Protocol for sequential extraction of organo-mineral associations

Purpose

This is a stepwise procedure to extract metals (and, subsequently, the carbon bound to metals). The sequential extractions remove increasingly crystalline metals. The KCl solution removes organic matter from the soils most easily exchangeable sites and prevents an over-estimation of organo-mineral associations. The Sodium Pyrophosphate solubilizes iron complexed with organic matter – typically considered the colloidal and chelated iron in soil. The Sodium Dithionate removes metals from the most crystalline iron-bearing minerals. By measuring the concentration of metals (Fe, Al, and Mn) and the concentration of DOC in the extractant solutions, we can estimate the relative fraction of carbon that is "stabilized" by organo-mineral associations in the soil. DOC is analyzed on a TOC analyzer and the concentrations of metals are analyzed on the AA.

References

Maietta, Christine. 2017. Soil microbial processes and community structure in natural and restored tidal freshwater wetlands of the Chesapeake Bay. Doctoral dissertation, University of Maryland.

Cloy, Joanna M., Clare A. Wilson, and Margaret C. Graham. “Stabilization of Organic Carbon via Chemical Interactions with Fe and Al Oxides in Gley Soils:” Soil Science 179, no. 12 (December 2014): 547–60.

Materials:

1. Soil samples– ground and sieved to 2mm
2. Standard soil
3. Oscillation shaker table
4. Serological pipettor
5. Super speed centrifuge (>10,000 rpm)
6. Conical centrifuge tubes for super-speed centrifuge
    a. 1 / sample
7. Clear HDPE bottles for filtered extractant
    a. 60 mL = 2 / sample
    b. 120 mL = 1 / sample
8. 0.45 μm nylon filters
    a. 3 / sample
9. 60 mL syringes
    a. many
10. 25 mL serological pipette tips
    a. 3 / sample

Reagents and chemicals:

1. Nano-pure water for rinsing, making reagents, blanks
    a. 60 mL / sample
2. 1 M potassium chloride (KCl), FW= 74.55 g / mol
    a. 60 mL/ sample
    b. dissolve 74.55 g in 1L nano-pure water
3. 0.1 M sodium pyrophosphate, pH 10 , FW = 265.90 g / mol
    a. 80 mL / sample
    b. dissolve 26.59 g in ~0.8 L nano-pure water. Add NaOH to raise pH and concentrated HCl to lower pH until pH = 10. Bring to 1.0 L with nano-pure
4. 0.5 M sodium dithionite, FW = 174.11 g / mol
    a. 30 mL / sample
    b. weigh 87.06g dithionite into 1L volumetric flask. Add nano-pure water to bring volume to 1.0 L
5. 0.05 M hydrochloric acid (FW 36.46 g / mol, 37% w/w)
    a. 30 mL / sample
    b. add 4.106mL to ~0.5 L nano-pure water. Bring volume to 1.0 L
6. Standards for DOC in extractant (suggested range: ____)
7. Standards for AA in extractant (suggested range: 0.1 – 50 mg/L Fe)

Using the Sorvall RC-26 PLUS Centrifuge for speeds > 10,000

1. Flip centrifuge on and allow instrument to internally calibrate
2. Knobs to open the inner container must be opened and closed sequentially
    a. open: loosen the inner (black) cap first, then loosen the outer (silver) cap
    b. close: the opposite.Tighten the outer cap first, then tighten the inner cap
    c. caps should be tightened snugly but not over-tightened
3. Use specific 60mL centrifuge tubes
4. 8 samples can be centrifuged at a time
5. Masses of liquids should be balanced by volume or weight (preferable)
6. The centrifuge tube should fit into slots easily
7. Tubes can be used with or without caps, but are positioned at a slight angle in the centrifuge and therefore cannot be overfilled if without a cap
8. Load samples into the rotor evenly balance weights. Only load an even number of samples in the rotor. Two samples should always be placed opposite each other to maintain balance. If an odd number of samples are required, make a counterbalance by filling a centrifuge tube with an equal mass of solution.
9. Set run controls for centrifuge
    a. Set desired speed, time, and temperature
    b. enter code for specific rotor being used
    c. turn on slow start
    d. turn on brake
10. Press “Start” button to begin centrifugation
11. When run has completed, rotor has come to a complete stop and unlocked, open the lid, unscrew the caps to the inner container
12. Carefully remove samples to prevent resuspension of sediments
13. Turn off with power switch between runs

Extraction procedure

Preparation (1-2 days)

1. Base wash all sample containers and leach for 24 hours with nano-pure water. Rinse 3 times with nano-pure water and allow to air dry
2. Pre-label all containers and lids
3. Using the analytical balance, add 0.5 ± 0.01 g air dried, ground soil into pre labeled tubes and record mass of each sample
4. Include 1-2 blanks per run.

Day 1 PM: Extracting water-soluble organic matter with 1M KCl (part I)

1. Between 3:30 and 5:00pm, add 25 mL of 1.0M KCl to each centrifuge tube of sample with the serological pipettor. Cap securely.
2. Secure to oscillation shaker table and shake on high for 16h
    a. example: start at 4:00pm, end at 8:00am the next day

Day 2 AM: Water-soluble organic matter (part II)

1. At 8:00 am, remove samples from shaker table and centrifuge at 10,000 rev/min for 6 minutes (see above protocol)
2. Carefully remove as much supernatant as possible with serological pipettor and the labeled pipette tip for that sample, being careful not to remove any soil sample. Record volume of supernatant removed.
3. Pipette supernatant directly into open 60mL syringe with new filter attached.
4. Gently place plunger back into syringe. Clear filter by dispensing ~5mL supernatant into waste. Filter the remaining sample into the labeled 60mL HDPE bottle.
    a. Keep filter and pipette tip labeled and with each individual sample.
    b. Rinse syringe 3x with nano-pure water between samples
5. Repeat extraction, centrifugation, and filtering procedure once with 25 mL 1.0M KCl except shake for only 1h.
    a. Two extractions should be sufficient; however if the extracted solution still has significant color then continue the extraction procedure until solution is clear or three total extractions.
    b. Filter supernatant and combine with the rest of the sample

Day 2 PM: Sodium pyrophosphate extraction (part I)

1. Prepare fresh 0.1M sodium pyrophosphate, pH 10
2. Between 3:30 and 5:00pm, add 25 mL of 0.1 M sodium pyrophosphate to each centrifuge tube of sample with the serological pipettor. Cap securely.
3. Secure to oscillation shaker table and shake on high for 16h
    a. example: start at 4:00pm, end at 8:00am the next day

Day 3 AM: Sodium pyrophosphate extraction (part II)

1. At 8:00 am, remove samples from shaker table and centrifuge at 10,000 rev/min for 6 minutes (see above protocol)

2. Carefully remove as much supernatant as possible with serological pipettor and the labeled pipette tip for that sample, being careful not to remove any soil sample. Record volume of supernatant removed.
3. Pipette supernatant directly into open 60mL syringe with new filter attached.
4. Gently place plunger back into syringe. Clear filter by dispensing ~5mL supernatant into waste. Filter the remaining sample into the labeled 120mL HDPE bottle.
    a. Keep filter and pipette tip labeled and with each individual sample.
    b. Rinse syringe 3x with nano-pure water between samples
5. Repeat extraction, centrifugation, and filtering procedure twice with 25 mL 0.1M sodium pyrophosphate except shake for only 1h.
    a. Three extractions should be sufficient; however if the extracted solution still has significant color then continue the extraction procedure until solution is clear or four total extractions.
    b. Filter supernatant and combine with the rest of the sample

Day 3 PM: Dithionite extraction (part I)

1. Prepare fresh 0.5M dithionite
2. **IN THE HOOD** Between 3:30 and 5:00pm, add 25 mL of 0.5 M dithionite to each centrifuge tube of sample with the serological pipettor. Cap securely.
3. Secure to oscillation shaker table and shake on high for 16h
    a. example: start at 4:00pm, end at 8:00am the next day

Day 4 AM: Dithionite (part II)

2. At 8:00 am, remove samples from shaker table and centrifuge at 10,000 rev/min for 6 minutes (see above protocol)
3. **IN THE HOOD**, Carefully remove as much supernatant as possible with serological pipettor and the labeled pipette tip for that sample, being careful not to remove any soil sample. Record volume of supernatant removed.
4. Pipette supernatant directly into open 60mL syringe with new filter attached.
5. Gently place plunger back into syringe. Clear filter by dispensing ~5mL supernatant into waste. Filter the remaining sample into the labeled 60mL HDPE bottle.
    a. Keep filter and pipette tip labeled and with each individual sample.
    b. Rinse syringe 3x with nano-pure water between samples
6. Repeat extraction using 25-mL 0.05M hydrochloric acid, centrifugation, and filtering procedure once except shake for only 1h.
    a. This is an acidifying step that will stabilize the dithionite for analysis on the AA.
    b. Filter supernatant and combine with the rest of the sample

Sample Analysis

1. Filtered supernatants can be stored in fridge for <2 weeks or freezer before analysis for Fe (Atomic Absorption Spectroscopy) and DOC (Shimadzu TOC/TON analyzer)
2. Remaining soil pellet can be dried, ground, and processed for C/N analysis

Calculations

Fe (in g / Kg soil) is the amount of Fe in each individual extractant multiplied by the volume of extractant and divided by the initial soil mass, corrected for any changes in units

*g Fe Kg^{-1} soil = [(measured concentration Fe * Dilution factor) * Total volume extractant] / Initial soil mass*

% Fe is g Fe Kg^{-1} / 10