Cornell Soil Health Laboratory 2022	Code: CSH 06
	Date: 1/6/2022
Title: **Respiration**	Page: 1 of 16
	Revision #: Final
Final revision: Zachary Batterman, Kirsten Kurtz, Daniel J. Moebius-Clune, Bob Schindelbeck	

Purpose and Justification

Many of the important processes that happen in the soil are accomplished or at least influenced by the soil biota. Nutrient transformations, mineralization, solubilization, and transport and delivery of nutrients to plants are key ecosystem services provided by soil microbes. Soil microbes and their activity contribute also to the formation and stabilization of aggregates, out of soil particles, limiting erosion, compaction, and crusting, while facilitating infiltration, aeration, and carbon sequestration. A robust and active soil microbial community can also improve plants' responses to water stress in fluctuating environments and can suppress the excessive growth of plant disease causing organisms and can cause plants to be more resistant to diseases. Several enzymatic and other biochemical assays are available which can allow us to assess various soil microbial functions and the rates of their activities. These vary in their resolution from very coarse to very fine.

A broad and integrative indicator of general microbial activity in soil is desirable for assessment of soil health. We use soil respiration rate as such an indicator. To measure soil respiration, we rewet air-dried, sieved soil, and trap the carbon dioxide that is released from it, as an indicator of overall metabolic activity.

(See extended note at the end for further details regarding the purpose and justification for measuring soil respiration as a soil health indicator)

Objective:

To assess the metabolic activity of the microbial community in soil samples through measurement by trapping of carbon dioxide released in a 4-day incubation following rewetting of an air-dried sample, and to use this as an indicator of biological soil health.

Description of Approach for CSHL respiration measurement

We use a method called sealed chamber alkali trap respirometry. In this method, we estimate metabolic activity by measuring the output of carbon dioxide (CO_2), a waste product of respiration (see also Zibilske 1994, and references therein).

An air-dried soil sample is rewet in a jar. Microbial activity, which has been very low in the sample due to the dry state, resumes rapidly as the soil rehydrates. CO_2 diffuses out of the soil sample and is prevented from escaping the jar by an airtight seal. A small beaker of potassium hydroxide (KOH) is sealed into the jar with the soil sample and serves as a trap for the CO_2. The properties of the KOH solution change in proportion to the amount of CO_2 trapped. It is this change in properties, or more specifically, the endpoint of this change, that we directly measure. The amount of CO_2 trapped is calculated from the property change, and this is used to estimate the amount of CO_2 evolved (released) from the sample, and by inference the metabolic activity of the microbial community in the sample.

To rewet the soil sample, in this protocol, we allow the sample capillarity draw to pull water up from below. This allows for maintaining continuity of air-filled porosity in the sample and avoids the effects of potential redistribution of finer particles from the surface of the sample if water were added from the top, which could clog pores and prevent diffusion of gases out of the sample into the jar's headspace. This has been shown to satisfactorily compare with methods that wet a sample to a predetermined moisture content (Haney & Haney 2010).

To determine the amount of CO_2 absorbed by the alkali trap, we measure the solution's electrical conductivity (EC). The EC of the trap solution drops in direct proportion to the amount of CO_2 trapped, so given two constants (the EC of 'raw' KOH having absorbed no CO_2, and the EC of a 'saturated' trap solution, were it to have absorbed its full capacity of CO_2) and a sample's measured EC, we can calculate the amount of CO_2 absorbed by the trap. We subtract the amount of CO_2 absorbed by a trap sealed in a jar with no soil ('blank'), to account for CO_2 coming from the air, and arrive at the amount emitted by the soil sample following rewetting during the incubation. This approach is substantially faster than titration and involves the use of fewer hazardous compounds (Wolf et al. 1952; Wollum & Gomez 1970).

References

Haney R.L. and Haney E.B. 2010. Simple and Rapid Laboratory Method for Rewetting Dry Soil for Incubations. Communications in Soil Science and Plant Analysis 41: 1493-1501.

Wolf J.M., Brown A.H. and Goddard D.R. 1952. An improved electrical conductivity method for accurately following changes in the respiratory quotient of a single biological sample. Plant Physiology 27: 70-80.

Wollum A. and Gomez J. 1970. A conductivity method for measuring microbially evolved carbon dioxide. Ecology 51: 155-156.

Zibilske L. 1994. Carbon mineralization. Methods of Soil Analysis: Part 2—Microbiological and Biochemical Properties. Soil Science Society of America, Madison, WI, pp. 835-863.

Procedure for Respiration Measurement

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

Materials

Pre-Setup:

Soil Samples (sieved to 8mm, air dried to constant mass, see CSH01 Soil Health Sample Intake and Processing)
Jars with Lids (wide mouth, 1 pint, Ball brand mason jars, with standard 2-part lids)
Labeling tape and Sharpie marker
Filter papers (55mm)
Weighing paper (3" x 3" or 4" x 4")
Pre-perforated aluminum weigh boats (with 9 holes in bottom, see note)
½" square double stick foam tape—Scotch Permanent Mounting Tape
Trap assembly (beaker on a pizza stool, see **Materials Preparation** section, below)
Blue jar rack (25 place)

Incubation initiation:

Jar or Beaker with distilled, deionized H_2O
(ddH_2O) Jar or Beaker with 0.5 M KOH (keep covered) Large Beaker to cover the KOH Jar or Beaker
Paper towels
10 ml pipettor
10 ml pipette tips
Marker
Gloves

Post-incubation reading:

Incubated samples in jars
EC meter with Computer
KimWipes
Gloves
Large (1/2 Gal.) jar for waste KOH
Wash tub with dH_2O for trap assemblies Trash receptacle for other jar contents
HCl to neutralize KOH
pH test strips
Stir plate and Stir bar

Sterilization solutions approved for use with Quarantined soil:

Bleach- 10% bleach solution within a labeled spray bottle must be left on contaminated equipment for 30 minutes before rinsing.

Ethanol solution (70%) within a labeled spray bottle must be left on contaminated equipment for 30 minutes before rinsing.

Additional Quarantine Protocols:

1. ***In the event of spilling quarantine soil, the soil should be swept up using a hand broom and dustpan and disposed of in an autoclave bag. The hand broom and dustpan as well as any surfaces contaminated with Q soil should be heavily sprayed down with 70% ethanol. The alcohol should be left on surfaces for 30 minutes.***
2. ***In the event of spilling bleach water, the water should be mopped or sponged up and bleached again with a 10% bleach solution for two hours. The mop head or sponge should be disposed of in an autoclave bag and autoclaved. The surface contaminated should be sprayed down with 70% alcohol. After two hours the bleach water can be disposed of down the drain.***

Additional Safety Considerations and Protocols:

1. When working with dry soil a mask should always be worn.
2. When working with bleach a mask, gloves and eye protection should always be worn.
3. Always work within a secondary container when working with quarantined soil.
4. Be aware of locations of PPE, first-aid kit and contact info for EHS and Lab management.

Prior to performing Respiration analysis, please make sure the following safety measures have been taken:

1. Proper training on Respiration methodology given in the following SOP
2. Completion of EHS 2555-Laboratory Safety
3. Completion of EHS 1475- Chemical Storage and Segregation
4. Completion of EHS 3355-Fume Hood Safety and Energy Conservation
5. Wear appropriate PPE (latex gloves, safety glasses, lab coat & Respirator when indicated)

Method

Start with sieved, air-dried soil

Gather clean, dry mason jars with lids, filter papers, labeling tape and sharpie, pre-perforated aluminum weigh boats, and weighing papers.

Have 2 blue 25-place jar racks ready to place jars with soil samples in after weighing

- Label a set of jars for the range of samples you will be weighing out (42 samples per set, one replication).
- For multiple replicates (2 jars per box of soil), append ‘a’ and ‘b’ to the sample number (e.g. L-123a and L-123b, 21 samples per set).

- Use labeling tape with sharpie rather than writing on jar, to facilitate jar cleanup.
- Place jars in blue rack in order, starting with a blank, then 10 samples (5 for multiple reps). Repeat. Include one blank for every 10 jars containing sample soil. Include one QC in every rack. See rack map below.

Rack 1

B1	1	2	3	4
5	6	7	8	9
10	B2	11	12	13
14	15	16	17	18
19	20	B3	QC1	

Rack 2

21	22	23	24	25
26	27	28	29	30
B4	31	32	33	34
35	36	37	38	39
40	B5	QC2	41	42

Once jars are labeled:

- Wearing latex gloves and a mask, which can be found in the supply cabinet:
- Add 2 filter papers to the bottom of each jar, offset from each other
  - Use long forceps if necessary, for adjusting placement of filter papers
- Weigh 20.00 g (19.97-20.03) dry soil into a pre-perforated aluminum weigh boat,
  - Use a weigh paper below the weigh boat on the balance pan to catch soil that falls through the perforations in the bottom of the boat.
  - Be sure to re-tare the balance when placing a new weighing paper on it
- Place the weigh boat into the jar
  - Use long, large forceps to pick up the weigh boat, holding with the tips far enough down to pick up the boat without it flexing.
  - Gently place weigh boat onto the filter paper in the jar,
  - Tap soil remaining on the weighing paper on the balance pan into pile of soil in weigh boat while transferring the weigh boat to the jar
- Jars can be stored with soil pre-weighed a few days ahead of time, cover with individual lids or with a sheet of kraft paper

Sterilize with sterilization solution after using. Discard any soil not used for test into autoclave bags and autoclave.

Prepare respirometer jars for incubation set up

- Gather trap assemblies (10 ml beakers stuck to plastic tripod ‘pizza stools’)
- Place one trap assembly into each respirometer jar, pressing the legs of the stool down into the soil sample to allow it to firmly stand, and to better conform the shape of the flat-bottomed weigh boat to the slightly domed jar bottom
- Set up a rack at a time (or a couple racks) of trap assemblies with soil samples to save time in advance

Set up incubations

- In addition to gloves, wear safety glasses when handling KOH as it is corrosive and can cause severe skin burns and eye damage. In case of a spill, neutralize with a dilute acid

solution and clean with plentiful water. In case of eye contact, flush eyes. Seek medical attention. If spilled on skin wash with plenty of water.

- Place fresh paper towels on a clean space on the bench, label one "KOH – 9ml" and the other "H_2O – 7.5ml"
- Place a fresh 10ml pipette tip on each of the labelled paper towels
- Draw distilled deionized water (ddH_2O from the deionization column tap) into a clean, dry beaker.

- Draw fresh KOH from the boxed bag into a clean, dry 500ml beaker. Fill to 500ml Cover this beaker while it sits on the bench in between uses with either a larger clean dry beaker (1L), or a sufficiently sized watch glass or dish, to prevent excessive air exposure
- Set out one row of jars from your blue rack onto your lab bench (a set of 11 at a time is convenient, 10 samples plus a blank):
    - Secure the "KOH" tip to the large (10 ml) pipette
    - Set volume to 9.00 ml by gently rotating knob
    - Add 9 ml 0.5 M KOH to trap beakers in jars
        - Double check volume setting prior to pipetting
        - Take extra care to avoid dripping any KOH on the soil sample (it will likely kill the microbes) or the other jar contents (it will react with the aluminum of the weigh boat, producing among other things, hydrogen gas)
    - When done with the set of e.g. 11 jars, remove large pipette tip and set aside on the paper towel marked "KOH – 9ml"
        - Do not confuse this tip with the tip used for water, in the next step.
    - Secure the other pipettor tip, from the paper towel labeled "H_2O – 7.5ml" to the large pipette
    - Set volume to 7.50 ml
    - Draw and dispense 7.5 ml ddH_2O into each jar.
        - Dispense the water gently onto the inside wall of the jar, holding the tip just a small distance away from the wall of the jar, as far down as you can safely and effectively hold the pipette tip without it contacting anything else in the jar.

      Strictly avoid any droplets spattering, as these can dilute the trap KOH solution, damaging the measurement.
        - Avoid dripping or dispensing onto the soil directly
    - Place a lid flat onto each jar, minimizing the amount of time that the jar is open, and the trap dispensed, to prevent unnecessary CO_2 absorption from the room air. Minimize the difference in amount of time that different jars are open and keep blanks in sequence with sample jars.
    - Close the jars securely with the screw top rings. Screw on tight enough to make an airtight seal. Keep in mind that opening the jars at the end of the incubation without jarring them enough that the traps spill will be necessary.
- Carefully moved closed jars back to the blue rack, avoiding tipping the trap assemblies inside (repeat previous steps starting by taking out your next set of 10-12 jars from the

blue rack).

- When done with a blue rack set it in a place where it will not be disturbed for four days, with a note indicating sample number range, date and time set up (note beginning time for batch, when traps began to be dispensed) and date and time to take down (4 days incubation time, start reading at the same time of day as the batch incubations were started – timing from when traps are dispensed)
- Discard used 10ml tips, paper towels, etc. Discard extra KOH after neutralizing or retain it in a 'used KOH' jar for rinsing the conductivity probe prior to measurements (See "clean-up" for further instruction on KOH neutralization). Do not set aside and use for further traps, other than those set up in an immediate tandem run. CO_2 absorption from the air, and evaporative concentration of the solution make it inadvisable to reuse this. Do not put it back in the stock.
- Incubate 4 days
    - Avoid major temperature fluctuations – particularly noting if there is a planned steam shutdown or hood shutdown planned and avoid having incubations running during these times. Cold temperatures will have a large effect on the respiration rate of samples.

Be sure to thoroughly disinfect work bench with sterilization solution.

Measure after incubation

- Gather a clean, dry beaker and fill partway with older KOH from the "used KOH" bottle. Label this beaker "used KOH". Get two more beakers with tap water for rinsing, and ddH_2O for final cleaning of EC probe. Label these beakers.
- Gather kimwipes for cleaning the probe off between samples.
- Ensure that computer is on and logged in, and that Orion EC meter is connected and on.
- Open a new excel workbook and fill in sample numbers in the order to be measured in a column on the left-hand side, including blanks. Insert a row or two for meter test numbers at the top of the worksheet. (If not using an excel workbook, manually record EC readings and Temperature for all samples, blanks, and QC's and manually calculate Respiration values. See **Calculation** section below for instruction).
- Place the probe in the 'used KOH' and wait for the EC meter reading to stabilize. Press the 'print' button on the meter. Make sure that the readout populates the spreadsheet appropriately and drops to the next line ready for another reading.
- Blot probe dry with kimwipe making sure to dry KOH off the metal temperature probe stud in the probe opening.
- Probe can be set dry on a paper towel in between measurements if it is already blotted dry. Do not let KOH dry onto the probe.
- Providing that the test numbers transferred correctly, and the cursor is in the appropriate cell to begin measuring, it is ready to read samples.
- Carefully retrieve blue rack with jars from shelf and move it to the bench.
- Gently remove a run of jars in order (10-12 jars), as when setting up the incubation.
- Double check the labels and make sure they are in the same order as in the worksheet.

- Triple check the labels, and when doing measurements, check again to make sure that the right reading is being recorded on the right line.
- Carefully remove the rings from the run of jars, leaving the flat lids in place – they should stay sealed on the tops of the jars.
- Pop the lid off the first jar and place the probe into the trap beaker, giving a brief gentle stir with the probe in the process, then letting it rest at the bottom of the trap beaker.
    - It is best to do this, even with the first jar, immediately after blotting it dry from the previous sample of KOH. In the case of the first jar, this should be the "used KOH" rather than a sample proper.
    - Not too much stirring is necessary or advised, a brief gentle stir to mix the upper layer with the rest of the trap is all that is needed.
- When the reading on the meter readout screen has stabilized, press the print button. The numbers will be recorded in the spreadsheet.
- As one sample reading is stabilizing, the previous sample jar can be set aside into a blue rack, and the next sample made ready.
- Blot the probe dry with a kimwipe in between samples and place it into the next sample with the same brief gentle stir.
- Double check that the order of labels on the jars still matches the spreadsheet the readings are being added to.
- If doing stretches of 11 or 12 jars, try to match the timing of reading with how long it takes to set up the set of jars initially, and avoid having jars sit open for very long.
- When the rack is finished, rinse the probe well in the tap water beaker, and then again in the ddH2O beaker. Set onto paper towel to dry.
- Save the spreadsheet with the readings. Identify the column with the conductivity (not conductance) readings. This will be the column to copy over into the calculation spreadsheet.
- Open the respiration calculation workbook template, and 'Save As' a workbook, naming according to the range of samples to be measured.
- Fill in the appropriate cells in the workbook's first sheet, with date, sample range, number of reps, et cetera.
- The list of samples to be quantified should auto-fill.
- Copy and paste EC readings into the appropriate cells. Double check to be sure they are being copied to the appropriate cells. Make sure the blank readings and check soil readings get copied to the appropriate spaces as well.
- Save the worksheet when readings have been added.
- Click on the last worksheet tab in the calculation workbook, which should now have the calculated respiration values by sample.
- If any samples are flagged as needing to be redone, make a note of these.
- Copy these results out into the results repository, clearing the values for those that need to be redone. Copy them also into a new worksheet, similarly omitting 'redo's'. This new worksheet should be saved with a name indicating the sample range, and that these are respiration numbers. Send this spreadsheet with today's readings to the appropriate

person.

Cleanup

- KOH neutralization is dangerous and should be done in fume hood wearing appropriate PPE
    - Lab coat
    - Respirator
    - Gloves
    - Safety glasses
- Empty the trap waste into a large ½ gal. jar for neutralization before drain disposal.
- Place jar on stir plate in fume hood with sash drawn low, and place a stir bar in the jar, turn stir function on at low to moderate speed. Wear gloves and eye protection.
- Neutralize with HCl while stirring, and test with litmus paper strip to ensure a pH of 7. Consult the table below for reference.
- When neutral, wash down drain with copious quantities of tap water.
- Separate trap beakers from pizza stools. This can be easier after they have soaked for some time in a tub of water, as small amounts of residual KOH will make the water basic and this will loosen the adhesive. Set beakers into a tub of distilled water to soak overnight before cleaning. Set pizza stools aside to be cleaned and reused.
- Empty jars into waste bin, discarding weigh boat and filter papers with the soil.
- Wash and rinse all glassware well, rinsing several times at the end with distilled water and then a final rinse in ddH_2O. Dry on clean drying rack or pegboard. If any residues become apparent after drying, wash again and rise well. Residues may interfere with further usage.
- Turn off meter and computer when not in use. Leave both plugged in.

Empty jars into buckets with autoclave bags and autoclave as soon as possible. Disinfect jars with sterilization spray for the required time and then rinse.

-

Calculation

Calculation of respiration values for samples, including determination of CO_2 quantity absorbed by the alkali trap, subtraction of mean blank values for the run, and averaging across soil sample lab reps, are handled automatically using the automated calculation workbook (Excel) on the lab computer.

To calculate respiration rate manually:

We know the trap capacity for CO_2 absorption based on its volume and concentration. 0.5 M KOH can absorb sufficient CO_2 to become 0.25 M K_2CO_3, when fully saturated (in practice, the absorption would likely become inefficient when close to the endpoint, but we calibrate the amount used so that this situation would be quite rare). One mole KOH can accommodate one half mole CO_2 (which, when trapped, accumulates as CO_3^{2-}). So, 9 ml of 0.5 M KOH can accommodate

0.009 L * 0.25 mol/L * 44.01 g/mol * 1000 mg/g = 99.025 mg CO_2

Of this total (theoretical) trap capacity of 99.025 mg CO_2, some fraction is absorbed. The proportion of the total trap capacity that is absorbed is equivalent to the proportion of the total conductivity drop that would be observed (between the EC of the 'raw' 0.5 M KOH and the EC of 0.25 M K_2CO_3) if the trap were saturated, which is observed. To restate this: the difference between the measured EC for a sample (or blank) and the EC of the 'raw' KOH is a quantity we can call the 'observed EC drop'. This quantity is some fraction of the total possible drop, which we can call 'full capacity EC drop'. Dividing the 'observed EC drop' by the 'full capacity EC drop' gives us a fraction that is equivalent to the fraction of the total trap capacity for CO_2 absorption that is used.

So:
Given all measurements at the same temperature (this is key),
If EC_{raw} is the electrical conductivity of pure 0.5 M KOH (about 112 microsiemens for your calculation you will want ECraw to be the average of your 5 blanks)
and EC_{sat} is the electrical conductivity of 0.25 M K_2CO_3 (about 42 microsiemens)
and EC_{sample} is the electrical conductivity of the trap associated with a particular sample, and P is the proportion of the trap capacity for CO_2 absorption that is used,
then
$((EC_{raw} - EC_{sample})/ (EC_{raw} - EC_{sat})) = P$ and
P*(trap capacity in mg) = amount of CO_2 in mg absorbed by the trap inquestion.

For each sample's trap, the amount of CO_2 absorbed must be adjusted to subtract out the amount of that absorbed CO_2 that came from the air sealed into the jar with the sample and trap, which is accomplished by measuring blanks which are set up just the same as the samples, but leaving out the soil, along with each run. The average blank value for each run is subtracted from each sample in that run, to arrive at the amount of CO_2 released from the soil samples.
If multiple reps are being run, average the values. If these differ from each other by more than a set threshold (we use an average deviation of 10% from the common mean), then the sample should be rerun.

Quality Control (QC) Methodology

- Air dry and sieve QC soil to 2mm. Use a soil splitter to homogenize the QC for use.
- Prepare 2 sets of respiration racks (98 samples) for analysis, following the protocol closely.
- Once racks are ready, put up a set of respiration samples on the same day one would normally plan on analyzing respiration (e.g. if respirations are normally put up on Mondays, Thursday and Fridays, follow this schedule in order to ensure normal lab conditions).
- Perform respiration take down according to protocol. Combine all QC results into one file.
- Using the results from your QC's, calculate the mean and standard deviation. Once this is done, apply +/-2SD from the mean to determine the acceptable range of the QC soil.

Materials Preparation

Weigh boat perforation

In batches beforehand, a sleeve of weigh boats at a time (each sleeve contains 144 weigh boats) Place weigh boat singly on top of a piece of single sheet thickness perforated cardboard. Using a dissecting pin (a plastic or wooden handle with a stout wire needle at the end), poke 9 holes in the bottom of the aluminum pan, starting in the center, and arranging the holes in a square. The needle should easily pierce the boats, to approximately the same depth each time. Keep boats and cardboard and pin clean and dust free while doing this.
Restack perforated boats in sleeve.

Trap assembly preparation

Gather clean, dry, 10ml glass beakers (borosilicate, Pyrex-type), double sided scotch foam mounting tape roll, scissors, paper towels, and 'pizza stools'
Cut several small squares of foam tape from roll. Peel one side and stick it to a pizza stool top (in the middle of the circular flat top), press firmly. Place a beaker open side down onto clean paper towels. Peel the remaining side of the foam tape square on a pizza stool and press it firmly onto the beaker bottom. Ensure that these are well stuck together (by pressing firmly) so that the beaker cannot slide on the stool while an incubation rack is being moved.

Note on Pipetting

While pipetting, draw in slowly, do not rapidly release plunger, keep the pipette upright while drawing in liquid, angled only slightly and gently touching the side of the beaker when dispensing. Depress plunger fully before putting tip below liquid surface, draw in fully and slowly before lifting tip out of liquid. Do not depress plunger past the initial stop when preparing to draw up a sample. When dispensing a sample, gently depress plunger smoothly to the first stop, while the tip is gently rested at a slight angle against the wall of the vessel you are dispensing into. After a pause at the end of this dispensing step, while holding the tip in place, if a small amount of liquid runs down and collects in the bottom of the tip, before having moved it away from the vessel wall, you can dispense this last bit of liquid by gently depressing the plunger past the first stop to the second stop. Once should be enough. This is the poorly named 'blow-out' function. Do not push hard enough to justify calling it that – it will not work nearly as well. Dispensing too fast will splash liquid, drawing in too fast will foul the filter or the pipette barrel. With the smaller volume pipettes and tips and with higher viscosity liquids, liquid adhesion to the walls of the pipette tip becomes greater relative to the volume being dispensed. Low retention tips should minimize this. Smooth dispensing to the first stop, waiting for it to pool, and smoothly pressing past the first to the second stop should deliver the whole sample.
Practice on non-samples and note the effects of tip contact angle with and distance from the wall of the well or beaker being dispensed into. You should not press hard against the wall with the tip, but gently placing it in contact at a slight angle or holding it a small distance away from the wall at a similar angle, should help. This distance from the wall should be about the diameter of the drop being dispensed if the total dispensing volume is small, or about the diameter of the droplet expected to be retained and then dispensed with the 'blow out' if the specific step requires this.

Detailed Materials List with Ordering Information

- Weighing paper
  - VWR PAPER WEIGHING 4X4IN PK500. Cat# 12578-165
- Aluminum weigh boats
  - VWR DISH ALUMINUM 57MM PK144. Cat# 16005-126
  - Pre-perforate these as described in above note
- Dissecting needle to perforate weigh boat
  - Forestry Suppliers.com Stock Number: 53850.
- Filter papers
  - VWR FILTER PAPER 5.5CM PK100. Cat# 28310-015
- KimWipes
  - WIPES KIMWPE 11.4X21.3CM PK280. VWR Cat# 21905-026
- Jars and Lids
  - 1-pint, wide mouth, Ball brand mason jars, these are sold at Agway, Wegmans, and other stores for canning. Lids are two part: a) flat metal disk lid, lined with a white resin coating on the inside surface, and a rubberized ring where the flat will sit on the jar lip, b) a threaded ring to hold flat lid onto jar. Lids generally come with jars, but may become bent, damaged or otherwise unusable, and are also available to purchase separately at the same stores that sell the jars in cases generally of 12.
- Small glass beakers
  - 10 ml size pyrex type (borosilicate) beaker, such as:
  - VALUEWARE BEAKER 10ML 12/PK. Fisher Cat# S00001
- Pizza stools
  - Plastic tripod ‘pizza saver’ stool shaped devices. These are available by the case of 1000 at Maines Food and Party Warehouse or other suppliers and may be otherwise found online.
- 0.5 M KOH
  - This is best purchased as a pre-made solution, since KOH pellets are liable to absorb both water and carbon dioxide from the air after opening, making solutions prepared in the lab less reliably precise. One can purchase either a 4L or 20L size from VWR – 20L size is recommended.
  - ***Be sure to allow a new batch to equilibrate to room temperature for several days prior to use, and to clean the area around the opening prior to opening the container, as it generally arrives dirty from shipping***
  - 20L size can be stored for added security in a plastic milk crate on its side
  - POT HYDROX (KOH) 0.5N STD SOL 4L. VWR Cat# AA35595-K7
  - Recommended: POT HYDROX 0.5N STD SOL 20L. Alfa Aesar Cat# 035595

- Distilled deionized water
    - A deionization column is plumbed to the distilled water tap in BF822
- Electrical conductivity probe
    - We use an Orion Versa Star meter with a Conductivity module and epoxy probe (013610MD Conductivity Cell)
    - This communicates with the lab laptop computer via USB cable connection and WinWedge Software.
    - An alternative meter and probe can be used, provided the measurable EC range is high enough, and that the probe is easily dried between samples. One we have used, which may be appropriate for additional research project use, particularly if traps are to be read multiple times at intervals, is the VWR expanded range conductivity meter, Cat# 89094-958
- HCl
    - Concentrated HCl (approx. 12.1 M), available at VWR Cat# BDH3028-2.5LG
- Litmus paper
    - BDH PH TEST STRIP UNIVERSAL RANGE 0-14. VWR Cat# BDH35309.606
- K_2CO_3
    - Not needed on an ongoing basis. Used to prepare a solution representing the fully saturated trap for measurement of trap EC endpoint at the temperature incubations are run at. This number is a constant and need only be measured once.
    - K_2CO_3 is generally available at the CU Chem Stockroom, or can be ordered from Sigma, VWR, or Fisher.
- 10 ml pipettor
    - RESEARCH PLUS 1-10ML TURQUOISE. Fisher Cat# 13690034
- 10 ml pipette tips
    - Racked 10 ml tips can be purchased as pre-filled racks or as bags of loose tips
    - EPPNDRF TIPS RACK 1-10ML 120PK. Fisher Cat# 05403117
        - or VWR Cat# 47747-988
    - PIPET TIP EP-TIP 10ML CS200 VWR Cat # 47747-992 (long tips)
    - PIPET TIPS 1-10ML CS200 VWR Cat# 47747-986 (normal)

Additional Supplementary Notes and Commentary on Purpose and Approach

Purpose

Soil microbial activity is central to several key soil functions:

- Nutrient cycling, as nutrients that are needed by plants for proper growth may be found in plant biomass that is left behind after harvest. To release these nutrients and make them available for further plant uptake, the activities of the soil biota are necessary to break down the complex structure of the residues.
- The liberation of nutrients from soil organic matter occurs as microbial activity degrades the organic matter. As lower trophic level organisms incorporate nutrients, higher trophic level organisms consume them and release excess nutrients back into the soil solution.

This mineralization of nutrients from soil organic matter makes a soluble nutrient pool available in the rhizosphere for plants and their associated symbionts to take up, allowing for incorporation into plant biomass, which is the general aim of soil nutrient management.

- Storage of nutrients in microbial biomass. As freely soluble nutrients are much more likely to be transported from the root zone, and the surface soil in general, than nutrients that are incorporated into higher molecular weight compounds such as biopolymers found in biomass and organic matter, it is preferable that nutrients cycle through the microbial biomass, thus increasing the residence time and decreasing losses from this part of the profile through an immobilization and mineralization cycle.
- Incorporation of residues left behind after crops are harvested, such as corn stover, as well as the decomposition of root biomass, which can be substantial, and is often recalcitrant. Without the breakdown of this material, the nutrients bound in these biomass sources would not be released, to become available to further plant growth, but additionally, materials would accumulate that would make agricultural soil management operation less effective.
- Incorporation of organic amendments, including manures, litter, green manure crop biomass, and even simpler organics such as urea. For the materials added that are intended for nutrient supply, or for soil organic matter for structural or water holding capacity improvement reasons, to be effective, these materials must be subject to the activities of an array of soil microbes.
- Soil structural development and stabilization, particularly aggregation, depend very strongly on the combined activity of multiple soil microbial organismal groups. Primary particles as well as particulate organic matter and preexisting microaggregates are bound together into macroaggregates in part by roots and root exudates, but even more effectively so by root associated fungal hyphae, which can form networked growth forms, in comparison with the simpler branched forms of roots, and exert stronger binding force than the weaker activity of polysaccharides such as those making up a substantial fraction of the root exudates. Following this macroaggregate formation, smaller microaggregates may form within macroaggregates through the activity of microbes such as bacteria, which align fine particles about themselves through wetting and drying and induce the formation of organomineral bonding.

- Additional important soil microbial activities include the solubilization of otherwise poorly available nutrients (as in the example of the phosphate solubilizing bacteria), the direct or indirect promotion of plant growth and vigor (the plant growth promoting rhizobacteria or PGPR), and the suppression through competition, antagonism, and other means of soilborne plant pathogens (the so-called ‘suppressive soils’)

Comprehensive measurement and assessment of the soil biotic community composition and detailed analysis of the various metabolic activities of key groups, or of individual separate processes would be highly valuable. However, to date these types of measurements are too complex, costly, and time consuming to include in a high throughput service lab which aims to process samples rapidly to aid in management decision making. Even were this feasible, an

integrative, or inclusive, more comprehensive measure of the overall activity level of the microbial activity would still be desirable. We use a measure of the overall metabolic activity of the whole soil (microbial) community as an indicator of the general functioning of these important processes, without at this point distinguishing between them.

Approach

Dry soil is relatively stable, with the microbial community in it in a state of relative quiescence. This community is not very metabolically active, and the soil is changing very little in this state. As we add water, the soil rehydrates, along with the cellular life within it. Enzymatic activities resume, inside and outside of cells, and metabolic rate ramps back upward steeply. Carbon dioxide is released by the oxidative metabolism as a waste product. We can trap this released carbon dioxide and measure its accumulation as an index of the rate of the processes it is released as a byproduct of.

Carbon dioxide is readily trapped from the gaseous phase (in air) using an alkali trap sealed in a chamber with the air in question. These alkali traps are generally solutions of either sodium hydroxide or potassium hydroxide, with the key being the presence of a large amount of hydroxide ions in solution. As carbon dioxide (CO_2) dissolves, it reacts with water (H_2O) molecules to form carbonic acid (H_2CO_3). This acid rapidly loses its two protons (H^+ions), which combine with two hydroxide ions (OH^-) to make water. The carbonate ion (CO_3^{2-}) is left in solution. The cations from the alkali do not participate in this reaction, and likewise remain in solution. So, if we seal just such a trap in an airtight jar with soil that we have just remoistened, we can trap the CO_2 released by the soil in the alkali trap, and just need to measure how much has been taken up by the trap to have a measure of metabolic activity.

There are multiple ways to accomplish this measurement of how much CO_2 has been absorbed by the trap. Two hydroxide (OH^-) ions combine with the two protons (H^+) released from the carbonic acid (H_2CO_3) produced by the reaction of each absorbed CO_2 molecule with water. Since we know the amount of hydroxide ion we start with, as we know the volume and concentration of the alkali used in the trap, we could measure how much remains and calculate the difference as what was ‘used up’ in deprotonating incoming carbonic acid to carbonate. This is a very commonly used approach, utilizing a time-consuming titration.

However, we know also that other measurable properties of the trap changes as it absorbs CO_2 and accumulates carbonate. One such property is the solution’s electrical conductivity (EC), which is proportional to the concentration of ions in the solution and their relative mobilities. As the trap solution’s hydroxide ion (OH^-) concentration decreases and carbonate ion (CO_3^{2-}) concentration increases, the trap’s electrical conductivity declines. This is due at least in part to the larger size and lower mobility of the carbonate ion relative to the hydroxide ion.

This electrical conductivity drop is linearly proportional to the accumulation of carbonate in the trap. So, if we know the conductivity of the trap solution when it is fresh, and the conductivity of a fully saturated trap, we can determine the conductivity decrease representing absorption of the full capacity of the trap for CO_2. By comparing the conductivity drop observed in a trap

sealed into a chamber with a soil sample, to this full-capacity conductivity drop, we can assess the fraction of the full capacity amount that each trap has absorbed. Put another way, we can measure the trap conductivity drop for each sample and compare that to the trap conductivity drop we'd see if the trap had become fully saturated and know from that the percentage of the total trap capacity that has actually been 'used' or 'filled.