13C and 15N Analysis of Solids by EA-IRMS

The SIF provides 13C and 15N isotope analyses of solid materials, such as soils, sediments, plant and animal tissues, etc., using an elemental analyzer interfaced to a continuous flow isotope ratio mass spectrometer (IRMS). We analyze both 13C and 15N in the same sample, or we can use a CO2 trap and measure 15N only in the case of very low N materials like wood.

Analysis

Solid materials are analyzed for 13C and 15N isotopes using a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK). Samples are combusted at 1020°C in a reactor packed with chromium oxide and silvered colbatous/cobaltic oxide. Following combustion, oxides are removed in a reduction reactor (reduced copper at 650°C) and the helium carrier flows through a water trap (magnesium perchlorate) and an optional CO2 trap (for N-only analyses). Nitrogen and CO2 are separated on a Carbosieve GC column (65°C, 65 mL/min) before entering the IRMS.

During analysis, samples are interspersed with several replicates of at least two different laboratory standards. These laboratory standards, which are selected to be compositionally similar to the samples being analyzed, have been previously calibrated against NIST Standard Reference Materials (IAEA-N1, IAEA-N2, IAEA-N3, IAEA-CH7, and NBS-22).

A sample's preliminary isotope ratio is measured relative to reference gases analyzed with each sample. These preliminary values are finalized by adjusting the values for the entire batch based on the known values of the included laboratory standards.

The final delta values, delivered to the customer, are expressed relative to international standards PDB (PeeDee Belemnite) and Air for carbon and nitrogen, respectively. For information on delta notation and the international references, please refer to a stable isotope reference such as Sharp, Z. (2005) *Princples of Stable Isotope Geochemistry* (Prentice Hall).

Pricing & Turnaround Time

Analysis	Instrument	Turnaround Time (weeks)	Price per Sample (USD)
13C only, enriched/tracer	Integra	8	$5.00
15N only, enriched/tracer	Integra	8	$5.00
Dual 13C & 15N enriched/tracer	Integra	8	$7.00
13C only, natural abundance sample	ANCA-GSL & PDZ Europa 20-20	8	$6.50
15N only, natural abundance sample	ANCA-GSL & PDZ Europa 20-20	8	$6.50
Dual 13C & 15N natural abundance sample	ANCA-GSL & PDZ Europa 20-20	8	$8.00
15N wood	ANCA-GSL & PDZ Europa 20-20 & CO2 Cryotrap	8	$7.50
^{34}S sulfur			no analysis

Samples are queued for each analysis in the order received. The turnaround times listed are approximate for *properly* prepared samples. Large sample sets (>1000 samples), poorly prepared samples, wood, salts, large filters, highly enriched, or samples that exceed our normal sample size range, etc. will delay sample analysis and data processing. The SIF does not offer ^{34}S sulfur analysis.

Sample Preparation

Small samples, such as leaf disks, sections of root, small insects, fish scales, etc., that meet the target weight can be encapsulated whole in tin capsules. Getting a representative sample of larger samples, like leaves, or coarse materials, such as soils or sediments, usually requires grinding and homogenizing.

The amount of sample required depends on the amount of carbon and nitrogen in the material. A sample

should contain between 20-150µg N and 200-2000µg C. See the table below for determining appropriate sample weights.

Target Weights for Solid Samples
Your actual weights will depend on your samples' C:N ratios

The Sample Weight Calculator will help you refine the required weight.*

Sample Type	Target Weight
Plant Tissue (15N only)	~3-10mg depending on %N content
Plant Tissue (13C&15N)	~2-3mg
Wood (15N only)	~20-30mg
Soil / Sediment	~10-75mg depending on organic matter content
Decaying plant litter	~4-6 mg
Animal, Fish, Invertebrate Tissue	~1mg +/- 0.2mg

For filter samples, while the amount of carbon and nitrogen required remains the same, the sample weight guidelines do not apply. Please minimize the amount of filter in each sample. This can be accomplished by increasing material loading and using only a portion of the filter or by cutting away the annular portion of the filter which contains no material. The largest whole filter that can be analyzed is 25mm. If possible include a few test samples. See also How to encapsulate samples for dimension restrictions.

Samples that exceed the target weights may not be analyzed because large samples saturate the mass spec detectors, producing unusable data. The SIF is not responsible for lost samples or poor data due to samples exceeding their target weights.

Organization

1) Organize samples into a clean 96-well tray. Please use all wells in a tray.
2) For small samples, ensure they remain in the wells during shipping by placing an index card (cut to size) or Parafilm over the tray before securing the lid. Do not use adhesive tape to cover the open wells. Tape the lid securely closed using tape on all four sides. Turn the tray over and gently shake to test if samples stay in the wells or if the capsules leak sample material. Re-encapsulate any leaky capsules before shipping.
3) Label each tray of samples with a unique name. Include this tray name in the Sample List. Results will include the unique tray name and well position (e.g., A1).
4) For **enriched samples**, arrange samples to avoid wide fluctuations in isotope content. Place non-enriched samples ahead of enriched samples within the same tray, or place non-enriched and enriched samples in separate trays.
5) IMPORTANT: Please label all trays containing soil, forest litter, wood or plant compost, humus, and earthworm castings. Most southeastern states and foreign countries have regulations regarding soil movement to prevent the spread of agricultural pests. We are responsible for proper disposal of any imported and restricted samples we receive. For more information please visit the USDA-APHIS Soil Circular or visit the USDA-APHIS website.

Shipping

1) If you are shipping *soils* from outside USA, contact David Harris for a copy of the Soils Import Permit.

2) Complete an Analysis Order Form and Sample List for your order. The sample list should include weights where appropriate.

Please complete both forms completely including:

- Your contact information (name, address, phone, e-mail)
- Billing information
- The type of analysis you are requesting
- A general description of the material being analyzed including approximate elemental composition
- Expected range of isotopic composition (particularly for enriched material)
- If more rows are required in the sample list, simply add the rows and continue the numbering in column A.

NEW 3) Email the completed forms to sif@ucdavis.edu ***and*** include a printed copy with your samples.

4) Carefully package the sample trays to protect them during shipping. Single trays should be wrapped with multiple layers of bubble wrap and can be shipped in a padded envelope or box. Multiple trays should be banded or taped together and shipped in rigid box filled with packing material. *Failure to properly package and protect trays often results in sample loss during shipping.*

Contact information

UC Davis Stable Isotope Facility
Department of Plant Sciences
One Shields Avenue, Mail Stop 1
Davis, CA 95616, USA

Phone:(530) 754-7517, Fax: (530) 752-4361
E-mail: sif@ucdavis.edu

Supplies

Here are a few part numbers and suppliers the SIF uses on a regular basis. Similar products can be found through other consumables catalogs.

Manufacturer / Part#	Description	Unit
Costech / 041061	Tin capsules for solid samples, 5x9 mm	100/pk
Costech / 041073	Tin capsules for large solid samples, 9x10 mm	100/pk
Elemental Microanalysis / D1008 or D1009	Tin capsules for solid samples, 5x8 mm	250/pk or 100/pk
Electron Microscopy Sciences / 70437-R1	96-Well Plate, Round-Bottom, With Lid	10/pk
Electron Microscopy Sciences / 70437-R5	96-Well Plate, Round-Bottom, With Lid	50/cs
BD Falcon / 320 353917	96-Well Plate, Round-Bottom, With Lid	100/cs

Tips for Preparing Your Solid Samples

Remove carbonates from calcareous soils before analysis for SOM-13C

Inorganic C in the form of carbonates can interfere with the measurement of organic 13C in soils. 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. Be careful not to lose material when crimping. Alternatively, place the whole capsule into a new tin capsule and crimp it closed.

For more information, 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.

Use KHSO4 for ammonia diffusion traps

Use KHSO4 rather than H2SO4 on the ammonia trapping disk to avoid rapid corrosion of the tin capsule. Adjust the volume of extract to obtain optimal mass of N on the disk, ideally 100 µg N.

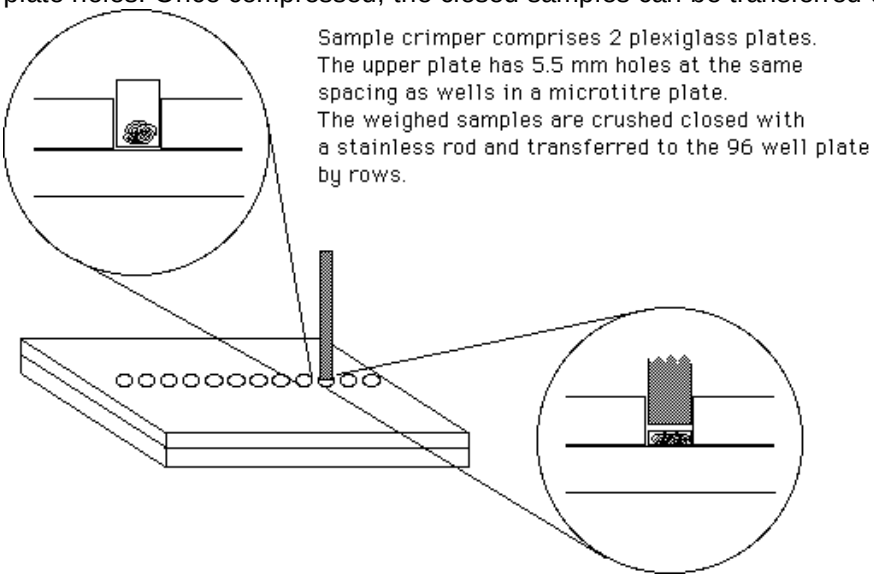
Use large tins for bulky samples

Large 9x10 mm tin capsules are helpful for encapsulating bulky items, like filter disks, which must be tightly packaged to maintain a compact form (no larger than 8mm wide X 8mm tall) in the autosampler. If samples are too large for a 96-well tray, ship them in a 24 or 48-well tray instead. Please do not force large samples into a 96-well tray, they will expand during shipping and we will not be able to extract these samples from their wells.

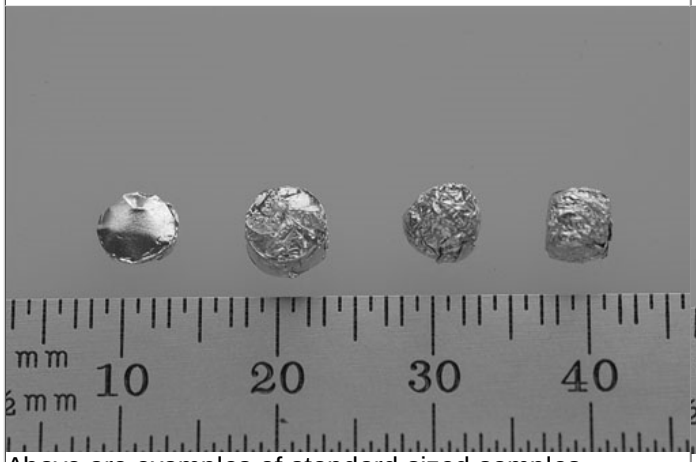
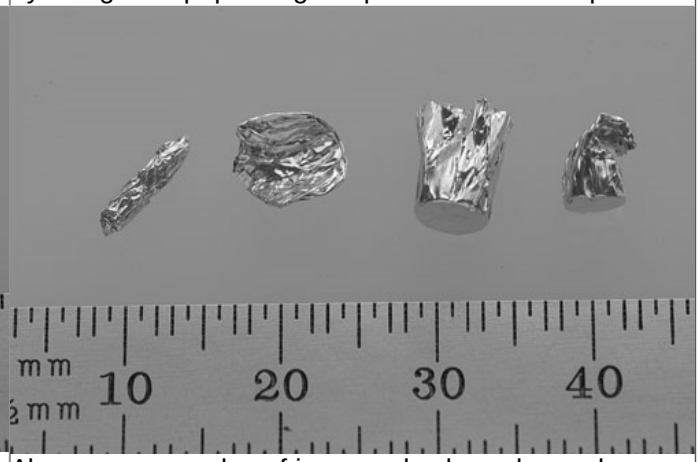
How to encapsulate samples

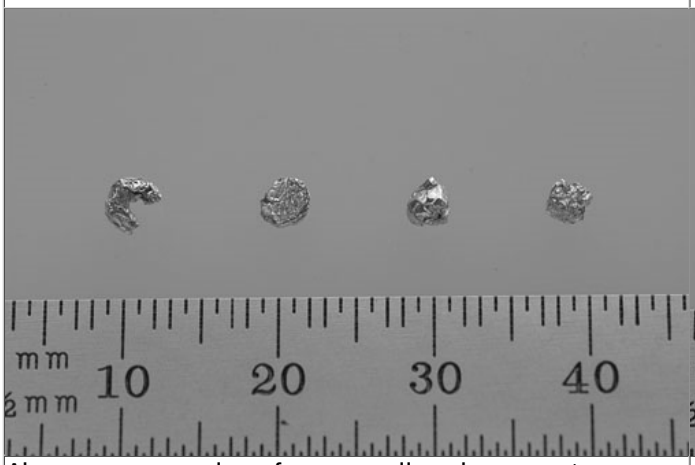
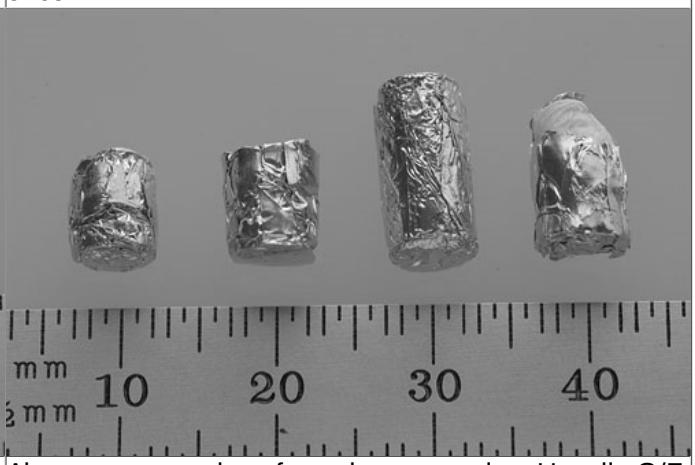
Please encapsulate solid samples in tin capsules so they remain intact and do not leak and contaminate other samples during shipping. Test your crimping-sealing method using a dummy sample in a tray. Shake and flip the tray to mimic agitation during shipping to see if the sample stays in its well or leaks from the tin. Very small or flat samples can escape their wells during shipping by slipping through the gap between the tray and cover. Do not underestimate the gap between the tray and cover! This is the number one cause of lost samples/data.

You can make a crimper plate (pictured below) to hold tins and use a metal dowel to compress them in the plate holes. Once compressed, the closed samples can be transferred directly into a 96-well tray.



Alternatively, you can manually seal the tins using two sets of forceps with blunt tips to form them into cubical or cylindrical shapes. First, pinch the top closed and fold it over. Then, press and hold the capsule top to bottom while using the other forceps to pinch the sides inward. Repeat until you have a compact tightly packed tin.

Do...	***Don't...***
- crimp samples into a compact spherical, cylindrical, or cubic shape, with maximum dimensions of 6 mm for 5x9 mm tins (or 8 mm 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 or Parafilm 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	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.

~5mm cubical sample	-The opening of this capsule has only been pinched closed. -The opening of this capsule has only been folded over once.
	
Above are examples of very small and compact samples. A tray of these samples will require an index card or Parafilm to cover the tray before taping on the cover. From left to right: ~1mm tall X 3mm wide crescent shaped sample ~1mm tall X 3mm wide cylindrical sample ~3mm spherical sample ~2.5 mm cubical sample	Above are examples of very large samples. Usually G/F filters From left to right: ~6mm tall X 5mm wide, this is an example of a "good" large sample. ~6mm tall X 6mm wide, this sample is too wide. It will fill the well of a 96-well tray, making it hard to retrieve, and may jam the autosampler. ~12mm tall X 5.5mm wide. While this sample is narrow enough, the height of the sample will cause the auto-sampler to chop off the top portion, therefore contaminating later samples and/or clogging the machine. -Over-stuffed with filter, this sample has burst. This may occur as you are closing your samples, or later during shipping as filter tends to expand after being compressed. Trimming off excess filter will reduce the volume of filter paper being packaged.

Updated April 24, 2009