Short communication

Sequential versus parallel density fractionation of silt-sized organomineral complexes of tropical soils using metatungstate

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Abstract

A comparison of two density fractionation methods used for studying soil organomineral complexes was carried out on the silt-size fractions of two highly weathered soils from NE Brazil. The difficulties commonly encountered in isolating organomineral complexes in highly weathered soils were overcome when sodium metatungstate solution in combination with centrifugation on an angular rotor was used. The sequential method caused a greater loss of C (8±10%) than the parallel method (3±5%); however, the sequential method permits direct measurements on C concentration, complexing cations (Fe and Al), and mineralogical composition. © 2001 Elsevier Science Ltd. All rights reserved.

Keywords: Density fractionation; Soil organic matter; Tropical soils

Physical fractionation of soil organic matter (SOM) includes mechanical dispersion (e.g. using ultrasonic vibration) to obtain soil size fractions and sedimentation of whole soil or its size fractions in heavy liquids. The information from physical fractionation was considered more relevant to the SOM function in situ (Christensen, 1992) than classical chemical fractionation. The light fraction of SOM, recovered by immersing the whole soil in a heavy liquid (e.g. NaI), has a fast turnover and serves as a labile nutrient pool in many agricultural soils (Bremer et al., 1994). Using metatungstate liquids, Cambardella and Elliott (1994) isolated a C- and N-enriched fine-silt fraction (2–20 μm) having a density of 2.07–2.22 g cm⁻³ from inside macroaggregates of a temperate prairie soil.

There are two contrasting approaches used in the density fractionation of SOM. The sequential separation used by Dalal and Mayer (1986) involves successive extractions with heavy liquids of increasing density. The parallel separation used by Cambardella and Elliott (1992) separates SOM from replicate samples with heavy solutions of different densities. The mass and C content of the intermediate fractions are calculated indirectly by taking the difference between two neighbouring (heavier or lighter) fractions (Cambardella and Elliott, 1992). Sodium metatungstate [Na₆(H₂W₁₂O₴₀)] was proposed as a new medium for density gradient centrifugation because of its low toxicity and high density (Plewinsky and Kamps, 1984). Although this inorganic salt has been successfully used for SOM fractionation (Baldock et al., 1990; Cambardella and Elliott, 1992; Shang and Tiessen, 1997; Six et al., 1999), the questions regarding C loss and fractionation efficiency remain unanswered.

The present study was to compare the two density fractionation schemes on tropical soil separates using sodium metatungstate and to investigate the suitability of this inorganic salt for tropical soil materials.

Two soils (a Ustalf and a Ustox) were collected under native vegetation in Pernambuco State, Brazil. The Alfisol was formed on granitic–gneissic parent material. The clay fraction of the Alfisol contained mainly kaolinite and illite; silts contained quartz, muscovite and K-feldspars as well as iron oxide minerals; sands were dominated by quartz and K-feldspar. The Oxisol was sampled on a plateau of Cretaceous sediments (Chapada de Araripe). The predominant minerals in this soil were quartz, iron oxides and kaolinite with only traces of 2:1 minerals. The A horizons (0–12 cm for the Alfisol and 0–20 cm for the Oxisol) were used in this study. Samples were air-dried and passed through a 2-mm sieve. The particle-size distribution, determined by H₂O₂–metaphosphate dispersion was: 66% sand, 16% silt and 18% clay for the Alfisol, and 79% sand, 2% silt and 19% clay for...
### Table 1
Comparison of sequential and parallel density separations on the silt-size separates of two highly weathered Brazilian soils (the average of two analyses from combined samples of three replicates)

<table>
<thead>
<tr>
<th>Density fraction (g cm(^{-3}))</th>
<th>Method</th>
<th>Alisol</th>
<th>Oxisol</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C conc. (^{a}) (mg g(^{-1}))</td>
<td>% of fraction mass</td>
<td>% of fraction C</td>
</tr>
<tr>
<td>&lt;1.8</td>
<td>Parallel</td>
<td>– (^{b})</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Sequential</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>1.8–2.0</td>
<td>Parallel</td>
<td>258</td>
<td>4.0</td>
</tr>
<tr>
<td></td>
<td>Sequential</td>
<td>214</td>
<td>5.1</td>
</tr>
<tr>
<td>2.0–2.2</td>
<td>Parallel</td>
<td>164</td>
<td>4.9</td>
</tr>
<tr>
<td></td>
<td>Sequential</td>
<td>171</td>
<td>3.2</td>
</tr>
<tr>
<td>2.2–2.4</td>
<td>Parallel</td>
<td>36</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>Sequential</td>
<td>72</td>
<td>3.8</td>
</tr>
<tr>
<td>&gt; 2.4</td>
<td>Parallel</td>
<td>6.0</td>
<td>81</td>
</tr>
<tr>
<td></td>
<td>Sequential</td>
<td>9.2</td>
<td>88</td>
</tr>
</tbody>
</table>

\(^{a}\) Calculated values were given for the C concentrations of intermediate density fractions by parallel method.

\(^{b}\) The <1.8 and 1.8–2.0 g cm\(^{-3}\) fractions were summed to give the <2.0 g cm\(^{-3}\) fraction because the 1.8–2.0 g cm\(^{-3}\) fraction was small.
the Oxisol. The organic C content of the Alfisol sample was 1.4% and that of the Oxisol 1.1%. More information on the two soils was given by Shang and Tiessen (1998).

Fifty grams of air-dry soil was pre-wetted overnight and ultrasonified in 300 ml of deionized water in a 400-ml beaker at 300 W for 8 min with a 1/2-inch (1.27 cm) probe. Oven-dry weight of soil samples was calculated with known moisture contents. Samples were cooled in an ice bath during sonication. The dispersed sample was passed through a 50-µm sieve to recover the sand, and the silt-size materials (2–50 µm) were separated from <2-µm clay by gravity sedimentation. Comparisons were carried out with silt and clay fractions since isolation of organic matter from sand fraction was straightforward (Shang and Tiessen, 1998).

In the parallel density separation, silt-size materials (0.5 g for the Oxisol and 1 g for the Alfisol) were suspended in 5 ml of metatungstate solutions with densities of 1.8, 2.0, 2.2 and 2.4 g cm\(^{-3}\)) in 15-ml centrifuge tubes. The suspension was stirred with a vortex mixer and then treated ultrasonically for 1 min at 50 W output using a 1/4-inch (0.64 cm) probe. Material adhering to the probe was washed into the centrifuge tube with 5 ml of heavy solution. The suspension was cooled for one hour at room temperature before centrifuging at 2000 g for 10 min using an angular rotor. The supernatant and floating matter were filtered through a Millipore membrane (0.10 µm pore size) using vacuum suction. The material trapped on the membrane was washed with 5 mM CaCl\(_2\), transferred into a pre-weighted aluminium tray and dried at 55°C. The heavy fractions were washed four times with 5 mM CaCl\(_2\) by centrifugation, and oven-dried.

In the sequential method, the residual soil (>1.8 g cm\(^{-3}\)) from the 1.8-g cm\(^{-3}\) separation was washed with dilute CaCl\(_2\) solution, oven-dried at 55°C and fractionated with metatungstate solution of 2.0 g cm\(^{-3}\) to obtain the 1.8–2.0 g cm\(^{-3}\) fraction and so on. Successive density fractions of 2.0–2.2, 2.2–2.4 and >2.4 g cm\(^{-3}\) were thus recovered using heavy solutions of 2.2 and 2.4 g cm\(^{-3}\). The organic matter fractions of three replicates were combined for analysis.

About 50% of total soil C were located in the silt-size fraction, which included silts and silt-size aggregates (Shang and Tiessen, 1998). Density separation by both the parallel and sequential methods produced a similar trend in C concentration for the Alfisol silt (Table 1), i.e. a decrease in C concentration with increasing fraction density. The sequential method gave a relatively lower concentration in the <2.0 g cm\(^{-3}\) fraction but higher concentrations in heavier fractions. The recovery of mass became less comparable between the two methods as the density increased; more mass was recovered in the >2.4 g cm\(^{-3}\) fraction by the sequential method (87.9%) than the parallel method (80.5%). The C distribution, as proportion of total fraction C, among density fractions followed the mass distribution.

In the case of Oxisol silt, the parallel method produced a low C concentration for the 1.8–2.0 g cm\(^{-3}\) fraction and a very high concentration for the 2.0–2.2 g cm\(^{-3}\) fraction. The irregularity of C concentration was likely caused by an over-recovery of mass in the <2.0 g cm\(^{-3}\) fraction by the sequential method (87.9%) than the parallel method (80.5%). The C distribution, as proportion of total fraction C, among density fractions followed the mass distribution.

In the case of Oxisol silt, the parallel method produced a low C concentration for the 1.8–2.0 g cm\(^{-3}\) fraction and a very high concentration for the 2.0–2.2 g cm\(^{-3}\) fraction. The irregularity of C concentration was likely caused by an over-recovery of mass in the <2.0 g cm\(^{-3}\) fraction by the 2.0 g cm\(^{-3}\) density separation. When subtractions were made, the greater mass of 1.8–2.0 g cm\(^{-3}\) fraction led to an underestimation of the 2.0–2.2 g cm\(^{-3}\) fraction. Consequently, the C concentration was lowered by dilution because the material of >2.0 g cm\(^{-3}\) density contained...
less C. Error accumulation in subtraction may also be a factor. Similar amounts of mass for the >2.4 g cm$^{-3}$ fraction were recovered by the two methods, but relatively large differences were found for intermediate density fractions. A similar trend was found for C content, expressed as the proportion of total fraction C.

On average 90–92% of C was recovered by the sequential method whereas the recovery of C by the parallel method was 94–96%. The greater C loss with the sequential method is likely related to the greater number of separation steps since carbon loss in general increased with the density of metatungstate solution (Fig. 1). The average C loss was 4% or less when the density of heavy liquids was ≤2.0 g cm$^{-3}$ (Fig. 1).

Separation of light from heavy materials in metatungstate solutions by gravity sedimentation, used on temperate soils and soil fractions by Cambardella and Elliott (1992, 1994), was also tested and a separation was not achieved due to the coagulation of materials. It seems that a quick separation is necessary to obtain an efficient fractionation.

The density fractionation of clay-size materials was not successful probably because of a narrow density range of clay particles (predominant kaolinite) or flocculation of clay particles in high ionic strength heavy solutions. The 1.7 g cm$^{-3}$ NaI solution, commonly used to extract light fraction of SOM, failed to recover any significant amount of light fraction (<1.7 g cm$^{-3}$) from the silt-size materials of both soils, and the reason was not clear.

The parallel separation was simple to use and time-saving, but the information for intermediate density fractions was derived indirectly by subtracting two neighbouring fractions. The sequential separation procedure used by Turchenek and Oades (1979) requires continuous flow centrifugation equipment, which is not commonly available. The sequential separation procedure of Dalal and Mayer (1986) is time-consuming but uses ordinary centrifuges and permits direct measurements such as C concentration, complexing cations (Fe and Al), mineralogical composition and some biochemical properties (Shang and Tiessen, 1998). However, a significant amount of organic matter can be partitioned into liquid phase in this repeated-separation scheme. Using metatungstate solution and centrifugation on an angular rotor, at least for the two highly weathered soils studied, is promising for isolating SOM stabilised in silt-size or larger aggregates.

References


