FEB 06, 2023

Protocol status: In development
We are still developing and optimizing this protocol

Created: Feb 06, 2023

Last Modified: Feb 06, 2023

PROTOCOL integer ID: 76475

Soil Organic Carbon Stocks and Change

SCarolina Córdova[1], Curtis Dell[2], Mark Liebig[3], michel.cavigelli[4], Phil Robertson[5]

[1]University of Nebraska, Lincoln;
[2]USDA-ARS-Pasture Systems Watershed Management Research Unit;
[3]USDA Northern Great Plains Research Lab;
[4]USDA Agricultural Research Service, Sustainable Agricultural Systems Lab (SASL), USDA GRACEnet and LTAR Network, Northeast Cover Crop Council;
[5]Michigan State University

SCarolina Córdova
University of Nebraska, Lincoln

ABSTRACT

The change in soil organic carbon (SOC) accumulation or decreases over time serves as an integrated indicator 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 CO_2 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 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 (normalized mass per unit area).

MATERIALS

Protocol

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) and in grazing systems post-senescence.

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 with a hydraulic probe.
Case 1: Two- and five-year rotations: sample every 10 years on the same crop phase.
Case 2: Three-, four- and six-year rotation: sample every 12 years on the same crop phase.
For plot-scale experiments, take at least three cores per plot and GPS-mark sampling positions to facilitate detection of changes in 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 at 4 ˚C until processing. If same day processing, store soils in shaded containers.
Surface soil sampling
For non-decadal sampling intervals, align with crop phase (e.g., five-year intervals samplings for two and four-year rotations). 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.

Archiving
SOC remains very stable in air-dried samples, so at least 500 g to 1,000 g of soil should be retained from each deep core and surface sample, and stored in hermetic containers to allow repeated analysis of any inconsistent samples in the future, as well as to expand the analysis of other chemical or physical soil properties.

Soil processing and characterization
Pre-label paper bags with sample information (e.g., date, plot, station, core#, depth section).
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.
Lay core horizontally on cutting board. Lift core slightly to remove end caps. Carefully cut open core liner using cutter.
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 to consider 0-5 cm depth where often SOC stock differences are found.

Record the fresh weight of each soil layer (WWsoil), and the actual depth of each soil layer.
Pass each soil layer through a 2 mm sieve to remove gravel and identifiable plant material > 1 cm.
Record the weight of the gravel and discard (WWgravel).
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.
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.

Soil total C concentration

Total soil C concentration should be determined by dry combustion gas chromatography (NRCS Technical Note 450-03; Nelson and Sommers, 1996) on an Elemental Analyzer (e.g., Costech ECS 4010 CHNSO Analyzer, Valencia, California, USA) calibrated with an analytical standard Acetanilide (C_8H_9NO) 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. Re-run samples if coefficient of variation (CV's) among analytical replicates exceeded 10% or if check standards are not within 10% of label values.

In the absence of inorganic C, total C is assumed to equal SOC. If locations are not equipped with an elemental analyzer, reach out to another LTAR location for help with these analyses.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 (as described below). If effervescence is detected, inorganic C must be either removed by acid fumigation prior to total C analysis (described below) or measured for subtraction from total C concentrations. To quantitatively measure inorganic C, refer to methodology outlined in NRCS Technical Note 450-03 or Sherrod et al (2002).

Gravel-free bulk densityshould be determined along with SOC concentration to facilitate 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).

Covariate metrics to be sampled 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).

Laboratory Materials

Pulverizer, such as SPEX SamplePrep 8530 Enclosed ShatterBox
Tin capsules (from Elemental Microanalysis: pressed, standard weight, 8 × 5 mm), otherwise other 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
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 HCl)

Procedure for Soil Carbon & Nitrogen Analyses

Prepare soil for analysis (Case: Shatterbox pulverizer):
Put about 50 g of the sieved and well-mixed dry soil into a grinding container specific to the pulverizer machine to be use.
Place one container in the center or three containers on the three outside pins in the shatterbox.
Close the lid, set timer for 1 minute and press start.
Transfer ~7 mL of pulverized soil from the grinding container into a labeled 7 mL plastic vial. Discard the remainder.
Test for carbonates if necessary:
Option 1. Check for effervescence
Put a few grams of pulverized soil onto a weigh boat or beaker.
Add 2-3 drops of HCl.
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).
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.

If carbonates are detected, they must either be quantified or removed prior to total C analysis. Refer to NRCS Technical Note 450-03 or Sherrod et al. (2002) for details on inorganic C measurement, and below for the acid-fumigation pre-treatment to remove carbonates when present (Harris et al. 2001).

Prepare sample (see below for additional acid fumigation steps as needed):
Always use forceps to handle capsules. Fingers and hands are sources of C and N contamination.
Place an empty capsule/container on the scale of the microbalance. Close the housing doors. Tare the capsule weight.
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.
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.
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.
Place the capsule in the sample tray well designated by the data spreadsheet (see below).
Always clean the forceps, spatula and marble block with lab wipes between samples.

Prepare analytical sample run:
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.
Always clean the forceps, spatula and marble block with lab wipes between samples.
Next add a “blank” consisting of an empty folded tin capsule (well #A2).
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 calibrate the analyzer (wells #A3-A6).
Follow with one blind soil standard, prepared as above for soil sample (well #A7).
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).
Store sample tray in desiccator until analyzed. Follow analyzer protocol.

Acid-fumigation pre-treatment to remove carbonates when present (after Harris et al. 2001):

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.
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.

Calculations

Estimation of soil gravimetric water content
Determine wet weight in each soil layer [g]
WW_{soil} = Total wet weight $soil_{layer}$ – Wet $weight_{gravel}$
Gravimetric soil water content (SWC) in each soil layer
$SWC = (WW_{soil} – DW_{soil}) / DW_{soil}$
where:
WW_{soil} = wet weight of a soil layer [g]
DW_{soil} = dry weight of a soil layer [g]

Estimation of total bulk density and gravel free bulk density
2.1 Total bulk density (includes gravel)
$BD_{total} = (DW_{soil} + WW_{gravel}) / V$
where:
V = volume of core section in cm^3 = cylindrical volume = (π) (r2) h
2.2 Gravel-free bulk density [g cm^{-3}]
$BD_{gravel\text{-}free} = (DW_{soil}) / V$

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.

Estimation of SOC stocks, change, and rate of change

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), giving mass of SOC per m^2(% C x 10 = [g C kg $soil^{-1}$] = [kg 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 of0-100 cm.

Rates of change are calculated by dividing the difference in SOC stocks by the number of management years (Mg SOC ha^{-1} $year^{-1}$).

4.2 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).

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:

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

Equation 1)

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}):

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

Equation 2)

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).

QA/QC

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

General principles

SOC is a crucial attribute of soil function. Change in SOC stocks in the soil profile over time provides an indicator of 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 scale. The approach requires the measurement of SOC by 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 the determination of 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 means 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 change, although more frequent sampling could add sensitivity to the detection of changes with time.

References

Gifford, R. M., & Roderick, M. L. (2003). Soil carbon stocks and bulk density: Spatial or cumulative mass coordinates as a basis of expression? *Global Change Biology, 9*(11), 1507-1514. doi:10.1046/j.1365-2486.2003.00677.x

Grossman, R.B. and T.G. Reinsch. 2002. Bulk density and linear extensibility. In: J.H. Dane and G.C. Topp, editors, Methods of soil analysis. Part 4. Physical methods. Soil Science Society of America, Madison WI USA. p. 201-225.

Harris, D., W. R. Horwath, and C. van Kessel. 2001. Acid fumigation of soils to remove carbonates prior to total organic carbon or carbon-13 isotopic analysis. Soil Science Society of America Journal 65:1853-1856.

Kravchenko, A. N. and G. P. Robertson. 2011. Whole-profile soil carbon stocks: the danger of assuming too much from analyses of too little. *Soil Science Society of America Journal*75:235-240. https://doi.org/10.2136/sssaj2010.0076

Necpálová, M., R. P. Anex, A. N. Kravchenko, L. J. Abendroth, S. J. Del Grosso, W. A. Dick, M. J. Helmers, D. Herzmann, J. G. Lauer, E. D. Nafziger, J. E. Sawyer, P. C. Scharf, J. S. Strock, and M. B. Villamil. 2014. What does it take to detect a change in soil carbon stock? A regional comparison of minimum detectable difference and experiment duration in the north central United States. *Journal of Soil and Water Conservation* 69:517-531.

Nelson, D.W. and L.E. Sommers. 1996. Total carbon, organic carbon, and organic matter. In: D.L. Sparks, editor, Methods of soil analysis. Part 3. Chemical methods. Soil Science Society of America, Madison WI USA. p. 961–1010.

Sherrod, L.A., G. Dunn, G.A. Peterson and R.L. Kolberg. 2002. Inorganic carbon analysis by modified pressure-calcimeter method. Soil Sci. Soc. Am. J. 66:299–305. doi:10.2136/sssaj2002.0299

USDA-NRCS Technical Note No. 450-03. 2019. Recommended soil health indicators and associated laboratory procedures.
Wendt, J. W., Hauser, S., 2013. An equivalent soil mass procedure for monitoring soil organic carbon in multiple soil layers. *European Journal of Soil Science*, *64* (1), 58-65.

Table 1. Summary of recommendations for measurement of soil organic C stock as an indicator of system C balance.

	A	B	C	D
	Metric name: Change in soil organic 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	2-3 years	5 years for surface samples, and 10 years for deep core samples	5- and 10-years sampling interval is sufficient for determining C balance. If more frequent sampling it needed for other objectives, inclusion of SOC stock determine with those measures could increase sensitivity of C stock determinations.
	Covariate metrics	None	None	
	Other			

Spatial scale = plot or field or both;
Frequency interval = once, weekly, monthly, annually (preferred season?), 5-year, etc.;
Covariate metrics = other metrics to sample concurrently.