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Soil organic carbon stocks and change protocol

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ABSTRACT

of carbon (C) balance in cropping systems and as an integral metric of soil health assessments that can be carried out at all LTAR locations. While measurement of C flux with micro-metrological methods, such as eddy-covariance, provides very precise measurements of carbon dioxide (CO₂) flux into and out of ecosystems. Measurement systems are expensive, require a high level of expertise to operate, and are not applicable for plot-scale measurements. Determination of SOC concentration and soil bulk density allows calculation of the mass of SOC accumulated in the soil, and changes in SOC over time reflect system C balance. In most soils, measurable SOC changes require a minimum sampling interval of 10 to 12 years, depending on local factors like existing SOC stores and rotation diversity. Thus, we recommend deep soil cores to 1 m depth taken at decadal intervals, and in intervening years (e.g., five-year intervals), surface soil samples at 0-25 cm or 0-30 cm depth, which includes the most active part of the rootzone. In general, soils should be sampled into the C horizon; in arable soils, a 1 m depth will often be adequate, but in some situations, deeper may be justified, and in many rangelands, soils are much shallower. We recommend minimum sample increments of 0-10, 10-25, 25-50, and 50-100 cm (or deeper / shallower as appropriate). Consistently sampling to the same depth with the same increments at known locations will greatly simplify the ability to detect small changes over decades reliably. In cropping systems, sampling intervals should be timed to the same crop phase and at the same time of year to minimize year-to-year variability in root and residue inputs; sampling post-harvest can minimize plant disturbance. A dry combustion CN

analyzer should be used to measure total C; for soils with detectable carbonate minerals, inorganic C must first be removed by acid fumigation or analyzed

independently for later subtraction. Estimation of SOC stocks can be calculated using two methods: spatial coordinate (includes bulk density), and equivalent soil mass

The change in soil organic carbon (SOC) over time serves as an integrated indicator

GUIDELINES

General Principles

(normalized mass per unit area).

Soil organic carbon (SOC) is a crucial attribute of soil function. Change in SOC stocks in the soil profile over time indicates C balance for cropping systems since the residual stock of SOC is the balance between C gains and losses in the cropping system. The approach can be applied at both the plot and field scales. The approach requires the measurement of SOC by an elemental analyzer and the determination of soil bulk density. Loss on ignition can also be used to estimate SOC, but it does not provide a direct measure of C and is not sufficiently precise for determining soil C stocks, so should be avoided. In most systems, changes in SOC due to management treatments are typically small compared to the initial stock of SOC, and spatial variation contributes significantly to measurement uncertainty. The combined effects mean that more than 10 years is usually needed before statistically

significant changes in SOC can be detected (Necpálová et al. 2014). Significant changes at depth, where carbon contents are lower and more spatially heterogeneous, may take much longer (Kravchenko and Robertson, 2011). Therefore, sampling on a decadal frequency is generally recommended to detect the change, although more frequent sampling could add sensitivity to the detection of changes with time.

Summary of recommendations for measurement of soil organic C stock as an indicator of system C balance.

A	В	С	D
Metric name: 0	Change in soil orga	anic C storage	
Attribute*	Preferred	Minimum	Comments
Spatial scale	Plot and field	Plot	The protocol could be replaced at the field scale by eddy covariance or other tower-based micro-met method if that technology is available.
Frequency	5 years	5 years for surface samples, and 10 years for deep core samples	5- to 10-years sampling interval is sufficient for determining C balance. If more frequent sampling it needed for other objectives, the inclusion of SOC stock determined with those measures could increase the sensitivity of C stock determinations.
Covariate metrics	soil pH	None	
Other			

Soil sampling & archiving:

- Soil core samples 2 cm diameter, 30 cm length
- 7 mL plastic vial
- Paper and plastic bags
- Plastic liners with caps
- Hermetic containers (e.g., mason jars)
- Labels

Lab Materials for Soil Organic C concentration analysis:

- Pulverizer, such as SPEX SamplePrep 8530 Enclosed ShatterBox
- Tin capsules (from Elemental Microanalysis: pressed, standard weight, 8 × 5 mm), otherwise capsules/containers specific for the dry combustion machine being used
- if no acid pre-treatment is needed, use tin capsules
- for acid pre-treatment, use silver capsules
- Forceps
- Micro spatula, scoop shaped
- Sample tray (e.g., 96-well plate, if using tin capsules)
- Electronic microbalance (resolution to 0.001 mg)
- Standards (acetanilide, atropine, or phenacetin*)
- Blind standard (stored soil standard)
- CHN analyzer

For carbonate pre-test:

- Option 1: Soil pH meter
- Option 2: small weigh boats or beakers, eye dropper or syringe, 1N HCl acid (~10 mL)

For acid pre-treatment if needed to remove carbonates:

- Microliter pipette
- Nanopure water
- Dessicator
- Concentrated hydrochloric acid (12M HCI)

Sample Collection

Collect whole-profile soil samples at 1 m depth at decadal intervals (or for shallower soils to the depth of parent material) or for surface soils only, at 0-25 cm or 0-30 cm depth at shorter intervals. In cropping systems, take samples post-harvest (late fall or early winter) or pre-planting (early Spring), and in grazing systems, post-senescence.

Note: In general, soils should be sampled into the C horizon; in arable soils, a 1 m depth will often be adequate, but in some situations, deeper may be justified, and in many rangelands, soils are much shallower. We recommend minimum sample increments of 0-10, 10-25, 25-50, and 50-100 cm (or deeper / shallower as appropriate). For No-till fields, we recommend considering 0-5 cm depth where often soil organic C stock differences are found. Consistently sampling to the same depth with the same increments at known locations will greatly simplify the ability to detect small changes over decades reliably.

1.1 Decadal one-meter depth soil sampling

Whole profile soil samples should be taken as intact cores in acrylic sleeves of at least 5 cm diameter to 1 m depth, using a hydraulic probe to avoid compaction.

The actual sampling interval will depend on the experimental design and rotation lengths of the treatments being compared. Samples should be taken post-harvest on the same (or similar) rotation phase at each interval.

Case 1: Two- and five-year rotations: sample every 10 years on the same crop phase.

A	В	С	D	E	F	G	Н	I	J	K	L
Two- year rotation											
Year	2023	2024	2025	2026	2027	2028	2029	2030	2031	2032	2033
Rotation	Corn	Soy	Corn	Soy	Corn	Soy	Corn	Soy	Corn	Soy	Corn
Year#	Baseline	1	2	3	4	5	6	7	8	9	10
Samplin g year	Х										Х

A	В	С	D	E	F	G	Н	I	J	K	L
Five-year rotation											
Year	2023	2024	2025	2026	2027	2028	2029	2030	2031	2032	2033
Rotation	Corn	Soyb ean	Whe at	Alfalf a	Fallo w	Corn	Soyb ean	Whe at	Alfalf a	Fallo w	Corn
Year#	Baseline	1	2	3	4	5	6	7	8	9	10
Samplin g year	Х										Х

Case 2: Three-, four- and six-year rotation: sample every 12 years on the same crop phase.

Three- year rotation							

Year	2023	202 4	202 5	202 6	202 7	202 8	202 9	203	203 1	203 2	203 3	203 4	203 5
Rotation	Corn	Soy bea n	Whe at	Cor n	Soy bea n	Whe at	Cor n	Soy bea n	Whe at	Cor n	Soy bea n	Whe at	Cor
Year#	Basel ine	1	2	3	4	5	6	7	8	9	10	11	12
Sampling year	X												X

Four-year rotation													
Year	2023	202 4	202 5	202 6	202 7	202 8	202 9	203 0	203 1	203 2	203 3	203 4	203 5
Rotation	Corn	Soy bea n	Whe at	Alfal fa	Cor n	Soy bea n	Whe at	Alfal fa	Cor n	Soy bea n	Whe at	Alfal fa	Cor n
Year#	Basel ine	1	2	3	4	5	6	7	8	9	10	11	12
Sampling year	X												Х

Six-year rotation													
Year	2023	202 3	202 3	202 3	202 3	202 3	202 3	202 3	202 3	202 3	202 3	202 3	202 3
Rotation	Corn	Soy bea n	Whe at	Alfal fa	Alfal fa	Fall ow	Cor n	Soy bea n	Whe at	Alfal fa	Alfal fa	Fall ow	Cor
Year#	Basel ine	1	2	3	4	5	6	7	8	9	10	11	12
Sampling year	Х												x

For plot-scale experiments, take at least three cores per plot and GPS-mark sampling positions to facilitate the detection of changes in soil organic carbon (SOC) stock in subsequent years at nearby sampling locations. Refill the holes with a mix of bentonite and sand to avoid sampling on the exact same locations next time. For field-scale experiments, a sufficient number of soil cores should be obtained to represent the field's variation in topography and yield. Transport and store soil cores in plastic liners and store at 4 °C until processing. If same-day processing, store soils in shaded containers.

1.2 Surface soil sampling

For non-decadal sampling intervals, align with crop phase. Surface soils can be taken in the same manner as above but without the need for intact cores / plastic sleeves and in most cases without the need for a hydraulic sampler. Samples should be taken to the same surface soil depth interval used for deep cores (0-25 or 0-30 cm) using a push core of 2 cm diameter.

Soil Processing

2	Pre-label paper bags with sample information (e.g., date, plot, station, core#, depth section).
2.1	OPTIONAL: Consider including barcodes in your labels if you have an automated system in
	place at your site.
3	Measure and record the length (in cm) of each horizon and the diameter (in cm) of the corer used; measurements are used to determine the volume of soil in each section. Make note of cores that are not full, often due to compaction during sampling, and adjust the depth horizon length accordingly.
4	Lay core horizontally on cutting board. Lift core slightly to remove end caps. Carefully cut open core liner using cutter.
5	Separate each soil core into multiple depth intervals that capture carbon stocks in both surface (A and Ap) versus subsurface horizons. For most cultivated sites the appropriate depth intervals will be 0-10 and 10-25 cm (or 10-30 cm) for surface horizons, and 25-50 cm (or 30-50 cm), and 50+ cm for subsurface. For No-till fields, we recommend considering 0-5 cm depth where often soil organic C stock differences are found.
6	Record the fresh weight of each soil layer (WWsoil), and the actual depth of each soil layer.
7	Pass each soil layer through a 2 mm sieve to remove gravel and identifiable plant material > 1 cm.
8	Record the weight or volume of the gravel (WWgravel or VWgravel) and discard. Gravel volume can be assess by displacement by following Grossman and Reinsch (2002).
9	Oven dry-sieved soil samples

- 9.1 Option A. Soil drying for SOC analysis, bulk density, and gravimetric water content:
 Oven-dry the sieved soil at 60 °C for at least 48h until dry (check for no additional mass loss) and record the dry weight (DWsoil). Both wet and dry weights of gravel-free samples can be used to calculate the gravimetric water content from each soil layer.
- 9.2 Option B. Soil drying for only bulk density and gravimetric water content determination: Oven-dry the sieved soil at 105 °C for at least 24h until dry (check for no additional mass loss) and record the dry weight (DWsoil). Both wet and dry weights of gravel-free samples can be used to calculate the gravimetric water content from each soil layer. This soil cannot be used for total %C and %N analysis as it may have lost C and N during drying at high temperatures.
- Pulverize a 50 g subsample to less than 250 microns in a shatterbox mill (or equivalent) with a hardened steel grinding container and puck (e.g., SPEX SamplePrep Shatterbox 8530, Metuchen, New Jersey USA). Store pulverized sample in a labeled ~7 mL plastic vial.

Archiving Soil Samples

- Soil organic C remains very stable in air-dried samples, save and store at least 500 g to 1,000 g of soil from each sample in hermetic containers for future analysis. All archive samples should be stored in the same room or storage facility, in properly labeled boxes or plastic totes (PI last name, Year, Field, Treatment, Replicate, Depth), and placed on the same shelf along with samples collected during the same sampling year and field. Additionally, always keep your archive database updated. Archived samples can help future researchers monitor SOC changes, among other soil properties.
- 11.1 Recommendation: In addition to the traditional way of labeling samples, consider using another automated labeling method with the barcoding of storage bins or archived samples.

Acid-Fumigation Pre-Treatment to Remove Carbonates whe.

- Prepare samples as above but with the following exceptions: a) use silver capsules instead of tin; b) transfer 40-60 mg of finely pulverized soil to the capsule; and c) leave capsule open, do not fold. Store sample tray in desiccator until ready to proceed.
- Add 90 microliters of nanopore water to each open capsule to wet the soil.

- Place sample tray in desiccator containing a beaker of concentrated (12M) HCl. Allow at least 6-8 hours for carbonate dissolution, but not more than 24 hours because extended exposure makes silver capsules brittle.
- Remove sample tray from desiccator and dry in 60 °C oven until dry, at least 24 hours.
- Fold capsules as described above. Silver capsules can become brittle after drying; be careful not to lose sample when folding. If brittle, enclose silver capsule in a tin capsule to avoid potential loss of sample. Discard sample if loss occurs.
- 17 Suggestions about wear on the elemental analyzer from acid-fumigated soils from the USDA Northern Great Plains Research Laboratory:
 - 1. Log that you are running fumigated samples on the analyzer. Fumigated samples consume more of the combustion and reduction reagents in the columns, so they will need to be replaced about twice as often.
 - 2. Only run ~80 fumigated samples per quartz insert, as they have more residue than a normal soil sample.
 - 3. Thoroughly check the elemental analyzer for evidence of corrosion near contact points and around seals when quartz insert is replaced.
 - 4. The analyzer should be baked out every 3 months at 110°C for 6 hrs (as opposed to every 6 months under normal operating conditions) when running acid fumigation.
- 17.1 NOTE: Post-fumigated soils will impact analyzer combustion tubes and seals differently than non-fumigated soils (not in a good way). Analyzers will need to be checked for routine maintenance more frequently when running soils fumigated for IC removal.

Soil Total Carbon Concentration - Using Elemental Analyzer..

- Total soil C concentration should be determined by dry combustion gas chromatography (NRCS Technical Note 450-03, 2019; Nelson and Sommers, 1996) on an Elemental Analyzer (e.g., Costech ECS 4010 CHNSO Analyzer, Valencia, California, USA) calibrated with an analytical standard Acetanilide (C₈H₉NO) or Atropine (C₁₇H₂₃NO₃), with three analytical reps per sample. Run a check standard (Low Organic Content Soil, Elemental Microanalysis, Marlton, NJ; 1.55 %C, 0.13 %N) every 12 samples per plate of 72 samples.
- **18.1 Other Equipment Options:** Elementar and Leco analyzers that can handle larger sample sizes (100-300 mg soil) and use crucibles rather than tins. So, details about the sealing of the

capsules and the amount of soil to use are analyzer specific.

- 19 In the absence of inorganic C, total C is assumed to equal SOC. Commercial labs are unlikely to provide sufficient precision or reproducibility.
- For soil layers where pH is greater than 7.2, quantification or removal of inorganic C may be required. To detect the presence of significant inorganic C, to a subset of representative samples add five drops of 1 N hydrochloric acid to 0.5 g of pulverized soil and check for effervescence. If effervescence is detected, inorganic C must be either removed by acid fumigation prior to total C analysis or measured for subtraction from total C concentrations (as described above in Step 12). To quantitatively measure inorganic C, refer to the methodology outlined in NRCS Technical Note 450-03 (2019) or Sherrod et al. (2002).
- **20.1 Option B:** For soils that in the past had a low but meaningful concentration of inorganic carbon, another method to track carbonates is by running a delta ¹³C isotopic analysis following (XYZ)
- If not equipped with an Elemental Analyzer, reach out to another LTAR location for help with these analyses.

Procedure for Soil Carbon & Nitrogen Analyses

- 22 Prepare soil for analysis (Case: Shatterbox pulverizer)
- **22.1** Put about 50 g of the sieved and well-mixed dry soil into a grinding container specific to the pulverizer machine to be used.
- 22.2 Place one container in the center or three containers on the three outside pins in the shatterbox.
- 22.3 Place one container in the center or three containers on the three outside pins in the shatterbox.

22.4 Transfer ~7 mL of pulverized soil from the grinding container into a labeled 7 mL plastic vial.

Discard the remainder.

23 Test for soil carbonates (if necessary)

- **23.1** *Option 1. Check for effervescence*
 - 1. Put a few grams of pulverized soil onto a weigh boat or beaker.
 - 2. Add 2-3 drops of HCl.
 - 3. If no bubbling is noted, stir gently to confirm (compare to soil with no carbonates to ensure bubbling is from carbonate dissolution and not from the physical release of entrapped bubbles).
 - 4. Record the presence or absence of carbonates and discard sample.
- Option 2. Check for soil alkalinity

 If soil pH is greater than 7.2, it is necessary to quantify or remove the inorganic C (e.g. carbonates) present in any soil sample.

24 Prepare Soil Sample

- **24.1** Always use forceps to handle capsules. Fingers and hands are sources of C and N contamination.
- 24.2 Place an empty capsule/container on the scale of the microbalance. Close the housing doors. Tare the capsule weight.
- 24.3 Remove tin capsule and place on marble block. Using a scooping spatula, transfer 15-20 mg of the finely pulverized soil in the sample vial to the capsule. Weigh the capsule to ensure it contains an appropriate weight of sample material.

Note: If you use Elementar equipment for this analysis, transfer 100-300 mg of soil finely

24.4	Return capsule to marble block and use forceps to first roll back the free end of the capsule and then to fold the capsule into a small ball, making sure that no sample leaks from the sealed capsule.
24.5	Place folded capsule on the scale, close the doors, and record the sample weight by pressing "print" on the balance instrument panel to link its mass via computer software to the selected cell on the data spreadsheet.
24.6	Place the capsule in the sample tray well designated by the data spreadsheet.
24.7	Always clean the forceps, spatula and marble block with lab wipes between samples.
25	Prepare Analytical Sample Run
25.1	Start the run sequence by preparing a "bypass" sample containing material with a C and N content anticipated for samples (well #A1). Record the well number for each sample on the data spreadsheet.
25.2	Always clean the forceps, spatula and marble block with lab wipes between samples.
25.3	Next add a "blank" consisting of an empty folded tin capsule (well #A2).
25.4	Follow with four replicates of a known standard, increasing the weight of sample with each replicate (0.2-0.3mg; 0.5-0.6 mg; 1.0-1.2 mg; and 2.0-2.8 mg). These samples are used to

pulverized into a crucible.

calibrate the analyzer (wells #A3-A6).

- 25.5 Follow with one blind soil standard, prepared as above for soil sample (well #A7).
- 25.6 Continue run sequence with samples to be analyzed, preparing three analytical replicates for each sample. Include a blind standard approximately every 12 capsules (well #A8 onward).
- 25.7 Store sample tray in desiccator until analyzed. Follow analyzer protocol.

QA/QC considerations #1

Re-run samples if the coefficient of variation (CV) among analytical replicates exceeded 10% or if check standards are not within 10% of label values.

Determination of gravel-free bulk density - Recommendation

Gravel-free bulk density should be determined along with soil organic C concentration to facilitate the calculation of C stocks. If there is no detectable soil compaction during soil core sampling, gravel-free bulk density can be determined from the dry weight of soil within the core volume of each soil layer. For soils with a significant gravel content, gravel as weighed earlier, can be used to adjust for the volume occupied by gravel to calculate total bulk density. Alternative methods for bulk density determination are described in Grossman and Reinsch (2002).

Calculations of Soil Organic Carbon Stocks, Change, and Ra..

- 28 Estimation of soil gravimetric water content
- **28.1** Determine wet weight in each soil layer [g]

WW_{soil} = Total wet weight soil_{laver} – Wet weight_{gravel}

28.2 Gravimetric soil water content (SWC) in each soil layer

where:

WW_{soil} = wet weight of a soil layer [g]

DW_{soil} = dry weight of a soil layer [g]

29 Estimation of total bulk density and gravel free bulk density

29.1 Total bulk density (includes gravel)

where:

V = volume of core section in cm³ = cylindrical volume = (π) (r2) h

29.2 Gravel-free bulk density [g cm⁻³]

30 Estimation of soil carbon concentration (%)

The CHN analyzer reports C and N as the percentage of C or N in the dried sample (g C or N per 100 g sample). Report the average values from analytical replicates. Total C is equivalent to total organic C (TOC) if inorganic C was not present in the sample or was removed via fumigation.

31 Estimation of SOC stocks, change, and rate of change

31.1 Spatial coordinate method suggested by Gifford & Roderick (2003)

Report SOC stocks as Mg ha⁻¹ per soil layer and per whole-profile (e.g., 0-100 cm). For this, use average gravel-free bulk densities by soil layer.

Calculate SOC stocks by multiplication of SOC concentration (kg C Mg soil⁻¹) by the averaged gravel-free bulk density (Mg soil m⁻³) by sampling depth (m), given mass of SOC per m²(% C x $10 = [g \text{ C kg soil}^{-1}] = [kg \text{ C Mg soil}^{-1}]$; [g cm⁻³= Mg m⁻³]), which can be transformed to hectares.

Change in SOC stocks is the difference between two sampling times. If SOC stocks were determined at multiple depths, the sum of SOC stocks across depth increments should be reported as cumulative SOC stock for the total sampling depth of 0-100 cm.

Rates of change are calculated by dividing the difference in SOC stocks by the number of management years (Mg SOC ha⁻¹ year⁻¹).

31.2 Equivalent soil mass method by Wendt and Hauser (2013)

The equivalent soil mass (ESM) assesses SOC stocks on a normalized soil mass per unit area basis to account for differences in soil masses caused by soil management

ESM procedures can be understood by visualizing soil profiles in terms of soil mass layers instead of soil depth layers (Figure 1).

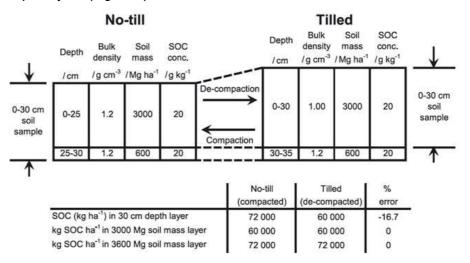


Figure 1 Error bias induced by quantifying soil OC stocks at fixed depths when differences in bulk density exist.

Source: Wendt and Hauser (2013).

Soil mass layers (such as 0-1000, 1000-2000, 2000-3000 Mg ha⁻¹) approximate are similar to soil depth layers (0-10, 10-20, 20-30 cm), but the mass of soil in a given depth layer will vary with bulk density, whereas the mass of soil in a soil mass layer, by definition, is fixed, and provides a consistent basis for comparing SOC changes and differences.

An exact correspondence exists between the mass of a soil sample depth layer (g) and its soil mass. Dimensionally, soil mass is mass per unit area, or Mg ha⁻¹. To calculate the soil mass represented by a soil sample depth layer (DL), one only needs to divide the dry sample mass (M_{SAMPLE}(DL), [g]) by the area sampled by the probe or auger, which is the cross-sectional area of its inside diameter, or $\pi(D/2)^2$. If multiple soil cores are combined to form one sample, then the area sampled is $\pi(D/2)^2 \times N$, where N is the number of cores sampled. If D is expressed in [mm], then the mass of the soil (M_{SOIL}(DL), Mg dry soil ha⁻¹) in each depth layer is calculated

as:

$$M_{\rm SOIL(DL)} = \frac{mass}{area} = \frac{M_{\rm SAMPLE(DL)}}{\pi \left(\frac{D}{2}\right)^2 \times N} \times 10000.$$

Source: Wendt and Hauser (2013).

The OC mass in the depth layer ($M_{OC}(DL)$, kg SOC ha⁻¹) is the product of its soil mass (M_{SOIL}) and SOC concentration, $C_{OC}(DL)$ (g SOC kg⁻¹):

$$M_{\rm OC(DL)} = M_{\rm SOIL(DL)} \times C_{\rm OC(DL)}$$

Source: Wendt and Hauser (2013).

The cumulative soil and OC masses are calculated by summing their respective depth layer masses, calculated in Equations (1) and (2) to any given depth; for example, the cumulative soil and OC masses to 30 cm, if cores were split into two equal depth increments, are the sum of their respective masses in the 0-15 and 15-30-cm layers, and the cumulative masses to 45 cm, if cores were split into three equal depth increments, are the sum of their respective masses in the 0-15, 15-30 and 30-45-cm layers.

A fitted curve, in this case a cubic spline, provides good estimates of cumulative SOC contents for any chosen reference soil mass in the profile. The cubic spline function fits a smooth curve to a series of points with a piecewise series of cubic polynomial curves.

An example of this calculation can be found in Wend and Hauser (2013).

Covariate Metrics to be Measured Concurrently

Total soil bulk density and gravel-free bulk density, gravel/rocks weight, where inorganic C is suspected, measure soil pH (especially in calcareous soils).

QA/QC Considerations #2

Certified standards should be used for the calibration of the elemental analyzer. The inclusion of a reference soil with known C concentration is recommended with each analyzer run.

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