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Soil Organic Matter Loss-on-Ignition and Combustion Analysis for Total C and Total N with Pressure Calcimetry for Soil Inorganic Carbon Determination	Page: 1 of 9
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Final Version: Robert Schindelbeck	

Soil Organic Matter Loss-on-Ignition and Total C and Total N with Pressure Calcimetry for Soil Inorganic Carbon Determination

Background / Strategy:

Soil Organic Matter is considered to be derived from living organisms (earthworms, soil organisms and plant roots), their exudates and nonliving biological residues. A soil sample is burned at 500C for two hours and the percent weight loss on ignition (LOI) is determined. This LOI is converted to a percent weight of soil organic matter (%OM) using an equation of the form:

$$\%OM = (\%LOI * 0.7) - 0.23$$

Total Carbon (Tot C) includes soil organic carbon and inorganic forms of carbon (carbonates). Generally, for soils with a pH below 7.2 that are free of carbonates the Tot C is considered to be organic carbon. Tot C in a sample is determined using dry combustion at 1100C to oxidize all of the carbon to CO₂ gas which is measured using Non Dispersive Infrared Detection (NDIR).

Total Nitrogen (Tot N) includes the organic (living and non-living) and inorganic (mineral) forms of soil nitrogen (ammonium and nitrate). A dry combustion furnace method following Dumas is used where all of the nitrogen is converted to N_xO_y gases using oxygen. The effluent gas is then moved to a reduction furnace where all nitrogen is converted to N₂. This gas is measured by Thermal Conductivity Detection (TCD).

The Total Carbon and Total Nitrogen analysis is performed using a Skalar Primacs SNC-100 Carbon and Nitrogen Analyzer.

Soil Inorganic Carbon (SIC) analysis is performed following a pressure calcimetry methodology (Fonnesbeck et al., 2013). Soil Organic Carbon (SOC) is then determined following Tot C – SIC = SOC.

Scope/Objective:

Air dried soil is sieved past 2mm. First, soil oven dried at 105C is placed into a furnace at 500C for 2 hours. Weight loss is called Loss on Ignition (LOI) and is converted to Percent Organic Matter. Another oven dried subsample is placed into a dry combustion furnace and the gaseous products are analyzed for Total Carbon and Total Nitrogen.

NOTE: For Quarantined soils, see labeled procedures in italics at the bottom of each section.

Materials and Equipment:

- Laboratory oven
- Ceramic LOI crucibles 10ml capacity
- Composite trays to accommodate crucibles
- Lab balance
- High temperature gloves

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Materials and Equipment (continued):

- Ceramic Total C crucibles 5ml capacity
- Holding tray for crucibles
- 125ml serum bottles
- Pressure transducer apparatus with needle
- Reagent grade CaCO₃
- 6 M HCl reagent acid containing 3% by weight FeCl₂ · 4 H₂O

Laboratory Procedure:

The steps outlined below start with soil samples that have been air dried and sieved past 2mm.

Table 1. Data sheet columns for soil LOI data, Tot C Tot N

sample set ID:							
TRAY ID (10ml LOI crucibles):				Date:			
RACK ID (5ml TotC crucibles):							
column A	column B	column C	column D	column E	column F	column G	column H
RACK	105Cwt	target	TRAY	Loss on Ignition (LOI)			
5ml crucible	of soil	sample	location	10ml	10ml cruc wt	10ml cruc wt	10ml cruc wt
location	TotC	ID	ID	cruc wt	+ 105C soil	+105C soil post smidge	after 500C
a1		QC	1				
a2		uu212	2				

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Laboratory Procedure (continued):

1. A set contains 50 samples. All sets are named by their Cornell Soil Health Lab ID (ex. uu212) sample contents.
2. Prepare and save data file (Table 1) with internal IDs of samples (column C), name of TRAY ID for 10ml LOI crucibles (column D) and name of RACK ID for 5ml TotC crucibles (column A).
3. Record internal sample ID in column C (Cornell SH ID).
4. Pre-weigh a tray of 10ml LOI crucibles and record tare weight in column E. Record **tray ID** at top of data sheet.
5. Grind soil samples for analysis. All grinding operations should be performed in the fume hood. Gloves, hearing protection, and eye protection are located in the PPE drawer of the lab bench.
6. Fill four LOI crucibles at TRAY positions 1, 20, 26, and 45 with about 4g of ground QC soil. There will be 4 QC samples and 46 target samples per TRAY.
7. For each of the 46 target samples, place one rounded tablespoon (about 15g) of air dried, sieved past 2mm material into a clean grinder. Pulse grind for 25seconds. Accept material that passes a 0.25mm sieve.
8. Collect one teaspoon (about 4g) of ground material and place into targeted, tared 10ml LOI crucible. Repeat for the 46 target samples. Use vacuum cleaner to clean soil grinder between each sample.
9. Dry in oven at 105C to constant weight. Remove TRAY of LOI crucibles to drying desiccator to cool. After 15 minutes, quickly record weight of LOI crucibles 1-25 (column F).
10. Return TRAY to 105C oven for 30 minutes.
11. Repeat step 8 for crucibles 26-50.
12. Leisurely place a smidgen of soil (about 1/16 teaspoon, 0.15-0.2g) from each LOI crucible into the corresponding 5ml TotC crucible in the correct RACK position.
13. Repeat steps 9-11 above for the target TRAY, recording 5ml TotC crucible weights in column G.

Grind, weigh and handle quarantined samples within a plastic tray to be disinfected upon completion of the batch. Sterilize all quarantine laboratory equipment that comes into contact with quarantined soil with an approved disinfectant. Disinfection methods for Quarantined samples are included at the end of this document.

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Laboratory Procedure (continued):

Loss on Ignition Procedure (follows step 13 in Laboratory Procedure above)

1. Lab coat, eye protection, and heat-resistant gloves are mandatory when opening the muffle furnace. Lab coats can be found hanging near the sample storage shelves, and eye protection and gloves are in the PPE drawer.
2. Place TRAY into preheated 500C muffle furnace for two hours.
3. Cool TRAY in 105C oven for one hour.
4. Remove TRAY from oven, quickly weigh samples 1-25, recording data in column H.
5. Return TRAY to 105C oven for 30 minutes.
6. Repeat step 4 above for crucibles 26-50.
7. Dispose of soil and clean crucibles in preparation for the next set.

Sterilize all quarantine laboratory equipment that comes into contact with quarantined soil with an approved disinfectant. Disinfection methods for Quarantined samples are included at the end of this document.

Total Carbon Combustion Procedure (follows step 13 in Laboratory Procedure above)

1. Calculate weight of soil sample in each 5ml crucible in the RACK by subtracting column G from column F and placing the result in column B.
2. Transfer RACK of 50 samples with a printed and digital EXCEL version of just columns A and Column B to Skalar Primacs SNC-100 Carbon and Nitrogen Analyzer for Tot C and Tot N determination in Bradfield room 813. Name file using target sample IDs in the name.
3. Create Primacs file with dedicated software, using the digital EXCEL file to identify the sample in each row and the correct 105C dry weight.
4. Fill Primacs carousel with corresponding internal QC samples in the required positions. Transfer crucibles from the RACK to the appropriate carousel positions.

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Pressure Calcimetry Procedure (follows step 13 in Laboratory Procedure above)

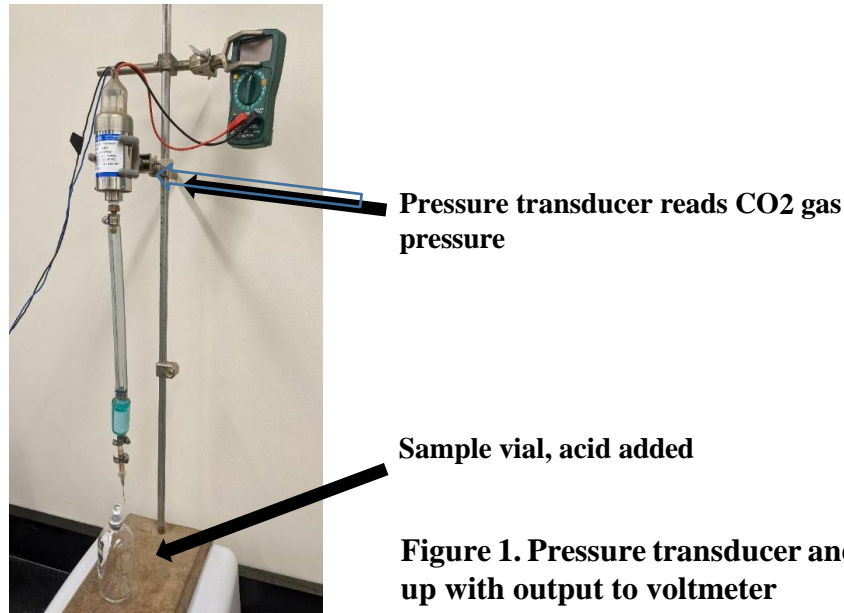


Figure 1. Pressure transducer and needle set up with output to voltmeter

Calibration carbon to voltage output

1. Use laboratory grade CaCO₃ and quartz sand to create a calibration curve for the test
2. bottle one: 2 grams sand
bottle two: 1.95g sand, 0.05g CaCO₃
bottle three: 1.9g sand, 0.1g CaCO₃
bottle four: 1.8g sand, 0.2g CaCO₃
bottle five: 1.6g sand, 0.4g CaCO₃
Scale carbonate levels in the calibration curve for the expected inorganic carbon content of your sample soils.
3. Weigh two grams of air dried and ground soil into each 125ml serum bottle. In Excel, convert these air-dried weights to oven dried weights using previously measured moisture % values for each sample.
4. Add 5ml water to each bottle using a bottle top dispenser.
5. Cap each bottle with a 20mm two-prong butyl stopper, then top each bottle with an aluminum tear-off cap. Crimp each bottle, then pull back the tab to expose the butyl stopper underneath.
6. In a fume hood, pour HCl solution into a beaker and return the bottle of HCl to the chemical cabinet. Using a 1 inch, 18 gauge hypodermic needle, carefully add 4ml of acid to each bottle. Swirl each bottle, then leave the sample tray in the fume hood for at least 2 hours to react.

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7. Use a pressure transducer with a multimeter to take readings on each bottle. Wait 9 seconds after inserting the hypodermic needle before recording the multimeter value.
8. In Excel, calculate inorganic carbon values by using the calibration curve.

Table 2. Excel sheet for carbon calibration with voltage

Sample ID	Rack #	Sand wt (g)	CaCO3 wt (g)	Reading (V)	baseline (V)	Reading Corr (V)	inorganic g CaCO3	SIC g	Sample ID	regression from this run		
$\text{inorganic} = \text{IF}(\text{k5} * (\text{12}/\text{100}) > 0, (\text{k5} * (\text{12}/\text{100}) - \text{h5} - \text{i5}) / (\text{r} - \text{s4}), 0)$												
$\text{SIC} = \text{IF}(\text{L4} * (\text{12}/\text{100}) > 0, (\text{L4} * (\text{12}/\text{100}) - \text{M9}/\text{E9} * 100 - \text{N9}/\text{Q9} * 100) / (\text{Q9} - \text{N9}), 0)$												
Regression formula												
CaCO3 0g	1	2.011	0	0.164	0.046	0.118	0.001	0.000	CaCO3 0g	6.68	0.11	1.00
CaCO3 0.05g	2	1.951	0.051	0.498	0.046	0.452	0.051	0.006	CaCO3 0.05g			
CaCO3 0.1g	3	1.904	0.116	0.924	0.046	0.878	0.115	0.014	CaCO3 0.1g			
CaCO3 0.25g	4	1.757	0.250	1.835	0.046	1.789	0.251	0.030	CaCO3 0.25g			
CaCO3 0.5g	5	1.509	0.505	3.530	0.046	3.484	0.505	0.061	CaCO3 0.5g			

Table 3. Excel sheet for calculation of SIC and SOC

Sample ID	Rack #	wet wt (g)	thimble correction	dry wt (g)	Reading (V)	baseline (V)	Reading Corr (V)	inorganic g CaCO3	SIC g	SIC (%)	Percent SIC of totC	SOC (%)	TotC (%)
$\text{SOC} = \text{C10} * (\text{1} - \text{D10})$													
$\text{SIC} = \text{IF}(\text{L4} * (\text{12}/\text{100}) > 0, (\text{L4} * (\text{12}/\text{100}) - \text{M9}/\text{E9} * 100 - \text{N9}/\text{Q9} * 100) / (\text{Q9} - \text{N9}), 0)$													
sample x	6	2.021	0.005	2.010	0.171	0.046	0.125	0.002	0.000	0.012	0.746	1.606	1.618

Sterilize all quarantine laboratory equipment that comes into contact with quarantined soil with an approved disinfectant. Disinfection methods for Quarantined samples are included at the end of this document.

Quarantine soil handling procedures:

- ***All excess soil must be autoclaved.***
- ***All water that comes into contact with soil must be bleached with a 10% bleach solution for 1/2 hour before disposing of bleach water down drain.***
- ***Any area or material that has come into contact with quarantined soil must be disinfected using sterilization solutions approved for use with Quarantined soil:***
 1. ***Bleach- 10% bleach solution within a labeled spray bottle must be left on contaminated equipment for 1/2 hour before rinsing. Note, this is the only approved method for handling water contaminated with quarantined materials.***
 2. ***Ethanol solution (70%) (stored in a labeled spray bottle) must be left on contaminated equipment for 30 minutes before rinsing***

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Data Collection and Calculations:

Table 4. Worksheet columns for soil LOI data, Tot C Tot N

I. Data output in column I – column J

sample set ID:									
TRAY ID (10ml LOI crucibles):					Date:				
RACK ID (5ml TotC crucibles):					Calculations				
column A	column B	column C	column D	column E	column F	column G	column H	column I	Column J
RACK	105C wt	target	TRAY	Loss on Ignition (LOI)				LOI%	OM%
5ml crucible	of soil	sample	location	10ml	10ml cruc wt	10ml cruc wt	10ml cruc wt		
location	TotC	ID	ID	cruc wt	+ 105C soil	+105C soil post smidge	after 500C		
a1		QC	1					$=((H9-E9)-(I9-E9))/(H9-E9))*100$	$=(J9*0.7) - 0.23$

1. The soil LOI% is calculated in column I, with the units of g of lost mass (assumed to be carbonaceous) per g of oven dry soil times 100 for percent of sample.
2. The Organic Matter percent is given in column J.

Quality Control/ Standards (LOI test):

The Quality Control (QC) standard soil (Lima silt loam, 0-6" depth, air dried to constant weight) is prepared according to the methodology in the **Laboratory Procedure** above. The soil is run in a set of 50 replicated samples to determine the reproducibility of the test.

Table 3 below lists the descriptive statistics from the 9-2020 standard soil Quality Control (QC) experiment. The run has 50 replicate measurements, and a mean is calculated. The grand mean of this QC set plus and minus two standard deviations is used as a criteria for determining the range of acceptability for any data set. If this standard result falls outside the expected range, the entire data set is rejected and is re-run.

Quality Control/ Standards (Total C Total N test):

The Quality Control (QC) standard soil (Lima silt loam, 0-6" depth, air dried to constant weight) is prepared according to the methodology in the **Laboratory Procedure** above. The soil is run in a set of 25 replicated samples to determine the reproducibility of the test.

Table 4 below lists the descriptive statistics from the 9-2020 standard soil Quality Control (QC) experiment. The run has 25 replicate measurements, and a mean is calculated. The grand mean of this QC set plus and minus two standard deviations is used as a criteria for determining the range of acceptability for any data set. If this standard result falls outside the expected range, the entire data set is rejected and is re-run.

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Table 3. Lima silt loam soil standard descriptive statistics for the Soil Organic Matter Loss on Ignition (LOI) test

<u>ID</u>	<u>LOI %</u>	<u>ID</u>	<u>LOI %</u>		
1	5.43	26	5.19		
2	5.45	27	5.27		
3	5.4	28	5.14		
4	5.46	29	5.18	Mean	5.21
5	5.35	30	5.12	Standard Error	0.02
6	5.27	31	5.01	Median	5.20
7	5.29	32	5.09	Standard Deviation	0.12
8	5.27	33	5.09	Range	0.52
9	5.03	34	5.15	Minimum	4.94
10	5.05	35	5.2	Maximum	5.46
11	5.33	36	5.23	Count	50.00
12	5.35	37	5.15	mean	5.21
13	5.26	38	5.19	mean MINUS 2 SD	4.96
14	5.4	39	5.14	mean PLUS 2 SD	5.46
15	5.39	40	5.21		
16	5.29	41	4.97		
17	5.33	42	5.16		
18	5.38	43	5.22		
19	5.17	44	5.18		
20	5.05	45	5.29		
21	4.94	46	5.3		
22	5.09	47	5.22		
23	5.18	48	5.15		
24	5.19	49	5.2		
25	5.12	50	5.13		

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Table 4. Lima silt loam soil standard descriptive statistics for the Total C Total N test

<u>ID</u>	<u>Tot C %</u>	<u>Tot N %</u>	<u>Total C %</u>	
1	2.05	0.22	Mean	1.99
2	1.85	0.19	Standard Error	0.03
3	1.70	0.17	Median	1.99
4	1.91	0.19	Standard Deviation	0.13
5	2.04	0.23	Range	0.62
6	2.32	0.18	Minimum	1.70
7	1.96	0.20	Maximum	2.32
8	1.93	0.20	Count	25
9	1.84	0.20	mean	1.99
10	2.03	0.21	mean MINUS 2 SD	1.74
11	2.04	0.22	mean PLUS 2 SD	2.24
12	2.00	0.20		
13	2.22	0.21		
14	2.01	0.21	<u>Total N %</u>	
15	1.94	0.20	Mean	0.201
16	1.99	0.21	Standard Error	0.003
17	2.16	0.21	Median	0.200
18	2.06	0.20	Standard Deviation	0.013
19	1.94	0.19	Range	0.058
20	1.89	0.18	Minimum	0.169
21	1.89	0.20	Maximum	0.227
22	2.05	0.21	Count	25
23	1.94	0.20	mean	0.201
24	2.01	0.20	mean MINUS 2 SD	0.175
25	1.98	0.21	mean PLUS 2 SD	0.227

References:

USDA NRCS Soil Survey Field and Lab Methods Manual. No.51, version 2, Rebecca Burt, ed. 2014. 5.1 Mineral Content. pp.314-316.

Primacs100 Analyzer Series, User Manual. 2018. Skalar International, The Netherlands. <http://www.skalar.com>