

BCAL Carbon-Nitrogen Sample Preparation Protocol

Blue Carbon Analysis Laboratory (BCAL) & Seagrass Ecosystems Research Laboratory (SERL)
Coastlines and Oceans Division, Institute of Environment, Florida International University
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Prior to Sample Preparation:

- 1a. Solid samples: Finely ground (homogenized) solid samples should be dried to constant weight in a 70 °C drying oven prior to being weighed for Carbon-Nitrogen analysis. Overnight drying directly before weighing is recommended.
- 1b. Filter samples: Filters should be frozen (-20 °C) and then lyophilized prior to being weighed for Carbon-Nitrogen analysis.
2. Print the BCAL Carbon-Nitrogen Data Sheet (available online at <http://seagrass.fiu.edu/bcal.htm>) and fill out the “Customer Information” section at the top. When weighing your samples, you will fill out Rows B-H and Cells 1-10 of the Data Sheet, which correspond to the positions within the 96-well plate. Leave Row A and Cells 11-12 in all rows (highlighted in gray) *EMPTY*. These cells will be filled in by BCAL technicians.
3. Obtain a 96-well plate to hold your samples. FIU labs can borrow a well plate from BCAL or you can purchase your own (see “Materials” section at the end of the protocol). Use label tape to label your tray with the same Tray Name written under “Customer Information” on your Data Sheet.

Sample Preparation:

4. Wearing gloves, clean the workspace (mirror or hard surface) and weighing tools (forceps and spatula) with 90% ethanol and Kimwipes to remove trace organic matter prior to weighing a sample.
5. Record the sample ID in the “Sample ID” column of the Data Sheet. Use tweezers to place a tin cup on the microbalance, close the microbalance doors and press the “Tare” button. NEVER touch anything inside the microbalance weighing chamber with your hands. Use tweezers only to place tin cups inside the chamber. If you spill anything inside the chamber, gently wipe the weighing tray with a Kimwipe.
- 6a. Solid samples: After the microbalance has tared, use the forceps to remove the tin cup from the weigh chamber and place it on the workspace. Use the spatula to place a small amount of ground sample material into the tin cup. Place the tin cup on the microbalance, close the doors, and check the sample weight. Add or remove sample from tin cup until the desired weight has been attained, but always do so on the workspace and not inside of the microbalance. Once the desired weight has been attained, record the weight (XX.XXX mg) in the “Sample Weight” column of the Data Sheet. Plant tissue should be weighed between 3-7 mg, animal tissue should be weighed between 1-3 mg, and soils should be weighed between 10-15 mg (but weigh peat soils rich in N at 3-7 mg).

6b. Filter samples: 25 mL GF/F filters can be folded into a large tin cup (10 x 10 mm). Larger filters will need to be cut or hole-punched to obtain a small enough volume to fit into a tin cup. If cut or hole-punching your filters, be sure to record the percentage (dry weight) of filter that is being run. This information, as well as the filter pre-weight prior to filtering, are necessary for producing Carbon-Nitrogen data as percent dry weight. Use forceps to place the filter into the tin cup. Place the tin cup on the microbalance, close the doors, and record the weight (XX.XXX mg) in the "Sample Weight" column of the Data Sheet.

7. Using tweezers, carefully fold the tin cup into a small circle or square so that no material leaks out. Place folded cup into appropriate hole in 96-well plate indicated on the Data Sheet. See the video prepared by EA Consumables at <https://eaconsumables.com/solid-sample-preparation> as an example of proper folding technique. As a general rule, the tighter you can fold up the tin capsule the better, since this prevents excess atmospheric N from interfering with the analysis. See the photo below from Northern Arizona University's Colorado Plateau Stable Isotope Laboratory website for an example of how properly folded capsules should look (smaller than you may think!):



8. Repeat steps 4-7 until all samples have been weighed, filling out the Data Sheet as you go. Don't skip step 4! Cleaning the workstation and tools must be done before EVERY sample to prevent cross-contamination.

9a. FIU researchers: Once you have finished preparing your samples, place the cover on the tray and secure with a rubber band (do not secure with tape around the sides). Fold the Data Sheet and secure it underneath the rubber band with the "Customer Information" facing up. Prepared samples should be placed in a desiccator until they are run on the Elemental Analyzer. Desiccator space is available in BCAL if your lab doesn't have any.

9b. Non-FIU researchers: Once you have finished preparing your samples, place an index card inside the cover and place these on top of the tray (the index card should prevent samples from "jumping" to other cells during shipment). Seal all four sides of the tray shut with label tape. If you can ship the tray with THIS END UP instructions on the box, that is helpful to further try to ensure that no samples "jump". Secure the Data Sheet to the tray with a rubber band with the "Customer Information" facing up. Send an e-mail to the BCAL Lab Manager, Sara at sawilson@fiu.edu to let us know your samples are on the way (don't forget to attach a BCAL Chain of Custody form). Ship to:

*Attn: Sara Wilson OE-148
Florida International University
11200 SW 8th St.
Miami, Florida, USA, 33199*

Materials:

BCAL Carbon-Nitrogen Data Sheet (available at <http://seagrass.fiu.edu/bcal.htm>)

96-well plate (FisherSci.com catalog # 12-565-501)

Tin cups 5 x 8 mm (EAConsumables.com catalog # D1008)

NOTE: Acidified samples will require silver cups (EAConsumables.com catalog # D1021)

NOTE: Filters will require 10 x 10 mm tin cups (EAConsumables.com catalog # D5055)

Stainless steel forceps/tweezers (EAConsumables.com catalog # E2016 and E2293)

Spatula (EAConsumables.com catalog # F5103)

Gloves, Kimwipes, label tape, sharpie

Microbalance – required for one-thousandth of a milligram weight precision (XX.XXX mg)