**SOIL 4234 Laboratory #7**

**Carbon, Nitrogen, and Organic Matter (20 points)**

 Student

 Lab

 TA

**Objectives**

1. Understand the relationship between total soil carbon and soil organic matter.
2. Identify the association between soil organic matter, soil carbon, and soil nitrogen and how that impacts seasonal nitrogen availability.
3. Recognize the advantages and disadvantages for determining soil organic matter/soil carbon using the loss on ignition and automated high temperature dry combustion methods, the Walkey-Black wet combustion method, and visible near infrared spectroscopy method.

**Introduction**

Organic matter is an important constituent of soils. It contributes to the nutrient retention capability of the soil through CEC and the ability to complex or chelate metals. It also has an important effect on soil physical properties. Organic matter serves as a binding agent to help aggregate soils. Thus organic matter influences soil structure and porosity. Structure and porosity in turn influence infiltration and percolation of water through the soil and the retention or storage of water in soils.

In addition to affecting soil chemical and physical properties, soil organic matter serves as a source of nutrients to plants and microorganisms. Soil organic matter interacts with pesticides and other organic compounds and affects the rate of herbicides or insecticides which must be used. Soil organic matter is also an important part of the global carbon cycle. In recent years there has been much interest in understanding the role soil organic matter plays in sequestering carbon from the atmosphere. Some scientists believe increasing soil organic matter may help decrease atmospheric CO2 levels and lessen the effects of this greenhouse gas on global warming/climate change.

There are a number of procedures that are used to measure soil organic matter. Generally they can be categorized as either wet or dry combustion techniques. Both wet and dry combustion involve the oxidation of organic carbon to quantify, directly wet or indirectly dry, the CO2  produced. These procedures are described in detail below.

 Most of the nitrogen (N) in Oklahoma soils is present as organic nitrogen in the soil organic matter. There are about 1,000 lbs/acre of N in this form for every 1% organic matter in the soil. However, since the soil organic matter is resistant to further decay, most of this N is unavailable during any given growing season. Normally each year about 2% of the N from soil organic matter will be released to mineral forms when soils are cultivated. This 20 to 40 lb/acre of mineral N is typical of the small amount present in unfertilized soils after cultivation and seed bed preparation.

Nitrogen Mineralization And Immobilization

 Because N release from organic matter is dependent upon decay by microorganisms, which themselves require mineral N, the amount of mineral N available for a crop is in constant flux. Unlike crops, which get their carbon (C) as carbon dioxide from the air, many microorganisms get their carbon by decaying organic matter. Nitrogen availability depends upon the relative amount of C and N in the organic matter, its resistance to decay, and environmental conditions to support microbialactivity. Figure 1 illustrates how nitrogen becomes more concentrated as soil organic matter decays.

Figure 1. Narrowing of carbon to nitrogen ratio as residue is decayed until mineral nitrogen finally becomes available.

 Note that N is not released during the first stages of decay. This is because N that is released is immediately consumed by active microorganisms. With time, remaining organic material becomes more resistant to decay, microorganisms die off, and there is more mineral N present than can be consumed by the few active microorganisms. This results in a final release of measurable mineral N in the form of ammonia (NH3). The ammonia readily reacts with soil moisture to form ammonium (NH4+). These two reactions can be stated simply as

 organic N → NH3 (gas) NH3 + H2O → NH4+ + OH- ammonia + water ammonium + hydroxide

 The process of converting or transforming N from organic compounds to inorganic compounds is called **mineralization**. This results in increasing N available for crops. When the reverse happens and available mineral N is absorbed by crops or microorganisms the process is called **immobilization** and results in a decrease in the amount of N immediately available for crops. These processes and their interacting nature with soil N for a typical field situation are illustrated in Figure 2.

 Approximately 98% of the soil nitrogen is unavailable for plant uptake. This large reservoir of organic N provides an important buffer against rapid changes in available N and plant stress. The small reservoir of mineral N can often be slowly replenished by mineralization (Fig. 2) when crops need additional N.



Figure 2. Interacting pools of soil nitrogen.

 Usually supplemental N as fertilizer must be added to support high, economic production levels. Immediately following fertilization with 120 lb N, the system may be illustrated by Figure 3a. Addition of fertilizer N will stimulate microorganism activity resulting in consumption of mineral N and breakdown of some crop residues (immobilization) as illustrated in (b). The immobilized N will be present as microbial tissue and other new material in the organic pool. As indicated by the two arrows pointing in opposite pathways, mineralization and immobilization are both taking place simultaneously. Immobilized fertilizer N will again become available in a few weeks if conditions favor crop uptake.



Figure 3. Relative amounts of organic and mineral nitrogen in soil immediately after fertilizing (a) and several days after active immobilization (b).

Techniques for Measuring Soil Organic Matter/Organic Carbon:

* + Dry Combustion
		- Loss on ignition using a kiln or muffle furnace
		- High temperature dry combustion using an automated analyzer
	+ Wet Combustion
		- Walkey-Black method
	+ Visible Near Infrared Spectroscopy

#### Loss on Ignition Method Using a Kiln or Muffle Furnace

When soil is heated to high temperatures the organic portion oxidizes to CO2 and H2O but the majority of soil minerals are left intact. Thus, most of the organic matter goes off as gas and the mass lost can be used as an estimate of organic matter content. The loss on ignition (LOI) method is advantageous because it is inexpensive, easy to perform with a minimal amount of equipment, and uses no chemicals. However, problems with this method arise when weight loss is due to something other than SOM. Minerals common in soils such as gypsum, montmorillonite, vermiculite, and gibbsite can be subject to moisture losses of 20-30% of their total weight at the temperatures used for organic matter oxidation. Another source of error common in calcareous soils is the loss of free carbonates. Soils high in calcite or dolomite will often give false-high SOM values with the LOI method because no distinction can be made between carbonate lost and OC oxidized. Therefore, when using the LOI method on calcareous soils, they must first be treated to remove free carbonates. The LOI method tends to work better in organic soils (mucks and peats) and soils low in clay. Several different procedures using different temperatures and heating times have been used for the LOI method, however, research has shown a heating time of 8 to 16 hours at 400°C results in maximum removal of organic matter while minimizing weight loss due to other minerals.

Calculating total soil organic matter from the loss on ignition method using a kiln or muffle furnace.



High Temperature Dry Combustion Using a LECO Automated Analyzer

Total C in soils is the sum of both organic and inorganic C. Soil organic matter is where most of the organic C is present in soil, whereas inorganic C is found in carbonate minerals. Organic C consists of the cells of microorganisms, decomposing plant and animal residues, stable humus, and highly carbonized compounds. Determining total C via high temperature dry combustion, oxidation of organic C and thermal decomposition of carbonate minerals (inorganic C) is achieved and the amount of CO2 produced is determined using gas chromatography. The total percent of C is determined by the following equation:

Total C (%) = [(g CO2 (sample) – g CO2 (blank)) / g of dry soil] x 0.2727 x 100

 Research in Pennsylvania determined that the organic matter content of surface soils may be accurately estimated by the following equation:

Organic matter (%) = 0.35 + [1.80 x Organic carbon (%)]

Note that high temperature dry combustion determines total C and the equation to calculate OM uses the percent of organic C. If inorganic C exists in the soil, a pretreatment of the soil must occur prior to determining total C using high temperature dry combustion. The pretreatment uses concentrated acid to oxidize the inorganic C into CO2 prior to the high temperature dry combustion procedure.

#### Wet Combustion

Historically, the wet combustion technique known as the Walkley-Black method, has been considered the standard method for estimating organic matter in soils. This procedure measures the easily oxidizable organic carbon (OC) by oxidation with dichromate. The reaction occurs as follows:

K2Cr2O7 is mixed with your soil and concentrated H2SO4 is then added.

a) K2Cr2O7 + H2SO4 → H2Cr2O7 + K2SO4 + excess H2SO4

 soil

 solution from above + carbon →

  **excess**

b) 3H2Cr2O7 + K2SO4 + 6H2SO4 + 3C → 2Cr2(SO4)3 + K2SO4 + 3CO2 + 8H2O + H2Cr2O7

 *orange solution green solution*

**or**….

 4Cr+6 + 12e- → 4Cr+3 and 3C0 – 12e- → 3C+4

The H2SO4 reacts with the potassium dichromate (K2Cr2O7) to form dichromic acid (reaction a). This is a powerful oxidizing agent which oxidizes the organic carbon, forming CO2 and chromous sulfate (reaction b). The change in oxidation state (reduction) of chromium is accompanied by a change in color of the solution from orange to green.

The quantity of H2Cr2O7 that was **not** reduced in the reaction (excess) is determined by titration with ferrous ammonium sulfate, Fe(NH4)2(SO4)2•6H2O (reaction c), using orthophenanthroline as an oxidation-reduction indicator. The ferrous iron (Fe2+) is oxidized to Fe3+, and the chromium is reduced from Cr6+ to Cr3+.

 **excess**

c) **6Fe2+ + Cr2O72- + 14H+ → 2Cr3+ + 6Fe3+ + 7H2O**

 ***green solution* *maroon solution***

Ferrous sulfate is fairly unstable and reacts with atmospheric O2 to form ferric sulfate. Therefore a blank solution is titrated to determine the normality of the ferrous sulfate, and this value is used in the calculations (the blank appears as B in the calculation section).

 The Walkley-Black method has been used extensively and has been experimentally determined to oxidize approximately 77% of the total organic carbon in mineral soils. Therefore, assuming that organic carbon has an average oxidation state of zero, dichromic acid extracts 77% of the soil OC, and SOM is 50% C, we can easily calculate SOM from measurements of dichromic acid-oxidizable OC. This value of 77% is an average correction value and the actual value will vary across different soils.

The Walkley-Black method has been criticized because of the dangers involved with generating high temperatures and the use of chemicals that are hazardous or pose disposal problems. There are also interferences by chloride, ferrous iron, and higher oxides of Mn have been known to cause incorrect predictions of OC. However, the value of this method becomes clear when estimating SOM in soils that contain <3% OM. These values are critical when decisions are made regarding the amount of pesticides to apply or when including SOM in N-fertilizer recommendations.

####  Calculating total soil organic matter from the Walkey-Black Method.

1. meq of chromic ion added = (N of K2Cr2O7) x (ml of K2Cr2O7)
2. meq of chromic ion not reduced by OC = (meq chromic ion added) x (S/B)

S = ml of Fe(NH4)2(SO4)2 used to titrate samples

 B = ml of Fe(NH4)2(SO4)2 used to titrate blank

1. meq of easily oxidizable OC = meq of chromic ion added – meq of chromic ion not reduced

 by OC

4. Wt of easily oxidizable OC = meq easily oxidizable OC x meq wt C

 meq wt C = (12 mg/mmol) / (4 meq/mmol)

***Note: divide by 4 since C0 – 4e- → C+4***

5. Wt. of Total OC = (wt of easily oxidizable OC) x (1 / 0.77)

***Note: 0.77 = recovery rate of OC by the Walkley Black method as determined by experimentation***

6. % total OC = (Wt. Total OC / sample wt (mg)) x 100

7. % total OM = % total OC x 2

***Note: OM is 50% OC***

Visible Near Infrared Spectroscopy

This method for determining soil organic matter and soil carbon has gained a lot of popularity in recent years. The method relies on visible and near-infrared reflectance values of whole soil profile scans. These reflectance values are then calibrated with known laboratory derived soil organic matter or soil C values. This method is highly advantageous because it is rapid and non-destructive. With some Vis-NIR equipment being field portable measurements can be collected in the field. Major disadvantages of this technique are that it is not as accurate as conventional laboratory methods and calibration models have to be created for each general area and soil type.



**Photo courtesy of CSA News**

**Questions** (20 pts total)

1. (6 pts.) List at least two advantages and two disadvantages for each method of determining soil organic matter/carbon.

Loss on ignition dry combustion

Automated high temperature dry combustion

Walkey-Black wet combustion

Visible-near infrared spectroscopy

1. (3 pts.) Assuming the C:N ratio of soil is 8:1 and soil has an organic C percent of 3%, what is the %N in this soil? (show calculations) Based upon the soil’s C:N ratio, would you expect nitrogen to be mineralized or immobilized within the first 8 weeks of spring warm-up?
2. (3 pts.) Based on the %N you calculated in question #2, what is the nitrogen content (lbs.) of an acre of your soil with a soil organic matter content of 4%? Assume an acre-furrow-slice weighs 2,000,000 lbs.
3. (4 pts.) Using the loss on ignition dry combustion method, calculate the percent soil organic matter of a soil sample which had an oven-dry weight of 10.50 g and a “burned” soil weight of 9.65 g. Estimate the percentage of total carbon assuming soil organic matter is 40% total carbon.
4. (4 pts.) You are working in a soil testing facility and a customer has contacted you about determining the soil organic matter content of his garden. First, using complete sentences with correct grammar and spelling, explain to the customer a strategy and the steps involved to accurately collect a soil sample which you can analyze. After receiving the oven dried and ground soil sample at the lab, you have weighed a 0.5001 g dry soil sample to run on the LECO automated analyzer. The CO2 in the sample was reported as 22.571 mg and the CO2 in the blank was 0.014 mg. Assuming the inorganic C content of this soil is 0.43%, determine the soil organic matter content (%) of this customer’s garden.