The Summer Soil Institute

SOIL ORGANIC MATTER & STABLE C ISOTOPE LABORATORY MODULE

M. Francesca Cotrufo[1], Catherine Stewart [1,2] & Dan Reuss[1]

[1] *Natural Resource Ecology Laboratory, Colorado State University, Fort Collins, CO*

[2] *Soil Plant Nutrient Research U.S. Dept. of Agriculture – ARS, Fort Collins, CO*

Introduction

Soil Organic Matter (SOM) is a complex system, composed of different fractions, each characterised by different physical and chemical properties, microbial degradability and turnover times (Paul 2007). Three major mechanisms: i) chemical recalcitrance, ii) physical protection and iii) organo-mineral association, in particular on surfaces of silicate clay minerals and Fe oxides, are widely accepted as being responsible for C stabilization in soils (Kogel-Knabner et al. 2008, Trumbore 2009).

SOM can be fractionated by physical methods, on the principle that SOM fractions with different size and/or density differ in terms of stabilization mechanisms/turnover times (Stevenson and Elliott 1989). Bigger sizes (> 53 μm) are characteristic of OM that is encapsulated in soil aggregates and therefore physically protected by microbial degradation (Six et al. 2002). Light fractions (LF < 1.8 g cm^{-3}) are predominantly undecomposed residues, whereas heavy and fine fractions (<53 μm) are generally characteristic of mineral-associated OM. An extensive description of SOM characterization methods (Fig.1) is provided in Denef et al. (2009).

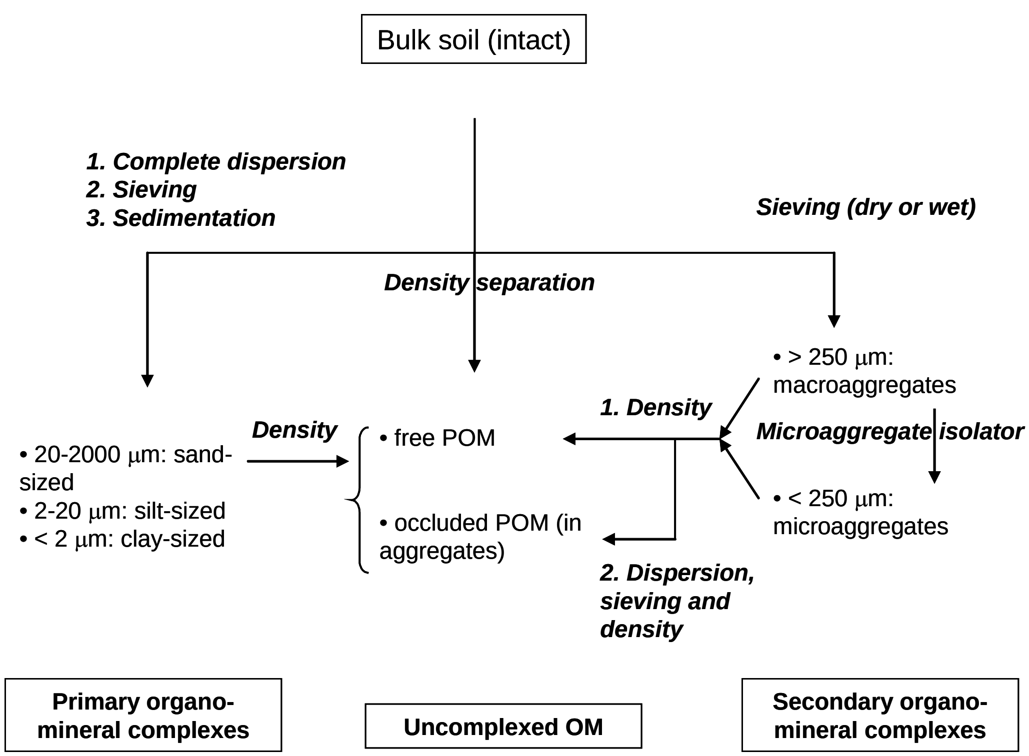

Figure 1: Schematic overview of the most common physical fractionation method (Denef et al., 2009)

The Carbon-to-Nitrogen (C/N) ratio of SOM varies with the state of degradation of the OM, since microbial biomass and bio-product have a lower C/N ratio of plant material (Schlesinger 1997). Additionally, organic N compounds appear to bond more strongly to minerals than organic C compounds, thus mineral associate OM is often characterized by lower C/N levels (Sollins et al. 2006). Soil organic matter maintains the stable carbon isotopic composition ($\delta^{13}C$) of the plant material it was derived from (Gleixner 2005). Therefore, ^{13}C analyses can be used to reconstruct past land use changes or quantify the contribution of the "new" *vs* "old" vegetation, ad example in systems where a C3 vegetation was replaced a by a C4 or *vice versa* (e.g. shifts from forest to savanna or the conversion to a C4 crop (Balesdent and Mariotti 1987, Del Galdo et al. 2003). While preserving the plant ^{13}C signal, SOM tends to enrich in ^{13}C with time, thus becoming progressively more enriched at depth (Balesdent et al. 1993) and from LF to the stabilized mineral associated forms. The reason for this enrichment is still a matter of debate (Ehleringer et al. 2000, Hoegberg et al. 2005).

In this laboratory experience, we will apply a physical fractionation protocol modified from (Denef et al. 2002, Plante et al. 2006, Stewart et al. 2008, Marzaioli et al. 2009) to isolate three SOC fraction: 1) the LF; 2) free particulate organic matter (POM); 3) mineral associated organic matter (Silt&Clay). Further we will analyze all soil fractions for C and N concentration, by elemental analyses (EA) and bulk soil samples for C and N concentration and $\delta^{13}C$ by a EA-Isotope Ratio Mass Spectrometry.

Timeline & Protocol

Upon return from field trip and soil collection (Monday or Tuesday evening):

Sieve a subsample of each collect soil sample to 2 mm, removing obvious litter and root fragments.

Assign a code to each of your samples, and use it to identify your samples in all your future steps.

Put 16 g of each sample in a glass beaker (of which you noted the weight empty) take the weight of beaker + soil on microbalance and then place in oven at 105˚C.

Make sure you have enough SPT (~45 ml per sample).

Make sure you have enough DRY CLEAN centrifuge tubes with lids (1 per soil sample).

Weigh out aluminum pans for LF, POM, and silt&clay (3 per soil sample).

Wednesday July 14, 1-5 pm:

Take soil samples out of oven and let cool in the desiccator (30 min).

Introduction to the SOM and Isotope lab module (30 min) in the Stable Isotope Lab, NESB A119.

After cooling, weigh out (on microbalance) beaker + soil together.

<u>Density fractionation:</u>

Fill FALCON centrifuge tubes with 30 ml 1.85 g cm^{-3} SPT.

Add 6 g dry soil sample and cap the centrifuge tube.

Add 12 glass beads to each tube. Shake vigorously and place lengthwise on reciprocal shaker (on LOW) for 18 hours (to disperse the iron minerals).

Bulk soil analyses of C%, N% and $\delta^{13}C$

Grind the remaining 10 g of soil in a ball mill and transfer samples to scintillation vials.

Weigh an aliquot of each soil sample into a tin cup for bulk soil analyses of C%, N% and $\delta^{13}C$ by EA-IRMS.

Run the samples in the EA-IRMS and record results.

Thursday July 15, 1-5 pm

Density fractionation:

Rinse cap and sides of centrifuge tube with SPT using a plastic pipette and fill up to 40 ml line.

Balance the tubes (one versus one) with SPT and centrifuge the samples at 20 ℃ for 60 minutes at 2500 rpm (use centrifuge in the lab upstairs).

Aspirate LF+SPT and filter the LF on a 20 µm nylon filter on the Millipore glass filter unit.

Rinse the tube 3 times under vacuum and rinse the LF thoroughly with water. When the flask is full, transfer the solution to the glass bottle for SPT recycling (unfiltered SPT).

Transfer the LF with minimal water to pre-weighed (on microbalance) aluminum pans and place the tray with pans into the oven at 50˚C (use the oven in the lab upstairs).

Recovery of heavy fraction (HF) and fractionation by size:

*Add water to the pellet in the centrifuge tube (up to 40 ml line), put lids onto tubes and shake vigorously (if necessary, use the automatic shaker). Take off caps and balance (one vs one) with water (don't forget to rinse the lids).

*Centrifuge at 20˚C for 10 minutes at 2500 rpm.

*Pour supernatant in glass bottle with unfiltered SPT-water mix.

Repeat steps * two times. For the final time add 3 drops of 0.25 M $CaCl_2$ and 0.25M $MgCl_2$, to each tube, shake vigorously, then balance with water and centrifuge for 15 minutes. Pour supernatant in sink.

Take the remaining pellet of soil from the centrifuge tube and disperse by shaking vigorously. Pour sample over a 53µm sieve in a large pan. Remove beads, rinse free POM (+ sand) and transfer into pre-weighed aluminum pans (microbalance). Take the smaller than 53 size fraction (silt+clay) and transfer into preweighed aluminum pans.

Place in oven at 50˚C overnight to dry.

Friday July 16, 1-5 pm

SOM fraction analyses of C%, N%

Weigh pans with the LF, the sand+freePOM, and the silt&clay fractions, and record weights. Transfer samples to scintillation vials. Grind all samples by the use of pestles. Weigh samples in tin cups for analyses of C and N concentrations by EA (Leco). Download and record results.

Instructions on data analyses

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