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IMPROVED METHOD FOR SEPARATING LIGHT- AND HEAVY-FRACTION ORGANIC MATERIAL FROM SOIL[1]

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Abstract

An improved method is presented for separating whole soil into a mineral-free organic fraction (light fraction) and an organomineral fraction (heavy fraction) by flotation in a dense inorganic salt solution. In the existing method, the heavy fraction is settled by centrifugation and the light fraction decanted. In the improved method, the light fraction is aspirated from the solution surface by suction after the soil suspension is allowed to settle. The improved method costs less, is more reproducible, and is practical in coarse-textured soils where heavy fractions do not form stable pellets during centrifugation.

Additional Index Words: **density fractionation, organic matter, C, N.**

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Soil organic matter is present in either a mineral-free form, which includes partly decomposed plant material and microbial biomass, or as an organomineral complex, in which the organic material may be adsorbed onto mineral surfaces or sequestered within microaggregates (Sollins et al., 1983). The mineral-free material (light fraction or LF) is less dense than the organomineral fraction (heavy fraction or HF) and can be separated by flotation in organic or inorganic solutions of varying density (Turchenek and Oades, 1974; Spycher and Young, 1977). Inorganic media may be preferred over organic when microbiological incubations follow because most organic media are highly toxic. Density fractionation has been used to study the composition of clay-sized organ-

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omineral complexes (Turchenek and Oades, 1974, 1979), to remove recently deposited organic material prior to soil ^{14}C-dating (D. Schimel, 1985, personal communication), and to identify factors controlling light- and heavy-fraction accumulation (Sollins et al., 1983).

In the separation method currently used (e.g., Spycher et al., 1983), whole soil is dispersed, the suspension centrifuged, and the LF decanted along with the supernatant. This method is slow, labor-intensive, and requires a large-capacity centrifuge. Moreover, because coarse-textured soils do not form stable pellets upon centrifugation, decanting the LF is impossible. We undertook to remedy these problems by first allowing the HF to settle by gravity, then removing the LF from the solution surface by suction. Results obtained with both methods are compared here.

Materials and Methods

Soil samples (0–15 cm depth) were collected from six sites in North and Central America that encompassed a range of soil types and textures (Table 1) and stored field moist at 4°C until needed. The HF and LF contents of each soil were determined in triplicate by each method.

Centrifuge Method

Thirty grams of field moist soil were dispersed by stirring (1800 rpm for 0.5 min) in 200 mL of NaI solution (density = 1.70 g cm^{-3}). Suspensions were immediately centrifuged at 4068 × *g* for 10 min. The supernatant containing the LF was decanted onto Whatman no. 50 filters (2.7-μm retention) and vacuum-filtered. The HF residue was resuspended twice in fresh NaI solution and the LFs were combined. Light and heavy fractions were then washed three times by vacuum filtration of 1.0 *M* NaCl (50 mL) through the sample and washed three times with deionized water (dIH_2O). Each fraction was then washed into preweighed tins with dIH_2O, dried at 100°C for 48 h, and weighed.

Suction Method

Soil samples were dispersed as described above. After stirring, the HF was allowed to settle for 48 h at room temperature. The top few centimeters of solution plus suspended LF were aspirated through Tygon hose (1.0 and 1.5 cm, inside and outside diameter) attached to a vacuum pump (Fig. 1) until about 5 cm of solution remained above the HF surface. The tubing fit snugly into a standard Buchner-funnel vacuum gasket on the receiving flask. The LF was then transferred to separate containers and kept at 4°C. Remaining LF material was extracted from the residual HF, and the LFs were combined. The HF and LF were washed, dried, and weighed identical to the centrifuge method.

Table 1. Selected properties of soils examined.

Site	Soil type	Vegetation type	Texture† Sand	Silt	Clay
Waldo, Florida	Ultic Haplaquod	Slash pine§	91.5	5.2	3.3
Woods Hole, Massachusetts	Typic Udipsamment	Oak, pine¶	80.6	15.4	4.0
H.J. Andrews, Oregon	Andic Haplumbrept	Burned clear-cut, formerly old-growth Douglas fir/western hemlock#	46.3	31.5	22.2
Cascade Head, Oregon	Typic Dystrandept	Conifer, red alder††	41.2	45.1	13.7
Konza prairie, Kansas	Pachic Argiustoll	Tallgrass prairie	19.8	60.0	20.2
La Selva, Costa Rica‡	Oxic Humitropept	Abandoned pasture	9.0	17.6	73.4

† Bouycous hydrometer method.
‡ Upper alluvial terrace (old alluvium).
§ *Pinus elliotii.*
¶ Oak, *Quercus* spp.; pine, *Pinus* spp.
# Douglas fir, *Pseudotsuga menziesii* [(Mirb.) Franca]; western hemlock, *Tsuga heterophylla* [(Raf.) Sarg.].
†† Red alder, *Alnus rubra* (Bong.).

Carbon and N Analyses

Oven-dried samples were ground (<0.25 mm) and analyzed for total C with a Leco WR 12 Automatic Carbon Analyzer. Nitrogen was measured as NH_4 on a Technicon Autoanalyzer after Kjeldahl digestion (0.5-g samples). Because the samples from Konza and La Selva contained insufficient LF for Kjeldahl digestion, these LFs were processed with a Carlo 1106 Erba CHN analyzer (Milan, Italy).

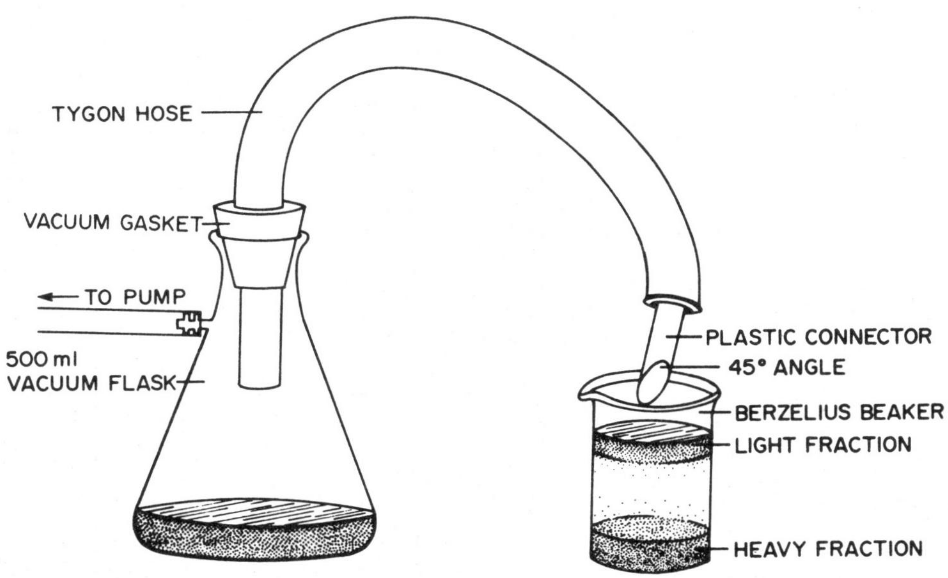

Fig. 1. Apparatus for removal of light fraction by suction.

Table 2. Comparison of suction vs. centrifuge methods for separating heavy- and light-fractions of six soils.

Soil and method†	Heavy fraction: Proportion of soil	Heavy fraction: C	Heavy fraction: N	Heavy fraction: C/N	Light fraction: Proportion of soil	Light fraction: C	Light fraction: N	Light fraction: C/N
	%	g/kg	g/kg		%	g/kg	g/kg	
Waldo, FL								
Suction	98.92*‡	26.7*	0.8*	32.8	1.07*	404.1	5.9*	68.6
(SE)	(0.04)	(1.0)	(0.1)	(3.3)	(0.04)	(39.4)	(0.2)	(9.4)
Centrifuge	96.29*	14.1*	0.5*	29.2	3.71*	418.3	7.1*	59.2
(SE)	(0.04)	(1.4)	(0.1)	(3.7)	(0.04)	(6.1)	(0.1)	(0.8)
Woods Hole, MA								
Suction	99.52*	16.9*	0.4	42.3*	0.48*	345.8	4.4	86.1
(SE)	(0.06)	(2.0)	(0.1)	(3.6)	(0.06)	(40.3)	(0.9)	(19.1)
Centrifuge	98.80*	9.1*	0.4	22.8*	1.20*	299.6	5.2	58.8
(SE)	(0.25)	(1.3)	(0)	(5.3)	(0.25)	(20.3)	(0.7)	(4.8)
H.J. Andrews, OR								
Suction	93.84*	33.8	1.5*	23.2	6.16*	293.4*	6.0*	49.1*
(SE)	(0.17)	(1.0)	(0)	(1.9)	(0.17)	(8.5)	(0.1)	(1.6)
Centrifuge	96.03*	36.4	1.6*	22.5	3.97*	307.2*	8.1*	37.8*
(SE)	(0.22)	(0.8)	(0)	(0.5)	(0.22)	(14.3)	(0.3)	(0.9)
Cascade Head, OR								
Suction	73.64	101.6	5.9*	17.2*	26.36	292.9	12.3*	23.9
(SE)	(1.96)	(3.1)	(0.1)	(0.2)	(1.96)	(17.1)	(0.2)	(1.5)
Centrifuge	78.13	94.0	5.1*	18.5*	21.87	341.2	15.6*	21.9
(SE)	(1.73)	(4.4)	(0.2)	(0.2)	(1.73)	(12.9)	(0.5)	(0.6)
Konza, KS								
Suction	99.53	30.1	2.2	14.0	0.47	339.4	10.4	33.3
(SE)	(0.05)	(0.4)	(0)	(0.5)	(0.05)	(2.6)	(0.9)	(3.3)
Centrifuge	99.48	30.0	2.4	13.0	0.52	293.4	9.0	36.8
(SE)	(0.03)	(0.3)	(0)	(0.4)	(0.03)	(2.0)	(1.6)	(8.2)
LaSelva, Costa Rica								
Suction	99.75*	53.7	4.7	11.5	0.25*	366.7	14.6	26.0
(SE)	(0.03)	(1.1)	(0)	(0.3)	(0.03)	(5.9)	(1.8)	(3.6)
Centrifuge	99.54*	52.7	4.6	11.5	0.46*	360.4	17.6	21.8
(SE)	(0.03)	(0.4)	(0)	(0.2)	(0.03)	(2.9)	(3.4)	(3.8)

† Mean and standard error (SE) for each method.
‡ For any soil and fraction, significantly different at the 5% confidence interval (Tukey's HSD test, n = 3).

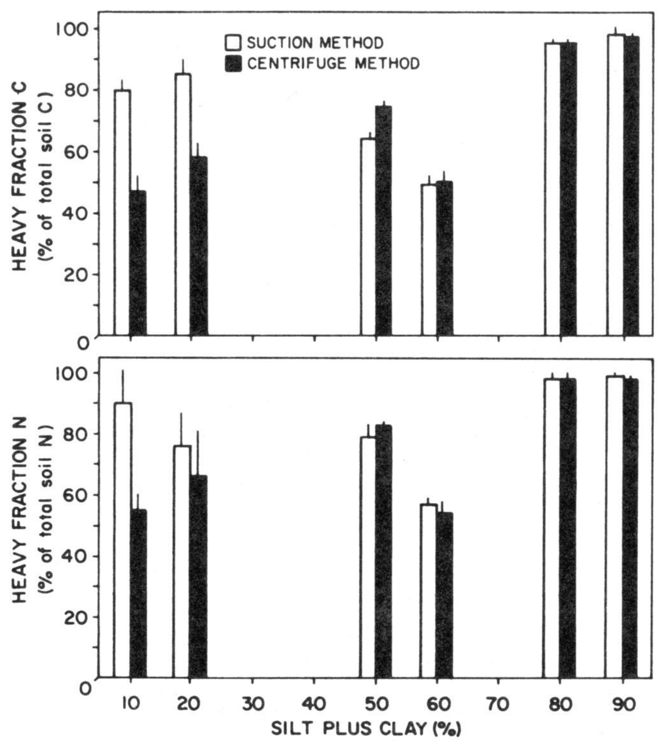

Fig. 2. Effect of soil texture on differences between HF C and N content (as % of whole soil C and N) as measured by the centrifuge and suction methods. Error bars are ± 1 standard error.

Results and Discussion

The two methods generally gave similar results. When differences were significant, the suction method was consistently more precise (Table 2). With both sandy soils (Waldo and Woods Hole), the suction method gave consistently lower values for percent LF than did the centrifuge method (Table 2), apparently because the supernatant could not be decanted without disturbing the unpelleted sand and silt. This pattern held also for the clay soil (La Selva). The two methods gave similar results for two of the three loam/silt loams (Cascade Head and Konza). For the third, Andrews, the suction method yielded more LF, for unknown reasons.

Carbon and N content were also affected by the separation method (Table 2). The suction method gave higher C and N content for Waldo HF, higher C content (and C/N ratio) for Woods Hole HF, and lower N content for Waldo LF. Effects of the separation method on the Andrews soil were apparent in the LF C/N ratio, which was much higher by the suction method than by centrifugation.

Soil texture may explain why the two methods produce different results. The difference in HF C and N (as a proportion of whole soil C and N) for the two methods was greater in the coarse- than in the fine-textured soils (Fig. 2). Apparently, during centrifugation the finer textured soils formed a more stable pellet, which reduced mixing of HF and LF during decanting.

Operationally, the suction method for LF collection is superior to the centrifuge method. We found that for most soils the suction method gives more complete separation and is more reproducible, less labor intensive, and less costly, requiring no centrifuge and only half as much NaI as the centrifuge method. Moreover, the suction method can be used with sandy soils, whereas the centrifuge method cannot.

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