Sample Preparation for C and N Elemental or Isotopic Analysis

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Introduction

Soil organic carbon (SOC) is a critical soil property for understanding ecosystem function. Soil carbon represents stored energy, introduced to the system by primary production, and used by heterotrophic organisms. The quantity of C in the soil relative to other essential elements such as nitrogen and phosphorous can indicate the health and fertility of the soil, the potential for certain metabolic processes, and is important to understanding the global C cycle and potential ramifications of changing climate.

Equipment Needed

<u>Soil Preprocessing Equipment</u>

Kimwipes and isopropyl alcohol for cleaning spatula and working surface
Soil grinder (e.g. Spex ball-mill or jar-mill)
Weigh paper (N-free) may be helpful but is not required
Spatula, small
Tweezers
(2) Specimen cup with lid or (2) large weighing boat for rinsing grinder materials
Coarse balance (0.01 g)
Oven @ 50°C for 24 hours
Scintillation vials or other archiving container

<u>Analysis Equipment</u>

CN analyzer (elemental analyzer, EA); optional Ion Ratio Mass Spec (IRMS) for isotopes
Capsules for soil samples, (5x9 mm for samples), aluminum

> **Note:** Depending on soil preprocessing and the method for inorganic carbon (IC) removal, additional capsules may be required (5x9 mm), silver (acid resistant).

Microbalance (0.001 mg) and utensils (microspoon and forceps)
Sample trays, 96-well plates

> **Note:** Here again, depending on the soil preprocessing and method for IC removal, additional trays may be required. If soils are acid fumigated, glass coated 96 wells are required (Fisher Sci. # 03-398-22) prior to final sample packing

Lab tape or rubber bands to close trays
Sample weight record sheet with designated standard locations for 96 well tray

Procedures

<u>Soil Preprocessing Procedure</u>

Because of the inherent heterogeneity of soils and also the small amount of soil subsampled for SOC analysis, it is essential to grind soils to break down soil aggregates and homogenize the sample.

1. If soils are moist, set bags out to air dry. Once samples are air dry, label scintillation vials with sample ID# and weigh out ~20 g soil into vials (no need to record weights). Do not cap vials yet but covering them with a large Kimwipe is a good idea to prevent dust contamination.

Note: When subsampling the soil, it is very important to get a well homogenized, representative sample. Therefore, prior to sampling, each bag should be sealed and turned over and churned to mix thoroughly. Be careful not to skim soil from the surface of the soil without also mixing with the spoon since the courser soil particles tend to float to the surface during shaking.

2. Place tray of scintillation vials containing soil into oven set @ 50°C for 24 hours to remove any residual water. Once the soils are dry, remove from oven and cap tightly. DO NOT DRY AT >50°C, you risk losing volatile compounds.
3. Next the soils are ground. In this protocol, use of a Spex ball-mill is described. You can also use a mortar and pestle to hand grind the soils to the consistency of talc making sure to clean with isopropyl alcohol between samples.
4. Empty the contents of the scintillation vial into a clean grinding crucible along with 2 ball bearings. Seal crucible and shake for 5 minutes.
5. Pour ground soil back into scintillation vial and cap tightly. You may need a piece of weigh paper to do this without spilling. Soil is now ready for IC removal or if not removing IC, then ready for total C and N analysis preparation.
6. Between samples, wash out grinding crucible with DI water and then a small amount of isopropyl alcohol. Wipe down crucible with a Kimwipe to remove any residual soil. Repeat for crucible lid.
7. Place ball bearings and O-ring from lid in specimen cup with isopropyl alcohol, cap, and shake. Remove items and place in clean isopropyl bath.
8. Wipe clean ball bearings and O-ring down with kimwipe. Allow a minute for any residual isopropyl alcohol to evaporate before processing you next sample.
9. If there is carbonate to be removed, follow the carbonate removal procedure protocol on the ground samples.

<u>Analysis Procedure</u>
Sample preparation for EA analysis assumes the soils have been ***<u>ground, homogenized, and are dry</u>***. If the soils have been sitting for several weeks or more, you should loosen the caps and place vials in the oven at 50°C overnight to drive off any residual moisture.

1. Wipe down microbalance, weighing tools, forceps, and the glass plate or weighing surface with isopropyl alcohol. Place weighing tools and forceps on an empty EA tin container or any small clean container so that the surfaces of the tools that will touch the soil are hanging over the edge, in contact with nothing. This of course minimizes the potential for contamination.
2. With gloved hands and using clean forceps, place a 5x9 mm capsule on the microbalance and hit “Tare.”
3. Remove capsule and place on glass plate. Using the small scoop or spatula, add soil to the capsule as neatly as possible. The amount of soil will vary depending on the analysis type and your soils. For EA analysis only (no IRMS) 20 mg soil is standard. For soils analyzed on the EA-IRMS, 40-60 mg soil is typical and depends on how much C and N is present; running a few test samples beforehand is always a good idea.
   Note: For soils that have already been acid rinsed or that do not require IC removal, use the 5x9 mm aluminum capsules. For those undergoing acid fumigation, us the 5x9 mm silver tins.

4. Reweigh capsule and adjust the soil weight up or down as needed.
5. Once the capsule is at the desired weight, remove from the balance and place on a clean spot of the glass plate. Then take a straight pair of forceps and clamp down on the capsule, approximately ¾ of the way from the bottom, leaving the capsule closed with a small (2-3 mm) tab at the top.
6. Using the flat side of another pair of forceps, flatten the tab down and fold it so the capsule is sealed. Continue to gently fold the tab in and down to better prevent the soil from escaping.
7. Use 2 pairs of forceps to gently work the capsule into a tighter ball. Make sure the capsule is more or less round and has no corners or tabs sticking out that will cause it to catch in the autosampler.
8. Once the sample is in a sealed ball, lightly drop it on the glass plate 2-3 times from about 4 inches and look for any spilled soil. This tests if there is a tear in the capsule. If no soil spills, place capsule back on the microbalance and ***record final weight*** in proper location on sample spreadsheet (note the 96-well tray column # and row letter).
   For Silver tins only: after recording the final weight, you must place the silver capsule into an aluminum capsule, repeating the folding process to make a smooth round capsule. The aluminum capsule will help the sample combust completely in the EA.
9. Place capsule in the appropriate well position and replace lid. NOTE: IF YOU SPILL THE SAMPLE TRAY, ALL YOUR WORK IS LOST. THAT'S DOZENS OR EVEN HUNDREDS OF HOURS!!! BE CAREFUL AND ALWAYS TAPE THE COVER CLOSED WHEN TRANSPORTING.
10. Clean all surfaces and utensils with isopropyl alcohol and a kimwipe and repeat for all samples.

References

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