How to Teach Soil Health Field Day:

Active Carbon Demo

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Introduction

Organic matter in the soil, which is mostly composed of organic carbon (C), can be divided based on chemical or physical susceptibility for microbial degradation:

- the ***recalcitrant C pool*** (complex structures or physically protected; slowly utilized by microorganism)
- and, the much smaller ***labile C pool*** (simple compounds; quickly and easily utilized and mineralized)

What is the demo? Measuring Active C in soil

- This method determines the amount of Oxidizable Carbon in the soil, which is considered an estimate of the Labile C pool
- the Labile C may also be referred to as Reactive Carbon, but most commonly referred to as Active Carbon

What does this demo tell us about the soil?

- Labile/Active C pool is source of energy and carbon for soil microbes;
- Results are also an indicator of microbial biomass, activity, and respiration
- Indicates the potential for nutrient cycling that produces nutrients readily available for plants

Why is Active C important for soil health?

- Active C pool is sensitive to management; therefore, changes in Active C pool, as a result of management, are more easily captured than the total carbon (TOC)
- Potential tool for predicting crop productivity responses to soil organic C management

What time of the year should this <u>demo</u> be done?

- For the purpose of teaching about Active C, this can be demonstrated anytime
- Demo at beginning (May) and end (August) of growing season can inform landowner about the amount of carbon likely to be mineralized or decomposed during the growing season, or the quantity remaining at the end of the season.; soil testing for results comparison should be the same time of each year

Overview of $KMnO_4$ method

- $KMnO_4$ is a strong oxidizer; when reacted with OM, C is oxidized, which reduces Mn (Mn^{+7} to Mn^{+4})
- The reduction of Mn changes the color; the bleaching of the purple $KMnO_4$ solution is proportional to the amount of oxidizable C in soil = the more Active C in soil, the lighter the color of the final solution (lower absorbance reading)
- **Assumption:** 1 mol MnO_4 is consumed (reduced) per oxidation of 0.75 mol (9000 mg) of C

Materials

- Potassium permanganate ($KMnO_4$)
- Calcium chloride ($CaCl_2$)
- Potassium hydroxide (KOH)
- Deionized water (DI)
- Dark bottle (1 L) for mixing solution; qty.2- 500 ml dark/amber bottles for traveling
- small dark colored container with lid for dispensing $KMnO_4$ solution at event
- Squirt bottle filled with DI water
- 50ml graduated polypropylene conical centrifuge tubes with caps
  *estimate 2 tubes per soil sample
- Tube racks
- Qty. 4 -50ml graduated tubes for standard solutions + Blank
- Color chart (lbs/Acre)
- Hand-held colorimeter (550 nm) + glass tube/cuvettes
- Aluminum foil OR 50 ml dark colored/amber bottles with lids (qty. 4)
- Laboratory tissues
- Disposable bulb pipettes (calibrated to 2.0 ml and 0.5 ml)
- Scale (grams; sensitivity to ±0.01); battery operated in case there in no access to electricity
- pH meter
- Plastic cup or beaker (for holding 50ml tubes on scale)
- Waste containers with lids: large for soil-solution, small for table top
- Dark colored tray, paper, or paper plates for air/sun drying moist soil samples
- Box to cover samples while reacting
- Timer (minutes)
- stickers or scrap paper and marker/pen[cil] for labeling samples that are drying
- Labtop with Excel spreadsheet set up for calculating:
  mg Active C / kg soil and/or **lbs Active C/Acre**
  *make sure fully charged in case no access to electricity
- Laboratory gloves
- Small graduated cylinder or bottle-top dispenser
  * to measure out DI water, if not using a scale to add water
- Containers for taking extra DI water to event

optional:

- Hand-out with Active C info for your state (e.g. regional averages or ranges for different management types and ecosystems
  **see example prepared by MU – Soil Health Assessment Center*
- How will you present results to landowner? on a slip of paper? written on hand-out?
- Sample information form to gather landowner's info: contact info and management info (some landowners may drop off samples for analysis, and want you to email their results)

Preparation before event

1. Make stock solution and standard solutions; label/date bottle, and keep cool

Stock solution: 0.2 *M* Potassium permanganate ($KMnO_4$) + 0.1 *M* Calcium chloride ($CaCl_2$)

Make by mixing 31.61 g $KMnO_4$ and 11.7 g $CaCl_2$ into 1L of Deionized (DI) water; adjust pH of solution to pH 7.2 if needed with 0.1 *M* KOH (0.561 g KOH/ 1L DI water; adding approx. 1-2 drops)

*adjusting solution pH is important for maintaining stability of the stock solution for 3-6 months

*solution should always be stored in dark bottle to protect from light

Standard solutions for calibration curve: 0.005, 0.01, 0.02, and 0.04 *M* $KMnO_4$:

1. Make standard stock solutions by adding 1.25, 2.50, 5.00, and 10 ml of the 0.2 *M* $KMnO_4$ stock solution to respective centrifuge tubes and diluting to 50 ml mark with distilled (DI) water.

Example: (1.25mL / 50mL) x 0.2 M = 0.005 *M* $KMnO_4$
(2.50 mL / 50mL) x 0.2 M = 0.01 *M* $KMnO_4$

*Wrap each tube with foil to protect from sunlight or store in 50 ml amber bottles

2. Make diluted standard solutions for reading on colorimeter by filling 50 ml tube with 49.5 ml of DI water, and 0.5 ml of the standard stock solution. Do for each concentration: 0.005, 0.01, 0.02, and 0.04 *M* $KMnO_4$. The absorbance values from these diluted solutions will be used to create standard curve.

* *See* **Set up Excel spreadsheet** to create standard curve and results equation
* Can save diluted standard tubes and label as: Excellent, Good, Fair, and Poor for display

2. Pre-fill several 50ml tubes with 49.5 ml DI water (can also do this at event as needed)

With scale, stabilize tube in a cup and tare scale (to zero). Add 49.5 ml DI water (= 49.5 g DI water); **OR** without scale, measure 50 ml DI water with graduated cylinder and remove 0.5 ml with pipette

3. Set up Excel spreadsheet

Active carbon (mg kg^{-1}) is determined for each sample using the following equation:

Active C (mg kg^{-1}) = [0.02 mol L^{-1} – (*a* + *bz*)] x (9000 mg C mol^{-1}) x (0.02 L solution / 0.0025 kg soil)

where 0.02 mol L^{-1} is the initial concentration of the $KMnO_4$ reactant, *a* and *b* are the intercept and slope of the standard curve, *z* is the sample absorbance.

1. First, mix up 0.01 *M* $CaCl_2$ (5 ml 0.1 *M* $CaCl_2$/ 45ml DI water) to use as Blank (ZERO)

2. Read absorbance of each standard and graph in excel (x-axis is absorbance; y-axis is the concentration) to obtain the equation of the line (slope and intercept).
Remember the equation of a line is: **y = intercept + (slope*x)**

Standards	**Absorbance (550 nm)**	**Concentration $KMnO_4$**
0.01 *M* $CaCl_2$ Blank (ZERO)	0	0
Standard 1 (0.005)		0.005
Standard 2 (0.01)		0.010

Standard 3 (0.02)		0.020
Standard 4 (0.04)		0.040

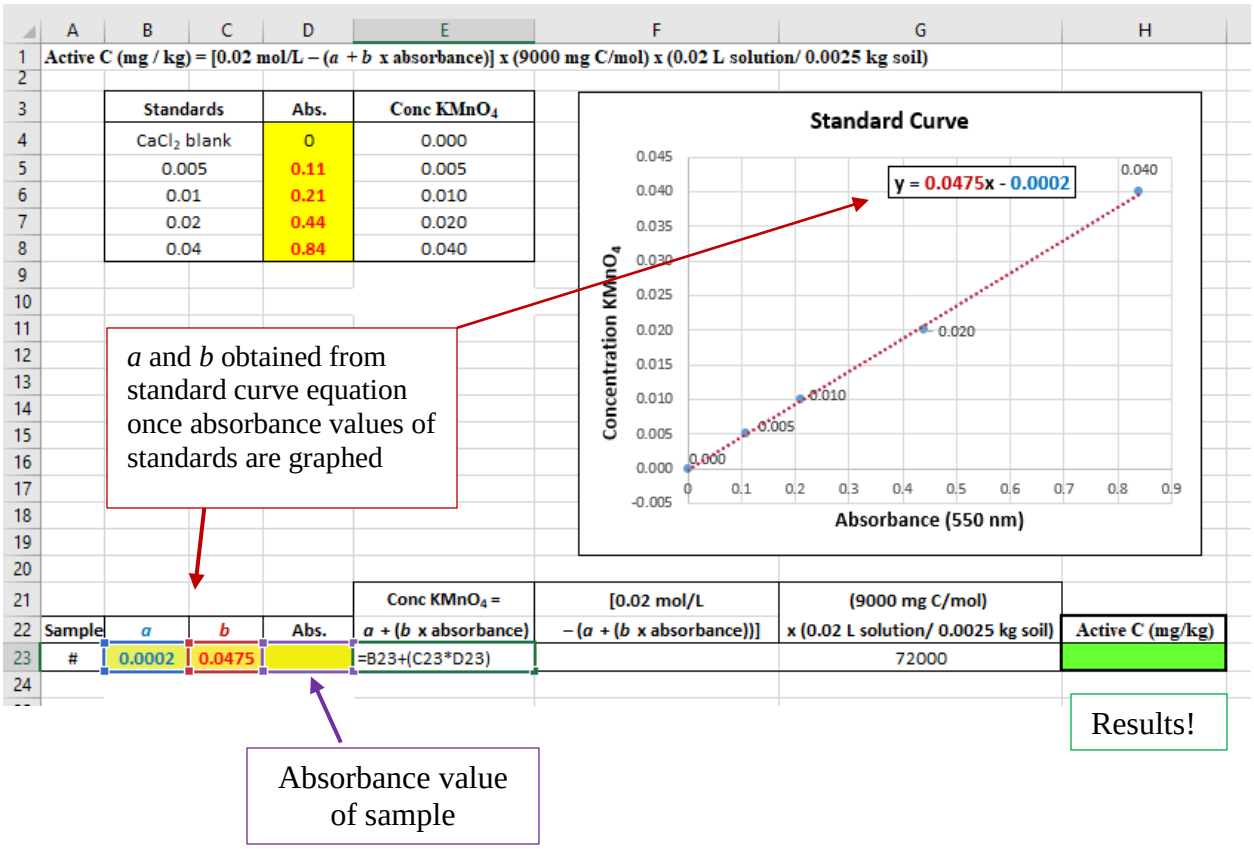
3. Create Excel spreadsheet with formulas; at the event, enter absorbance and the mg/kg (and/or lbs/A) are quickly calculated

Example:
* Typical absorbance readings for the standard solutions using a HACH 550 nm colorimeter are: 0, 0.11, 0.21, 0.44, and 0.84, which give the standard curve equation:

conc. = -0.0002 + 0.0475 x abs.

*Therefore a typical calculation (within rounding error) for Active C would be:

Active C (mg/kg) = [(0.02) - (-0.0002+0.0475 x Abs)] x 72000



Conversions

Mg kg^{-1} = parts per million (ppm)

ppm x 2 = pp2m = lbs/acre

Conducting the Active C Analysis

1. If soil sample is moist, then lay out a small sample (> 2.5 g) onto a dark surface and set in sun to dry (periodically, gently shake soil to expose moist bottom layer)

2. Using scale, weigh out 2.5 g of air-dried soil into tared 50ml tube

3. Add 18ml DI water and 2ml of the 0.2 *M* $KMnO_4$ stock solution to soil; cap tube

4. Using timer, shake continuously tube(s) for 2 minutes

5. Then, place tube(s) under box to protect from sunlight and keep undisturbed for 10 minutes; during this time, the soil is settling to the bottom of tube

*this is a timed reaction! the longer the soil and $KMnO_4$ solution are together, to more it will react, so follow step 6 immediately after the 10-minute settling period.

6. Using plastic pipette or syringe, remove 0.5ml aliquot sample from the upper 1cm portion of reacted solution and add it the second 50ml tube containing 49.5 ml DI water; cap tube and mix

*be careful to not collet soil from the bottom of tube or OM debris floating on top

*once aliquot is added to DI water, the mixture is stable and can be read at any time

*either use a new pipette for each sample **OR** can use the same pipette on subsequent samples by: wiping off outside of tip with lab tissue, rise out pipette with new sample by collecting 0.5 ml and discarding, then collect another 0.5 ml of new sample to be added to 50ml tube of DI water

7. Transfer the diluted (soil-free) solution to glass tube / glass cuvette and read absorbance value with Hand-held colorimeter (550 nm); input absorbance value to excel formula to obtain results in mg kg^{-1} or further convert to lbs/A

*make sure there are no air bubble in tube/cuvette and no fingerprints on the outside when reading absorbance (wipe off with lab tissue)

*immediately after reading value, rinse out tube/cuvette with DI and completely dry before next sample; any water droplets remaining in tube/cuvette will dilute results of next sample

8. Allow landowner to compare their tube with Color chart and/or Example standard tubes of known concentrations (write on the tube the estimated range of mg/kg or lbs/A Active C) ; provide landowner with results

Clean up:

- Dump all soil-solution into bucket (the concentrated solution must be reacted with OM, such as compost before it is safe to dump outside).
- $KMnO_4$ solution (without soil) must be extremely diluted in order to discard down drain
- Thoroughly rinse out tube and caps; let air dry or wipe out before next use.
    *tubes and cap must be completely dry before next use or the water droplets will dilute solution and skew results

References

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Weil, R.R., R.I. Kandikar, M.A. Stine, J.B. Gruver, and S.E. Samson-Liebig. 2003. Estimating active carbon for soil quality assessment: A simplified method for laboratory and field use. Am. J. Alternative Agric. 18(1):3–17.