SOLID SAMPLE PREPARATION

DRYING METHODS

1) Oven Drying – This should be done at NO MORE than 60°C and for NO LONGER than 2 weeks. Do not try to increase temperature above 60°C to speed up drying time; it may volatilize lighter organics in your sample!
2) Freeze-Drying – Viable option for those who have access to a freeze drier. This method is particularly useful for samples that are harder to dry, like a chunk of animal tissue. Freeze-dried samples are often easier to grind and produce a finer, more homogenous powder.

STORAGE

Keep your samples safe in a container that seals well. I prefer samples arrive in micro-centrifuge tubes, centrifuge tubes, or small vials. The key is that the sample containers are clean and seal tightly.

GRINDING AND HOMOGENIZING METHODS

The goal of grinding up samples is to have a homogeneous powder so that when scooping out a sample, you are getting a complete representation of your original sample. Also, as you grind, make sure you keep your instruments clean. We use ethanol on our forceps, spatulas, and mortar and pestle to dissolve any organics and avoid cross-contamination between samples.

1) Mortar and Pestle – sometimes, if the material is stubborn, you can use liquid nitrogen. Just pour out a little bit to submerge your sample and make it brittle while you grind.
2) Homogenizers or motorized grinders. Can be expensive, but often produce a very fine, homogenous powder. Basically, anything you can think of that you can keep clean and avoid contamination and will give you a fine powder. Some people have used coffee grinders and other types of amalgamators.

ACIDIFICATION

If you are interested in ONLY organic carbon and do not want your data to include any contribution from inorganic carbon (i.e. carbonate sand, shell fragments, biomineralization, etc.), you will need to acidify your sample. There are many acid treatment methods in the literature. Whichever method you use, be aware that an acid treated sample will degrade the tin capsule. Acid treated samples should be run soon after encapsulation. Notify the lab when submitting acid treated samples.

"In **low carbonate materials** (less than ~3% carbonate), you can remove inorganic C by acid fumigation. Weigh soil samples into silver capsules (tin decomposes when exposed to acid) and arrange samples in a 96-well tray. Add a small amount of water to each open capsule to wet the soil. Place the whole 96-well tray in a desiccator containing a beaker of concentrated (12M) HCl. Carbonates are released as CO2 in 6 to 8 hours. Dry the samples at 60°C and carefully crimp-seal the capsules. The capsules become brittle after drying, resulting in leaks; be careful not to lose material when crimping. We recommend placing the whole capsule into a new tin (Sn) capsule and crimp it closed. The additional tin capsule is an important combustion catalyst, so it is advantageous to use tin capsules for re-encapsulating leaking samples.

High carbonate samples must be pulverized and acid washed until the material stops bubbling. Rinse thoroughly to remove residual acid and hygroscopic salts (access to a micro-centrifuge is helpful), and encapsulate in silver (Ag) capsules. The formation of hygroscopic salts does mean the material can be very difficult to keep dry. Please ship acidified samples with a fully sealed desiccant pouch (loose desiccant on the 96-well plate is a contaminant). Due to hazardous waste disposal, we strongly advise against shipping blue desiccant that contains cobalt chloride ($CoCl_2$). Please use a desiccant that is safe for standard garbage disposal.

For more information on acid fumigation to remove carbonates, please refer to: Harris, D., Horwath, W.R., and van Kessel, C., 2001. Acid fumigation of soils to remove carbonates prior to total organic carbon or carbon-13 isotopic analysis. Soil Science Society of America Journal 65: 1853-1856."
-UC Davis website (http://stableisotopefacility.ucdavis.edu/13cand15ntips-Difficult.html)

WEIGHING & ENCAPSULATION

Samples for carbon and nitrogen analysis must be enclosed in tin (Sn) capsules for analysis. The tin is an important combustion catalyst. While the use of silver (Ag) capsules may be necessary for acid treated materials, the use of any alternative capsule (e.g. nickel (Ni) capsules) is unacceptable.

The amount of sample to be weighed depends on the amount of carbon and nitrogen in the dried material. The ideal detection range of our EA is 15-350µg of nitrogen (N) and 50-1500µg of carbon (C). The lower detection limits are 0.04% N and 0.20% C.

Examples of %N and %C of sample materials:

Sample Material	%N	%C
Plant - leaves	2-3%	42-50%
Plant - roots	0.8-1.3%	36-40%
Plant - stems	0.4-0.8%	46-48%
Plant - wood	0.02-0.06%	40-44%
Plant - grain flour (wheat, rye, rice)	1.5-3.5%	43-47%
Plant - grass, alfalfa	2.5-5%	45-47%
Soil - low OM	0.1-0.15%	0.8-1.2%
Soil - medium OM	0.2-0.3%	2.5-3.5%
Soil - high OM	0.3-1.0%	10-14%
Sediment - bulk	0.05-0.15%	0.5-1.5%
Animal, Fish, Invertebrate tissue	10%	40%
Human Hair	14%	47%

The capsules we consistently use are distributed by EA Consumables (https://eaconsumables.com) and come in the following sizes: 5x3.5mm, 6x4mm, 9x5mm, 10x9mm. Do not use anything larger, as it may not fit in the EA! EA Consumables offers a sample prep kit of tin capsules, trays, and tray seals (EA4000). We offer a discount for samples that arrive weighed and wrapped (see "you prepare" on the pricelist).

The following is a guide to weighing and encapsulating samples. See the images of properly wrapped samples. You can adapt this method to suit your sampling style.

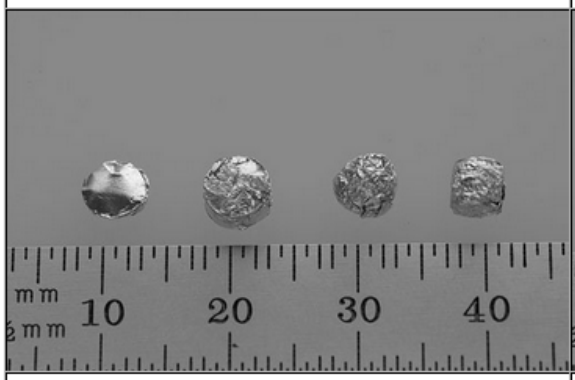
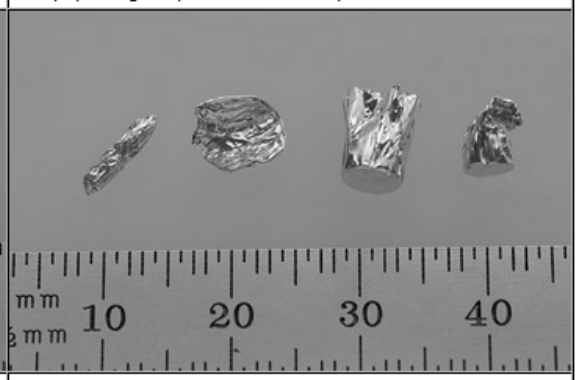
1) Wear gloves to prevent contamination when handling tools or the sample. Make sure all of your utensils and surfaces are clean! I clean everything with ethanol and wipe it dry with a kimwipe. Useful tools include a stainless steel spatula, forceps (straight and curved), and a clean flat surface.

2) Place a tin capsule (straight from the box…we don't usually find contamination or a difference between methanol cleaned capsules and unaltered capsules) on a scale. Tare the capsule.
3) Remove capsule from scale and place a small amount of material into capsule. Place on scale to see tentatively how much material you put in. Remove or add material until it is in the desired range. **Be sure to record and submit the precise sample mass.**
4) Use the supplied Sample Submission Worksheet to log your samples.
5) Once you have the amount you need, use a forceps to pinch closed the top of the capsule as you remove it from the scale. Fold the top 2-3 times to secure the sample. Next, flatten the capsule and fold into a smooth, compact ball or a cube with forceps and spatula. My samples usually look like tight, compact balls with no leaks.
NOTE: Capsules should not be flat packets or envelopes. This may cause the sample to get stuck in the autosampler during analysis.
6) Place in secure container and make sure you know what is where and how much there is. For example, I use microtitre plates (96 wells). Cover sample wells with parafilm, plastic wrap, or an index card before securing the plate cover. This will prevent samples slipping into other wells during shipping.

It is good practice to include replicates to check precision, especially for complex matrices
We run calibrated standards to confirm the precision of our analyses. Client replicates are recommended to check the precision of your samples, especially for samples that exceed our detection limits. The number of replicates you provide is your discretion. We recommend providing a few replicates per batch (e.g. 1 replicate per 8-12 samples).

-UCDavis Stable Isotope Lab website (http://stableisotopefacility.ucdavis.edu/13cand15ntips.html)

Tips for Preparing Your Carbon (^{13}C) and Nitrogen (^{15}N) Solid Samples

Do...	Don't...
- crimp samples into a compact spherical, cylindrical, or cubic shape, with maximum dimensions of 5.5 mm for 5x9 mm tins (or 8mm for 9x10 mm tins) - make sure tin capsule openings are folded over more than once if you can't compress the samples - place an index card over the tray before securing the cover if you have small samples - use clean equipment to handle the samples and tins.	- shape your samples into very flat disks (<1mm) or thin tube/cigar shapes. - ship capsules that only have their openings pinched closed or folded once. - ship samples that are leaking. - over-fill capsules. Excess filter paper can be trimmed off to reduce volume. - contaminate samples by handling with bare hands or by using sandpaper to grind plant or wood samples.
	
Above are examples of standard sized samples. From left to right: ~1mm tall X 5.5 mm diameter cylindrical sample ~ 4mm tall X 5.5 mm diameter cylindrical sample ~5mm spherical sample ~5mm cubical sample	Above are examples of improperly shaped samples. From left to right: - Tube/cigar shaped sample >5mm long. - Very flat, flake- shaped sample, less than 1mm tall and over 5.5mm wide. - The opening of this capsule has only been pinched closed. - The opening of this capsule has only been folded over one time.