Colorado Plateau Stable Isotope Laboratory

Sample submission - organics - $\delta^{13}C$ and $\delta^{15}N$ via EA-CFIRMS

***[Please note:** the information on this page is available for download by clicking here (pdf version). **Also**, our sample-submission sheet (for prepared samples) is available by clicking here (pdf version), or here (MS Word version).]*

Drying, grinding, and weighing

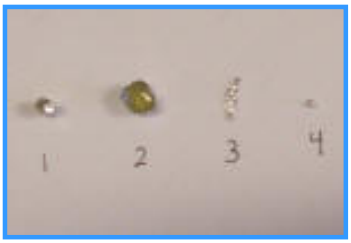
Samples must be dried, ground, and weighed prior to isotope analysis.

[*Please note: soils should be sieved to a consistent particle size prior to grinding and weighing. **Also**, soils containing carbonate materials may exhibit erroneous (organic) $\delta^{13}C$ values unless acid-treatment is performed prior to analysis.*]

Drying of plants, animals, and soils can be accomplished via a drying oven at 50-60°C for 24-48 h, or via a freeze drier. Grinding can be achieved via a mortar and pestle, a ball-mill grinder, a mixer mill, etc. The goal is to improve sample homogeneity (i.e., isotopic homogeneity) by way of pulverizing the sample into a fine powder or flour with a consistent particle size. After drying and grinding, samples need to be weighed into small tin capsules prior to isotope analysis. Capsule size will depend on sample weight. For example, 4x6-mm tin capsules are preferred when sample weights are less than 7 mg; 5x9-mm tin capsules are used for sample weights between 7 mg and 40 mg; and 9x10-mm tin capsules are used for samples greater than 40 mg. Tin capsules can be purchased from COSTECH Analytical Inc. (800-524-7219), CE Elantech (888-232-4676), Elemental Microanalysis (978-526-8517), or in Canada from Isomass Scientific (800-363-7823), or from any other company supplying products and services for isotope-ratio and/or elemental analysis.

Using a micro-analytical balance, the mass of a sample should be determined to 3 decimal places on a milligram. For example, you should be able to record the mass as "2.104 mg". *[**Please note:** if you are unable to acquire a balance that measures to 3 decimal places on a milligram, but instead can only use a balance that measures to 2 decimal places (e.g., 2.10 mg) or to 1 decimal place (e.g., 2.1 mg), it is important to realize that some precision may be lost when measuring %C and %N (but not C/N, or $\delta^{13}C$ and $\delta^{15}N$). This is because %C and %N are calculated using the recorded weight of the sample. Isotope measurements are not affected by small weighing inaccuracies because their determination is not weight-dependent... beyond the assurance that a large enough sample has been combusted in the EA. Thus if one can live with slightly lower precision on %C and %N (e.g., ± 2.0% for carbon as opposed to ± 0.5%) then acquiring a balance that records to 3 decimal places on a milligram is not essential.]*

Prior to weighing out the sample, tare the balance to exclude the initial mass of the tin capsule. Once the appropriate amount of sample has been placed in the tin capsule, the capsule needs to be "crushed" into a small ball or square (see figure below - you want the final sample to look like #4). This can be accomplished by gently applying force using a pair of forceps, or by rolling the sample gently between your thumb and index finger.



At no point in time should you touch the sample or the tin capsule with your bare hands. Powder-less latex gloves should be used if you plan on "crushing" the capsule between your fingers. I usually use 2 pairs of forceps to handle the sample, so that I never have to touch it with my bare hands. Please note that if a capsule containing a sample falls on the floor, it should be thrown away. Also, please be sure that material will not leak from the capsule after it has been crushed, as the loss of material can affect both the isotope and elemental data (through incorrect mass determination and subsequent sample-to-sample contamination). Once the tin capsule has been crushed, please re-weigh the sample to confirm and record the final mass.

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Mass requirements

The required (dry) mass for simultaneous analysis of $\delta^{15}N$ and $\delta^{13}C$ depends mostly on the sample type, but also on the nitrogen content of each sample. Since nitrogen occurs in lower concentrations than carbon (for most organic materials), the nitrogen content becomes the limiting factor in dual-isotope measurements. Plants (i.e., 2-5% nitrogen) and soils/sediments (0.1-1.0% nitrogen) have much lower nitrogen concentrations than animals (i.e., 8-12% nitrogen), so more material is needed per sample for plants and soils than for animals***. The preference in our isotope lab is to weigh out 60 micrograms of nitrogen per sample (regardless of the sample type)***. This amount of material usually results in a nitrogen peak of sufficient size for accurately measuring stable isotopes, but it does not result in a peak so large that it will exhaust the chemicals too quickly. Since there is usually much more carbon than nitrogen in organic materials, there should always be enough carbon per sample for $\delta^{13}C$ analysis (so long as samples have been properly prepared for $\delta^{15}N$ analysis).

***[Please note**: there is both a **LOWER** limit and an **UPPER** limit to the amount of sample that should be submitted in each tin cup. Please follow the guidelines below for different sample types, and if you have any questions, do not hesitate to contact the lab manager for more information.]*

To achieve 60 micrograms of nitrogen per sample, one needs to know the approximate nitrogen content of the material being analyzed. As mentioned above, plants usually contain less nitrogen than animals, so to obtain 60 micrograms of N for plants with a nitrogen content of approximately 2%, one would need to weigh out approximately 3.000 milligrams of dried plant material. For animals (e.g., fish, birds, mammals, invertebrates, etc.) with a nitrogen content of

approximately 10%, one would need to weigh out approximately 0.600 milligrams of dried material to obtain 60 micrograms of N. For ease of measurement, we usually ask clients to weigh out plant material between 4.000 and 6.000 mg. For animal material, we ask that you weigh out samples between 0.600 mg and 1.200 mg. Below, there is a small table outlining required sample masses, given the approximate % nitrogen content of different sample materials:

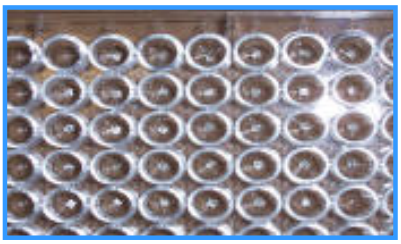
Milligrams of dried material to be weighed per sample	%N of sample	Micrograms of N needed per sample for isotope analysis	Possible sample types
60.000	0.1%	60	soil
12.000	0.5%	60	soil
4.000 to 6.000	1.5%	60	decaying plant litter
3.000 to 4.000	3.0%	60	fresh leaves, algae
2.000 to 3.000	5.0%	60	legumes
0.600 to 1.200	10.0%	60	all animals
0.300	20.0%	60	ammonium salts
0.150	40.0%	60	urea

If you are unsure of the nitrogen content of your sample (e.g., bacteria, soils, sediments, FPOM, etc.), it would be best to send a preliminary sample of the material (in a vial) to our lab so that we could analyze it for you and recommend an appropriate mass. Another alternative is for you to send ALL of your samples to our lab to be weighed here, at which point we would be able to run repeats for you on larger/smaller samples if necessary.

Once weighed, crushed, and re-weighed, samples should be placed into a 96-well cell plate (with cover) for easy storage and shipping. The following two images show samples that are poorly packed into cell plates and those that are well packed.

These are poorly packed samples. Note the scattered bits of organic material all over the tray...

These are nicely packed samples. Very compact with no tears or leaks...



We use 96-well polystyrene plates. Cell plates can be ordered (in bulk) from companies such as Fisher-Scientific Inc or VWR International. For smaller quantities, plates can be purchased from COSTECH Analytical Inc. Each well in the cell plate has an alpha-numeric position (rows A through H, columns 1 through 12). Thus, the first well is A1, the next A2, and so on. Once you

are ready to weigh out your samples, please download our sample-submission sheet so that you can provide us with enough information to successfully analyze your samples.

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Cost of simultaneous $\delta^{13}C$ and $\delta^{15}N$ analyses via EA-CFIRMS

Sample costs are:
$9 per sample (if **you** grind and **you** weigh)
$12 per sample (if **you** grind and **we** weigh)
$15 per sample (if **we** grind and **we** weigh)

For this price, you get $\delta^{15}N$, $\delta^{13}C$, %C, %N, and C/N for each sample.

An additional $3 per sample will be included for any other tasks that need to be performed by our lab prior to isotope analysis (e.g., sieving soils, pre-combustion of sediments to remove organics, acid-treatment to remove inorganic carbon, grinding samples to a fine powder, weighing samples, etc.). Initial costs are reduced to $7.50 per sample if submitted in bulk (n > 500).

For additional prices on other types of analyses conducted at CPSIL, click here.

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Turn-around Time

Once the samples arrive in our lab, we will confirm receipt of the shipment via email. Turn-around time in our lab depends mostly on the state of preparedness of your samples. For example, if your samples arrive weighed out and crushed into small tin cups, then analysis should be complete within 3-4 weeks of our receiving the shipment (depending on the size of the shipment). However, if the samples need to be prepped in our lab before analysis (e.g., ground, weighed, acid-treated, etc.), then the turn-around time could be on the order of 4-8 weeks.

Once the samples have been analyzed, we will send you a copy of the data via email. A hardcopy will follow by regular mail along with the invoice for services. Included in the data file will be the results of quality assurance and quality control (QA/QC). We use four IAEA standards (IAEA-N1, N2, CH6, and CH7) to correct (i.e., normalize) the raw isotope data. We then use a series of elemental standards and various NIST standards to correct the %C and the %N data. These standards also serve as secondary checks on isotope data from day to day, and are logged into a datafile which serves as a long-term QA/QC report. Standards are interspersed throughout each daily run, to ensure data integrity over the entire analysis. As you will note on the sample-submission sheet, we ask that you weigh out duplicates of some samples as a check on sample homogeneity and reproducibility. If for some unknown reason (e.g., power failure, exhaustion of chemicals, computer error) a sample is lost during analysis, we will request that you re-submit the material. Any repeats that requested by our lab will be analyzed free-of-charge.

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Shipping

To send samples to our lab, please click here for our shipping address.

Additional Information

We run samples for $\delta^{15}N$, $\delta^{13}C$, %C, %N, and C/N, in continuous-flow mode using a Thermo-Finnigan Delta^{plus} Advantage gas isotope-ratio mass spectrometer interfaced with a Costech Analytical ECS4010 elemental analyzer. We also have a Carlo Erba 2100 elemental analyzer which can be run in tandem on our Delta^{plus} XL mass spectrometer. Helium flow rate is usually set at 110-130 ml/min. Oxygen flow rate is at 80 ml/min. A standard 3-meter GC column is used (set at 55°C) for peak separation, in combination with one quartz (combustion) tube filled with chromium oxide and silvered cobaltous/cobaltic oxide (set at 1020°C) and one quartz (reduction) tube filled with reduced copper (set at 650°C). Dual analysis of $\delta^{15}N$ and $\delta^{13}C$ usually takes about 7-8 minutes per sample. The average daily run contains approximately 120 samples (incl. standards). Data are normalized using 4 internationally-accepted isotope standards (IAEA CH6, CH7, N1, and N2). Our main working standard is peach leaves (NIST 1547). External precision on these standards is ± 0.10 ‰ or better for $\delta^{13}C$ and ± 0.20 ‰ or better for $\delta^{15}N$. $\delta^{13}C$ and $\delta^{15}N$ data are expressed relative to VPDB for carbon, and to AIR for nitrogen.

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Send mail to Richard.Doucett@nau.edu with questions, comments, and/or corrections about this web site.
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