STANDARD OPERATING PROCEDURE

Preparation of solid samples for C&N analysis by EA/IRMS

1. METHOD OBJECTIVE: To prepare solid samples for stable isotope analysis of $\delta^{13}C$ and $\delta^{15}N$, and the total carbon and nitrogen percentage. Samples of various materials such as wood, soils and sediment as well as plant and animal tissue can be prepared by this technique.

2. METHOD VARIATIONS: Due to the variety of sample types, degrees of isotopic enrichment and variation in the conditions of analysis, slight modifications to the method may be made for different samples.

2.1 SAMPLE TYPE: 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 ug N) and a lower limit (about 20-25 ug N) for ^{15}N samples (optimum is 100 ug N), different masses of material must be weighed out for different sample types (different %N content):

SAMPLE TYPE (%N CONTENT)	SAMPLE WEIGHT
Sediment (0.5 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 (0.3 to 2 %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

2.2 ENRICHED SAMPLES: If samples are from a stable isotope labeling experiment (e.g., $^{15}NH_4$ 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 $\delta^{15}N$ 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. Clearly indicate if any samples are enriched on the microplate.

2.3 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.

3. SAFETY PRECAUTIONS: Although proper training is required to conduct these preparations, one must remain mindful of the inherent dangers in some types of samples such as inhalation of fine particles and fibers.

4. EQUIPMENT, MATERIALS AND REAGENTS:

4.1 EQUIPMENT: Spex Ball-Mill, Microbalance accurate to 0.001 mg, lyophilizer, 50C drying oven

4.2 MATERIALS: Scintillation vials, Encapsulating tins (holder optional), 96 well microtitre plates for storing encapsulated samples, forceps, micro-spatula, weigh plates and tools.

5. PROCEDURE:

5.1. Overview: The method details procedures to address the following:

5.2. Dry

5.3. Grind

5.4. Encapsulate & Weigh

5.2. Dry organic matter samples

5.2.1. By lyophilization: Freeze samples until completely solid and place in lyophilizer for at least 24 hours

5.2.2. By oven: Place in drying oven at 50 C for at least 48 hrs

5.3. Grind

5.3.1. Grind sample to a uniform fine-grained (talc-like) texture using a Spex ball-mill; more details on this can be found in protocol CAIS-037-1.1 Grinding Solid Samples for Isotope Analysis

5.3.2. Clean the mill with ethanol between samples

5.4. Encapsulate and Weigh

5.4.1 Initial check and evaluation of balance precision: Two different certified weights are placed onto the balance and recorded as to whether they measure within the specified range for each weight. Note either PASS or FAIL in log book along with date and technician's initials.

5.4.2 If PASS proceed with weighing samples. If FAIL, repeat with another set of certified weights. If FAIL again, contact Laboratory Supervisor for troubleshooting balance.

5.4.3. Place tin capsule on balance accurate to 0.001 mg and tare

5.4.4. 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

5.4.5. Place tared tin capsule on bench and carefully add material using small spatula using the weighing criteria below

5.4.6. Place capsule back on balance (using forceps) to assess added dry mass (or remove and add more material if mass is too low) according to weighing notes below

5.4.7. 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). If using weigh plate kit, place tin in well, fold over top to close, crimp down sides, and compress using crusher tool.

5.4.8 Using forceps, place sealed tin back on balance and record final dry mass.

5.4.9. 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

6. QUALITY CONTROL: Check to see that there is no leakage of material by dropping tin packet (from a height of 2 inches) onto white surface

7. DATA MANAGEMENT: All sample identifications, client name, enrichment, prepared by whom and preparation date should be recorded on official weight sheets and submitted with the plate.