**Thain Family Forest Soil Dry Composition**

**Standard Operating Procedure 1.1**

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1. **PURPOSE AND SCOPE:**

The Thain Family Forest (TFF), a 20 ha stand within NYBG, Bronx, NY, is the largest old growth forest in New York City and the epicenter for 125 years of urban forest inventory and ecosystem monitoring. By building on prior analyses and aligning with international standards, NYBGs ongoing forest monitoring will reinforce the forest’s status as the world’s premier urban research forest with high quality, transparent and repeatable data across the spectrum of forest structures and functions.

This standard operating procedure details procedures for determining the dry mass and bulk composition of samples from the floor of the TFF, with the goals of (1) aligning with an international standard and (2) establishing a scalable framework for repeatedly measuring multiple features of soil structure and function. Doing so will (A) maximize comparability with other research forests and (B) inform analyses of (i) change in soils from key historic datasets, (ii) belowground impacts of current and proposed management and (iii) ongoing monitoring of soil health, especially related to carbon stocks and fluxes.

To accomplish these goals, this protocol adapts the Smithsonian Institution Forest Global Earth Observatory (ForestGEO) protocol (Smithsonian institution n.d.) with more intensive sampling of points that overlap with CFI plots. This SOP also emphasizes lab procedures tailored to resources available at NYBG. The Thain Family Forest Field Soil Field Sampling Protocol (TFF\_soil\_field\_SOP\_v1.1) describes field protocols for collecting representative samples of leaf litter and soils at different depths (0-10 cm, 10-20 cm, 20-50 cm and deeper in 50 cm increments) for temporary storage in a 4 degree C refrigerator. This protocol describes complementary laboratory techniques to determine the dry mass and bulk composition of stored samples of litter and soil.

The development of this SOP was supported with resources from the Forest Ecosystem Monitoring Consortium. Data collection activities are indicated by the symbol “○” with named fields on the datasheet labeled in **bold underline.**

1. **SAFETY:**

This section highlights some, but not all, of the safety concerns you should be aware of while working in the lab. Using required PPE and immediately reporting any concerns or injuries to Supervisory Staff is crucial for maintaining a safe work environment.

1. **Personal Protective Equipment (PPE)**

It is REQUIRED that all personnel working on this study:

1. wear long pants

2. wear closed-toed shoes (preferably boots)

3. wear disposable gloves while handling samples, which may contain poison ivy leaf litter or roots

1. **Reporting an Injury** All injuries or safety concerns must be reported to on-site to Brad Oberle or Senior Lab Manager Tynisha Smalls. Personnel must complete a Personnel Report of Accident/Injury form for all reported injuries.
2. **Lab safety** Use of laboratory drying ovens entails fire hazards. Always ensure open circulation around samples, leaving gaps between samples and the oven walls. Personnel without C14 training may only use lab facilities during regular business hours (9-6 pm Monday -Friday).
3. **Lab etiquette** Many research groups use the Pfizer lab for procedures that are sensitive to dust and dirt. Any process that could generate dust or spread dirt, including sieving soils or transferring dried soils between containers, must take place either in the autoclave room on the first floor or in the bulk prep room through the growth chamber room.

**3. Outline of Procedures**

1. **Personnel**
2. **Materials**
3. **Measurement and Collection Procedures**
4. **Summary**
5. **Litter dry mass**
   * 1. **Prepare equipment and supplies**
     2. **Dry litter samples**
     3. **Record dry mass and store litter samples**

**3. Soil Bulk Density sample moisture content**

1. **Prepare equipment and supplies**
2. **Wet mass and composition by sieving**
3. **Record dry mass and store dried SBD samples**

**4. Soil Composition**

1. **Prepare equipment and supplies**
2. **Wet mass and composition by sieving**
3. **Record dry mass and store dried components**
4. **Data entry**
5. **Personnel**
   1. **Brad Oberle** is the project PI responsible for data collection, developing and revising SOPs, training personnel and conducting quality assurance activities. Brad is the supervising personnel responsible for lab-based activities.
   2. **John Zeiger** is the Thain Family Forest Manager who is responsible for implementing the Thain Family Forest Management Plan. John is the supervising personnel responsible for field-based activities.
   3. **Tynisha Smalls** is the Senior Laboratory Manager of the Pfizer lab. Tynisha is responsible for day-to-day laboratory management and safety training.
   4. Record **Personnel** and **Date**
6. **Materials**

**PPE**

* 1. Nitrile gloves for handling roots
  2. Lab goggles for any steps that may generate dust

**Field collected samples in labeled, sealed plastic bags stored in fridge**

* 1. Soil Bulk Density (SBD) [or in sample rings if processed directly from the field]
  2. Soil composition
  3. Litter
  4. Field datasheets (in hanging folder in phytochem lab)

**For weighing samples**

* 1. Leveled calibrated ohaus scout balances
     1. 0.01 g precision in phytochem lab or bulk sample prep for aluminum tray, dry paper bag, soil, litter and rock masses.
     2. 0.001 g precision in phytochem lab for dry coin envelope and dry root masses
  2. Hexagonal weigh boats for weighing sample wet masses and dry rocks (in phytochem lab above balances)

**For separating sample components**

* 1. Large tray for protecting bench from sieve motion (in outer room of bulk prep area)
  2. 6-12 clean dry 2 mm (#10) sieves for separating soil from SBD and sorting rocks and large diameter roots from soil C samples (in bulk prep area)
  3. 3-6 clean dry 250 um (#60) sieves for separating fine roots from C samples (in bulk prep area)
  4. Forceps for handling fine roots and spatulas for separating sieves (in outer bulk prep area shelf)

**For drying samples**

* 1. Active drying
     1. 105°C drying oven in phytochem lab for drying soil bulk density moisture content samples and rocks
     2. 60°C drying oven in phytochem lab for drying paper bags with litter, and coin envelopes with roots
     3. Dessicators with silica gel for storing pre-dried envelopes and paper bags (in phytochem lab)
  2. Containers
     1. Clean aluminum trays for pre-weighing, labeling and drying SBD samples at 105°C (in phytochem lab under bench)
     2. Aluminum tray lids for moving samples (in phytochem lab under bench)
     3. New pre-dried paper bags for pre-weighing, labeling and drying litter samples at 60°C (in phytochem lab under bench)
     4. New paper bags for labeling and air-drying sieved soil C samples (in bulk prep room under bench)
     5. New pre-dried coin envelopes pre-weighing, labeling and drying litter samples at 60°C (in phytochem lab under bench)
     6. New or used paper bags for drying sieved stones (in bulk prep area)
     7. Four rectangular cardboard ventilators for separating layers of aluminum trays with SBD samples in drying oven (in phytochem lab)

**For storing and organizing dried, sieved samples**

* 1. Large containers for storing and organizing oven-dried litter by plot (in bulk prep area)
  2. 50 mL conical tubes for storing sieved oven-dried SBD soils (in bulk prep area)
  3. Large envelope for storing and organizing oven-dried soil C roots in coin envelopes by plot (in office)
  4. Large containers for storing and organizing air-dried soil C soil by plot (in phytochem lab)
  5. 3.5 gallon bucket for storing dried stones (in phytochem lab)

**For cleaning and moving**

* 1. Kim wipes for cleaning processing materials (in phytochem lab)
  2. Dust pan for cleaning bench tops (in both labs)
  3. Standing broom for cleaning floors (in bulk prep lab)
  4. Lab cart (in phytochem lab)

**c. Measurement and Collection Procedures**

**1. Summary**

The outputs from this procedure are (1) measurements of (a) soil bulk density and (b) dry mass of biological tissues ([i] litter and [ii] roots) and (2) organized, stabilized, dried samples stored for further homogenization, subsampling and chemical analysis. Generating these outputs requires processing three different kinds of samples from the field; leaf, soil bulk density and soil composition. All samples are stored in labeled, sealed plastic bags in the phytochemistry lab fridge. To facilitate workflow, all samples of a particular type are processed together, rather than samples of different types from the same plot. Doing so requires careful management of the datasheets associated with the samples from one or more plots which are stored in a hanging folder below the large capacity balance in the phytochem lab.

Litter samples generate litter dry mass data and are the easiest to process. All steps can occur in the phytochemistry lab. Litter dry mass is determined after drying in paper bags for 3-5d at 60°C. Dried, bagged litter samples are retained for homogenization and C content analysis. To facilitate workflow and accuracy, pre-dry bags for 24 hours at 60°C, weigh dry bags to a precision of 0.01 g, label with the bag dry mass, load weighed bags with the litter sample from the field, add sample plot and point information, dry, reweigh dry and store in a plastic bag. Additional steps are necessary for subsample litter. Refer to the field datasheets.

Soil bulk density samples are used for determining soil moisture content and bulk density and require an intermediate level of processing. The process begins by thoroughly homogenizing the sample in the bag by kneading it and breaking up any clumps, pre-weighing and labeling an aluminum dish for collecting and drying soils, measuring the total wet mass of the homogenized sample in the dish, separating loose soil from roots and rocks by passing the sample through a 2 mm sieve, reweighing the wet mass of the passed soil on the pre-weighed dish, drying for 24-48 hours at 105°C (this is the only step that occurs in the phytochemistry lab), and weighing the mass of the dried soil in the dish. The dried, sieved soil from the SBD sample is transferred to a labeled 50 ml plastic conical for long-term storage and possible organic matter determination by loss-on-ignition.

Soil composition samples are used for collecting an air-dried soil sample for advanced chemical characterization and for measuring the proportion of samples consisting of roots, stones and soils adhered to either other component. Homogenized samples are weighed wet. A 2 mm sieve is weighed dry and clean and then the sample is passed through the sieve, with the soil which passes through collected into a labeled paper bag for air drying on a shelf in the bulk sample prep room, and the sieve with retained roots, soils and stones reweighed. After reweighing the sieve with the coarse material, a fine sieve (250 um) is placed beneath the coarse sieve and thoroughly rinsed under a sink. Roots larger than 2 mm diameter are collected from the coarse sieve and placed into a pre-weighed coin envelope. Roots smaller than 2 mm diameter are collected from the coarse and fine sieves and placed into a second coin envelope. Both root envelopes are dried for 3-5d at 60°C and weighed dry and reserved for homogenization and chemical characterization. Stones from the coarse sieve are placed in a different coin enveloped dried for 3-5 d at 105°C, weighed dry and retained in a bucket for density estimation.

Personnel working in one or more groups of at least 2 people can perform all of the steps necessary to process all three kinds of samples in two 6 hour lab days per week. **plotIDs are created from two numeric values corresponding to the CFI transect and central plot or substitutes determined for soil plots not centered on CFI transect. Sample IDs are concatenated from the plotID + pointID [{N,C,S}x{E,C,W} or outer] + the level of the uppermost stratum of the sample [litter = “L”, soil = “0, 10,20,50,100…”] and the type of soil sample as needed [bulk density = “B”, composition = “C”]**

Lab Day 1: *Check ovens and fridge for all material and datasheets below*

1. Weigh and store existing dried material (approximately 2.5 h for a team of 2 in phytochem lab)

* Maximum 26 envelopes with stones to 0.01g dried at 105°C and transferred to bucket for storage.
* Maximum 52 weighed envelopes to 0.001g with narrow or wide roots dried at 60°C and placed in sealed bag for storage.
* Maximum 26 envelopes with other material to 0.01g, dried at 60°C and transferred to bucket for storage.
* Maximum 8 weighed bags with litter to 0.01g dried at 60°C and placed in a carboard box in the bulk prep room for storage

2. SBD sample processing (approximately 2.5 h for team of 2 in 1st floor lab):

* Maximum 48 SBD samples, with trays weighed to 0.01g, samples homogenized and weighed wet, sieved, reweighed and sieves cleaned.
* Weighed, sieved samples loaded into 105°C as 5 stacks of 5 trays separated by cardboard.

3. Prep containers for day 2 (approximately 0.5 h for team of 2 in phytochem lab):

* + Weigh and write dry masses on 8 paper bags to 0.01g from 60°C

If more than 2 personnel are available, soil bulk density processing can happen in parallel and/or teams can begin processing soil composition samples (see day 2)

Lab Day 2: *Check ovens and fridge for all material and datasheets below*

1. Weigh and store existing dried material (approximately 1.5 h for a team of 2 in downstairs lab)

* 25-48 h 105°C SBD samples
  + - Remove SBD samples, add lids and transfer to downstairs lab
    - Remove lids and weigh dried samples to 0.01g in trays
    - Transfer samples from trays to 50 ml conicals.
    - Store conicals in box in bulk prep area
    - Rinse trays
  + Soil composition samples
    - Check soil samples for dryness, move to second shelf if still moist, move others to plastic bin in bulk prep area

2. Composition processing (approximately 4-6 h for a team of 2 in downstairs lab)

* Homogenize soil composition samples, weigh sample to 0.01g, weigh sieve, collect passed soil into labeled paper bag, reweigh sieve with material.
* Stack sieves and rinse adhered soil from roots and stones
* Sort roots into diameter classes and place in pre-weighed coin envelopes
* Collect stones into reused coin envelopes for drying

3. Drying (approximately 1 h for a team of 2 in phytochem lab)

* Place sieved composition soils in labeled paper bags on shelves in SBD room to air dry
* Place sorted root samples in new coin envelopes, in cardboard box in 60°C
* Place litter samples in pre-weighed paper bags in 60°C
* Place stone samples in re-used bags, in cardboard box in 105°C
* Place 8 new paper bags in 60°C to pre-dry

If more than 2 people are available, soil composition processing can happen in parallel in the autoclave room and bulk sample prep areas.

1. **Litter dry mass**

Measuring the dry mass of litter samples requires drying for [3-5 days at 60°C](https://doi.org/10.1007/s10457-013-9596-y). Litter drying must occur within 28 days of field collections. In between field collections and drying, all samples must remain in sealed, labeled plastic bags in the lab refrigerator at 4 degrees C.

* + 1. **Prepare equipment and supplies (day 2, in phytochem lab)**
  1. Set the oven to [60°C](https://doi.org/10.1007/s10457-013-9596-y)

Prior to drying samples, make sure that the lab drying oven has space and nothing is stored on top of the oven.

* + - 1. Flip the power switch on the bottom of the drying oven to the “on” position.
      2. Press the far right arrow key until the bottom LED displays “SP”
      3. Use the up and down arrows to change the value of SP to “60.0”
      4. Press the far right arrow key to enter the selection
  1. Predrying and weighing paper bags
     + 1. Place a maximum of 8 new paper bags in 60°C for 2 plots
       2. Allow to dry for 24 hours
       3. Weigh and label bags 1 at a time

Weigh to a precision of 0.01 g

Write the bag dry mass in sharpie on the bag

* + - 1. Place the dried, weighed bags in a desiccator
    1. **Dry litter samples (day 2, in phytochem lab)**
  1. Remove litter samples from fridge and gather associated field datasheets. Choose a litter sample to begin processing.

○ ON THE ASSOCIATED FIELD DATASHEET record the number of and day the litter samples were removed from the fridge for processing in **L#X4d\_d**

○ ON THE LAB BL DATASHEET check the names of the samples to be processed and the number of sheets used if processed in parallel by two teams

1. Remove a pre-dried, labeled paper bag from the desiccator

○ Record the **sampleID**

○ Write the sample name on the pre-weighed bag and record the recorded bag dry mass in **L\_bM\_g.gg.**

1. Transfer litter samples from plastic bags to paper bags, recording wet mass of subsampled litter

🡪if the litter sample was subsampled, remove the subsample from the plastic bag, and place the entire litter subsample into the weighed paper bag. Weigh to a precision of 0.01 g and record the combined mass of the dried bag and wet litter subsample in NOTES

1. Place the bagged litter sample in the drying oven
   * + - 1. Fold the open end of the paper bag over to enclose the sample
         2. Place the sample in the oven, careful to leave space for circulation between bags and all 4 walls.

○ Record the day and hour the sample was placed into the oven in **L\_in60\_dh**.

1. Repeat a-c for all litter samples and leave in drying oven for 3 to 5 days
   * 1. **Record dry mass and store litter samples (day 1, in phytochem lab)**
   1. Remove dried litter samples from drying oven

○ Record the day and hour the sample was removed from the oven in **L\_out60\_dh**.

* 1. Weigh the dried litter sample in its bag to a precision of 0.01 g

○ Record the dry mass in **L\_bM\_g.gg.**

* 1. Repeat a-b for all dried litter samples, place all samples from a plot in a container and transfer containers to designated storage space in the bulk sample prep area.

**3. Soil Bulk Density sample and composition subsample moisture content**

Measuring the moisture content of the soil bulk density sample (0-10, 10-20 cm) and composition subsamples (20-50, 50-100,100-150) requires weighing the wet mass and the portion that passes through a sieve. The portion that passes through a sieve is dried for 24-48h at 105°C and reweighed dry. Processing must occur within 28 days of field collections. In between field collections and drying, all samples must remain in sealed, labeled plastic bags in the lab refrigerator.

1. **Prepare equipment and supplies (day 1, phytochem lab🡪first floor)**
   1. Set the oven to [105°C](https://doi.org/10.1007/s10457-013-9596-y)

Prior to drying samples, make sure that the lab drying oven has space and nothing is stored on top of the oven.

* + - 1. Flip the power switch on the bottom of the drying oven to the “on” position.
      2. Press the far right arrow key until the bottom LED displays “SP”
      3. Use the up and down arrows to change the value of SP to “60.0”
      4. Press the far right arrow key to enter the selection
      5. Remove SBD & composition moisture content samples from fridge and thaw if necessary

○ ON THE ASSOCIATED FIELD DATASHEET record the day the bagged B samples and were removed from the fridge for processing and the number in **B#X4d\_d**

○ ON THE LAB BDL DATASHEET check the names of the samples to be processed and the number of sheets used if processed in parallel by two teams

6. Only 20 samples will safely fit in the drying oven. Do not process more than 20 at a time.

7. Transfer samples to first floor lab

1. **Wet mass and composition by sieving (day 1, first floor lab🡪phytochem lab)** 
   1. Select a sample

**○** Record the **sampleID**

* 1. Homogenize SBD samples by using your hands to break up clumps of soil in the sealed plastic bag
  2. Place aluminum tray on the 0.01 g balance and tare

○ record the tared bulk density sample wet mass **B\_WM\_g.gg**

* 1. Weigh and label aluminum trays for capturing the sieved SBD sample
     + - 1. Use a sharpie to write the sample name on the bottom of the tray
         2. Weigh the tray to a precision of 0.01 g

○ record the tray mass **t\_M\_g.gg**

* 1. Sieve the SBD sample into the weighed, labeled tray
     + - 1. Carefully fit a clean, dry 2 mm sieve into the labeled tray
         2. Dump the homogenized sample from the hexagonal weigh boat onto the sieve
         3. Place the sieve in the tray on a protective tray over the bench
         4. Agitate the sieve with the tray for about 1 minute until only stones, roots and adhered soil remain above the sieve

🡪if soils are cohesive, use a glove hand to push the soil through the sieve. Be sure to clear the bottom of the sieve into the tray.

* + - * 1. Weigh the passed-through soil to a precision of 0.01 g

○ record the tray with SBD wet mass **t\_tsBD\_WM\_g.gg**

* + - * 1. Rinse used sieve and set aside to dry
  1. Repeat a-e for up to 20 B samples
  2. Dry the samples at 105°C for 24-48 hrs
     + - 1. Place lids over the samples and transport to phytochemstry lab then remove lids
         2. Load samples into 105°C to a maximum of 5 stacks of 5 trays separated by cardboard with each tray being exposed to the circulating air and no trays contacting the dryer walls (See figure)

○ record the time (day hour) the samples are fully loaded into the oven **tsBD\_in105\_dh**

1. **Record dry mass and store dried SBD samples (day 2 phytochem 🡪 first floor lab)**
2. After 24-48 hours, used gloved hands to remove trays and add lids

○ record the time (day hour) the samples are removed from the oven **tsBD\_out105\_dh**

1. Transfer covered samples to downstairs lab
2. Remove lids and weigh dry samples

○ record the tray with SBD dry mass **tsBD\_DM\_g.gg**

1. Transfer samples from trays to 50 ml conicals

Label a 50 ml conical with the sample name

Fit a clean funnel into the top of the conical

Pour the dried sample from the tray through the funnel into the conical

Discard any material that does not fit into the conical

Wipe the funnel with a Kim-wipe

1. Repeat c-d for all samples
2. Rinse trays and funnels
3. Place labeled conicals with samples in storage box in bulk prep area

**4. Soil composition**

Determining the composition of the soil sample requires weighing the homogenized sample wet, air drying the portion that passes through a coarse sieve, weighing the portion that remains above the sieve, rinsing and separating roots from stones, drying those components and storing each in different ways. Measurements must occur within 28 days of field collections. In between field collections and drying, all samples must remain in sealed, labeled plastic bags in the lab refrigerator at 4 degrees celsius.

1. **Prepare equipment and supplies (in phytochem lab, previous week)**
   1. Ensure that one drying oven is at 60°C and the other is at 105°C
   2. Gather materials sufficient for the samples to be processed
      * 1. 1x new paper bags for collecting soil composition (~14 per plot)
        2. 2x new envelopes for root samples (~28 per plot, 56 per 2 plots)
        3. 2x used envelopes for stone and other samples (~28 per plot, 56 per 2 plots)
        4. Sharpies
        5. New composition (LAB C) datasheets (1 per plot)
        6. Incomplete field datasheets (1 per plot)
2. **Wet mass and composition by sieving (day 2, phytochem🡪downstairs🡪phytochem)**
   1. Remove composition samples from the fridge and allow to thaw if necessary

○ ON THE ASSOCIATED FIELD DATASHEET record the day the soil composition samples were removed from the fridge for processing in **C#X4d\_d**

**○** ON THE LAB C DATASHEET check the names of the samples to be processed and the number of sheets used if processed in parallel by two teams

* 1. Take all sample datasheets, envelopes and 0.01 g balances to a downstairs lab Select a sample

○ Record the **sampleID**

* 1. Place an aluminum tray on the 0.01 g balance and tare

○ record the tared composition sample wet mass **C\_WM\_g.gg**

* 1. Homogenize Composition sample by using your hands to break up clumps of soil in the sealed plastic bag
  2. Weigh a clean, dry 2 mm (#10) sieve to a precision of 0.01 g

○ record the sieve mass **C\_sM\_g.gg**

* 1. Sieve the Composition sample into clean paper bag labeled with the sample ID
     + - 1. Place the 2 mm sieve into the bag
         2. Dump the homogenized sample from the hexagonal weigh boat onto the sieve
         3. Agitate the sieve holding the bag below it for about 1 minute until only stones, coarse roots and adhered soil remain above the sieve, being careful not to tear the bag beneath the sieve.

🡪if cohesive soils do not pass through the sieve, use your gloved hand to push the soil through the sieve into the bag until only dirty stones and roots remain above the sieve

* + - * 1. Fold the top of the paper bag closed, place the bag in a used aluminum tray and place on the < 1 week shelf in the bulk sample prep room to air dry for 7 days – 14 days
  1. Reweigh the sieve with the roots, stones and adhered soil to a precision of 0.01 g

○ record the sieve mass with sample **C\_sRS\_g.gg**

* 1. Place the 2 mm sieve with the sample on top of a 250 um (#40) sieve and press lightly together (snapping the sieves together makes them very difficult to separate and risks losing material.)
  2. Turn on the sink and adjust the water pressure to a low to intermediate level (high pressure risks washing material out of the sieve)
  3. Place the stacked sieves under the running water until all adhered soil is rinsed from the stones and roots on the 2 mm sieve.
  4. Separate the components from the rinsed sieve set
     + 1. Roots above 2 mm sieve

1. Coarse roots represent any and all belowground plant tissue where the smallest segment is larger than 2 mm and will not fit through the sieve.
2. Fine roots represent any and all belowground plant tissue where the larges segment is smaller than 2 mm and will fit into a #10 sieve aperture when separated from a coarse root
3. Label weighed coin envelope with the sample ID and cR for “coarse roots” and fR for “fine roots” as needed
4. Use forceps to remove and separate coarse and fine roots
5. Place all coarse root segments into the labeled cR envelope
6. Place all fine roots segments into the labeled fR envelope
7. Stones above 2 mm sieve
8. Stones represent any rocky material that is too large to pass through the 2 mm sieve
9. Label a coin envelope (may be reused) with the sample ID and S for “stones”
10. Use a forceps to transfer the rinsed stones from the 2 mm sieve to the labeled envelope
11. Fine roots above 250 um sieve
12. Use a soil spatula to carefully separate the 2 mm from the 250 um sieves
13. Use forceps to collect any additional fine roots that passed from the 2 mm sieve to the 250 um sieve and transfer to the labeled sample id fR envelope with any fine roots separated from the 2 mm sieve material
14. Other material
    * 1. Other material is anything in the sieved rinsed sample that is not a root, seed or animal

🡪if no other material is present, strike the corresponding cells on the datasheet

* + - 1. Remove all animals, living or dead from the sample and set aside to discard outside

○ concisely describe the other material as **O\_TYP** (e.g. ”glass” “seeds” “litter” etc.)

* + 1. Label a coin envelope (may be reused) with the sample ID and O for “other”

1. Rinse sieves and set aside to dry
   1. Repeat a-j for all Composition samples
   2. Transport the materials back to the phytochem lab
   3. Place the coin envelopes with roots of each size class and other material into corresponding carboard boxes the 60°C oven to dry for 3-5 days

○ record the time into the oven as **R\_in60\_dh**

o. Place the coin envelopes with stones into a box in the 105°C oven to dry for 3-5 days

○ record the time into the oven as **S\_in105\_dh**

1. **Component dry mass and storage (in phytochem lab on day 1 following week)**
   * + 1. Allow samples to dry
       2. Remove dried root envelopes from oven and place in a desiccator

○ record the time out of the oven as **R\_out\_dh**

* + - 1. Weigh dry roots to a precision of 0.001 g

○ record the mass of the dried coarse roots in the envelope as **cR\_DM\_g.ggg**

○ record the mass of the dried fine roots in the envelope as **fR\_DM\_g.ggg**

* + - 1. Place roots back into labeled, weighed root envelopes and then into a sealed bag for the plot for storage in the phytochemistry lab.
      2. Weigh stones to a precision of 0.01 g
         1. Place a hexagonal weigh dish on a 0.01 g balance and tare, remove the weigh boat from the balance
         2. Empty the stone envelope into the tared weigh boat

**○** record the mass of the stones as **S\_DM\_g.gg**

c.Empty dried stones into the 3.5 gallon bucket for storage

d. Strike the sample ID and set aside envelope for reuse

* + - 1. Weigh other material to a precision of 0.01 g
         1. Place a hexagonal weigh dish on a 0.01 g balance and tare, remove the weigh boat from the balance
         2. Empty the other envelope into the tared weigh boat

**○** record the mass of the stones as **O\_DM\_g.gg**

c.Empty dried stones into the 3.5 gallon bucket for storage

d. Strike the sample ID and set aside envelope for reuse

**d. Data Entry**

1. Scan the completed datasheet and upload it to the google drive folder

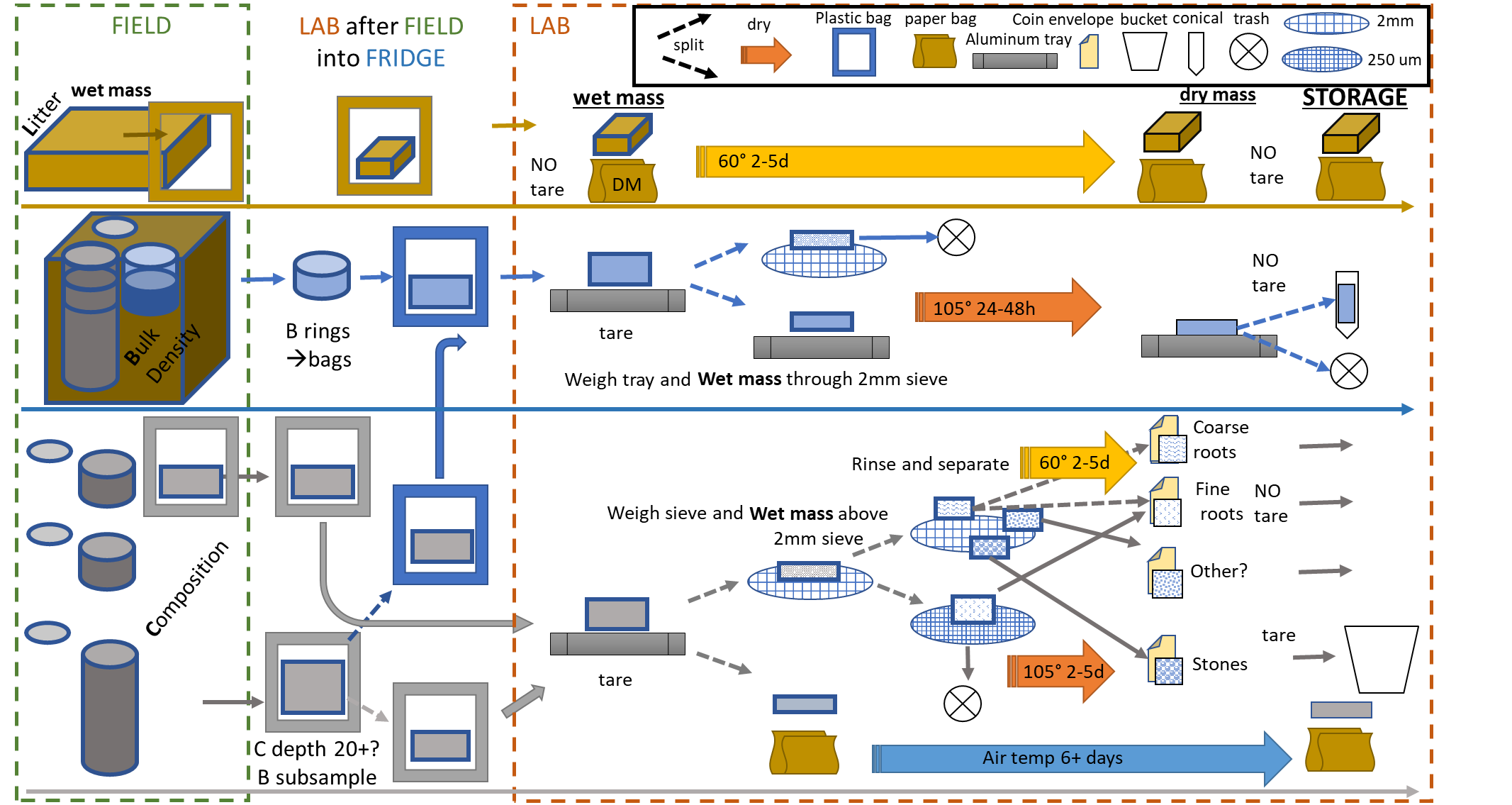
SOILS/DATA/DATA\_SCANS/TFF\_soil\_lab

2. Within 28 days of sampling, the data should be entered into a google sheet

Version notes

1.0 added pushing step when cohesive soils remain stuck above the 2 mm sieve. Edited sample codes to include B for bulk density steps. Modified datasheets slightly to include new ids.

1.1 eliminated envelope pre-weights (too noisy). Added other dm to datasheet.

Appendix 1: workflow

Appendix 2: datasheets

Bulk density and litter datasheet



Composition datasheet

