



Protocol for preparing organic matter for stable isotope analysis

Laboratory Equipment and Materials:

Lyophilizer (or drying oven)

Spex Ball-Mill

Microbalance accurate to 0.001 mg

Scintillation vials

Encapsulating tins (holder optional)

96 well microtitre plates for storing encapsulated samples

Micro-scupula and forceps

DRY & GRIND

1. Dry organic matter samples via lyophilization for at least 48 hrs. Mild oven drying at 50C is also acceptable, but not preferred.
2. Grind sample to a uniform fine-grained (talc-like) texture using a Spex ball-mill
3. Clean the mill with ethanol between samples and thoroughly dry

ENCAPSULATE & WEIGH

4. Place tin capsule on a micro-balance accurate to 0.001 mg and tare
5. Clean all surfaces and utensils by wiping with ethanol. It is best to work over an area that is white (a piece of white plastic tape can be placed down on the table) so that you can see if any material is spilled
6. Place tared tin capsule on bench and carefully add material using small spatula using the weighing criteria below
7. Place capsule back on balance (using forceps) and remove or add more material according to weighing notes below
8. Remove tin capsule from balance and place on clean white surface to crimp tin down to small packet (fold over top to close, then crimp sides down so that all dimensions are < 2 mm)
9. Place capsule packet back on balance (using forceps) to record the final dry mass
10. Check to see that there is no leakage of material by dropping tin packet (from a height of 2 inches) onto white surface



11. Tin packet is then placed in one well location (use 96-well microtitre plates) and the well location (e.g., A1...A12, B1...B12, etc), sample type, sampling station, and dry mass is recorded using the same tared balance

ANALYSIS

12. Combust at 1100C and deliver gases via continuous-flow for analysis of $\delta^{13}C$, $\delta^{15}N$, percentage carbon and percentage nitrogen using an isotope ratio mass spectrometer (IRMS)
13. Determine C:N ratio from percentage element weight

WEIGHING NOTES:

Because organic material will generally contain plenty of C for detection by the mass spectrophotometer, it is more important to assure that there is enough N in each type of sample when analyzing for C and N simultaneously. Most labs have an upper limit (300 mg N) and a lower limit (about 20-25 mg N) for ^{15}N samples (optimum is 100 mg N), different masses of material must be weighed out for different sample types (different %N content):

<u>SAMPLE TYPE (%N CONTENT)</u>	<u>WEIGHT</u>
Sediment (0.05 to 0.15 %N):	3 – 30 mg*
Leaves (0.5 to 2 %N):	1 – 2 mg
Roots/Stems (0.4 to 1.3 %N)	2 – 3 mg
Wood (0.02 to 0.06 %N):	10 – 15 mg
Fine organic material (FOM) or soil (0.3 to 2 %N):	1 – 2 mg
Suspended organic material (SOM) (0.5 to 3 %N):	1 – 2 mg
Biofilm (0.5 to 3 %N):	1 – 2 mg
Plant grains (1.5 to 3.5 %N):	1 – 2 mg
Grass/algae (1.5 to 5 %N):	1 – 2 mg
Animal, Fish & Invertebrates (~10 %N):	1 – 2 mg

*starred sample types can vary by more than an order of magnitude in N content. We recommend analyzing some test samples before weighing all samples.

ENRICHED SAMPLES



- If samples are from a stable isotope labeling experiment (e.g., $^{15}\text{NH}_4$ uptake in plants), process samples in order of least enriched to most enriched (background/control samples of all types first, then least enriched to most enriched of each type; for example, it is expected that wood would be first because they are expected to have the least ^{15}N and fast-assimilating tissue like algae last because they would likely have the highest ^{15}N values).
- The background samples of all types should be grouped together first in the well plates (e.g., first 1 or 2 columns of wells - locations A1 through A12 and B1 through B12) and then the ^{15}N -enriched samples from least to most enriched. This helps to avoid carry-over effects with the mass spectrometer. Generally, the differences in $\delta^{15}\text{N}$ will be greater between sample types than with increasing distance from the labeling source. The order of ^{15}N enrichment for sample types in streams, for example, is likely to be wood < FOM < leaves < SOM < biofilm < algae, so you would group them in this order in the microplates.

SAMPLES ON FILTERS

- For samples retained on GFF filters (FBOM, SPOM, epilithon), carefully remove only organic material from filter to add to tin.
- For samples that do not have a thick “cake” on filter (epilithon, SPOM), be as careful as possible to minimize inclusion of filter fibers within the sample (e.g., use a sharp scalpel or small knife for scraping material from filters).
- If it is not possible to scrape material from filter without inclusion of filter material in the sample, then it may be possible to encapsulate the entire filter, but record only the dry mass of material on the filter (subtract the filter tare mass) as the sample dry mass. The well plates with samples can then be stored for up to several months before shipment to the ^{15}N analytical lab.

References:

Mulholland et al. 2004. LINX II STREAM ^{15}N EXPERIMENT PROTOCOLS

<http://www.faculty.biol.vt.edu/webster/linx/linx2proto-rev5.pdf>

UC Davis Stable Isotope Facility:

<http://stableisotopefacility.ucdavis.edu/sample-weight-calculator.html>