Standard Operating Procedure

Soil Inorganic Carbon by Modified Pressure Calcimeter

Scope

This document outlines the measurement of inorganic carbon (IC) concentrations in mineral soil samples by pressure calcimeter. This method is very well described in Fonnesbeck, Boettinger and Lawley, (2013). The measurement procedure is described in general terms, as apparatus-specific operations will vary. Soil sample preparation is the same as for total carbon and nitrogen by dry combustion, and the same ground sample can be used for both analyses. Inorganic C concentration should be reported on an oven-dry (105 °C) soil mass basis.

Equipment

125 mL Wheaton serum bottle
Two-prong, gray butyl rubber septum
Aluminum tear off seal, 20 mm
Bottle seal crimper, 20 mm
Bottle top dispenser
2.5 L bottle
5 mL syringe with a 22-gauge, 1 in. (2.54 cm) long hypodermic needle
18-gauge, 1 in. (2.54 cm) hypodermic needle
Pressure transducer, 0-15 PSIG range, 0.03 to 5.03 V DC
Multimeter
24 V AC transformer
Chemical resistant tubing, 0.25 in. inner diameter (¼”), 0.375 in. outer diameter
0.6 µm particulate filter
Weigh boat

Reagents

Calcium carbonate, $CaCO_3$ reagent grade, dried @105 °C
3 M hydrochloric acid for fizz test
6 M hydrochloric acid containing 3% $FeCl_2.4H_2O$ by weight
Deionized (DI) water
Silica sand, lab-grade
Soapy water solution (~30 mL of commercial dishwashing liquid mixed into 1 L water)

Procedure

Part 1) Identification of samples requiring inorganic carbon testing.

Soils should be air-dried and ground to pass through a 2 mm sieve as described in the total carbon standard operating procedure. If the soil sample does not have a pH analysis, that is ok, a simple fizz test works on any soil. We suggest a prior pH reading to reduce labor in a high throughput situation.

1. OPTIONAL. Analyze soil pH with the 1:2 H_2O method (one part soil to two parts water by weight). Chose only samples with pH greater than 6.5 for the fizz test.
2. Perform a fizz test on the dried and ground soil sample by dropping 1N HCL from a dropper bottle into a small (quarter-sized soil sample). Observe visually and by sound, to see if the soil reacts to the acid. Record the level of effervescence as “None”, “Slight”, “Moderate”, or “Strong” effervescence. Samples with “None” do not require the inorganic carbon test as part of soil organic carbon determination.
3. For samples with “Strong”, “Moderate”, and “Slight” effervescence, weigh a 1.00, 2.00, or 10.00 g sample (Table 1) and proceed with Part 2. (Note: samples containing inorganic carbon in the form of dolomite [$CaMg(CO_3)_2$] may not fizz upon addition of hydrochloric acid. If dolomite is suspected based on soil parent material or amendment history, proceed assuming “Slight” effervescence). A list of counties containing dolomitic parent material in the top 30 cm is located in Appendix A.

Table 1: Amount of soil to use based on effervescence level and associated masses of $CaCO_3$ standards. These standards can be made in batches ahead of time and used according to the reference level of each soil sample to be measured for inorganic carbon.

Effervescence Class	Amount of dried ground sample (g)	$CaCO_3$ Standards (g)
Strong Effervescence	1	0.020, 0.040, 0.10, 0.20, 0.40, 0.60, 0.80, and 1.00
Moderate Effervescence	2	0.025, 0.05, 0.10, 0.20, 0.50, and 1.00
Slight Effervescence	10	0.025, 0.05, 0.10, 0.20, 0.50, and 1.00§

§Lab-grade silica sand should be mixed with carbonate standards to achieve a total mass of 10.00 g to match the volume of soil in the 10.00 g samples.

Part 2) Pressure calcimeter configuration

The setup for this measurement requires creating a pressure calcimeter. This setup is inexpensive, straightforward and requires little maintenance. The pressure calcimeter needs a multimeter, pressure transducer, filter, and hypodermic needle (Figure 1). Parts and specs for building the calcimeter can be found in Sherrod et al. (2002) and briefly described below.

The pressure transducer is connected to a power supply (24 V DC) with 14-gauge wire. The digital voltmeter is wired inline to monitor transducer output. Attached to the base of the pressure transducer is 20 cm of clear laboratory tubing (0.25 in. inner diameter) connected to an 18-gauge hypodermic needle with a particle filter (0.6 µm for removing particles from carrier gas) spliced in the middle of the tubing to prevent any reflux from reaching the pressure transducer. A 125 mL Wheaton serum bottle (Wheaton Science Products, Millville, NJ) serves as the reaction vessel for soils.

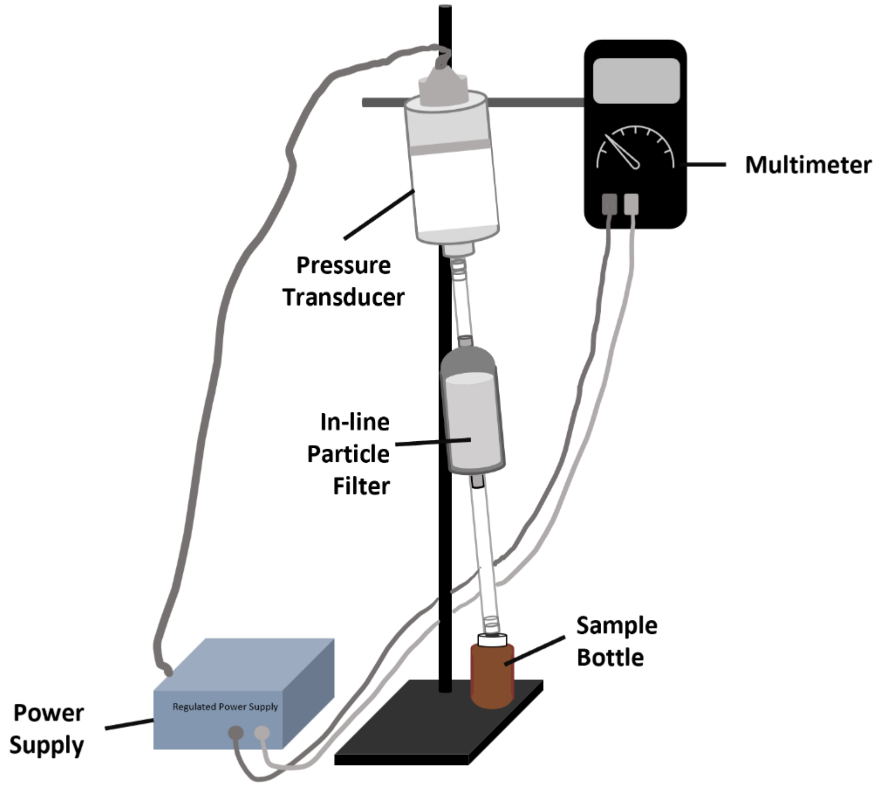

Figure 1. Setup of a modified pressure calcimeter.

Part 3) Procedure for creating a calibration curve for sample quantification

1) Group samples by effervescence level for streamlining the following steps.
2) Homogenize each sample and transfer the correct weight of soil based on effervescence level (Table 1) to tared weigh boat. Record weight to 0.0001 g and transfer to a numbered serum bottle. You may choose to analyze many samples (30 to 90) in a batch.
3) Include a set of control samples that have been created with the corresponding level of effervescence of the samples being analyzed and $CaCO_3$ concentration (Table 1), at the beginning, at an interval of every 30 samples, and at the end of the samples. Record the weights of the controls.
4) For slight effervescing samples, weigh approximately 0.50 g $CaCO_3$ after the last control and record the weight to 0.0001 g. Then add lab-grade silica sand to the weigh boat from Step 3 until the final weight is 10 g and transfer to a numbered serum bottle.

5) Include three empty serum bottles as blanks at the beginning of the batch and one at the end. The readings from the blanks will be included later when generating the standard curve.
6) Weigh and record $CaCO_3$ weights (g) for standards corresponding to the correct effervescence level (Table 1).
7) Using a bottle top dispenser, add 5 mL DI water to each bottle (including standards). Soil should be completely moistened.
8) Insert into each serum bottle a two-leg stopper, ensuring that one leg is lined up with the bottle number.
9) Cap with aluminum tear-off cap and secure using crimper, ensuring that the cap will open to the left while looking at the bottle number, on all bottles.
10) Swirl contents.
11) Peel the center of the cap to the left exposing the septum.
12) Place bottles in fume hood, and using a syringe, inject 4 mL of acid reagent into the serum bottle. Insert the needle into the septum through the “front leg” to reduce the chance of leakage.
13) Remove the needle quickly to reduce loss of pressure.
14) After each injection, turn the serum bottle 90 degrees to signify the next sample.
15) Using a transfer pipet, place a drop of the soapy water solution on the injection site to identify a leak. If bubbles form, reweigh and repeat steps beginning with Step 2.
16) Swirl bottles after injection and then every hour after for a total reaction time of 6 hr.
17) Turn on the multimeter at least 15 min before reading pressures.
18) After 6 hr, insert the needle connected to the pressure transducer into the septum through the “back leg”. Start with the blanks, standards, and then read and record samples.
19) Start all readings with the meter set to 2000 mV DC. If the meter reading rises then shows only lines, move the meter setting to 20 V DC for the higher range.
20) Reading will stabilize in less than 10 seconds. Record the reading.
21) After all samples have been read, insert the venting needle to release any remaining pressure.
22) Remove aluminum caps and septum and pour contents with rinsing into a 3-gallon plastic bucket placed in a sink.
23) Neutralize content by slowly adding sodium bicarbonate until effervescence stops.
24) Plot the weights of the standards as X, and their corresponding voltages as Y, including the mean voltage of the 4 blanks whose $CaCO_3$ weight is 0 g. The resulting equation will then be applied to the unknowns.
25) The generated curve should be linear and appear in the form of $y = bX+a$ where b is the slope of the line and a is the intercept.
26) Determine the concentration of unknown samples using the linear regression equation generated in the previous step by solving for X ($CaCO_3$ g).
27) Calculate the ratio of air-dried (AD) to oven-dried (OD) soil weights and multiply by the $CaCO_3$ weight calculated in Step 26. Multiple by 100 to derive % $CaCO_3$.
28) Multiple % $CaCO_3$ by 0.12 to determine % inorganic carbon.

Calculations

1. AD/OD = (mass of air-dry soil and tin – mass of empty tin) / (mass of oven-dry soil and tin – mass of empty tin). Commonly referred to as a moisture correction. If the sample submitter requests that this value be included in their data report, round to a minimum of three decimal places (e.g., 1.028).
2. Y=bX+ a

   where:
   Y = voltage from unknown sample
   b = slope from linear regression equation
   X = $CaCO_3$ (g) from unknown sample
   a = intercept from linear regression equation
3. % $CaCO_3$ = ($CaCO_3$ (g)/sample weight (g)) x AD/OD x 100
4. % Inorganic carbon = (% $CaCO_3$) x 0.12

QA/QC

1. The standards should create a very straight line, if there is noise around the line this gives you an idea of the quality of the standards and if there are leaks in the system. If the points about the line make a nonlinear trend, check the standards, and assess possible temperature gradients (remember ideal gas law PV=NRT).
2. Make sure there is no extrapolation, sometimes the fizz test will over or underestimate carbonate concentration in a soil sample. If the pressure of the soil sample is outside the range of your calibration line, redo that sample with the appropriate set of standards.
3. Report the R^2 and RMSE of the calibration line used to determine soil inorganic carbon.
4. After every 20 samples, run a duplicate analysis on one of the preceding 20 samples selected at random. Include data for QC duplicates in final data report. The difference in IC concentration between duplicate readings of the same sample should be less than 20% of the average of the two readings.
5. In addition to instrument-specific calibrations, which may not use soil materials, analysis of a minimum of four soil standard reference materials is recommended every six months.

References

Sherrod, L.A., G. Dunn, C.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

Fonnesbeck, B.B, J.L.Boettinger, J.R. Lawley, 2012. Improving a simple pressure-calcimeter method for inorganic carbon analysis. Soil Sci. Soc. Am. J. 77:1553-1562. doi 10.2136/sssaj2012.0381

NOTE

This SOP was developed by SHI, for SHI communication, and developed after the references listed above. For any specific questions, contact erieke@soilhealthinstitute.org

Appendix A

List of counties containing carbonate from dolomitic parent material in the upper 30 cm of soil.

State	County	State	County
Alabama	Pickens	Montana	Wheatland
Alabama	Sumter	Nevada	Churchill
Arizona	Coconino	Nevada	Clark
Arizona	Mohave	Nevada	Douglas
Arkansas	Baxter	Nevada	Elko
Arkansas	Boone	Nevada	Esmeralda
Arkansas	Fulton	Nevada	Eureka
Arkansas	Madison	Nevada	Lander
Arkansas	Marion	Nevada	Lincoln
Arkansas	Newton	Nevada	Mineral
Arkansas	Randolph	Nevada	Nye
Arkansas	Searcy	Nevada	Pershing
Arkansas	Stone	Nevada	White Pine
California	Inyo	New Jersey	Sussex
California	Mono	New Mexico	Cibola
California	San Bernardino	New Mexico	Dona Ana
Colorado	Archuleta	New Mexico	Eddy
Colorado	Conejos	New Mexico	McKinley
Colorado	Gunnison	New Mexico	Otero
Colorado	Moffat	New Mexico	Rio Arriba
Colorado	Saguache	New York	Albany
Connecticut	Fairfield	New York	Cayuga
Connecticut	Hartford	New York	Columbia
Connecticut	Litchfield	New York	Dutchess
Connecticut	Middlesex	New York	Fulton
Connecticut	New Haven	New York	Greene
Connecticut	New London	New York	Herkimer
Connecticut	Tolland	New York	Jefferson
Idaho	Bannock	New York	Lewis
Idaho	Bear Lake	New York	Livingston
Idaho	Bingham	New York	Madison
Idaho	Butte	New York	Montgomery
Idaho	Caribou	New York	Oneida
Idaho	Cassia	New York	Onondaga

Idaho	Custer	New York	Ontario
Idaho	Franklin	New York	Orange
Idaho	Oneida	New York	Orleans
Idaho	Power	New York	Otsego
Illinois	Boone	New York	Putnam
Illinois	Carroll	New York	Saratoga
Illinois	Cook	New York	Schenectady
Illinois	DuPage	New York	Schoharie
Illinois	Grundy	New York	Schuyler
Illinois	Hancock	New York	Seneca
Illinois	Henderson	New York	Tioga
Illinois	Jo Daviess	New York	Tompkins
Illinois	Kane	New York	Ulster
Illinois	Kankakee	New York	Wayne
Illinois	Kendall	Ohio	Adams
Illinois	LaSalle	Ohio	Allen
Illinois	Ogle	Ohio	Ashland
Illinois	Stephenson	Ohio	Clark
Illinois	Warren	Ohio	Delaware
Illinois	Will	Ohio	Erie
Illinois	Winnebago	Ohio	Fayette
Iowa	Allamakee	Ohio	Franklin
Iowa	Benton	Ohio	Greene
Iowa	Black Hawk	Ohio	Hancock
Iowa	Bremer	Ohio	Hardin
Iowa	Buchanan	Ohio	Highland
Iowa	Butler	Ohio	Huron
Iowa	Cedar	Ohio	Logan
Iowa	Cerro Gordo	Ohio	Lucas
Iowa	Chickasaw	Ohio	Madison
Iowa	Clayton	Ohio	Mercer
Iowa	Clinton	Ohio	Miami
Iowa	Delaware	Ohio	Montgomery
Iowa	Dubuque	Ohio	Morrow
Iowa	Fayette	Ohio	Ottawa
Iowa	Floyd	Ohio	Pickaway
Iowa	Franklin	Ohio	Preble
Iowa	Hardin	Ohio	Richland
Iowa	Henry	Ohio	Ross
Iowa	Howard	Ohio	Sandusky
Iowa	Humboldt	Ohio	Seneca

Iowa	Jackson
Iowa	Jefferson
Iowa	Jones
Iowa	Linn
Iowa	Mahaska
Iowa	Marion
Iowa	Mitchell
Iowa	Plymouth
Iowa	Tama
Iowa	Van Buren
Iowa	Winneshiek
Iowa	Woodbury
Iowa	Worth
Kansas	Barber
Kansas	Linn
Kansas	Miami
Kentucky	Bath
Kentucky	Clark
Kentucky	Estill
Kentucky	Fleming
Kentucky	Lee
Kentucky	Lewis
Kentucky	Madison
Kentucky	Marion
Kentucky	Mason
Kentucky	Montgomery
Kentucky	Nelson
Kentucky	Powell
Kentucky	Washington
Massachusetts	Berkshire
Michigan	Chippewa
Michigan	Delta
Michigan	Dickinson
Michigan	Mackinac
Michigan	Marquette
Michigan	Menominee
Minnesota	Dakota
Minnesota	Fillmore
Minnesota	Goodhue
Minnesota	Houston
Minnesota	Olmsted
Ohio	Union
Ohio	Warren
Ohio	Wood
Ohio	Wyandot
Oklahoma	Beckham
Oklahoma	Caddo
Oklahoma	Comanche
Oklahoma	Cotton
Oklahoma	Creek
Oklahoma	Greer
Oklahoma	Harmon
Oklahoma	Harper
Oklahoma	Jackson
Oklahoma	Kay
Oklahoma	Kiowa
Oklahoma	Lincoln
Oklahoma	Noble
Oklahoma	Pawnee
Oklahoma	Payne
Oklahoma	Stephens
Oklahoma	Tillman
Oklahoma	Washita
Oklahoma	Woods
South Dakota	Custer
South Dakota	Fall River
South Dakota	Lawrence
South Dakota	Meade
South Dakota	Pennington
Texas	Baylor
Texas	Childress
Texas	Collingsworth
Texas	Cottle
Texas	Culberson
Texas	El Paso
Texas	Fisher
Texas	Foard
Texas	Hardeman
Texas	Haskell
Texas	Hudspeth
Texas	Jones
Texas	King

Minnesota	Wabasha	Texas	Knox
Minnesota	Winona	Texas	Lampasas
Mississippi	Chickasaw	Texas	Llano
Mississippi	Clay	Texas	Oldham
Mississippi	Kemper	Texas	Potter
Mississippi	Lowndes	Texas	Reeves
Mississippi	Monroe	Texas	Runnels
Mississippi	Noxubee	Texas	San Saba
Mississippi	Oktibbeha	Texas	Stonewall
Missouri	Barry	Texas	Taylor
Missouri	Bates	Texas	Wheeler
Missouri	Bollinger	Texas	Wichita
Missouri	Camden	Texas	Wilbarger
Missouri	Cape Girardeau	Utah	Box Elder
Missouri	Carter	Utah	Cache
Missouri	Cedar	Utah	Juab
Missouri	Christian	Utah	Millard
Missouri	Crawford	Utah	Rich
Missouri	Dallas	Utah	Sanpete
Missouri	Dent	Utah	Tooele
Missouri	Douglas	Utah	Uintah
Missouri	Franklin	Utah	Washington
Missouri	Gasconade	Virginia	Rockbridge
Missouri	Greene	Wisconsin	Brown
Missouri	Henry	Wisconsin	Buffalo
Missouri	Hickory	Wisconsin	Calumet
Missouri	Howell	Wisconsin	Columbia
Missouri	Iron	Wisconsin	Crawford
Missouri	Jefferson	Wisconsin	Dane
Missouri	Laclede	Wisconsin	Dodge
Missouri	Lewis	Wisconsin	Door
Missouri	Lincoln	Wisconsin	Dunn
Missouri	Madison	Wisconsin	Fond du Lac
Missouri	Maries	Wisconsin	Grant
Missouri	McDonald	Wisconsin	Green
Missouri	Miller	Wisconsin	Green Lake
Missouri	Montgomery	Wisconsin	Iowa
Missouri	Oregon	Wisconsin	Jefferson
Missouri	Osage	Wisconsin	Juneau
Missouri	Ozark	Wisconsin	Kewaunee

Missouri	Perry	Wisconsin	La Crosse
Missouri	Phelps	Wisconsin	Lafayette
Missouri	Pike	Wisconsin	Manitowoc
Missouri	Polk	Wisconsin	Marinette
Missouri	Pulaski	Wisconsin	Milwaukee
Missouri	Reynolds	Wisconsin	Monroe
Missouri	Ripley	Wisconsin	Oconto
Missouri	Shannon	Wisconsin	Outagamie
Missouri	St. Clair	Wisconsin	Ozaukee
Missouri	St. Francois	Wisconsin	Pepin
Missouri	St. Louis	Wisconsin	Pierce
Missouri	Ste. Genevieve	Wisconsin	Richland
Missouri	Stone	Wisconsin	Rock
Missouri	Taney	Wisconsin	Sauk
Missouri	Vernon	Wisconsin	Shawano
Missouri	Washington	Wisconsin	Sheboygan
Missouri	Wayne	Wisconsin	St. Croix
Missouri	Webster	Wisconsin	Trempealeau
Missouri	Wright	Wisconsin	Vernon
Montana	Beaverhead	Wisconsin	Walworth
Montana	Cascade	Wisconsin	Washington
Montana	Fergus	Wisconsin	Waukesha
Montana	Flathead	Wisconsin	Waupaca
Montana	Gallatin	Wisconsin	Winnebago
Montana	Glacier	Wyoming	Crook
Montana	Golden Valley	Wyoming	Fremont
Montana	Judith Basin	Wyoming	Lincoln
Montana	Lewis and Clark	Wyoming	Natrona
Montana	Madison	Wyoming	Park
Montana	Meagher	Wyoming	Sublette
Montana	Pondera	Wyoming	Teton
Montana	Teton	Wyoming	Weston