CN Sample Preparation Protocol

Thanks for choosing the Aridlands Ecology Service Center for your Carbon and Nitrogen analyses. The current price per sample is $3.50. Included in the cost of sample analysis is the cost of consumables associated with sample analysis (sample vials, sample tins, etc.), sample analysis by a Barger Lab Technician, and the delivery of data in an excel spreadsheet format. We will provide training and tools on how to prepare, weigh, and pack your own samples. Some equipment (sample splitters, sieves, mortar and pestle, wiley mill) is available for check out in the Barger Lab. The sections below are an overview of sample prep steps that will ensure that you are provided with representative and high quality data.

I. Sample Preparation:

Estimate %C for your samples based on previous runs or literature values. This will determine how much sample you will weigh into the tins. Here is a general rule of thumb:

%C	Sample Weight (mg)
0.6-2.4	25-40
2.5-5	15
40-70	2-3

If you have no idea what the %C is for your sample then you can determine weights based on whether it is plant or soil material:

%C	Weight (mg)
Plant	2-3
Rock	60
Mineral soil	50
Most Colorado Soils	10-15
Desert soil	30-40

Note: These values DO NOT have to be exact. Don't spend extra time trying to get an exact number. It is more important that the correct weight is recorded. There are a number of ways you can reduce error in the sample weight, see text below. Consult with the Barger Lab before deciding on a sample weight.

A. Soil samples

1. Soil samples should be dried in the 60° C oven for 48 hrs.
2. Once soils have been dried, pass entire soil sample through a 2 mm sieve. Separate the materials that do not pass through the sieve. Air dried samples can be sieved and then oven dried as well.

3. Oven dried soil should then be split with a sample splitter to the desired sample size. Soil may need to be split several times in order to obtain the desired "split amount" of ~3g.
4. Grind the 3 g of soil in the mortar and pestle. It is important that the ground sample is homogenous because we are analyzing such a small sample size. The goal is to analyze a representative sub-sample, for this reason it is critical that this step is done consistently for all samples.
5. Place ground soil into flip-cap vial.
6. Store samples in a desiccator prior to packing in tins.
7. Take the 3 g sample to the Sartorius microbalance.
8. Make sure that the microbalance is leveled and calibrated. Ask the Barger people how to do this.
9. Press the blue button to take microbalance out of standby.
10. Place a tin on the microbalance, close the draft shield, and tare.
11. Place the tin into the holder and flare the top to make it easier to load sample.
12. Load sample using the small spatula.
13. Pick up the tin with the forceps and give the forceps a tap to compact the sample within the tin.
14. Crimp the tin starting from the bottom and moving toward the top of the tin. Fold over top edges and roll the top down. The shape should be a ball or a box.
15. Weigh the sample and record on the data sheet.
16. Make sure there is no soil leaking out of the sample or there are no tears in the tin.
17. Place sample in correct cell in 96 well plate and make sure the number matches the data sheet.
18. Between samples use a dry Kimwipe to clean the spatula. Either water or methanol can be used to clean the spatula if necessary; however, spatula must be completely dry before touching the sample.
19. Enter data into an excel file. Include the **well plate position, sample ID,** and **sample mass**. Send this file to the Barger Lab Technician.
20. The remaining ground soils should be archived in case samples need to be re-run.

B. Plant Samples

1. Plant samples should be dried in the oven at 60°C for 48 hours.
2. Once samples are dried, grind them with the grinding mill or mortar and pestle to a fine powder. It is important to have a homogeneous sample since we are analyzing such a small sample size. The goal is to analyze a representative sub-sample, for this reason it is critical that this step is done consistently for all samples.
3. Store samples in a desiccator prior to packing in tins.
4. Take the ground plant sample to the microbalance.
5. Press the blue button to take microbalance out of standby.
6. Place a tin on the microbalance, close the draft shield, and tare.
7. Place the tin into the holder and flare the top to make it easier to load sample.
8. Load sample using the small spatula.

9. Pick up the tin with the forceps and give the forceps a tap to compact the sample within the tin.
10. Crimp the tin starting from the bottom and moving toward the top of the tin. Fold over top edges and roll the top down.
11. Weigh the sample and record on the data sheet.
12. Make sure there is no plant material leaking out of the sample or there are no tears in the tin.
13. Place sample in correct cell in 96 well plate and make sure the number matches the data sheet.
14. Between samples use a dry Kimwipe to clean the spatula. Either water or methanol can be used to clean the spatula if necessary; however, spatula must be completely dry before touching the sample.
15. Enter data into an excel file. Include the **well plate position, sample ID,** and **sample mass**. Send this file to the Barger Lab Technician.
16. The remaining ground soils should be archived in case samples need to be re-run.

Before using the Satorius Microbalance, it is important that the balance is leveled. The balance should also be calibrated if you are moving it to a different location than the designated weighing bench.

C. Sample Map in a 96 well plate:

This is only if you are packing your own calibration curve and chk standards

1. Bypass (By) samples are atmospheric samples (no tin, no sample). Start the tray with two empty (By) well.
2. Blanks (Bl) are empty tins that are folded up in the same way a sample tin would be. Two empty tins should be run prior to the calibration curve.
3. Weigh out one conditioning sample (C). These are just soil or plant material weighed into capsules at the suggested weights to be run before generating a calibration curve.
4. Weigh out four atropine standard samples, ex. 1) Cal1=0.2 mg 2) Cal2= 0.5 mg 3) Cal3=1.0 mg and 4) Cal4=1.5 mg. Note: All these weights are approximate. You do not have to have these exact amounts. But exact weights must be recorded on your data sheet. The calibration curve that you build will depend on the concentrations that you expect to see in the samples. If you are expecting low concentrations make sure to include the smaller weights in the calibration curve.
5. Check Standards (Chk) are atropine samples of known weights and should be run every 10^{th} sample. Chk's should span the range of the calibration curve, but again these don't have to be exact weights.

****Atropine is a potentially harmful chemical. Make sure that no dust gets in your eyes and that you wash hands immediately after handling it****

6. Once you have weighed these out you can start weighing samples to be analyzed (U=unknown).
7. Run an atropine standard (0.5 mg) at the beginning of each row and finish the run with a standard.

8. Once a well plate is full or all the samples are weighed out, place the lid over the plate and **tape the edges**. If you drop the well plate the samples will remain in their respective wells if the top is taped.

Map of 96 well plate.

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
A	By	By	Con	Bl	Bl	Cal1	Cal2	Cal3	Cal4	Chk1	U	U
B	U	U	U	U	U	U	U	U	Chk2	U	U	U
C	U	U	U	U	U	U	U	Chk3	U	U	U	U
D	U	U	U	U	U	U	Chk4	U	U	U	U	U
E	U	U	U	U	U	Chk5	U	U	U	U	U	U
F	U	U	U	U	Chk6	U	U	U	U	U	U	U
G	U	U	U	Chk7	U	U	U	U	U	U	U	U
H	U	U	Chk8	U	U	U	U	U	U	U	U	Chk9