and embedded in Epon's medium. Ultrathin sections were stained successively in uranyl acetate and lead citrate solutions. Polysaccharides were revealed by Thiery's reaction (Thiery, 1967). Ultrathin sections were examined with a Zeiss EM 95-2 at 60 Kv.

RESULTS

Weight of size fractions

Weight recovery of the size fractions was 100%, irrespective of the method used. A comparison of the fractionation methods (*see* Figure 2) indicated that the increase in disruptive energy (method A < method B)

Figure 1 **Stages in fractionation methods A and B**

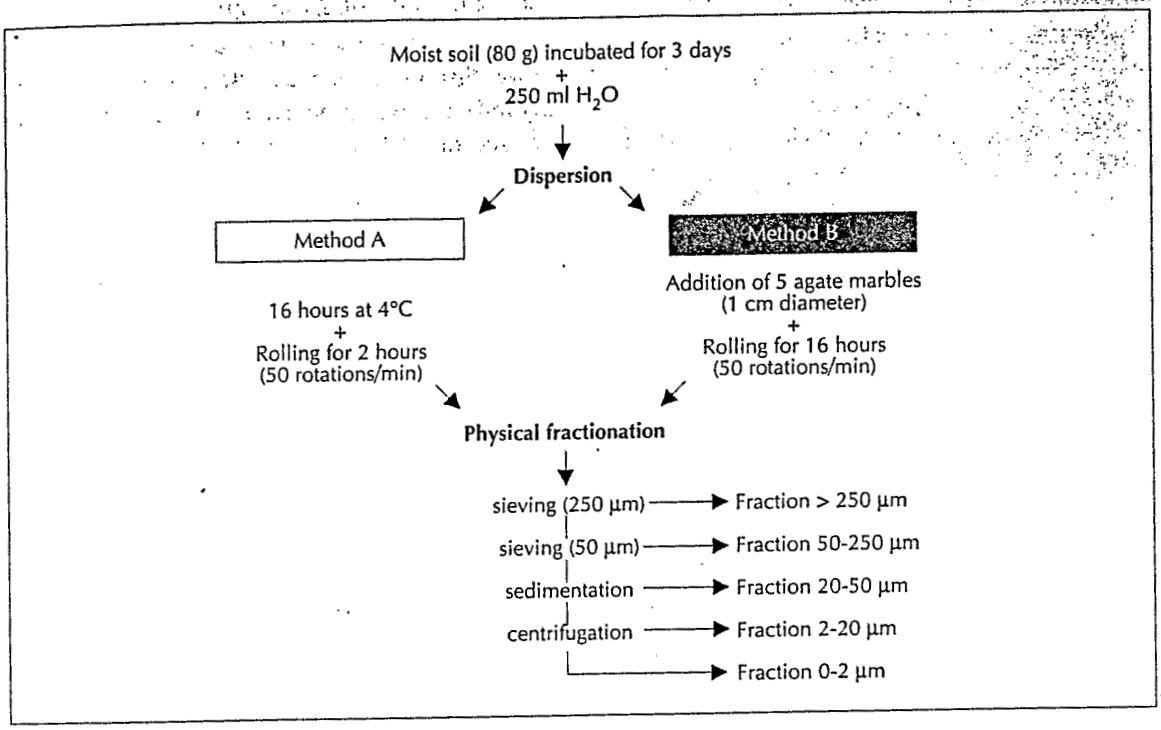
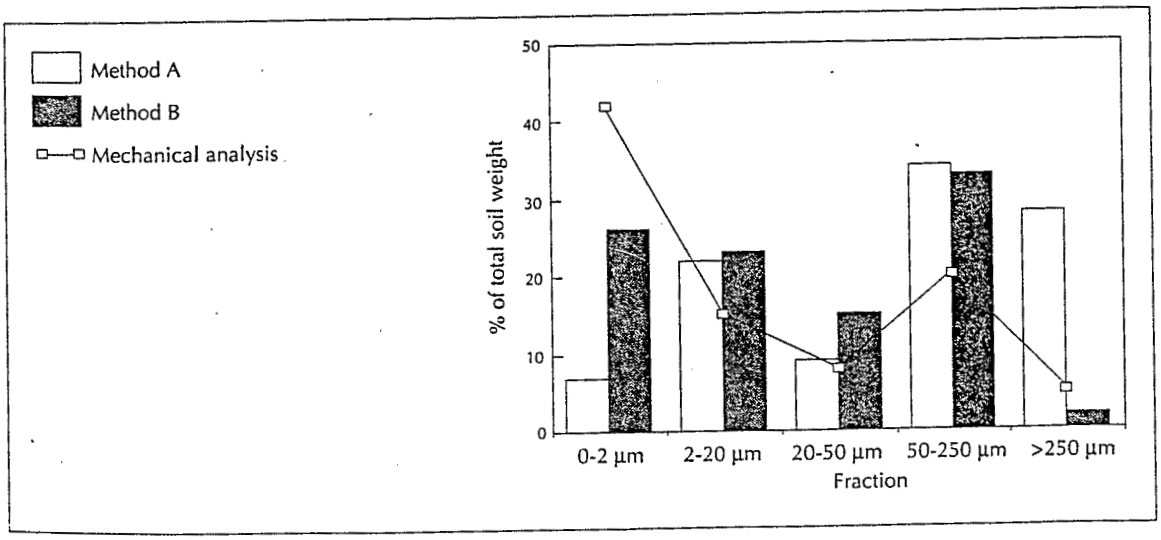


Figure 2 **Weight distribution of size fractions separated after dispersion by methods A and B, compared with values obtained by mechanical analysis**



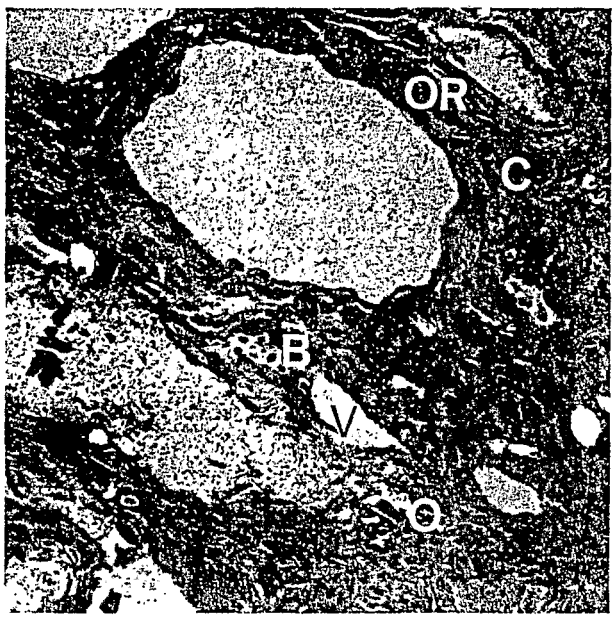
modified the weight distribution of isolated size fractions, except for those in the 50-250 µm and 2-20 µm range, which did not appear to change (34% and 23%, respectively, of the unfractionated soil).

However, the measured variations were not identical for all size fractions. The variation of the 20-50 µm size fraction which had been isolated by method A was lower than the parallel size fraction obtained using method B (9% and 15%, respectively, of the unfractionated soil). The weight variations of the > 250 µm and 0-2 µm size fractions were the highest. When method B was used the >250 µm size fraction almost completely disappeared because of the increase in disruptive energy. The > 250 µm macro-aggregated fraction, isolated by method A, represented 28% of the total soil weight, whereas for method B it amounted to only 2% of the total soil weight. Similarly, the 0-2 µm size fraction weight increased as a result of the increase in disruptive energy. Although the weight of the < 2µm size fraction increased from 7% for method A to 26% for method B, it was still lower than the weight obtained when classical mechanical analysis (total dispersion after the destruction of soil organic matter) was used. The increase in disruptive energy did not destroy all the micro-aggregates.

Morphological characteristics of size fractions

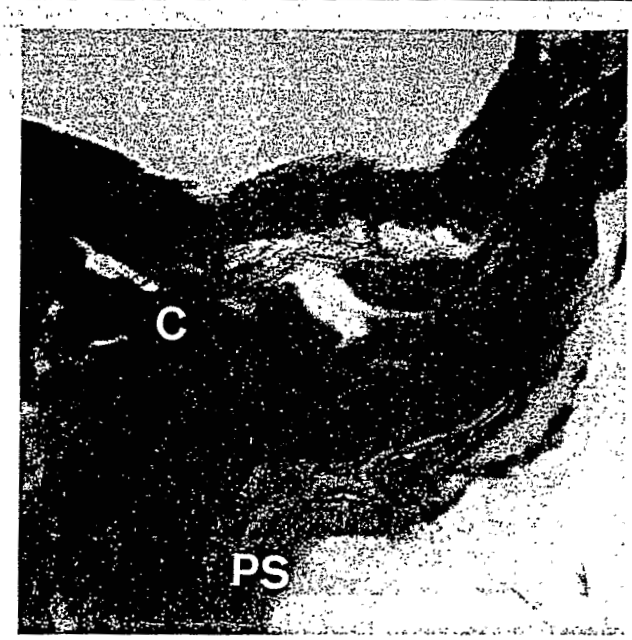
Only the > 250 µm and 0-2 µm size fractions were observed. The > 250 µm size fraction, isolated by method A, was composed of organic residues and coarse sands coated with a clay matrix (*see* Figure 3). There were numerous microorganisms (bacteria in the photograph) located in the matrix. The cohesion of this organo-mineral association was achieved, in part, by polysaccharides of bacterial origin (*see* Figure 4). The use of

Figure 3 **TEM view of > 250 µm macro-aggregated size fraction isolated by method A (x 5600)**



Organic residues (OR) and quartz particles (Q), coated in a clay matrix (C), are colonized by bacteria (B). V = void (uranyl acetate and lead citrate stain)

Figure 4 TEM view of > 250 µm macro-aggregated size fraction isolated by method A (x 5600)



Polysaccharides (PS) revealed by Thiery's reaction (Thiery, 1967) and coated clay particles (C)

method B resulted in the total destruction of this mineral matrix and the release of organic residues and sands. The > 250 µm size fraction obtained by method B was therefore composed of organic residues and dispersed sands.

Using ultramicrostructural analysis, it was not possible to determine a significant influence of the dispersion method on micromorphological features of the 0-2 µm size fraction. This size fraction contained clay particles which were either dispersed or associated with bacterial residues.

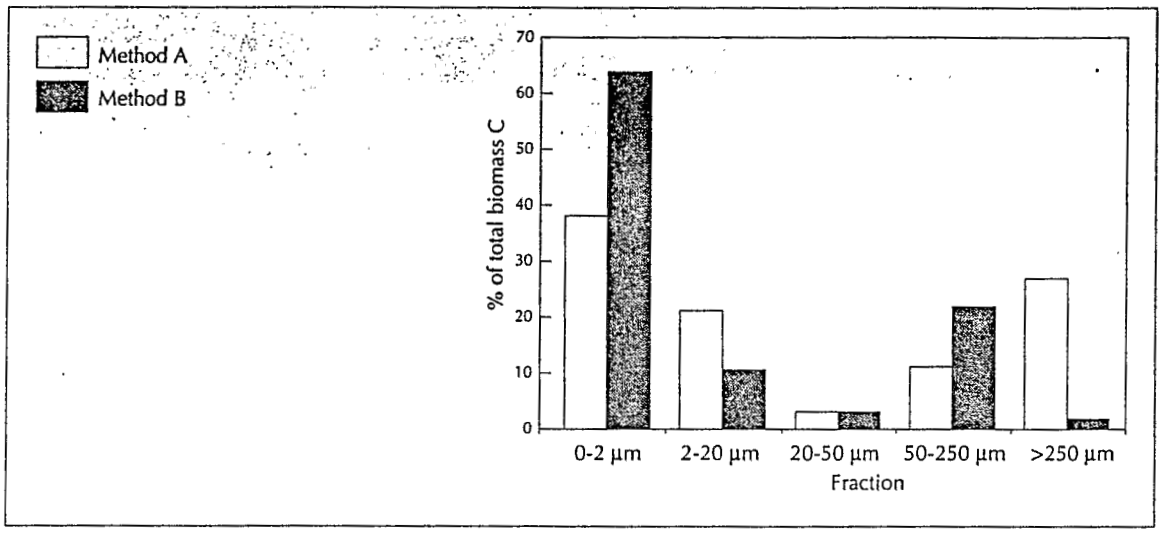
Distribution of microorganisms in size fractions

Biomass C of the unfractionated soil was equal to 1400 µg/g soil (4 % of the total soil organic C). The biomass C recovery of particle size fractions for methods A and B was 90% and 107%, respectively. A comparison of the two dispersion methods indicated that the distribution of microorganisms in all the size fractions apart from 20-50 µm was susceptible to change when the macro-aggregates were disrupted.

The biomass C content of the > 250 µm and 2-20 µm size fractions decreased with an increase in disruptive energy (*see* Figure 5 *overleaf*). The former, isolated by method A, contained 27% of the total biomass C of the soil, whereas in method B only 2% of the biomass C was located in this fraction. An increase in disruptive energy led to a 50% reduction in the number of microorganisms located in the 2-20 µm size fraction (for methods A and B, 21% and 9% of biomass C in the unfractionated soil, respectively).

The microorganisms located in the 50-250 µm and 0-2 µm size fractions were more numerous when isolated by method B than by method A. Biomass C of the former doubled (for methods A and B, 11% and 22% of total

Figure 5 **Biomass C distribution in the different size fractions separated after dispersion by methods A and B**



biomass C, respectively). Biomass C of the 0-2 µm size fraction ranged from to 38% of biomass C of total soil (method A) to 64% of biomass C of total soil (method B).

CONCLUSION

The increase in disruptive energy resulted in the disaggregation of the macro-aggregates and the dispersion of clay particles and associated microorganisms. These macro-aggregates need to be further studied because they represent one of the sites of microbial activities. The results of this study provide clear evidence of the influence on microbial activities of the method used to characterize soil aggregation. The optimum energy level for such soil fractionation needs to be determined.

References

Adu, J.K. and Oades, J.M. 1978. Physical factors influencing decomposition of organic materials in soil aggregates. *Soil Biol. and Biochem.* 10: 109-15.

Ahmed, M. and Oades, J.M. 1984. Distribution of organic matter and adenosine triphosphate after fractionation of soils by physical procedures. *Soil Biol. and Biochem.* 16: 465-70.

Amato, M and Ladd, J.N. 1988. Assay for microbial biomass based on ninhydrin-reactive nitrogen in extracts of fuigated soils. *Soil Biol. and Biochem.* 20: 107-14

Brewer, R. (ed) 1964. *Fabric and Mineral Analysis of Soils.* New York, USA/Chichester, UK: John Wiley.

Bruckert, S. 1979. Analyse de complexes organo-minéraux des sols. In Duchaufour, Ph. and Souchier, B. (eds). *Pédologie. 2. Constituants et Propriétés du Sol.* Paris, France: Masson.

Elliott, E.T. 1986. Aggregate structure and carbon, nitrogen, and phosphorus in native and cultivated soils. *Soil Sci. Soc. Am. J.* 50(3): 627-33.

Elliott, E.T. and Coleman, D.C. 1988. Let the soil work for us. *Ecol. Bull.* 39: 23-32.

Gupta, V.V.S.R. and Germida, J.J. 1988. Distribution of microbial biomass and its activity in different soil aggregate size classes as affected by cultivation. *Soil Biol. and Biochem.* 20(6): 777-86.

Gray, T.R.G. and Williams, S.T. 1971. *Soil Microorganisms.* Edinburgh, UK: Oliver and Boyd.

Hattori, T. 1988. Soil aggregates as microhabitats of microorganisms. *Rep. Inst. Agr. Res. Tohoku Univ.* 37: 23-36.

Jocteur Monrozier, L., Ladd, J.N., Fitzpatrick, R.W. Foster, R.C. and Raupach, M. 1991. Components and microbial biomass content of size fractions in soils of contrasting aggregation. *Geoderma* 49: 1-10.

Kanazawa, S. and Filip, Z. 1986. Distribution of microorganisms, total biomass, and enzyme activities in different particles of brown soil. *Microb. Ecol.* 12: 205-15.

Seifert, J. 1964. Influence of the size of soil structural aggregate on the degree of nitrification. *Folia Microbiol.* 9: 365-77.

Thiery, J.P. 1967. Mise en évidence des polysaccharides sur coupes fines en microscopie électronique. *J. Microscopie* 6: 987-1017.

Villemin, G. and Toutain, F. 1987. Méthode de fixation d'échantillons organo-minéraux de sols pour la microscopie électronique à transmission. In Fedoroff, N., Bresson, L.M. and Courty, M.A. *Micromorphologie des Sols.* AFES.