**Aggregate size carbon fractionation**

This method is used to separate soil into different aggregate size classes, which we want as a measure of stability. The size classes include: large macroaggregates, small macroaggregates, microaggregates, silt and clay, and floating particulate organic matter. This procedure is based on Six et al. 2000 and communication with C. Maietta, 2017.

Video of fractionation procedure:
https://www.youtube.com/watch?v=VOaae2bDDCY

**Supplies needed**

- 4.75 mm sieve
- 2 mm sieve
- 250 µm sieve
- 53 µm sieve
- Aluminum pie tin
- Aluminum turkey tin
- 3 aluminum bread tins
- 2 small aluminum circular tins
- 2 or 3 dish basins
- Ultrapure DI and squirt bottles of ultrapure DI
- Ziploc bags
- Coin envelopes

**Procedure**

1. Sieve the ~200 g of soil sample from the field that were transported back to the greenhouse and dried for 3 days through a 4.75 mm sieve and into a labeled aluminum pie tin.
    a. If the soil is too dry, use a squirt bottle of ultrapure DI to moisten the soil. Be sure to note that water was added.
    b. Label the pie tin with the sample ID, date it was sieved, and if water was added to aid in sieving.
2. Dry the 4.75 mm sieved soil in the greenhouse for at least another 4 days.
3. Pour dried 4.75 mm sieved soil from the aluminum tin into a labeled Ziploc bag.
    a. Label Ziploc bag with sample ID, 4.75 mm, and the date it was 4.75 mm sieved.
    b. *Recommendation*: save the labeled pie tin for step 5
4. Invert Ziploc about 2-3 times to homogenize sample.
5. Weigh the soil that is in the labeled Ziploc and record this weight.
    a. *Recommendation*: tare the used the aluminum pie tin from step 3 and pour the Ziploc bag of soil into the pie tin
    b. This is done so that in case a sample needs to be redone, you know how much soil you have left.
6. From the total soil just weighed, pour exactly 100.00 g of that soil into a weigh boat and record the weight.
7. Label all aluminum tins that will be used for the fractionation with the sample ID, size class, and the date the sample is wet sieved.
    a. *Recommendation*: 1 turkey tin for silt and clay (sc), 3 bread tins for large macroaggregates (LM), small macroaggregates (sM), and microaggregates (m)
8. Weigh all labeled tins and record the weights.

9. Place a 2 mm sieve into a dish basin. Fill the dish basin with ultrapure DI until the water level is about 1 cm above the sieve mesh.
    a. *Recommendation:* use a ruler to mark 1 cm above the 2 mm sieve mesh
10. Sprinkle the 100.00 g of soil evenly on the 2 mm sieve and wait 5 minutes
    a. Waiting 5 minutes is because the pressure from the water will build up within the aggregates and cause them to break apart naturally. The sequential sieving (slaking) determines if aggregates are water-stable at various aggregate size classes.
11. After 5 minutes, set a metronome to a pace of 25 beats per minute (bpm). Sieve soil for 2 minutes by moving the sieve 50 times up and down in the dish basin with a slight angle to ensure that water and small particles pass through the mesh (sieve every time your hear the beat).
    a. *Recommendation*: download the app "Pro Metronome" and set the temp to 25 and T.S. to ¼
12. After 2 minutes, hold the sieve above the water in the dish basin and rinse of the outside and bottom of the sieve with a squirt bottle of ultrapure DI.
    a. Squirt the perimeter of the mesh on the bottom of the sieve until the water runs clean.
    b. Be very careful rinsing the bottom of the sieve as you don't want to squirt aggregates back into the dish basin.
13. Transfer the contents on the sieve into the pre-weighed aluminum tin for **large macroaggregates (LM).**
    a. Backwash the sieve into the aluminum tin with sufficient ultrapure DI. Everything on the sieve should end up in the LM tin.
14. Place a 250 µm sieve into a new dish basin.
15. Pour the water and aggregates in the dish basin that passed through the 2 mm sieve onto the 250 µm sieve. Use a squirt bottle to thoroughly clean the basin so there is nothing left.
16. Repeat sieving procedure (Step 11 – there is no longer a 5 minute wait).
17. After the 2 minutes, follow Step 12.
18. Transfer the contents on the sieve into the pre-weighed aluminum tin for **small macroaggregates (sM).**
    a. Backwash the sieve into the aluminum tin with sufficient ultrapure DI. Everything on the sieve should end up in the sM tin.
19. Place a 53 µm sieve into a new dish basin.
20. Pour the water and aggregates from the dish basin that passed through the 250 µm sieve onto the 53 µm sieve. Use a squirt bottle to thoroughly clean the basin so there is nothing left.
    a. This step may need to happen in 2 batches if the soil is a silt loam. This is discussed below in "Common issues".
21. Repeat sieving procedure (Step 11). It's very important to be able to see the mesh screen of the 53 µm sieve before beginning to sieve. If you cannot, then that's an indicator of needing to sieve in 2 batches.
22. After the 2 minutes, follow Step 12.
23. Transfer contents on the sieve into the pre-weighed aluminum tin for **microaggregates (m).**
24. Pour the water and aggregates in the dish basin into the pre-weighed turkey tin for **silt and clay (sc).**
25. Now that the LM and sM tins have had time to settle, look to see if there is any floating particulate organic matter (fPOM). Depending upon your system and your question, fPOM may only be important in the LM and/or sM size class.
26. If there is fPOM, label and weigh small aluminum circular tins for the aggregate size classes that have fPOM

   a. If the fPOM is from the LM size, label the tin with the sample ID and fPOM_LM
   b. If the fPOM is from the sM size, label the tin with the sample ID and fPOM_sM
27. Decant fPOM.
   a. *Recommendation*: use a transfer pipette. Cut the end of the pipette if there's large fPOM.
28. Once all the tins are filled with their respective aggregate size, leave them on a counter or an open space so water can evaporate.
29. After all of the standing water has evaporated, cover tins with aluminum foil and place in a 60 deg C forced air-drying oven for 4 days minimum.
   a. *Recommendation*: fold a corner of the aluminum foil when placing tins into drying oven.
30. After 4 days, weigh tins (without the aluminum foil cover) and record the weight
31. Transfer the oven dried LM and sM samples to a 250 µm sieve over a mortar and grind the sample until all aggregates pass through the sieve. The rocks that remain on the sieve weigh and record the weight.
   a. This is done so that the sample is fine enough for C/N analysis.
   b. This is not done for the other size classes because they are fine enough.
   c. Place rocks in a labeled coin envelope with the size class they were from
32. Transfer ground aggregates to a labeled coin envelope
33. Transfer fPOM, m, and sc to a coin envelope and label the envelope
   a. This is best done by scraping the tins
34. Take a subsample from the 53 µm and 250 µm samples for sand content.
   a. The remainder of sample in the coin envelope can be used for any analyses such as C/N.

**Common issues**

- When transferring aggregates to a 53 µm sieve, there are too many microaggregates and silt and clay to see the 53 µm sieve mesh. When the mesh can't be seen water cannot pass through the sieve, which means silt and clay cannot properly pass through the sieve. When this occurs you will need to 53 µm sieve twice.
   - Pour half of the dish basin contents that passed through a 250 µm sieve onto a 53 µm sieve. Be sure to rinse off the corner of the dish basin that the aggregates were poured from.
   - Follow the 2 minute sieving procedure. Rinse the outside and bottom of the sieve well and then backwash the aggregates into the **microaggregate (m)** tin. Backwashing does not have to be extensive because you are going to add the rest of the dish basin onto the 53 µm sieve.
      - *Recommendation*: pour half of the contents from the dish basin that the 53 µm sieve sits in into the **silt and clay (sc)** tin.
   - Pour remaining aggregates and water onto the 53 µm sieve and be sure to rinse out the entire dish basin.
- You may have too much water in the last dish basin of silt and clay that it won't all fit into one turkey tin – weigh and record the weight of a second empty labeled (**sc2**) turkey tin and pour remainder into there.

**References**

Six, J., K. Paustian, E.T. Elliott, C. Combrink. 2000. *Soil Structure and Organic Matter: I. Distribution of Aggregate-Size Classes and Aggregate-Associated Carbon*. Soil Science Society of America Journal. 64:681-689.

**Example of data sheet**

| Sample ID | Date of field soil collection | Date soil sieved through 4.75mm | Water added to pass 4.75mm? (Y/N) | Date of sieving | Wt of soil in 4.75mm bag (g) | Wt of soil being sieved (g) | Wt of empty labeled tin (g) | Date in drying oven | Date of OD wt | OD wt (g) | Total wt in tin (g) | Wt of rocks (g) | Total wt of aggs (g) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ID1_LM | | | | | | | | | | | | | |
| ID1_sM | | | | | | | | | | | | | |
| ID1_m | | | | | | | | | | | | | |
| ID1_sc | | | | | | | | | | | | | |
| ID1_sc2 | | | | | | | | | | | | | |
| ID1_fPOM_LM | | | | | | | | | | | | | |
| ID1_fPOM_sM | | | | | | | | | | | | | |
| ID2_LM | | | | | | | | | | | | | |
| ID2_sM | | | | | | | | | | | | | |
| ID2_m | | | | | | | | | | | | | |
| ID2_sc | | | | | | | | | | | | | |
| ID2_sc2 | | | | | | | | | | | | | |
| ID2_fPOM_LM | | | | | | | | | | | | | |
| ID2_fPOM_sM | | | | | | | | | | | | | |

*Note*:
“wt” = weight
“aggs” = aggregates
“sieving” = fractionation