

Cornell Soil Health Laboratory
Comprehensive Assessment of Soil Health
Standard Operating Procedures
February 2016



Acknowledgments



Robert Schindelbeck, Cornell Soil Health Laboratory Coordinator
2006-present

This laboratory manual contains the Standard Operating Procedures (SOP'S) for the suite of physical and biological soil analyses offered in the Standard Soil Health Analysis from Cornell University's Comprehensive Assessment of Soil Health Laboratory for 2016.

Correct citation:

Cornell University Comprehensive Assessment of Soil Health Laboratory Standard Operating Procedures, February 2016. Schindelbeck, R.R., B.N. Moebius-Clune, D.J. Moebius-Clune, K.S. Kurtz and H.M. van Es.

Special acknowledgment for the development of the laboratory procedures is due to the early work of the Cornell Soil Health Team beginning in 2003. This list of contributors includes: Beth Gugino, O.J.(John) Idowu, Harold van Es, David Wolfe, Bianca Moebius-Clune, Janice Theis, George Abawi and W. Dean Hively.

The second generation Cornell Soil Health team members includes Kirsten Kurtz, Daniel Moebius-Clune, Joseph Amsili, Michael Rutzke and other members of the Cornell Nutrient Analysis Laboratory staff.

Throughout the years numerous talented staff and students worked in the Soil Health Laboratory performing the analyses through each phase of the development of the procedures. Through the tenure of each employee we attempted to cultivate a fertile atmosphere for investigating the soil processes described herein.

Unless otherwise noted, photos were taken by authors or soil health team members.

This laboratory manual provides an outline of the testing procedures summarized in the Comprehensive Assessment of Soil Health Training Manual third edition:

Moebius-Clune, B.N., D.J. Moebius-Clune, B.K. Gugino, O.J. Idowu, R.R. Schindelbeck, A.J. Ristow, H.M. van Es, J.E. Thies, H. A. Shayler, M. B. McBride, D.W. Wolfe, and G.S. Abawi, 2016. Comprehensive Assessment of Soil Health – The Cornell Framework Manual, Edition 3.0, Cornell University, Geneva, NY.

For additional information related to this project please visit <http://soilhealth.cals.cornell.edu>.



Cornell Soil Health Laboratory

Standard Operating Procedures (SOPs) 2016

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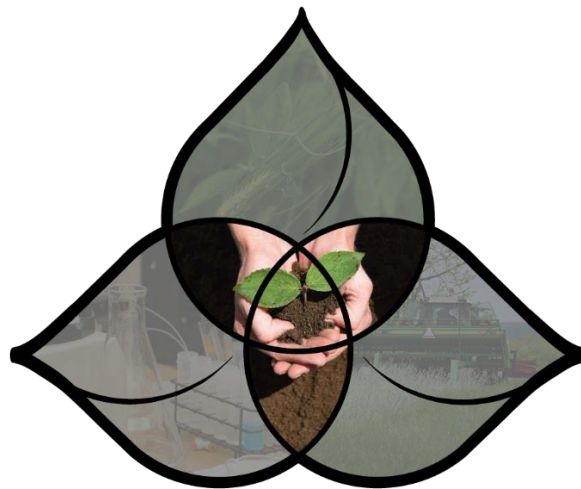
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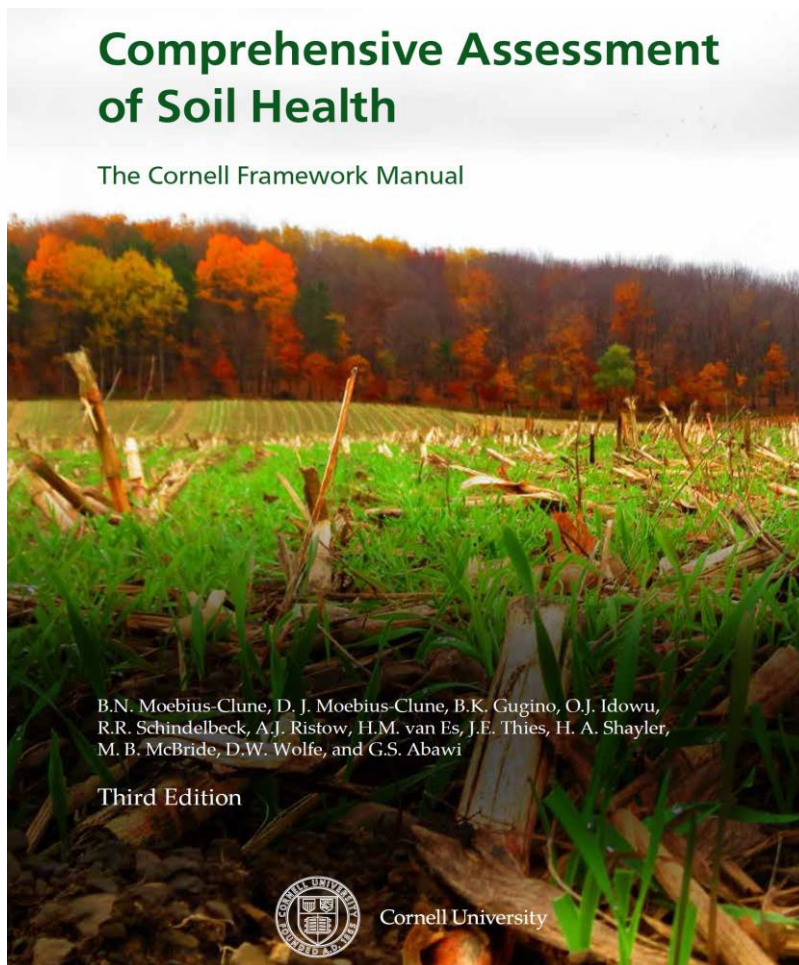
Purpose and Justification:

The Cornell Soil Health Laboratory receives soil samples for analysis year round. Rapid shipping of the sample to the lab is recommended. Once a Soil Health sample arrives in the lab, it is either processed immediately or stored at 4° C (40° F) until the earliest opportunity. All soil is air dried to constant weight before entering into laboratory processing. Correction is made to return reported soil test results to oven dry weight. The received sample is broken into sub-samples for all of the Soil Health laboratory tests.

The Cornell Soil Health website <http://soilhealth.cals.cornell.edu> also has updates on laboratory tests offered (**typically as packages of analyses** but also as individual tests). Current-year soil sample submission forms are also available at this site.

Note: The suite of soil analyses in the Cornell Assessment of Soil Health packages in the following pages are all available as individual tests.

To order tests individually, please use Cornell Nutrient Analysis Laboratory [Form S](#).



Refer to the 2016 Cornell Soil Health Training Manual for appropriate field sampling techniques:


<http://www.css.cornell.edu/extension/soil-health/manual.pdf>

The manual provides further information on the individual laboratory tests. Information is also provided to interpret the laboratory values and show how these values are scored.

The Training Manual also outlines the strategies for soil health management planning using the information returned in the Cornell Soil Health Assessment Report.

Comprehensive Assessment of Soil Health

From the Cornell Soil Health Laboratory, Department of Soil and Crop Sciences, School of Integrative Plant Science, Cornell University, Ithaca, NY 14853. <http://soilhealth.cals.cornell.edu>



Grower: 1
 Bob Schindelbeck
 306 Tower Rd.
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Agricultural Service Provider:
 Mr. Bob Consulting
 rrs3@cornell.edu

Sample ID: LL8

Field ID: Caldwell Field- intensive management

Date Sampled: 03/11/2015

Given Soil Type: Collamer silt loam

Crops Grown: WHT/WHT/WHT

Tillage: 7-9 inches

Measured Soil Textural Class: silt loam

Sand: **2%** - Silt: **83%** - Clay: 3 4

	Group	Indicator 2	Value	Rating	Constraints 5
	physical	Available Water Capacity	0.14	37	
	physical	Surface Hardness	260	12	Rooting, Water Transmission
	physical	Subsurface Hardness	340	35	
	physical	Aggregate Stability	15.7	19	Aeration, Infiltration, Rooting, Crusting, Sealing, Erosion, Runoff
	biological	Organic Matter	2.5	28	
	biological	ACE Soil Protein Index	5.1	25	
	biological	Soil Respiration	0.5	40	
	biological	Active Carbon	288	12	Energy Source for Soil Biota
	chemical	Soil pH	6.5	100	
	chemical	Extractable Phosphorus	20.0	100	
	chemical	Extractable Potassium	150.6	100	
	chemical	Minor Elements Mg: 131.0 / Fe: 1.2 / Mn: 12.9 / Zn: 0.3		100	

6 **Overall Quality Score: 51 / Medium**

ABOVE: Sample Soil Health Assessment Report with (1) Background information, (2) Measured indicator, (3) Indicator Value, (4) Indicator Score, (5) Constraints, and (6) Overall Quality Score.

Descriptions of Package Analyses Offered:

BASIC Package

Organic Matter Content:

The pool of organic matter (OM) in soil includes the carbon-containing solid materials which are, or are derived from, living organisms including plants and soil microorganisms. Soils with high OM content tend to require lower inputs of nutrients and are more resilient to drought and extreme rainfall. The percent OM is determined by measuring the mass loss on ignition at 500°C in a furnace. At these extreme temperatures, carbonaceous materials are burned off (i.e., oxidized to carbon dioxide (CO₂)), while non-organic, mineral materials remain.

pH and Nutrient Testing:

A traditional soil fertility test analysis designed for application in the Northeastern U.S. is used to determine soil pH and estimate plant nutrient availability. Results are interpreted within a general framework to evaluate deficiencies or excesses, but are not crop specific. Soil pH is measured in water, and macro- and micro-nutrients are determined using a Modified Morgan extractant.

Wet Aggregate Stability:

Wet aggregate stability is a measure of the extent to which soil aggregates resist falling apart (i.e., slaking) when wetted and exposed to the force of rain drops. This physical soil property is measured using a rain simulator, which rains for 5 minutes (delivering 1.25cm of water) on a sieve containing a known weight of soil aggregates between 0.25-2.0 mm. Unstable aggregates slake and pass through the sieve, while the fraction of soil remaining on the sieve is used to calculate the percent wet aggregate stability. Soils with high wet aggregate stability stable show increased water infiltration, water and air movement, and water storage. Such soils are more resistant to water and wind erosion and show better overall soil health (e.g., less crusting, increased root growth, promotion of biological activity, etc.).

Soil Respiration:

Soil respiration is a measure of the metabolic activity of soil microbial populations. Respiration is determined by capturing and quantifying the amount of carbon dioxide (CO₂) released from a re-wetted sample of dried, 8 mm sieved soil stored at room temperature in an airtight jar for four days. Greater release of CO₂ is indicative of a larger, more active soil microbial community participating in nutrient cycling and OM decomposition.

Surface and Sub-Surface Hardness:

Surface and sub-surface hardness are indicators of the soil's compaction status, which decreases water, air, solute, and root movement through the soil while potentially increasing the likelihood of surface runoff and soil erosion. Surface and sub-surface hardness are both measured as penetration resistance in pounds per square inch (psi) using a field penetrometer or soil compaction tester at two depth increments (surface: 0-6", sub-surface: 6-18"). The Cornell Soil Health Lab can provide clients with a soil penetrometer by mail to collect hardness ratings in the field; these data can then be included for the interpretation of overall Soil Health score

STANDARD Package (BASIC Package Plus)

Rapid Soil Texture:

The non-organic, solid material in soils is composed of a mixture of mineral particle sizes, the relative proportion of which determine the soil texture. Textural class affects many of the important physical, biological, and chemical processes in the soil, but is not easily altered by management practices because it changes little over time. Although soil texture itself is not a soil health indicator per se, knowledge of the textural class informs the interpretation of soil health indicators. This Rapid Texture procedure involves dispersion of soil particles using sodium hexametaphosphate followed by the 1) isolation of the sand fraction using a 0.053 mm sieve, and 2) separation of the silt and clay fractions by settling.

Active Carbon:

Active Carbon measures the portion of soil organic matter that can be easily oxidizable and can serve as a readily available food and energy source for the soil microbial community, thus helping to maintain a healthy soil food web. To measure Active Carbon, soil is reacted with a potassium permanganate solution which has a deep purple color. As the solution reacts with the sample, it loses some of its color in direct proportion to the amount of Active Carbon in the soil sample. The color change is determined by using a spectrophotometer and is calibrated against standards of known concentration.

Soil Protein:

The Autoclaved-Citrate Extractable (ACE) Protein Index is used as an indicator of the fraction of the soil organic matter pool that is present as proteins or protein-like substances. Soil protein content is an indicator of the biological and biochemical quality of the soil, and is very well associated with the overall soil health status because it represents the largest pool of organically-bound nitrogen in the soil. Soil proteins are determined using a sodium citrate extraction under autoclaving (high temperature and pressure); extracted proteins are quantified using the colorimetric bicinchoninic acid assay (BCA) as calibrated against protein standards of known concentration.

Available Water Capacity:

Available water capacity is the amount of plant available water the soil can store. In the field, a soil is at the upper range of soil water storage when water that it cannot hold against the force of gravity has drained – this is called ‘field capacity.’ The lower end of the range is referred to as the ‘permanent wilting point,’ which occurs when only hygroscopic water remains (i.e., water tightly held so tightly onto soil particle surfaces that it is inaccessible by plant roots). Available water capacity is determined from measuring the soil’s water content at both field capacity and permanent wilting point in the lab and calculating the difference.

EXTENDED Package (STANDARD Package Plus)

Soluble Salts:

Problems with high salt concentrations can occur naturally, but are most prevalent under irrigated agriculture in semi-arid and arid areas. Although this is most commonly encountered in western regions of the U.S., salinity issues are also associated with high tunnels used for season extension in the Northeast. High concentrations of soluble salts in soil have a severe impact on growing crops by limiting the ability of plant roots to take up water; in severe cases, this can cause plants to wilt and die. Electrical conductivity is measured on a 1:1 saturated paste extract of the soil sample.

Heavy Metal Screening:

Testing for heavy metals is available for situations where soil contamination is suspected, or as a precautionary measure. Heavy metals accumulate in soils naturally at low levels, but toxic levels can be reached as a result of mining, manufacturing, and industrial activities. Contact with heavy metals is dangerous for humans and animals, but can also have adverse effects on plants and soil organisms. Metals falling under this category include cadmium, chromium, lead, copper, nickel, and zinc. To extract heavy metals, a soil sample is completely digested using acid; quantification of metals in the extract is then performed using Inductively-Coupled Plasma (ICP) atomic emission spectroscopy.

Bean Root Bio-Assay:

Root pathogen pressure is a measure of the degree to which sensitive, test-plant roots show symptoms of fungal disease pressure when grown in a soil sample for a set amount of time under controlled conditions. This assay is qualitative and reports either the absence or presence of symptoms of damage from a variety of fungal (e.g., *Fusarium*, *Rhizoctonia*, and *Thielaviopsis*) and oomycete (e.g., *Pythium*) pathogens which can cause major crop retardation or death if not kept in check. The observed pathogenic pressure is given a rating from 2 to 9, with higher numbers indicating greater levels of pathogen-induced damage.

Other Add-on Test Options:

Hot Water Soluble Boron:

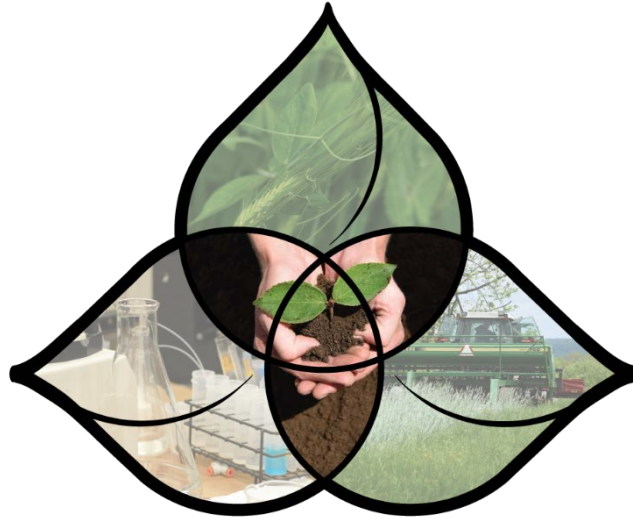
Boron is an essential plant micro-nutrient especially important for many fruits, vegetables, and hops. Boron availability is expected to be low in soils with high pH, low organic matter content, low moisture, and limited nitrogen availability. Hot water soluble boron is extracted from the soil by boiling with 0.125% barium chloride solution. The extract is then filtered and analyzed for Boron concentration using Inductively-Coupled Plasma (ICP) atomic emission spectroscopy.

Potentially Mineralizable Nitrogen:

Potentially mineralizable nitrogen (PMN) represents the portion of the organic nitrogen pool which is most easily decomposed by soil microorganisms and converted into ammonium via mineralization. PMN is generally higher in soils with high accumulations of organic matter and total N, such as those in low lying landscape positions which inhibit aerobic decomposition. Soil PMN is determined by measuring the amount of ammonium produced during a seven day anaerobic incubation at 30 °C and comparing this with the pre-incubation background concentration of ammonium.

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Soil Health Sample Intake and Processing



Background / References:

The Cornell Soil Health Laboratory receives soil samples for analysis year round. Rapid shipping and inclusion of blue ice packs with the sample during the hottest months protects the sample from damage due to high temperatures. Once a Soil Health sample arrives in the lab, it should either be processed immediately or stored at 4° C (40° F) until the earliest opportunity. Soil samples need to be friable before they are ready for processing i.e. the sample should readily crumble or break into smaller fragments without smearing as it passes through the sieve. The received sample is broken into sub-samples for all of the Soil Health laboratory tests. **NOTE:** Refer to the 2016 Cornell Soil Health Manual for appropriate sampling techniques.

Objective:

Upon receipt of the Soil Health sample, data from the Submission Form should be recorded into the Cornell Soil Health Lab Sample Sign-In Sheet (Table 1). Initial crumbling, mixing, sieving and partitioning of the sample is performed before oven drying. If the sample arrives too wet to process, it can be spread out on a large pan and allowed to dry enough to be friable. After this initial processing, sub-samples are routed through the laboratory according to the requirements of the particular analysis (see II.2 below and Figure 1 below). The Cornell Soil Health Assessment package 2016 provides for determination of: *Rapid Soil Texture, Wet Aggregate Stability, Available Water Capacity, Active Carbon, Autoclaved Citrate Extractable (ACE) Protein Assay, Soil Respiration, and Standard Nutrient extraction and analysis.*

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

Materials and Equipment:

Analytical Balance
 8mm sieve
 2mm sieve
 Wire brush
 Indelible marker
 Tared aluminum cans (2 sets)
 Falcon tubes with 42ml of 3% Hexametaphosphate (soap) solution
 Weigh boat
 Fan
 Aluminum pan
 Funnel/Acetate sheet
 Scintillation vial
 Plastic cups and lids
 Plastic bags
Plastic bin

Sterilization solutions approved for use with Quarantined soil:

Bleach-10% Bleach solution within a labeled spray bottle must be left on contaminated equipment for 1/2 hour before rinsing.

Green Shield-Disinfectant and Algicide, .08% solution within a labeled spray bottle must be left on contaminated equipment for 5 minutes before rinsing.

Ethyl Alcohol 70% within a labeled spray bottle must be left on contaminated equipment for 1 minute before rinsing.

Procedure:

I. Sample sign-in and storage:

- Before processing the sample, assign a sample ID to the sample. Record this ID on the sample bag and on the master data sheet (Table 1 below). Maintain sample at 4° C (40° F) until it is ready to be processed.
Quarantine soil permit holder must sign in samples according to permit specifications. All other following quarantine procedures may be done by properly trained laboratory technician. Autoclave all packing materials that the quarantined samples arrived in. Place a large “Q” on each quarantined sample bag. Put a red “Q” next to each sample ID in the Soil Health sign in book and highlight entire line with yellow highlighter.

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Table 1. Cornell Soil Health Lab Sample Sign-In Sheet

Cornell Soil Health Sample Sign-In Sheet 2016															
Once sample is signed in (ex. N1), write that # on the sample bag.															
Sample Number ¹	Date Sampled	Date Received	Sample ID	Agent Contact	State	Moisture Correction (Thimble)			Hand Texture (F, M, C)	Add On					No. Excess
						wet wt. with can	dry wt. with can	Moisture Can ID		Boron	Metals	Salts	Bean Root Bio	PMN	
N															
N															

- If the sample is not friable, it will need to air dry for several days. Spread the soil out into a deep aluminum pan and place in front of a fan. As much surface area should be exposed to the air as possible to maximize drying rate – occasionally turn soils by hand to facilitate drying. The soil should crumble apart by hand and not smear when sent through the sieve. **DO NOT BEGIN PROCESSING SAMPLES UNTIL THEY ARE FRIABLE.**

Keep quarantined samples covered if it is necessary to air dry. Sterilize all quarantine laboratory equipment that comes into contact with quarantined soil with an approved disinfectant.

II. Initial sieving of samples (large 8mm sieve):

- Once the sample is friable, pass entire sample through the 8mm sieve. Discard any material that does not pass through the sieve. Use the wire brush to remove all soil from the sieve and ensure that it is clean for the next sample. Thoroughly mix the soil to ensure that the sample is homogenized, using separate containers if necessary to mix properly. Re-assemble the entire sample into one unit before continuing.
Use quarantined sieves and quarantined wire brushes for all sieving. Discard any soil or stones that will not pass through 8mm sieve into autoclave bags and autoclave. Do all laboratory procedures inside a plastic tub and disinfect with an approved quarantine disinfection solution before rinsing.
- Roughly ~4 cups of sieved soil is needed (about 1 L) for all analyses, distributed accordingly:
 - 0.5 cup is air dried for Available Water Capacity and Texture.
 - 1 cup is air dried for Wet Aggregate Stability and Active Carbon.

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- c. 75-100 grams (3/4 cup) is air-dried for the Soil Respiration test and Soil Protein assay.
- d. 0.5 cup for Nutrient Analysis.

Put the remaining portion of soil back into the bag and archive at 40° F.

Quarantined samples do not receive the Root Bioassay or the PMN analysis. Do all laboratory procedures inside a plastic tub and sterilize any laboratory equipment that comes in contact with quarantined soil. Put remaining soil into boxes clearly labeled as containing quarantined samples and archive at 40° F.

III. Available Water Capacity (AWC), Texture, Nutrient Analysis

1. These tests use air dried soil.
2. Put ½ cup soil into cardboard box (with the sample ID recorded on it) for standard nutrient analysis, dry in 50C oven until completely dry. This sample is now ready to pass through 2mm for standard nutrient analysis.
3. Weigh about 2 tablespoons of 8mm sieved soil into a pre-tared aluminum can. Record can ID and total wet weight on the sample sign-in sheet (Table 1 above).
4. Place can in oven at 105° C for a few days, stirring soil in evening before leaving. Allow 2-3 days in oven to reach complete dryness. (Determine complete dryness by weighing sample, recording weight, date/time weighed and then reweighing two hours later. If the sample has not lost any weight it is then completely dry and ready for further processing).
5. Record dry wt. of soil with aluminum can on the sample sign-in sheet. The moisture loss in this “thimble” of soil is used to correct the lab analyses to oven dry soil weight.
6. Pass soil thru 2mm sieve. Discard what does not pass through sieve.
7. Place ~14g soil (+/- 0.10g) into Falcon centrifuge tube that contains 42 ml of Sodium Hexametaphosphate solution (see Rapid Soil Texture procedure – CSH 02) for textural analysis specifics. Record exact weight on the Texture data sheet.
8. Collect about 60g of soil (4 tablespoons) into a labeled plastic bag for Available Water Capacity determination. See Available Water Capacity procedure – CSH 04) for specific laboratory protocols for this analysis.
Sterilize all quarantine laboratory equipment that comes into contact with quarantined soil with disinfection solution. Discard any soil that does not pass through sieve into an autoclave bag and autoclave as soon as possible. Label all sub-samples with a “Q”. Discard cardboard boxes and any

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remaining soil after testing soil for nutrient analysis into autoclave bags and autoclave as soon as possible.

V. Active Carbon and Wet Aggregate Stability

1. These tests use air dried soil.
2. Place about 30g (2 tablespoons) soil onto 2mm sieve, force sample through the sieve, crushing aggregates so that they fit through. Save any material that doesn't fit through the sieve.
3. Use a funnel or acetate sheet to pour the <2.0mm aggregates into a 20 ml scintillation vial labeled with the sample ID. Cap and store in a dry place until ready for Active Carbon analysis. See Active Carbon procedure – CSH 05) for this protocol.
4. Place remainder (including the soil that did not pass through the 2mm sieve during processing for Active Carbon analysis) of dried soil on stack of 2mm and 0.25mm sieves with a catch pan.
5. Soil is placed atop the sieve stack that is shaken for 15 seconds on a Tyler Coarse Sieve Shaker.
6. Soil in the catch pan and on top of the 2mm sieve can be discarded. Note: If there is not enough soil in the 0.25 sieve, grind soil that did not pass through the 2mm sieve by hand and repeat steps 5 through 7.
7. Gently shake (by hand) soil in 0.25 sieve to remove any fine dust.
8. The **0.25-2.0mm aggregates** will be lying on top of the 0.25mm sieve – save this fraction in a cup for the Wet Aggregate Stability test. Note that this test requires 20-30g of this material for each sample.
9. See Wet Aggregate Stability procedure – CSH 03) for the protocol for this test.
10. Store Wet Aggregate Stability soil in labeled, deep-dish plastic trays that hold 16 individual cups.
11. Order in the tray and sample ID should correspond to data sheet for Wet Aggregate Stability.

Discard cardboard box used for drying sample into autoclave bag. Discard any soil that does not pass through 2mm sieve into autoclave bag. Autoclave materials in bag as soon as possible. Saturate any laboratory equipment that comes in contact with quarantined soil with an approved disinfectant solution and let sit for required time before rinsing. Label all sub-samples with a "Q".

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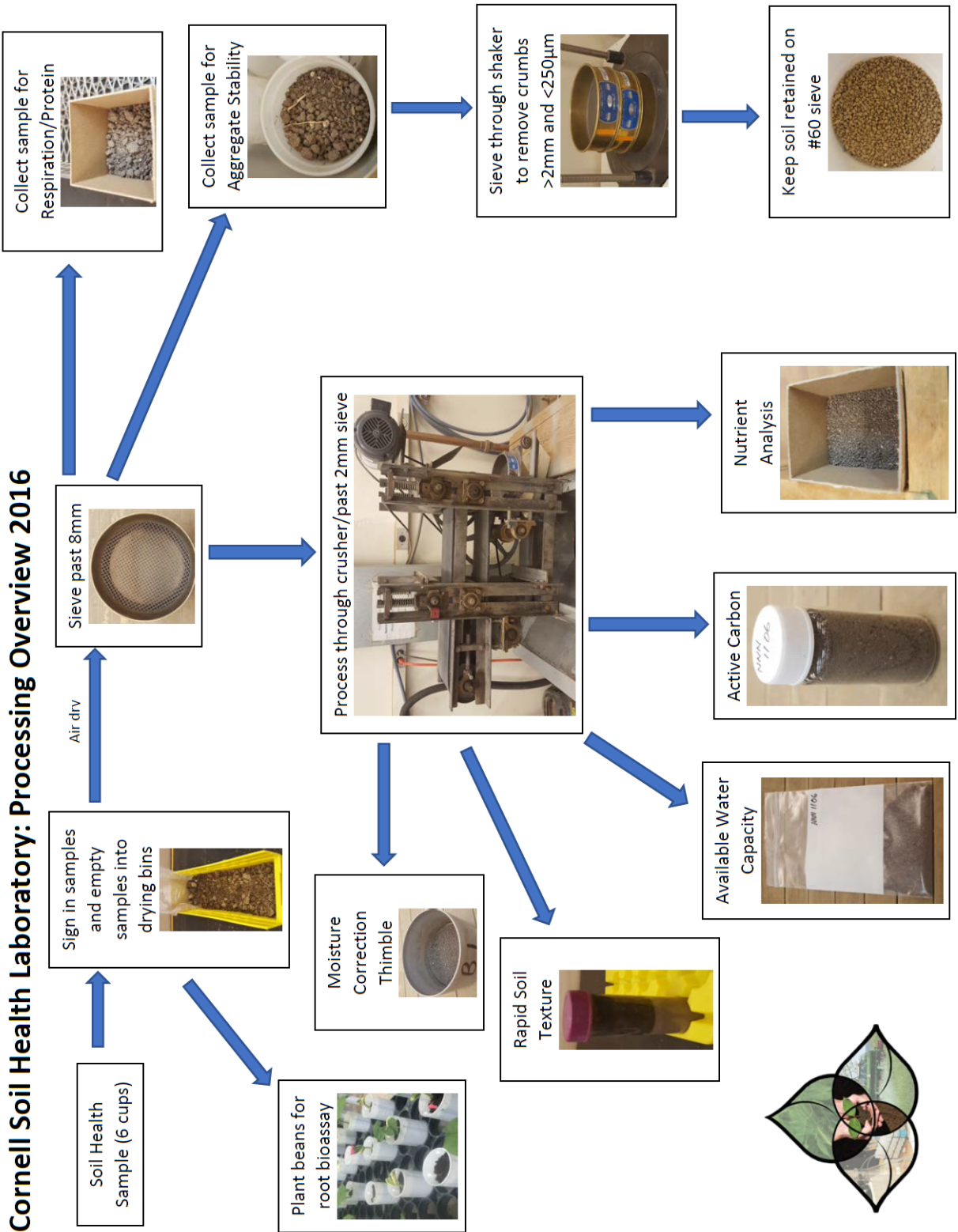
VI. Root Bio-assay (included in the Extended Package or as an optional Add-on Test)

1. Return the remainder of the 8mm sieved soil to the original sample bag for later transport to Greenhouse for planting. See Root Bioassay procedure –(CSH 07) for this protocol.
2. Store at 40° F if not immediately planting soil.
Quarantined samples do not receive Root Bio-Assay.

Calculations and Data Entry:

After all data is filled from sample the sign-in sheet and other data sheets, transcribe into a spreadsheet for a later generation of Soil Health Report. See other Standard Operating Procedures (CSH 02- 07) for specific calculations.

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Rapid Soil Texture

Purpose and Justification:

The non-organic, solid material in soils is composed of a mixture of mineral particle sizes, the relative amounts of which determine a soil's texture. Textural class affects many of the important physical, biological, and chemical processes in the soil, but is not easily altered by management practices because it changes little over time. Although soil texture itself is not a soil health indicator per se, knowledge of the textural class informs the interpretation of soil health indicators. The Rapid Texture procedure involves dispersion of soil particles using sodium hexametaphosphate followed by the 1) isolation of the sand fraction using a 0.053 mm sieve, and 2) separation of the silt and clay fractions by settling.

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Background / Reference:

The rapid soil texture method is designed for processing large volumes of samples with accuracy comparable to more sophisticated tests. For original published procedure see:

Kettler, T.A., Doran, J.W., Gilbert, T.L. *Simplified Method for Soil Particle-Size Determination to Accompany Soil Quality Analysis*. Soil Sci. Soc. Am. J. 65:849-852 (2001).

Scope/Objective:

Soap solution is used to disperse soil aggregates. Using a 0.053mm mesh sieve, sand is wet sieved from the soil sample, dried and weighed. Silt fraction is allowed to settle out of a water column in two hours and is removed, dried and weighed. Clay fraction is determined based on the difference from the original soil sample weight.

Note that that this procedure is designed to NOT remove Organic Matter with peroxide before testing. Mineral soils containing less than 5% OM will have the organic debris collected with the sand fraction on the 0.053mm sieve. A soil texture method is being developed for soil/media samples with higher amounts of OM where the dried material collected on the 0.053mm sieve is burned at 500C to constant weight to remove the organic material. This result gives the texture of the mineral component of the soil/ media sample.

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

Materials and Equipment:

Analytical Balance

“A, B or C” aluminum drying cans

Falcon Tubes with 42ml, 3% Hexametaphosphate (soap) solution

Shaker

20cm diameter 0.053mm sieve

20cm diameter Funnel

Catch basin

600ml beakers

Drying oven at 105° C

Squeeze bottles with water

Sterilization solutions approved for use with Quarantined soil:

Bleach- 10% bleach solution within a labeled spray bottle must be left on contaminated equipment for ½ hour before rinsing.

GreenShield- Disinfectant and Algicide, 0.08% solution within a labeled spray bottle must be left on contaminated equipment for 5 minutes before rinsing

Ethanol solution (70%)- within a labeled spray bottle must be left on contaminated equipment for 1 minute before rinsing

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Procedure:

1. Prepare Falcon tube racks with about 42ml of 3% HMP (soap) solution added to each tube. (Fill tubes from a HMP carboy - release the pinch clamp until liquid arrives at the 42ml mark on the centrifuge tube.) Label lids of Falcon tubes within Falcon tube racks with sample IDs.
Wear gloves (that are to be autoclaved after using) throughout entire test.
2. Fill out Soil Health Texture data sheet (Table 1 below) with Sample IDs. Insert a control sample in the last spot on the Falcon rack.
3. Use air dried soil for this test. Sieve about 2 tablespoons of soil past 2mm and record sample ID onto soil texture data sheet. Weigh and record 14g (+/- 0.1g) of soil and carefully add soil to the correct Falcon tube with soap solution and cap and shake vigorously to bring soil into suspension.
Weigh soil in weigh boat placed on top of a sheet of velum on the scale. Sterilize both with sterilization solution after using. Sterilize sieves used for sieving soil with sterilizing solution after soil is used. Discard any soil not used for test into autoclave bags and autoclave. Put a "Q" on each quarantined texture lid.
4. Place up to 3 racks onto a shaker for 2 hours at 150 rpm. Samples can be stored for several weeks before or after shaking.
Place texture tubes in a plastic bin within the Shaker. Bleach shaker with sterilization solution after using.
5. On Texture data sheet (with sample IDs and weights) record ID of pre-tared aluminum cans into the sand can and silt can positions.
6. Once shaken, re-suspend soil (shake by hand until no soil is stuck to the sides or bottom of the Falcon tube) into solution before uncapping. Use a squeeze bottle to rinse ALL material onto the 20mm diameter 0.053mm sieve assembly (place sieve in funnel over 1000ml beaker inside catch basin).

Make sure that a 1000ml beaker is in place inside catch basin.

Process all soil in the Texture test within a plastic bin that is to be disinfected after test is complete.

7. Rinse contents of Falcon tube (including inside the cap) onto the sieve assembly. Use less than 1000ml water to rinse tube and force all soil particles (while wearing gloves, using fingers and water) through the mesh. When the contents of the Falcon tube rinse clean, collect sand grains and OM to a corner of the sieve. Decant OM. Flush the sand into the arranged SAND aluminum can. Dry at 105° C until sample reaches

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complete dryness. Record aluminum can identity onto the Soil Texture data sheet. Place the 1000ml beaker on a stable table (in order based on rack position of tubes). Re-suspend all soil particles by emptying contents from labeled beaker to a temporary beaker and back into the labeled beaker. Allow a 2 hour settling period.

Do all procedures within a plastic bin that will be sterilized after use. Place beakers within a bin for the required settling time. Spray outside of water bottles with sterilization solution and let sit for the required time before rinsing.

8. At the end of the settling period, decant (and waste) the water and clay in beaker above the settled silt particles. Use caution when decanting the suspended clay from the silt layer at the bottom of the beaker. These silt particles at the bottom of the beaker are rinsed into the SILT aluminum can. Dry at 105° C until samples reach constant weight.

Decant quarantined water into buckets with 10% bleach and let sit for a half hour before dumping down drain. Do decanting within a plastic bin that along with the outside of the water bottles will be sterilized with sterilization solution.

9. When samples have dried, record the weight of the dry aluminum sand can with sand. Record the weight of the dry aluminum silt can containing silt on the Soil Texture data sheet.

Weigh cans on top of a sheet of velum. Bleach velum with 10% solution for a half hour before rinsing.

10. Clean, rinse and store tin cans upside down in order on the metal trays.

Place all soil in autoclave bags and autoclave as soon as possible. Disinfect cans in sterilization solution for the required time and then rinse.

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Table 1. Soil Texture datasheet

DATE:

Soil Texture Data 2016

rack-position

rack-position

A						B						C						D						E						F											
texture						dry						sand						silt						dry wt.						dry wt.						Theta M					
tube ID						soil wt.						can						can						sandcan						siltcan						thimble					
1																																									
2																																									
3																																									
4																																									
5																																									

Data Sheet calculations:

$$\text{Sand \%} = ((D - B) / (A/1 + F)) * 100$$

$$\text{Silt \%} = ((E - C) / (A/1 + F)) * 100$$

$$\text{Clay \%} = 100 - (\text{Sand \%} + \text{Silt \%})$$

Where:

$$\text{Sand \%} = (\text{oven dry sand mass} / \text{original sample mass}) * 100\%$$

$$\text{Silt \%} = (\text{oven dry silt mass} / \text{original sample mass}) * 100\%$$

$$\text{Clay \%} = 100 - (\text{Sand \%} + \text{Silt \%})$$

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Quality Control/ Standards:

Each year the standard soil (lima silt loam, 0-6" depth, air dried to constant weight, sieved past 2mm) is run in a set of 16 replicated samples to determine the reproducibility of the test. From Table 1 above a datasheet is created where sample number 25 in each set of samples is this standard. If this standard result falls outside the expected range, the entire data set is rejected and is re-run.

Table 2 below lists the descriptive statistics from the 2015 standard soil. The mean plus and minus three standard deviations is used as a criteria for determining the range of acceptability for any single measurement.

Table 2. Lima silt loam soil standard descriptive statistics for the Rapid Soil Texture test

<u>REP</u>	<u>%sand</u>	<u>%clay</u>	<u>%silt</u>		<u>% sand</u>	<u>%silt</u>
1	38.8	16.1	45.2	Mean	38.27	46.21
2	37.6	15.6	46.7	Standard Error	0.15	0.24
3	38.6	16.4	45.0	Median	38.26	46.18
4	38.1	15.9	46.0	Mode	#N/A	#N/A
5	38.4	14.4	47.2	Standard Deviation	0.62	0.97
6	38.6	16.6	44.8	Sample Variance	0.38	0.94
7	39.2	15.3	45.5	Kurtosis	-0.78	-1.42
8	37.7	14.9	47.4	Skewness	0.18	-0.27
9	37.6	16.5	45.9	Range	2.15	2.78
10	38.8	14.9	46.3	Minimum	37.27	44.66
11	37.6	15.5	46.9	Maximum	39.42	47.44
12	39.4	13.4	47.2	Sum	612.29	739.28
13	38.1	14.6	47.3	Count	16.00	16.00
				Confidence		
14	38.5	16.8	44.7	Level(95.0%)	0.33	0.52
15	38.1	16.0	45.9	mean	38.3	46.2
16	37.3	15.5	47.2	mean MINUS 3 SD	36.4	43.3
				mean PLUS 3 SD	40.1	49.1

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Wet Aggregate Stability Test

Purpose and Justification:

Wet aggregate stability is a measure of the extent to which soil aggregates resist falling apart (i.e., slaking) when wetted and exposed to the force of rain drops. This physical soil property is measured using the Cornell Sprinkle Infiltrometer (the rain simulator), which steadily rains on a sieve containing a known weight of soil aggregates between 0.25-2.0 mm for five minutes. Unstable aggregates slake and pass through the sieve, while the fraction of soil remaining on the sieve is used to calculate the percent wet aggregate stability. Soils with high wet aggregate stability are more resistant to water and wind erosion and show better overall soil health (e.g., infiltration, root growth, biological activity, etc.).

Each soil sample is placed on a 20cm diameter soil sieve which receives 1.25cm water depth (as drops) in 5 minutes. About 11,700 drops of 4mm diameter with a total weight of 0.393kg are delivered. These drops reach a velocity of 3.1m/s as they fall the 50cm distance (terminal velocity for this sized drop is 8.8m/s). The total Kinetic Energy (KE) delivered in 5 minutes is:

$$KE = \frac{1}{2} m * v^2$$

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$$KE = \frac{1}{2} (0.393\text{kg})(3.1\text{m/s})^2$$

$$KE = 1.9 \text{ Joules}$$

Procedure reference: B.N. Moebius, H.M. van Es, R.R. Schindelbeck, O.J. Idowu, J.E. Thies, D. J. Clune. (2007) Evaluation of Laboratory-Measured Soil Physical Properties as Indicators of Soil Quality. Soil Science Vol. 172, No. 11, pp. 895-910.

Poster description of the WAS test used in the Cornell Soil Health Assessment (see Figure 1 below): Wet Aggregate Stability Test of Soil Structure in the Cornell Soil Health Assessment 2009. Robert R. Schindelbeck (rrs3@cornell.edu), Bianca N. Moebius-Clune, Omololu J. Idowu, Harold M. van Es

Objective:

The wet aggregate stability of soil health samples is tested on soil air dried to constant weight using the aggregate size fraction 0.25mm-2mm. From the total weight of dry aggregates tested, the weight of slaked soil and the weight of stones retained on wetted sieve are measured and the difference determines the percent of stable soil as aggregates.

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

Materials and Equipment:

Cornell Rainfall Simulator
Stopwatch
Filter Paper
16, 0.25mm sieves
Drying oven
Aluminum trays
Plastic cups
Soil sieve brush
Analytical Balance
Two-tablespoon scoop
4-cone portable platform
Sink with sprinkler hose
Squeeze bottles with water
Reverse osmosis system and water storage tank with pump

Additionally for quarantine soils:
2, 5-gallon buckets
Bleach
Spray bottle with 10% bleach solution
Latex or Nitrile gloves to protect hands
Large basin to bleach quarantine sieves

Procedure:

I. Filter preparation:

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1. Weigh a series of dry filters and group them in rounded tenths of grams. For example, if a filter weighs 9.32 grams, group it under 9.3 grams, if it weighs 9.26 grams, group it under 9.3 grams. These filters can be stored for future use.
2. Once ready for testing, get out the 4-cone portable platforms and lay them out together on a table.
3. Place the filters into the cones, noting rounded filter weight on the Aggregate Stability data sheet. NOTE: Make sure there are no tears or punctures in the folds of the filter. It may be helpful to squirt a *small* amount of water onto the filters to help them open up - be careful to not over water, as filters are susceptible to failure.
4. The cones and filters are now ready to receive sieves.

II. Sieve/Aggregate preparation:

1. Set out the 16 pre-weighed, numbered, 200mm diameter, 0.25mm mesh sieves on a bench near the analytical balance.
2. Confirm that the weights of the sieves match what is labeled on the data sheet.
3. Aggregate Stability samples should be stored in groups of 16 in deep-dish trays labeled something like "J1", "N1" or "F2".
Quarantined samples should be clearly marked with a "Q" on the lid.
4. Using a 2-tablespoon sized scoop, spread about 20 grams of 0.25-2.0mm aggregates in a single layer onto the surface of the 0.25m sieve, with order of samples corresponding to numbers labeled on each sieve. Spread the soil evenly on the sieve by shaking the soil scoop about six inches above the sieve.
Spread soil on sieves inside a bin. Discard any soil into an autoclave bag and autoclave as soon as possible. After using scoop and bin sterilize with a disinfectant solution and let sit for the required time before rinsing.
5. Weigh each sieve and record the total weight of the sieve + aggregates onto the Wet Aggregate Stability data sheet (Table 1 below).
Weigh sieves on top of a velum paper, and after using apply sterilizing solution and let sit for the required time before rinsing.
6. Place sieves onto the filters that are resting within the 4-cone portable platforms. NOTE: Make sure that sieves are resting within the cone so that any material falling through the sieve will fall into the filter and not behind it, and that the sieves are resting as parallel to the floor as possible
7. **MAKE SURE ALL SIEVES HAVE BEEN WEIGHED BEFORE PLACING THEM UNDER THE SIMULATOR.**
8. Do not disturb the soil on top of the sieve. Try to maintain an even distribution of aggregates across the sieve surface. Samples are ready to be placed under the rainfall simulator.

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III. Rainfall Simulator Calibration/Preparation:

1. Make sure the rainfall simulator tank contains enough water to run the amount of samples you will prepare.
2. To fill simulator tank, confirm there is enough deionized (reverse osmosis) water in the storage tank located below the workbench.
3. Through the large opening on top of the simulator tank, insert the clear plastic hose, flip the pump switch over to turn it on. Water should begin to flow.
4. Fill the rainfall simulator tank as much as possible without completely emptying the storage tank. Turn the pump switch upside down to turn off the pump.
5. Cap rainfall simulator with large rubber stopper.
6. You must perform a practice run to check the amount of rainfall delivered in a 5 minute period.
7. DO NOT place samples under the simulator UNTIL AFTER CALIBRATION
8. Pull out the large rubber stopper and let dripper run for a few minutes. (If you have just filled the tank, this step can be avoided).
9. After a few minutes, pull out the small rubber stopper and replace the large rubber stopper. Let water flow for a few more minutes. Ensure that the dripper is suspended 0.5m above the soil on the sieve.
10. Make sure the stop-watch is zeroed and press the start button and simultaneously note the water level on the ruler glued to side of simulator tank. This is measurement one.
11. After 5 minutes, note water level. This is measurement two.
12. Record difference between measurement one and two on the Wet Aggregate Stability data sheet (Table 1 below). This difference should be as close to 1.25cm as possible.
13. If substantially higher or lower (should be within + or - 0.1), then the rate may be adjusted by sliding the small diameter tube inside the tank up or down. Get a faster rate by sliding the tube up, and a slower rate by sliding it down.
14. Minor adjustments are only necessary if there has been an extreme difference in temperature between the last testing and the current testing event.

IV. Wet Aggregate Stability Test:

1. MAKE SURE ALL SIEVES HAVE BEEN WEIGHED BEFORE PLACING THEM UNDER THE SIMULATOR.
2. Check to make sure stop watch has been "zeroed".
Place catch basin out flow tube into 5 gallon bucket with 1.5 liters of bleach to collect all runoff water. When buckets have approximately 4 gallons move out flow tube to another bucket prepared with 1.5 liters of bleach inside. Let buckets sit for 30 minutes before discarding bleach water down the drain.

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3. With rainfall simulator running, place the first platform of cones, filters, and sieves 0.5m under the tank, directly under it such that all areas of the sieves are receiving drops.
4. Immediately after placing platform under dripper, start the stopwatch.
5. Throughout a 5-minute period, it is necessary to rotate the tank of the simulator (around its vertical axis) and to allow it to swing very slightly (< 1cm swing) to ensure random delivery of drops to the entire sieve surface. Simulator should be rotated ~90 degrees once every 15-30 seconds.
6. At the 5-minute mark, carefully remove the platform from under the dripper and place sieve rack into bin to trap waste water from the draining sieves.
7. Re-zero stopwatch and repeat the test for the other three 4-cone platforms. Let sieves sit while performing the rest of the tests to allow water in filters to drain, leaving just soil inside the filter.
Spray any water that may have dripped on the floor with an approved Quarantine disinfectant solution and rinse away after the required time.
8. Carefully remove sieves and gently slide filters sideways so retained water drains out of the filter and is not 'trapped' by retained soil.

V. *Rinsing the sieves:*

1. A sink with a sprinkler hose and regular faucet work best for washing sieves.
2. Set up sprinkler hose and faucet so that they are running simultaneously and hitting the sieve with retained soil in roughly the same area.
Place bin with a 5 gallon bucket inside it into sink. Catch all water used for sieve rinsing in bucket, add 10% bleach and discard after 30 minutes.
3. Wash any remaining material THROUGH the sieve such that only clean sand too large for the mesh is left behind (do not lose any sand during washing). Use fingers to rub all stable soil particles less than 0.25 mm through the sieve and into sink.
Catch all water used for sieve rinsing in bucket, add 10% bleach and discard after 30 minutes.
4. Using a squeeze bottle with water, starting with a stream of water at one side of the remaining material, slowly move across the sieve, washing the retained material into a tared and labeled aluminum can to be dried at 105C. Decant excess water and organic matter from the can.
Decant water into 5 gallon bucket with 1.5 liters of bleach in it. Bleach any laboratory equipment that comes in contact with quarantined soil.
5. Place the cup in the correct order in a labeled metal tray. Record label of metal tray on the Wet Aggregate Stability data sheet (Table 1 below). Fold drained filters and place into appropriate aluminum cans. These are called soil "tacos" on the data sheet.

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6. Wash any remaining rocks and organic matter out of sieves; shake off excess water and place sieves in 105° C oven for 10-20 min. or until dry
Disinfect sieves with an appropriate solution for the required time period, then rinse and dry in oven
7. Repeat entire procedure for next batch of sieves.
8. MAKE SURE THAT SIEVES START OUT DRY! Use round sieve brush to clean top and bottom area of dry sieves before sprinkling on new aggregates.
9. At the end of the day, put trays into the 105° C oven to dry overnight. Complete analysis 12-24 hours later.
Disinfect any laboratory equipment as well as any surfaces that come in contact with quarantined soil.

VI. Weighing rocks and slaked soil:

1. After drying to constant weight in the 105° C oven, remove trays from the oven one at a time.
Place into bin.
2. Weigh filter and aluminum can together on the analytical balance, record weight in the “filter and oven dry soil and taco can” column of the Wet Aggregate Stability data sheet (Table 1 below).
Weigh sub-samples on a weigh boat on top of a sheet of velum to catch crumbs.
3. Weigh aluminum can with stones, record weight in “sand can and oven dry stones” column of the Wet Aggregate Stability data sheet (Table 1 below).
Weigh sub-samples on a weigh boat on top of a sheet of velum to catch crumbs.
4. Discard filter and stones into compost bucket after they are weighed and recorded.
Discard sub-samples into autoclave bags and autoclave as soon as possible. Bleach any laboratory equipment as well as any surfaces that come in contact with quarantined soil.
5. Continue with all filters and cups in tray and repeat for other dried trays.
Discard sub-samples into autoclave bags and autoclave as soon as possible. Bleach any laboratory equipment as well as any surfaces that come in contact with quarantined soil.

VII. Data Collection Sheet and Calculations:

After all data is recorded on the Wet Aggregate Stability data sheet in the laboratory, transcribe into an Excel spreadsheet (Table 1 below). In each set of 16 samples that are run, sample 16 is a soil standard which is run as a check or quality control. Columns are dedicated to identifying the samples and their locations during the testing. Other columns list the sieve used and the tare weight of the sieve that occupies each location. Record the collected laboratory data into the blank cells (columns 2-5) in each row for the automatic

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calculation of percent stability of soil aggregates. Note that column 5 will be completed before testing. Columns 6 and 7 are completed during the data analysis stage from a saved file of tare weights of the aluminum cans. Highlighting the cell in column 8 of each row in an Excel table would reveal the formula used in the calculation:

Percent Stability of Aggregates =

$$\frac{((\text{initial dry material on sieve (g)} - \text{sieve wt (g)}) / (1 + \text{thimble theta M})) - (\text{dry rock and organic material in can (g)} - \text{can wt (g)})}{((\text{initial dry material on sieve (g)} - \text{sieve wt (g)}) - (\text{dry filter and failed soil (g)} - \text{filter wt (g)} - \text{can wt (g)}))} * 100$$

$$\frac{((\text{column 2} - \text{column 1}) / (1 + \text{column 6})) - (\text{column 4} - \text{column 7}) - (\text{column 3} - \text{column 5} - \text{column 6})}{((\text{column 2} - \text{column 1}) - (\text{column 4} - \text{column 7}))} * 100$$

Percent Stability of Aggregates =

$$\frac{((\text{column 2} - \text{column 1}) / (1 + \text{column 6})) - (\text{column 4} - \text{column 7}) - (\text{column 3} - \text{column 5} - \text{column 6})}{((\text{column 2} - \text{column 1}) - (\text{column 4} - \text{column 7}))} * 100$$

$$((\text{column 2} - \text{column 1}) - (\text{column 4} - \text{column 7})) * 100$$

Table 1. Wet Aggregate Stability datasheet and calculator

WET AGGREGATE STABILITY Data Sheet
(2 - 0.25mm diameter crumbs)

DATE:
2016

Operator:

sample ID	Sample number	dry crumb TRAY	Sieve ID	Sieve tare Wt (g)	Sieve+ Dry Soil wt (g)	column 2		column 3		column 4		column 5	column 6	column 7	column 8	column 6	
						spoon on	dry taco	filter + oven dry soil + taco can (g)	sand can + oven dry stones (g)	filter wt.	drying tray ID						z can taco can ID
MM1	1		1	354.2													
MM2	2		2	542.6													
MM3	3		3	350.4													
MM4	4		4	351.6													
MM5	5		5	343.4													
MM6	6		6	343.8													
MM7	7		7	542.1													
MM8	8		8	353.3													
MM9	9		9	539.5													
MM10	10		10	545.9													
MM11	11		11	541.4													
MM12	12		12	543.3													
MM23	13		13	347.0													
MM24	14		14	358.4													
MM25	15		15	347.7													
QC	16		16	344.0													

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Figure 1. Wet Aggregate Stability Test of Soil Structure in the Cornell Soil Health Assessment. 2009. Robert R. Schindelbeck (rrs3@cornell.edu), Bianca N. Moebius-Clune , Omololu J. Idowu, Harold M. van Es

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Wet Aggregate Stability Test of Soil Structure in the Cornell Soil Health Assessment

Robert R. Schindelbeck (rrs3@cornell.edu), Bianca N. Moebius-Clune , Omololu J. Idowu, Harold M. van Es



Wet Aggregate Stability: Physical parameter used in the Cornell Soil Health Test (<http://soilhealth.cals.cornell.edu>) to quantify soil slaking potential, surface sealing, water infiltration, erosion, and soil workability. Useful in evaluating effects of soil management on soil structure (tilth).

- Procedure:**
- Soil sample dried at 40C, collect crumbs > 0.25mm, < 2mm
 - Spread ~30g material onto soil sieve with 0.25mm mesh
 - Uniform water drops of 4mm diameter delivered from 0.5m height at a constant rate of 1.25cm/ 5mins. Total energy delivered to crumbs is 2J
 - Failed soil is collected in paper filter, dried. Sand particles are washed from sieve, calculate % dry weight of intact sample crumbs

Measuring effects of varied soil management on identical soil

Williamson silt loam

Soil structure is affected by different soil management. The Wet Aggregate Stability is one indicator of soil functional behavior in the Cornell Soil Health test.

**Moldboard plow
Intensive cropping**

Long-term sod



CORNELL SOIL HEALTH TEST REPORT				
Moldboard plow Intensive cropping Williamson silt loam				
	Indicators	Value	Rating	Constraint
PHYSICAL	Aggregate Stability (%)	18.0	19	erosion, infiltration, rooting
	Available Water Capacity (mm)	8.13	20	water retention
	Surface Hardness (psi)	95	51	
	Subsurface Hardness (psi)	360	23	Subsurface Pan/Deep Compaction
BIOLOGICAL	Organic Matter (%)	1.6	5	energy storage, C sequestration, water retention
	Proteins/Polysaccharides Carbon (%)	260	3	Soil Biological Activity
	Plant Available Nitrogen (ppm)	6.2	21	N Supply Capacity
	Soil Health Rating (0-10)	2.0	25	
CHEMICAL	pH (see CNM Report)	6.6	100	
	Extractable Phosphorus (see CNM Report)	30.0	44	
	Extractable Potassium (see CNM Report)	158.0	100	
	Minor Elements (see CNM Report)		100	
OVERALL QUALITY SCORE (OUT OF 100)		51.3	Low	
Soil Tested @ Date: 10/10/09 SAND (%) 86.8 SILT (%) 11.8 CLAY (%) 1.4				

CORNELL SOIL HEALTH TEST REPORT				
Long-term sod Soil-building rotation Williamson silt loam				
	Indicators	Value	Rating	Constraint
PHYSICAL	Aggregate Stability (%)	75.0	100	
	Available Water Capacity (mm)	8.18	66	
	Surface Hardness (psi)	95	82	
	Subsurface Hardness (psi)	151	63	
BIOLOGICAL	Organic Matter (%)	4.8	25	
	Proteins/Polysaccharides Carbon (%)	844	89	
	Plant Available Nitrogen (ppm)	16.8	71	
	Soil Health Rating (0-10)	3.0	75	
CHEMICAL	pH (see CNM Report)	6.4	100	
	Extractable Phosphorus (see CNM Report)	13.0	100	
	Extractable Potassium (see CNM Report)	90.0	100	
	Minor Elements (see CNM Report)		100	
OVERALL QUALITY SCORE (OUT OF 100)		88.2	Very High	
Soil Tested @ Date: 10/10/09 SAND (%) 86.8 SILT (%) 11.8 CLAY (%) 1.4				

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Active Carbon

Purpose and Justification:

Active Carbon (also known as Permanganate-Oxidizable Carbon or POXC) measures the portion of soil organic matter that can serve as a readily available food and energy source for the soil microbial community, thus helping to maintain a healthy soil food web. To measure Active Carbon, soil is reacted with a potassium permanganate solution which has a deep purple color. As the solution oxidizes, it loses some of its color. This loss of color upon reaction is directly proportional to the amount of Active Carbon in the soil sample, which is determined by using a spectrophotometer and calibrated against standards of known concentration.

Background / References:

This method is intended as a rapid and repeatable approach to estimating a labile portion of soil organic carbon, which is sensitive enough to detect different management practices across soil types and climatic zones. An alkaline, KMnO_4 solution oxidizes easily accessible soil carbon fractions, with greater diminishment of purple color – due to conversion of Mn(VII) to Mn(II) – corresponding to increasing concentrations of “active carbon”, measured as absorbance at 550 nm.

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See:

Weil, R., Islam, K. R., Stine, M.A., Gruver, J.B., Samson-Liebig, S.E. *Estimating active carbon for soil quality assessment: A simple method for laboratory and field use*. Amer. J. Alternative Agric. 18(1):3-17 (2003).

Stiles, Cynthia A., et al. "Validation testing of a portable kit for measuring an active soil carbon fraction." *Soil Science Society of America Journal* 75.6 (2011): 2330-2340.

Objective:

Duplicate soil samples are air dried to constant weight, shaken with 0.02 KMnO₄ solution, allowed to settle, diluted and absorbance measured at 550nm.

Materials and Equipment:

KMnO₄
 CaCl₂
 KOH and HCl
 Distilled H₂O
 50ml Falcon tubes w/ caps
 Falcon tube racks
 Bottle-top solution dispenser
 pH meter and buffered calibration solutions
 Analytical balance (3 digit)
 Colorimeter (w/ 550 nm setting)
 Kimwipes ®
 100-1000 µl pipettor and disposable tips
 Platform shaker
 Stop watch
 Stir plate
 Stir bar
 1000 ml volumetric flasks, beakers and graduated cylinder
 Amber bottle

Procedure:

Preparation of 0.2M KMnO₄ stock solution

1. Dissolve 11.09g CaCl₂ in ~750ml distilled water in a beaker. Dissolve completely, using stir plate with stir bar (final concentration, 0.1M).

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- Note:** CaCl₂ is included in the stock solution as a flocculant agent, which is intended to eliminate the need for centrifugation.
- Add 31.61 g KMnO₄ to the solution and a further ~200 ml of distilled water. Allow to dissolve completely (about one hour), covering solution and stir plate with an opaque box or paper bag.
 - Ensure that the pH meter is properly calibrated.
 - Measure solution pH (final pH should be 7.2).
 - Depending on pH measure, make a dilute (~0.1M) acid or base solution using HCl or KOH. Using a pipettor, slowly add acid or base, while monitoring pH, until constant at 7.2.
 - Pour solution into a volumetric flask and bring to 1000ml with distilled water. The solution is light sensitive - add to an opaque bottle, label and date. Solution should remain stable for 3-6 months.

Standard curve (see **Figure 1 below** for schematic of methodology)

- Ensure that the colorimeter is set to 550 nm and zero with distilled water.
- Dispense 45 ml distilled water into each of three Falcon tubes.
- Add additional distilled water to the tubes in the following volumes:
 - tube 1, 3.75 ml
 - tube 2, 2.50 ml
 - tube 3, 0.00 ml
- Then add 0.2M KMnO₄ to the tubes in the following volumes:
 - tube 1, 1.25 ml
 - tube 2, 2.50 ml
 - tube 3, 5.00 ml

Final concentrations of 50 ml KMnO₄ solutions are now 0.005M, 0.01M, 0.02M. Cap and shake for 10 seconds.
- Dispense 20 ml distilled water into 9 Falcon tubes – three for each standard solution.
- Add 0.2 ml of each standard to each respective triplicate set. Cap and shake for 10 seconds.
- Read and record the absorbance of each triplicate standard, filling the cuvette with one volume of standard and cleaning the outside with a Kimwipe to remove any liquid or smudges before each reading.

Measuring Active Carbon in Soil Samples (see **Figure 2 below** for laboratory schematic)

- Soil samples should have previously been air dried to constant weight (see procedure CSH 01).

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2. Each soil sample is run in duplicate, requiring 2 Falcon tubes with 18 ml distilled water and 2 Falcon tubes with 20 ml distilled water. Samples are generally run in groups of 20 per rack (10 duplicate soil samples).
3. Dispense enough distilled water into Falcon tubes for as many as ten soil samples and set aside.
4. Into Falcon tubes, measure two 2.5 g replicates for each soil sample. (± 0.005 g)
5. Dispense 0.2M KMnO_4 solution into a beaker in small amounts as needed (~50ml volumes) and cover with an opaque container to block light.
6. In sequence, add 18 ml distilled water to each tube containing soil. Then, in same sequence, begin redox reaction by adding 2 ml of 0.2M KMnO_4 to each tube. Cap tightly.
7. Place tubes and rack on the shaker at 120 rpm, start stopwatch and allow to shake for 2 minutes.
8. After 2 minutes (do not stop stopwatch), remove samples from the shaker and 'slosh' solution in tubes to ensure that soil is not stuck to the cap or top of the tube. Uncap tubes. On bench-top, allow settling and reaction to continue for a further eight minutes.
9. After 10 minutes of total reaction time, remove 0.2 ml from each reaction tube and transfer to a Falcon tube with 20 ml distilled water. **Note:** dispensing this 0.2 ml aliquot from the reaction tube into 20 ml distilled water is a 100x dilution; *this ends the reaction.*
10. After all reactions have been stopped, cap diluted sample tubes and shake by hand for 10 seconds.
11. Read and record absorbance as described above.
12. **Note:** Repeat duplicates with a difference in absorbance greater than 5%.
13. Clean all materials (particularly colorimeter cuvette, using distilled water).

Calculations:

The bleaching (loss of purple color; reduction in absorbance) of the KMnO_4 is proportional to the amount of oxidizable C in the soil sample. It is assumed that 1 mol MnO_4 is consumed (reduced from Mn^{7+} to Mn^{2+}) in the oxidation of 0.75 mol (9000 mg) of C.

Standard curve:

Concentration = a + b * (absorbance). Determine the slope (b) and y-intercept (a) of a linear regression equation with concentration as the dependent variable (y) and absorbance as the independent variable (x).

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Active carbon:

Active C (mg/kg) = [0.02 mol/L - (a + b * absorbance)] * (9000 mg C/mol) * (0.02 L solution/0.0025 kg soil).

Where:

0.02 mol/L is the initial solution concentration,

(a + b * absorbance) is the post-reaction concentration,

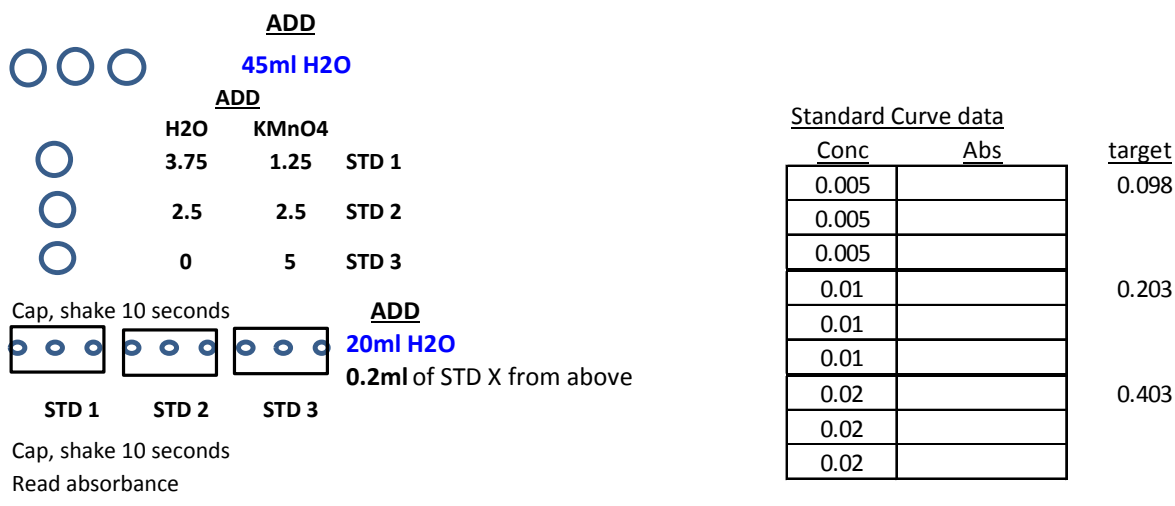
9000 mg of C (0.75 mol) is assumed to be oxidized by 1 mol of MnO₄ changing from Mn⁷⁺ to Mn²⁺,

0.02 L is the volume of KMnO₄ solution reacted, and

0.0025 kg is the weight of soil used.

Figure 1. Schematic of bench top protocol for KMnO₄ dilution for development of a standard curve in the Active Carbon test

Active Carbon Standards Diagram



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Figure 2. Laboratory schematic of Active Carbon testing procedure/data collection

Duplicate readings for each sample (10 samples = 20 tubes) in each rack

ADD

Each tube **18ml H₂O**

2.5g sample (remember to duplicate)

Finish filling all tubes, THEN add **2ml KMnO₄**. Cap quickly. Put rack on shaker.

Start stopwatch. Shake for 2 mins on shaker at 120rpm. Keep stopwatch running.

Uncap tubes, wait 8 more minutes for 10 minute total reaction time.

WHILE SHAKING, Prepare 20 more tubes with **20 ml H₂O**

End reaction by adding **0.2ml of reaction solution** to tube with 20 ml H₂O.

Just rinse pipette tip between samples.

Cap diluted sample, shake 10 seconds, read absorbance.

<u>ID</u>	<u>Rep</u>	<u>Abs.</u>
	1	
	2	
	1	
	2	
	1	
	2	
	1	
	2	

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Available Water Capacity

Purpose and Justification:

Available water capacity reflects the quantity of water that a disturbed sample of soil can store for plant use. It is the difference between water stored at field capacity and the wilting point, and is measured using air pressure chambers and ceramic pressure plates. In the field, a soil is at the upper end of soil wetness when water that it cannot hold against the force of gravity has drained – this is called ‘field capacity.’ The lower end of the range is referred to as the ‘permanent wilting point,’ which occurs when only hygroscopic water remains (i.e., water tightly held so tightly onto soil particle surfaces that it is inaccessible by plant roots). Available water capacity is determined from measuring the soil’s water content at both field capacity and permanent wilting point in the lab and calculating the difference.

Background / References:

Reynolds, W. D., & Topp, G. C. (2008). Soil water desorption and imbibition: tension and pressure techniques. *Soil sampling and methods of analysis*. 2nd ed. CRC Press, Boca Raton, FL, 981-997.

Scope/Objective:

Soil is air dried and sieved past 2mm prior to placing on a pressure plate apparatus for one week to remove soil water. Each sample has a subsample equilibrated to 0.1 bar (10 kPa) and a subsample equilibrated to 15 bar (1500 kPa).

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

Materials and Equipment:

Pressure plates
Rubber rings
Pressure chambers
Compressor
Pressure regulation system
Analytical balance
Spatula
Wash bottle
Spoon
Bleach/10% Bleach solution in spray bottle

Procedure:

1. Saturate 1 bar plates and 15 bar plates using a wash bottle to deliver water. Place 14 rubber rings on each.
Work inside a large plastic tub to collect any water run-off and bleach water with 10% bleach solution for 30 minutes before discarding. Clearly label throughout test as quarantine with a sign or a large "Q" on samples.
2. Fill a rubber ring on each of two plates (one 1 bar and one 15 bar) with about 15 g of soil (as much as will fit into each ring). Add enough water to the plate to allow soil to saturate itself through suction. Let soils sit until they look fully saturated.
3. Using the back of a spoon, carefully pat the soils on the 1500kPa plate (but NOT on the 10kPa plate), so that they are well packed and saturated.
Bleach any laboratory equipment that comes into contact with quarantined soil with 10% bleach solution for 30 minutes before discarding.
4. Stack pressure plates into the appropriate pressure chamber (three plates, with 42 total samples in each chamber), using plastic cylinders as spacers between plates. Connect outflow tubes of plates to pressure chamber outlet tubes, set pressure chamber outlet tubes into beakers.
Bleach all water in beakers with 10% bleach solution for 30 minutes before discarding water.
5. Tighten lids down using the appropriate bolts.
6. Bring both chambers up to pressure slowly over 2 minutes.
7. Equilibration requires ~1 week.

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8. After equilibrium, the samples are placed in tared moisture cans and weighed.
Bleach pressure plates and any equipment that comes into contact with quarantined soil with 10% bleach solution for 30 minutes before rinsing.
9. These cans are placed in an oven at 105C overnight and weighed again.
Bleach cans with quarantined soil with 10% bleach solution for 30 minutes before rinsing. Discard soil into autoclave bags and autoclave as soon as possible.

Calculations for each subsample which has been equilibrated to 0.1 bar and 15 bar:

Theta M = ((wt. wet soil + can) - (wt. dry soil + can)) / ((wt. dry soil + can) - wt. of can)

For sample X

$AWC_{\text{sample x}} = \text{Theta M}_{0.1 \text{ bar}} - \text{Theta M}_{15\text{bar}}$

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Soil Respiration Protocol

For Quantification of Soil Respiration

Cornell Soil Health Assessment

Daniel J. Moebius-Clune, PhD

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.

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A number of 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 size and 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 CSHA 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 (CO₂), 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. CO₂ 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 CO₂. The properties of the KOH solution change in proportion to the amount of CO₂ trapped. It is this change in properties, or more specifically, the endpoint of this change, that we directly measure. The amount of CO₂ trapped is calculated from the property change, and this is used to estimate the amount of CO₂ 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's capillary 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 CO₂ 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 CO₂ trapped, so given two constants (the EC of 'raw' KOH having absorbed no CO₂, and the EC of a 'saturated' trap solution, were it to have absorbed its full capacity of CO₂) and a sample's measured EC, we can calculate the amount of CO₂ absorbed by the trap. We subtract the amount

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of CO₂ absorbed by a trap sealed in a jar with no soil ('blank'), to account for CO₂ coming from the air, and arrive at the amount emitted by the soil sample following rewetting, during the course of 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.

(See extended note at the end for further details regarding the approach used in these measurements)

Procedure for Respiration Measurement

Materials

Pre-Setup:

- Soil Samples (sieved to 8mm, air dried to constant mass)
- 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)
- Trap assembly (beaker on a pizza stool, see note)
- Blue jar rack (25 place)

Incubation initiation:

- Jar or Beaker with distilled, deionized H₂O (ddH₂O)
- 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

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Marker
Gloves

Post-incubation reading:

Incubated samples in jars
EC meter with Computer
KimWipes and cut strips of filter papers
Gloves

Large (1/2 Gal.) jar for waste KOH

Wash tub with dH₂O for trap assemblies
Trash receptacle for other jar contents

HCl to neutralize KOH
pH test strips
Stir plate and Stir bar

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 a blue 25-place jar rack 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.
- Include 2 replicates per soil sample (2 jars per box of soil), appending 'a' and 'b' to the sample number (e.g. L-123a and L-123b).
- Use labeling tape with sharpie rather than writing on jar, to facilitate jar cleanup.

To a set of several jars for which you have soil samples ready to be weighed

- 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 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 by the back tab, holding with the tips far enough down to pick up the boat without it flexing
 - Gently place weigh boat onto the weighing papers in the jar,

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- 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

Prepare respirometer jars for incubation set up

- Start with a set of jars with soil samples pre-weighed in them
- 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

- Place fresh paper towels on a clean space on the bench, label one "KOH – 9ml" and the other "H₂O – 7.5ml"
- Place a fresh 10ml pipette tip on each of the labelled paper towel
- Draw fresh KOH from the boxed bag into a clean, dry 500ml beaker – label the beaker "KOH 0.5M"
- Draw distilled deionized water (ddH₂O from the deionization column tap) into another clean, dry beaker. Label this beaker "ddH₂O"
- 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.
- To a few jars at a time (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 "H₂O – 7.5ml" to the large pipette
 - Set volume to 7.50 ml
 - Draw and dispense 7.5 ml ddH₂O into each jar.

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- 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 CO₂ 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 to the blue rack, avoiding tipping the trap assemblies inside.
- Set blue rack on shelf, with a note (tape sticks around better than a post-it) 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. Do not set aside and use for further traps, other than those set up in an immediate tandem run. CO₂ absorption from the air, and evaporative concentration of the solution make it inadvisable to reuse this. Whatever you do, don’t put it back in the stock.
- Incubate 4 days
 - Avoid major temperature fluctuations – particularly noting if there is a planned stem shutdown or hood shutdown planned, and avoid having incubations running during these times. Definitely don’t have incubations running on the campus-wide no-steam days that happen each year. The cold temperatures in Bradfield will have a large effect on the respiration rate of samples.

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 ddH₂O for final cleaning of EC probe. Label these beakers.
- Gather paper towels, kimwipes, and several filter papers’ worth of strips cut from filter papers with scissors, to use for blotting (not wiping) the probe off between samples.
- Ensure that computer is on and logged in, and that Orion EC meter is connected and on.

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- 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.
- 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 and use a filter paper strip to blot KOH off of the metal temperature probe stud in the probe opening. Blot, don’t wipe.
- 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, 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. Check again.
- 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 of 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 kimwipe and filter paper strip 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.

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- 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 and triple 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 'redos'. 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 (Bob).

Cleanup

- 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.
- 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 ddH₂O. Dry on clean drying rack or pegboard. If any residues

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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.

Calculation

Calculation of respiration values for samples, including determination of CO₂ 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 in BF822.

To calculate respiration rate manually:

We know the trap capacity for CO₂ absorption based on its volume and concentration. 0.5 M KOH can absorb sufficient CO₂ to become 0.25 M K₂CO₃, when fully saturated (in practice, the absorption would likely become fairly 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 CO₂ (which, when trapped, accumulates as CO₃²⁻). So 9 ml of 0.5 M KOH can accommodate

$$0.009 \text{ L} * 0.25 \text{ mol/L} * 44.01 \text{ g/mol} * 1000 \text{ mg/g} = 99.025 \text{ mg CO}_2$$

Of this total (theoretical) trap capacity of 99.025 mg CO₂, some fraction is actually 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 K₂CO₃) if the trap were saturated, which is actually 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 CO₂ absorption that is actually 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

and EC_{sat} is the electrical conductivity of 0.25 M K₂CO₃

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 CO₂ absorption that is actually used ,

then

$$((\text{EC}_{\text{raw}} - \text{EC}_{\text{sample}})/(\text{EC}_{\text{raw}} - \text{EC}_{\text{sat}}))=P$$

and

$$P * (\text{trap capacity in mg}) = \text{amount of CO}_2 \text{ in mg absorbed by the trap in question.}$$

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For each sample's trap, the amount of CO₂ absorbed must be adjusted to subtract out the amount of that absorbed CO₂ 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 CO₂ released from the soil samples.

In addition, multiple reps of each sample should be run, and the values averaged. If these differ from each other by more than a set threshold (we use an average deviation of 5% from the common mean), then the sample should be rerun.

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

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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. Don't push hard enough to justify calling it that – it won't 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
 - This needle is the type typically found in an introductory biology dissection kit, with a straight plastic handle and a steel needle with sharp point
- 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

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- 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 0.5N STD SOL 4L. VWR Cat# AA35595-K7
 - Recommended: POT HYDROX 0.5N STD SOL 20L. VWR Cat# AA35595-M4
- 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 the CU Chemistry Stockroom
- Litmus paper
 - BDH PH TEST STRIP UNIVERSAL RANGE 0-14. VWR Cat# BDH35309.606
- K₂CO₃
 - 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.
 - K₂CO₃ is generally available at the CU Chemical Stockroom, or can be ordered from Sigma, VWR, or Fisher.

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- 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

Soil microbial activity is central to a number of 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 fairly 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. In order for the materials added that are

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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

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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) by the use of 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 fairly 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 actually 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'.

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Protein Protocol

For Extraction and Quantification of Autoclaved-Citrate Extractable (ACE) Soil Proteins
 Cornell Soil Health Assessment
 Daniel J. Moebius-Clune, PhD

Purpose and Justification:

The purpose of measuring soil proteins is essentially twofold. Firstly, as an index of the size of a pool of organically-bound N in the soil, consisting of the major compound class of soil organic matter derived from expected biomass inputs that is likely to contribute to N mineralization and subsequent plant uptake. Secondly, as a general indicator of the (re-)coupling of C and N cycling in the soil ecosystem. Of the major compound classes that make up the biomass contributing to the soil organic matter, protein is likely to contribute to the immobilization storage and remineralization of plant-available N, as it is present in abundance, is relatively high molecular weight and stability, enzymatically degradable by a wide variety of microbes, with a

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C:N ratio that is low relative to numerous other compound classes in the same mixed materials. N cycling in systems with a healthy active soil biological community is strongly coupled to C cycling. The microbial activity in these systems is fueled by energy stored in organic compounds, which, when broken down by microbial activity, release N if the C:N ratio is sufficiently low. The organic N stored in such systems is to a large extent stored in the microbial biomass, much of it as protein. The presence of a substantial fraction of the soil N in protein form indicates that the processes involved in the storage and release of N in organic form are functioning.

Objective:

The objective of this procedure is to extract protein from the organic matter in soil samples using a neutral sodium citrate buffer to disaggregate soil and dissolve soil protein with high heat and pressure in an autoclave, and to quantify the protein content of such an extract using a bicinchoninic acid protein assay. The extraction procedure used is a modification of an approach used to extract proteins from fungi and from soil (Keen & Legrand 1980, Wright & Upadhyaya 1996) which has been shown to extract proteins of numerous sources. The quantification assay used is a well-established procedure and chemistry, run at high temperature for an extended time to increase protein sensitivity and decrease variation by protein type (Walker 2002).

Summary of Approach

In this procedure, proteins are extracted from soil samples in a sodium citrate solution adjusted to neutral pH with citric acid. The sodium and citrate ions contribute to soil dispersal, which is further achieved through mechanical agitation by shaking of the mixture. Soil is disaggregated or slaked and the soil organic matter particles are exposed to the extractant solution. The mixture is exposed to high heat and temperature by holding in an autoclave at 121 °C to further solubilize proteins. After cooling, aliquots of the mixture are clarified by centrifugation, to settle soil particles. The concentration of dissolved proteins in the clarified extract is determined by reaction in a bicinchoninic acid assay at 60 °C, and quantified, against a bovine serum albumin (BSA) standard curve, by colorimetry using a 96-well spectrophotometric plate reader.

References:

- Keen N.T. and Legrand M. 1980. Surface glycoproteins - evidence that they may function as the race specific phytoalexin elicitors of *Phytophthora megasperma* f. sp. *glycinea*. *Physiological Plant Pathology* 17: 175-192.
- Walker J.M. 2002. The bicinchoninic acid (BCA) assay for protein quantitation. In: Walker J. M. (ed), *The Protein Protocols Handbook*. Humana Press, Totowa, NJ.
- Wright S.F. and Upadhyaya A. 1996. Extraction of an abundant and unusual protein from soil and comparison with hyphal protein of arbuscular mycorrhizal fungi. *Soil Science* 161: 575-586.

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Procedure for protein extraction and quantification:

Materials:

Soil (air-dried, sieved to 8mm)
Glass extraction tubes with caps
Extraction buffer (20 mM sodium citrate, pH 7.0)
Microcentrifuge tubes
Storage tubes in racks
Transfer pipettes
Pipettors and Tips
(1000 ul ClipTip pipettor, large and small volume 8 channel ClipTip Pipettors)
(1000, 200, 20 ul tips in boxes)
Pipetting reservoir
96-well clear flat bottom chimney well polystyrene plate
Tape seal for plate
BCA reagents A and B
Standards set
50 ml tube for mixing working reagent

Method

Part 1: Extraction

- Weigh soil into tubes
 - Label tubes in advance. Weigh out two reps for each sample. Label these by appending 'a' and 'b' after the sample designation. Use labeling tape rather than marking straight on the glass tube
 - Weigh 3.00 g air-dried soil onto clean weigh paper
 - Transfer to glass extraction tube
 - Curling the paper into a funnel, and tapping on the back of it with a fingernail several times should leave no measurable mass of soil behind on the paper. You can use the same paper to weigh out a second rep of the same soil sample, which gives an opportunity to check to make sure that the paper weighs 0.00 g when put back on the balance (no measurable amount left on the paper). But use a clean weighing paper for each new sample.
 - Cap tube gently
- Add extractant
 - Add 24.00 ml extractant (20 mM sodium citrate, pH 7.0), using the bottle top dispenser

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- Dispense two or more times into a waste beaker to prime the dispenser. Make sure no bubbles are in the dispensing tube, as these will impact volume dispensed. Dispense additional aliquots into the waste beaker if there are bubbles or other volume-impacting issues with the dispenser. Waste an aliquot or two rather than dispense a poorly measured aliquot into a tube with a sample.
- Always have the next tube under the spout when drawing the dispenser plunger up, as a small part of the volume comes out when you hit the top. This is factored into the volume setting for the dispenser. Make the draw up and the dispensing push down smooth and not stuttered as this will affect the volume dispensed. Likewise ensure that your fingers are not in a position to be trapped under the plunger as it is pushed down. Drawing up with thumb and middle finger on the sides of the plunger cap, and gently keeping pressure on the plunger as it descends with your index finger works best.
- Cap tubes tightly after adding extractant
- Shake to slake
 - Place an inverted empty rack over the filled rack of tubes, and fill the small gap above the filled, capped tubes with a stack of several paper towels and a rectangle of corrugated cardboard. Hold the racks to each other with several rubber bands.
 - Place the double rack on the shaker rack assembled to hold them on the shaker bed. This rack has threaded rod sides, and can accommodate two racks of 24 extraction tubes. Use additional rubber bands to hold the threaded rods snugly against tube racks.
 - Shake at 180 rpm for 5 min
 - Remove from shaker, and swirl mixture to consolidate solids
 - Extractant and soil may be left on the sides and around the collar of the tubes following shaking. With the caps on the tubes, and holding the rack upright, swirl once or twice rapidly, with a slight lift to part of the swirling motion. This should wash these trailing amounts of soil back down into the extractant in the bottom of the tube, consolidating the contents.
 - Remove the rubber bands and upper rack.
 - Loosen caps so they are not airtight, but still placed on tube to protect contents
 - Unscrew the caps while holding down on the top with one finger, until the cap snaps down into place level after climbing the tube threads. This should ensure that the caps are **fully** disengaged (needed to avoid any pressure differential inside and outside of the tubes in the following, autoclave, step). Leave disengaged caps on top of tubes.
- Autoclave 30 min
 - Preheat autoclave while samples are shaking

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- Add dH₂O to autoclave chamber, to marked line, making sure that the drain valve is off
- *Leaving autoclave open*, turn on the heaters by turning the timer knob past the 5 minute mark. Do not preheat autoclave with door shut, or it will have to cool and vent before the door can be reopened.
- Turn off preheating before placing racks in autoclave, to avoid splattering water
- When ready to autoclave extraction tubes, place two racks of tubes into autoclave, on metal tray, not touching sides. Tray should be well supported by rails that are built into the autoclave. If the tray seems poorly supported by rails, adjust it prior to placing racks of tubes on it.
- Close autoclave door securely, paying attention to the gasket seating correctly, and by pivoting lever, rather than pushing, to avoid moving autoclave on bench.
- When door is securely closed, turn knob to the 35 minute mark (timing empirically determined to expose to full temp for just 30 min)
- Be certain that the vent/exhaust switch is in the slow/liquid position, as fast exhaust would cause severe boil-over. This switch should remain taped in this position, as the autoclave we use is dedicated to this extraction, and is not used routinely for dry materials sterilization.
- When the pressure gauge reads zero, and the temperature gauge reads 100, open the autoclave door, taking care to avoid the steam which will come out. Wear leather protective hot gloves. Autoclave surfaces are very hot and the steam can burn exposed skin quite badly.
- Remove racks gently and slowly, taking care to not jostle caps loose or bump sides of autoclave.
- Set aside to cool to room temperature before clarification

Part 2: Clarification

- Label, ahead of time, a set of 2.2 ml microcentrifuge tubes, and one or more racks of sample storage tubes (1.1 ml open top tubes, in strips of 8, racked in 96-place format) to accommodate the sample range to be clarified.
- Close caps on glass extraction tubes again, and resuspend solids by shaking for 1 min, then swirl to consolidate as above. Loosen or remove caps.
- Withdraw approximately 1.75 ml of mixture using a disposable plastic transfer pipet, and place this in a clean, *labeled* 2 ml microcentrifuge tube. Close microcentrifuge tube cap.
- Use fully labeled tubes because of high likelihood of sample order mix-up in these steps, moving from one rack format to another!

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- Place labeled microcentrifuge tubes in microcentrifuge rotor slots, distributing to ensure rotor balance if less than a full rotor
- Place lid on microcentrifuge rotor, and close centrifuge cover.
- Centrifuge at 10,000 xg for 3 min (make sure that the settings are for 10k gravities, not 10k rpm – these are quite different)
- Gently remove tubes one at a time, and transfer 1 ml of the cleared extract liquid layer to a storage tube (microtiter tube) in a 96-well format rack, using a 1000 ul pipettor with a new, clean, tip. Avoid dislodging the pellet of solids at the bottom of the tube.
 - See additional notes about pipetting
- Be certain to keep good track of placement of tubes in this rack, and orientation of 8-tube strips. Label the first tube of the strip to ensure this.
- Place clean cap strip on tubes, keeping the orientation of the tab on the cap strip facing the same direction each time.
- Set aside rack with tubes in refrigerator overnight if not quantifying on the same day.

Part 3: Quantification

- Remove sample tubes in 96-well format microtiter tube racks from refrigerator, as well as rack with standards, also in microtiter tube strips.
- Allow tubes to equilibrate with room temperature before quantifying
- Ready a reaction plate, inspecting bottom to avoid scratches, and avoiding getting plate dusty. Dust or fibers or hairs will interfere with quantification
- Preheat the heat block to 61.5 degrees, but without turning on the timer. Check to be sure there is no debris in the heating block that could scratch the bottom of the plate or prevent it seating well. This should remain covered generally.
- Make ready a plate sealing tape sheet pad, and the plate sealing roller, before filling the plate
- Prepare the BCA working reagent in a 50 ml ‘Falcon’ tube. Make enough for 200 microliters per well, plus enough extra ‘for the reservoir’ and to avoid pipetting bubbles. 20 ml is just barely enough if you’re quite steady with the pipettors, 25.5 ml is a safe and convenient amount.
- The working reagent is a 50:1 mixture of two parts: A, a clear reagent mixture in a larger bottle, and B, a blue-green copper sulfate solution. For 25.5 ml put 0.5 ml (500 microliters) of the blue-green reagent B into a falcon tube or clean small beaker, and then add 25 ml of reagent A to it, and stir or swirl to mix. A cloudiness that appears initially and then dissipates is normal. Make sure the parts are well mixed, and avoid getting dust or fibers into the liquid. Set this aside, covered, while preparing the plate with samples and standards

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- Remove strip caps from tubes with standards (0, 125, 250, 500, 750, 1000, 1500, and 2000 micrograms per milliliter BSA), being careful not to splatter any. A small droplet of carryover would have a very large effect on all further use of these standards. This bears repeating – remove caps gently, and with a kimwipe or other protection, to avoid any splatter or droplet carryover between tubes of the standards
- Using the 8 channel, small volume, multichannel pipettor and the ClipTip size 20 tips, pipette 10 microliters of the standards into the first column of the reaction plate. Using a new set of tips, draw another 10 microliters and place in the 7th column (just past the middle) of the plate. Dispense this droplet slowly, and carefully, onto the bottom of the wells, at the edge of the sidewall, maintaining contact with the side of the plate. **DISPENSE SLOWLY!** (see additional notes on pipetting) This is a small volume to pipette precisely, and precision is important here, so practice with water and a practice plate if necessary before doing this part.
- Recap standards with new cap strips and set aside.
- Change to new gloves before opening samples.
- Uncap samples with care, and pipet samples into the available columns of the plate. Keep careful track of which samples are where on the plate, and go in order.
- Pipet two replicate columns of each strip of 8 sample tubes into the plate wells. The best arrangement for this is to have the plate's left and right halves be copies of each other. Columns 1 and 7 should be standards, columns 2 and 8 should be the same, columns 3 and 9 the same, and so forth. Our general practice is to use two reaction replicates per extraction, with two extractions per soil sample. So 4 wells on the plate will represent each box of soil.
- When all samples (and standards) have been placed in the appropriate wells of the reaction plate, recap the samples and set aside
- Retrieve the pre-mixed working reagent, and transfer it to a clean, dry, multichannel pipettor reservoir
- Using the larger volume multichannel pipette and the 200 microliter tips, add 200 microliters of working reagent to each well of the reaction plate. Pipette onto the sidewalls of the well, sufficiently below the top edge as to not spill, but up away from the filling liquid level in the well. Keep the tip of the tips gently in contact with the sidewall of the wells, and pipette gently and smoothly and **SLOWLY**. The swirling action should mix the liquids in the wells, but if you pipette too vigorously, it will splash, making the plate unusable. Further mixing will happen naturally while the reaction is heating, due to convective motion in the wells.
- When the plate is filled, seal with a tape seal, using the roller to press the sealing tape to the well tops as well as upper surface of the plate. After this use your thumbs to make sure the seal is sound. Don't let the plate skid around on the surface of the bench as you do this or other step, as this could scratch the bottom, interfering with the optical clarity.

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- When plate is sealed, place gently in heat block and cover.
- Start timer, which should be pre-set for 60 min.

To Read:

- When plate has incubated for 60 min, gently remove from heat block and place on benchtop to cool for at least ten minutes undisturbed.
- Turn on plate reader and computer, make sure plate reader is plugged into computer with USB cable. Start the plate reader program (Gen5) on the computer, and see that it is detecting the plate reader attached (this should happen automatically).
- Select the appropriate protocol from the Gen5 menu, to read the plate at 562nm wavelength without any shaking or other special effects.
- When plate has cooled, ensure that the sealing tape is well in place still, using you thumb or the roller or both. Invert and re-right the plate to incorporate the droplets which will have collected on the tape seal. Be gentle.
- Carefully remove the tape seal without letting the plate be jarred by the motion. Hold it securely by the plate edges or skirt and smoothly peel back the tape without letting droplets cross contaminate wells.
- Place plate in tray of plate reader
- Click the read plate button. The tray will automatically retract.
- When plate is done being read, save the file generated by the plate reader software, and say yes to the question about exporting to excel. Save the excel document newly created with an informative name including the sample range quantified on the plate.
- Remove plate from tray of reader, and set aside to dry in hood. If this is the last plate for the day, press the small black button to close the tray, and power down the reader.
- *Do not close the reader and power it down with a plate in the tray!*
- Open the protein quantification workbook template, answer the questions relating to sample range and replicates and standards, and copy and paste (paste values only) readings from the auto-generated excel sheet into the appropriate location in the calculation workbook.
- Save a copy of the calculation workbook.
- Copy and paste (values only) into the protein data repository. Save.
- Note any samples for which the concentration was too high to calculate (these will need to be re-quantified after an aliquot is diluted to quarter strength), or those for which extraction reps deviate by too much (these will need to be re-extracted). Don't include values in the protein data repository for these 'redo' samples. Make a note of these and include them with the next batch of extractions.

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Calculation

Calculation of protein concentrations from A_{562} readings in 8x12 (RxC) format from the plate reader output (or for values input individually for readings from single channel spec with flow-through cuvette), is handled automatically using the auto-calculation workbook on the computer in 822 Bradfield, including handling of variable number of reaction reps, standard sets, and in-run check samples, 2nd order standard curve calculation, replicate averaging and deviation checks.

To calculate protein concentrations manually from absorbance (A_{562}) readings, follow the process outlined below:

Separate out standards readings from samples, placing them in different columns in an excel worksheet.

Arrange standards concentrations in the left most column of a block of columns, stacked into a single column (concn), regardless of the number of replicate sets. In the column to the right of this, calculate the square of the concentration value ((concn)²). In the third column, copy the appropriate absorbance values.

Use an array formula (linest) to obtain coefficients for the parabolic (second order) regression line of best fit. The "LINEST" formula will do this if entered the correct way. Label three empty consecutive columns a, b, and c. Drag to select a 3 x 3 block of cells below these labels, and while these are selected (they should be highlighted in grey) type in the formula =linest(While typing, excel should allow click-and-drag selection of Ys and Xs for the formula. Select the absorbance values for Ys, as a column of values only (leave the header unselected), type a comma, and the prompt should now ask for 'known Xs'. Select as a block both the 'concn' and 'concn²' columns' values (values only, leaves the headers unselected) for Xs. The last two arguments to LINEST() should be TRUE and TRUE. After typing the closing parenthesis, hold down CTRL and SHIFT while pressing ENTER. This enters LINEST as an array formula, rather than as a regular formula, allowing the output to span an array of cells rather than a single cell. The a, b, and c values for the parabolic best fit line should now show up in the first row of the array output. These are the coefficients for the line $y=ax^2+bx+c$, where $y=A_{562}$ and $x=concn$. The r^2 value should be displayed in the 1st column of the 3rd row of the output array. If this r^2 value is greater than or equal to a set threshold for standard curve quality (say, 0.99), use this equation to calculate protein concentrations from observed absorbance values, otherwise, re-do the reaction plate (after checking that data were input correctly).

Provided that the standard curve is acceptable, use the a, b, and c coefficients in an excel formula to calculate protein concentration from absorbance, using the quadratic formula.

The excel formula below is one way:

=IF(H2="", "", (((-1*\$K\$3)+(SQRT((\$K\$3^2)-(4*\$K\$2*(\$K\$4-H2)))))/(2*\$K\$2)))

In the above formula, column H contains Absorbance values, and the focus is currently on row 2 (hence the reference reads H2). Cells K2, K3, and K4 contain coefficients a, b, and c, respectively.

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We average absorbance values for multiple reaction reps of the same extract, prior to calculating protein concentration, and average concentration values across replicate extracts of the same soil sample. If the relative average deviation of replicates from their mean exceeds 5% the sample is flagged for re-running.

Reagent preparation

We mix 20 L at a time of 20 mM sodium citrate pH 7.0

Prepare the 20 L carboy by emptying it and rinsing it thoroughly. Make sure the final rinses are with distilled deionized water, and that the spigot as well as the inside of the carboy are well rinsed.

In a clean, dry, glass 1 L beaker, place an appropriately sized stir bar.

Add 115.19 g Tribasic sodium citrate dihydrate (m.w. 294.10), and 1.603 g citric acid.

Gently add ~ 500 ml ddH₂O, stirring at moderate speed.

When salts are fully dissolved, pour solution into carboy, being sure to retain the stir bar with the beaker (use another magnet held outside the beaker to hold back the stir bar).

Rinse the beaker three times, with ddH₂O to full, with the stir bar in it, into the carboy, to ensure complete transfer of the citrate salts

Add ~10 L more ddH₂O, then cover and agitate the carboy to mix contents. Fill the rest of the way (to 20 L total), using ddH₂O, until the water level just reaches the line at the top of the raised panel of graduation marks on the front of the carboy. Cover again and agitate to mix.

Draw off a gallon through the spigot into a clean jug, and pour back into top of carboy, repeat several times

An alternative approach, which would be appropriate if using different water or preparing smaller quantities is to make a solution of strictly 0.020 M sodium citrate, and another of 0.020 M citric acid, and adjust the citrate solution pH to 7.0 using the citric acid solution. The masses above have been empirically determined, given our water purification system, to yield a final pH of 7.0.

When filling 4 L bottles with citrate, or when moving the repeating dispenser between bottles, take extra care to be sure that the glass pieces are well supported, and avoid disturbing the set screw that sets the volume to be dispensed. This has been set carefully by water mass dispensed to ensure accurate delivery of the right volume of reagent.

Expanded Materials List with Notes and Catalog Numbers

- Soil
 - Air-dried to constant mass

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- Sieved to 8 mm
- Weighing papers
 - VWR PAPER WEIGHING 4X4IN PK500, Cat # 12578-165
- Glass extraction tubes
 - “Disposable” Plain Centrifuge Tubes, Glass, Kimble Chase, 50 mL, 29 x 137 mm, VWR Cat # 21020-695, Supplier Part # 73785-50
 - Caps for glass extraction tubes:
 - Black Phenolic Screw caps, with White Rubber Liner, Kimble Chase, size 24-400, VWR Cat # 89046-840, Supplier Part # 75204G-24400
- Racks for glass tubes
 - VWR Epoxy Coated 50 mL Centrifuge Tube Rack, Rack, Cat # 60916-101
- Labeling tape
 - VWR General Purpose Laboratory Labeling Tape, 1/2 IN, Cat # 89097-920
- Sodium citrate
 - Sodium Citrate Tribasic Dihydrate, Sigma Cat # C8532-1KG
- Citric acid
 - Citric Acid, Sigma Cat # 251275-500G
- Rubber bands
- Transfer pipettes
 - VWR Disposable Transfer Pipettes, Cat # 414004-001
- Microcentrifuge tubes
 - 2.2ml tube, flat cap, 500/UN, LPS Cat # L250803
- Pipette tips
 - Clip-Tip 20 Reload Stacks (PK960), VWR Cat # 89348-030
 - Clip-Tip 200 Reload Stacks (PK960), VWR Cat # 89348-034
 - Clip-Tip 1000 Reload Stacks (PK768), VWR Cat # 89348-038
- 96-well plates
 - 96 Well Non-binding Microplate, Greiner Bio-One, Polystyrene, Flat Bottom, Chimney Style Wells, VWR Cat # 89131-676
- Sealing films for plates
 - Qiagen Tape Pads, Cat #27112600
 - Roller for sealing tape: VWR Cat # 60941-118
- ‘Falcon’ tubes
 - Polypropylene flat capped centrifuge tube, VWR Cat # 89004-364
- Multichannel reservoirs
 - BTX RESERVOIR 25ML PS WHT ST, VWR Cat # 89511-196
- BCA reagents A and B
 - Pierce BCA Protein Assay Kits, Thermo Scientific, VWR Cat # PI23225

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- Can order individual parts (Reagents A and B) separately if needed, on the lookup page under the above part number. Fisher also supplies this, filed under the same part number, if needed
- Protein standards
 - Pierce Pre-Diluted Protein Assay Standard Set, Thermo Scientific, VWR Cat # PI23208
 - Bovine Serum Albumin (BSA)
 - Each set comes with 3 mL each of the following seven dilutions: 125, 250, 500, 750, 1000, 1500, and 2000 µg/mL
- Microcentrifuge tube racks for bench
 - VWR 96-Place Microtube Racks, Cat #82024-494
- Microtiter tubes with racks and caps
 - 1.1 ml polypropylene tubes in 96 well format boxes, VWR Cat #89005-584
 - Caps in 8-strips, VWR Cat # 89005-724
- Block Heater
 - VWR Block Heater, Cat # 12621-088
 - Block for 96-well Plates: VWR Cat # 13259-295

Additional Supplementary Notes and Commentary on Purpose and Approach

Purpose

The purpose of measuring soil proteins is essentially twofold. Firstly, as a direct measurement of a desirable compound class, and secondly, as an indicator of important processes. Organic matter in the soil is made up of a numerous compounds, derived from a number of sources. Plant residues which are left behind after harvest, such as roots or stover, cover crops, green manures and mulches which are either grown in place or brought in as amendments, and manure, composted, and other organic materials added primarily for their nutrient content, all contribute to the accumulation of organic matter in the soil.

Much of this material is plant biomass, with additional contribution from fungal and bacterial biomass, and in the case of manures, digestive residues from animals. In addition, soil microbes and soil dwelling micro- and meso-fauna contribute biomass to the pool of organic matter in the soil when they die, leaving their bodies or cellular debris behind.

A large fraction of the dry matter in these residues is cellulose, which may contribute to the accumulation of soil organic matter and the storage of carbon in soils, but does not contain N and therefore does not contribute directly to N cycling. Similarly, hemicelluloses and lignin, also found in cell walls, do not contain N or contribute directly to its cycling. However, all cells contain protein, and protein can make up a significant fraction of the organic matter found in soil. Proteins are fairly high in N, relative to other large (high molecular weight) compounds

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contributing to biomass and to the organic matter in soil. Proteins, when broken down by soil biotic activity, can contribute to the available (free, or mineral) N pool in the soil through mineralization. Mineralization in this case happens as organisms consume organic compounds (through extracellular digestion and absorption, or in the case of those higher in the ‘food chain’, by consuming smaller organisms) as both an energy and nutrient supply, and release soluble nitrogenous compounds as a waste product. It is both the relative contribution of C and N in an organic material, as well as the relative quantities of compounds like cellulose and lignin, that serve to determine whether the net effect of incorporation of these materials into the soil organic matter pool will result in net mineralization or immobilization. It is the growing understanding that in natural systems, the N cycling rate is limited by the depolymerization (or breakdown) of high molecular weight N containing biopolymers (primarily protein). We can manage agroecosystems to rely to a greater or lesser degree on nutrient storage in soil organic matter (in contrast with application in soluble low molecular weight form and storage only on the soil’s exchange complex). Much of the sustainable agricultural paradigm rests at least in part on this management objective.

This brings us to the second major purpose in measuring soil proteins, as an index of the coupling of carbon and nitrogen cycling in the system. The storage and release of nutrients in the organic matter and the soil microbial biomass is an important functional process in soil. Where nutrients, particularly N, are stored in the biomass and organic matter, they are present in the system, but are not as readily subject to loss as when they are in a more soluble mineral form. As plants grow in the agricultural system, they can access nutrients from the smaller available pool, over the course of their growth, with this pool being replenished by further turnover of nutrient containing organic matter, which the plants can in fact stimulate through the secretion of root exudates, which encourage the growth and metabolic activity of rhizosphere microorganisms that accomplish the breakdown of the organic matter. This is in contrast with management of nutrients by application of larger amounts of nutrients in the early part of the season in soluble form, and the reliance on sufficient quantities of these nutrients to remain available throughout the plants’ growth. Growing concern for the vulnerability of the latter approach to losses of nutrients through leaching, runoff, and denitrification, along with the understanding that the combined presence and activities of the microbes and organic matter heavily involved and presumably increased by the former approach contributes substantially to not only improved nutrient storage and release functionalities but also to other important soil processes such as aggregation and aggregate stabilization, plant growth support and promotion, and carbon sequestration, argues in favor of management that relies to a greater extent on the former.

If we wish to manage systems with a greater reliance on N incorporation into organic matter and subsequent release from that organic matter, then we are seeking to couple N cycling with C cycling in that system to a greater degree. We describe elemental cycles in ecosystems through stocks and flows, or pools and fluxes. Where N is stored in organic form, it is (by definition)

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bound to C, and the compounds that represent the N stock also are a C stock. The fluxes into and out of this pool are biotically mediated, as both the incorporation of N into biomass by plants and microbes and its release through mineralization are metabolically driven activities, necessarily using stored energy. The energy use in these fluxes utilize energy stored in and released from reduced C compounds. The C cycle, as driving force, and the N cycle, are here coupled to each other. This tight coupling of C cycling and the cycling of nutrients that can take an organic form, is a general feature of natural ecosystems, but are de-coupled to a large extent in systems that rely on nutrient storage in mineral form. As we seek to design and manage agroecosystems to better take forms that are modeled after natural systems, we seek to recouple these C and N cycles.

The presence of a substantial pool of organic N in a system materially indicates the process of coupled C and N cycling in that system. As we consider a soil system to be in a healthier state if the process of N storage and turnover in organic matter is functioning well, and as the activity of the microbial community, that is essential for the cycling of N into and out of the biomass pool and soil organic matter, accomplishes numerous functional processes that are necessary for optimal soil functioning, we use the pool of soil proteins as an indicator of soil biological health.

Approach

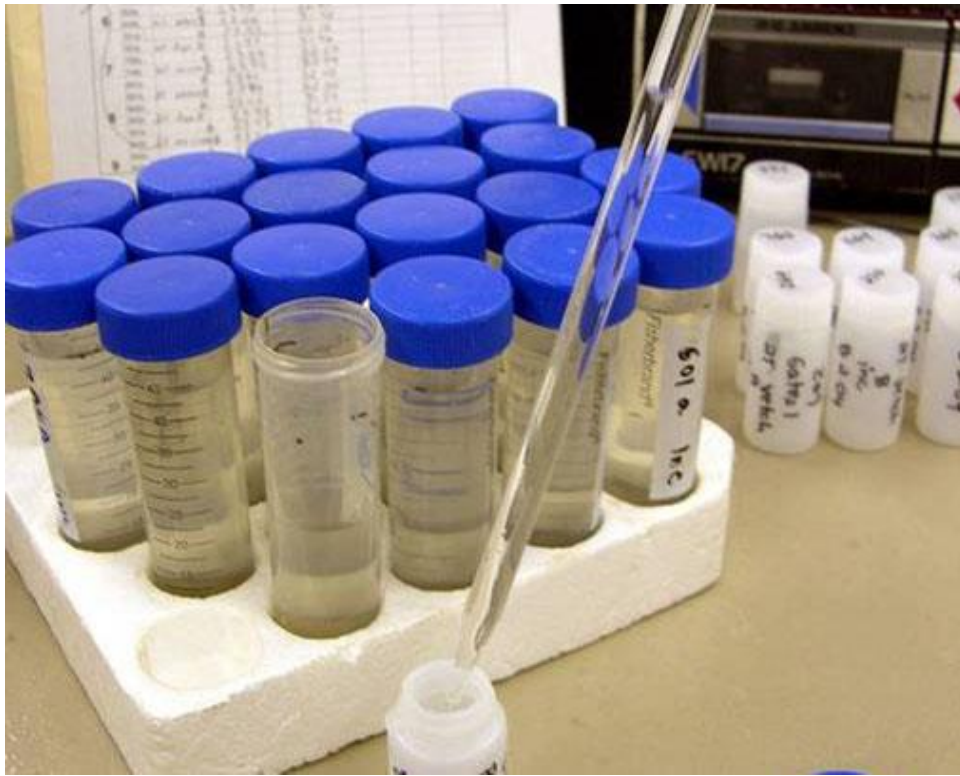
The approach we use to extracting and quantifying soil proteins is extraction in a citrate buffer assisted by shaking and autoclaving, followed by quantification using a standard, widely used chemical assay for protein content, modified to better quantify mixtures of various kinds of protein. The basic extraction approach is a modified form of an approach that has been used by various soil researchers since the mid-1990s. A number of studies have indicated that the amount of protein retrieved in such a way is related to management, and is generally higher where tillage intensity is lower, aggregate stability is higher, and biological activity is high. These studies generally grew out of work tracking the presence and activity of particular fungal groups in the soil, including antibody driven approaches that appeared to preferentially identify biomass of these fungi. While the antibody-driven approach sought specific binding of a monoclonal antibody to antigenic material extracted from these fungi, and from soil in which they presumably were active, a more rapid and straightforward quantification of total proteins in a crude citrate extract was used in most of these studies wither in addition to or instead of the antibody-based quantification. As this extraction process retrieved a substantial quantity of material that reacted with the antibody in use, the quantification of total proteins extracted was frequently taken to be synonymous with or nearly synonymous with (or at least a sufficient proxy for) quantification of the immunoreactive material, and the same terminology was subsequently applied to both pool quantities. However, the quantification of protein concentration in a crude citrate extraction is not specific to any one protein component of the protein mixture extracted. The autoclaved citrate extraction likely works through the enhanced solubilization at highly elevated solvent temperature, with sodium ion enhanced disaggregation

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of soil particles and citrate ion chelation of cationic species that could otherwise counter this. Some improved retrieval of proteins from soil samples using sodium pyrophosphate solution instead has been reported, but for consistency with the larger body of information regarding soil protein pool size and the relationship of this with soil quality, we retain the citrate buffer approach. We found that this extraction protocol retrieves proteins from organic matter sources that are unrelated to the fungal groups these earliest studies were predominantly focused on, and that quantification of total protein in the extracts should be taken as a measure of proteins from numerous sources. The numbers reported in this earlier soil protein work for the total protein on citrate extracts remain comparable to proteins quantified with our approach, but the source of extracted proteins should be considered to be any proteinaceous material in the soil sample, rather than any particular microbial group.

As the protein pool extracted is of a mixed nature, we use a quantification chemistry that has a reduced sensitivity to differences in protein type or amino acid composition. There are numerous standard and widely used protein quantification approaches, and the bicinchoninic acid assay we employ is fairly rapid, and when carried out at elevated temperature quantifies protein by reaction of copper ion with the protein peptide backbone, reaction of the products of this with an indicator compound, and subsequent light absorbance wavelength shift in the reaction solution. This is in contrast with approaches such as the Bradford assay, which quantifies proteins by direct reaction of a dye compound with amino acid side groups, and differentially so with proteins of differing amino acid composition. Other approaches require one or more distillation steps or brief, carefully timed sequential reagent additions, which are less appropriate for the needs of a higher throughput service-lab protocol.

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Add-on Potentially Mineralizable Nitrogen

Purpose and Justification:

This procedure estimates the capacity of a soil to generate plant available mineral nitrogen from the pool of soil organic nitrogen not directly accessible to plant roots. Soil is incubated under saturated, anaerobic conditions, to stimulate microbially mediated mineralization of ammonium-N and inhibit nitrification (microbially-mediated conversion of ammonium to nitrate under aerobic conditions). After incubation, the concentration of ammonium-N is determined.

Drinkwater, L., Cambardella, C.A., Reeder, J.D., Rice, C.W. *Potentially Mineralizable Nitrogen as an Indicator of Biologically Active Soil Nitrogen* in, *Methods for Assessing Soil Quality*, 217-229. SSSA. Madison, WI (1996).

Objective:

Ammonium-N is extracted from a fresh soil health sample and frozen. A duplicate sample is incubated under anaerobic conditions for a one week period after which

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ammonium-N is extracted. Concentration is measured on an auto-analyzer and the difference in ammonium-N concentrations between to the two samples is used to estimate potentially mineralizable nitrogen in the sample.

Materials and Equipment:

Sodium Hypochlorite
EDTA
NaOH
Na₂HPO₄·7H₂O
Na-salicylate
Na-nitroprusside
100 ppm NH₄⁺ solution
Triton x-100
2.0 M KCl
2.67 M KCl
#6 rubber stoppers, electrical tape
Nanopure H₂O
50ml Falcon tubes
N₂ source
Analytical balance, weigh boats, spatulas
Incubator at 37°C
Refrigerator/Freezer
Continuous flow autoanalyzer (Alpkem)
Rotary shaker
Centrifuge
pH meter and buffer solutions
Bottle top, solution dispenser
100-1000 µl and 1000-5000 µl pipettors and disposable tips
100, 500, 1000ml beakers and volumetric flasks
Stop watch
Stir plate

Procedure:

Ammonium-N Extraction and Soil Incubation

1. Prepare two concentrations of KCl solution. Weigh 149.12g KCl and dissolve completely in ~800 ml nanopure water using a stir bar and stir plate. Transfer to a 1000ml volumetric flask and bring to 1L to yield a 2.0 M KCl solution.
2. Prepare a 2.67 M KCl as above, weighing 199.08g KCl/L.

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3. Previously, 2, ~8.0g replicates of fresh soil sample were weighed into Falcon tubes and kept at 4°C upon receiving the soil sample (CSH 01). To one of the replicates, dispense 40ml, 2.0M KCl solution directly into the Falcon tube, cap tightly and place on a rotary shaker at 150 rpm for 1 hour.
4. After shaking, gently ‘slosh’ liquid in tubes to ensure that no soil remains in the cap or top of the tube. Centrifuge tubes for 10 minutes at 1500 rpm, and pour off approximately 20 ml into labeled scintillation vials, taking care not to introduce any floating debris from the Falcon tube, and freeze samples. (NOTE: do not fill vials to the very top).
5. To the second replicate, dispense 10ml nanopure water into the tube, purge the headspace by introducing N₂ directly into the partially capped tube for 45 seconds. Immediately use a # 6 stopper to cover tube, seal with electrical tape, and place in an incubator at 37°C for exactly seven days. (NOTE: preparation of incubation replicates can occur while first set of replicates is on the shaker).
6. After seven day incubation, remove samples from the incubator and add exactly 30ml of 2.67M KCl to each tube, cap tightly and extract in the same manner as the first set of tubes, taking care to label scintillation vials to indicate these samples were incubated. Place in the freezer until ammonium-N determination.
7. If Falcon tubes are re-used, they should be thoroughly cleaned, removing all soil into a separate disposal container, not down the drain. Do not use soap to rinse tubes and caps, but allow to soak for ~1 hour in a 10% HCl solution – be sure to wear proper safety equipment. After soaking, rinse caps, and tubes, along with stoppers, three times with DI water and allow to dry. Store in a clean, dry place before next use.

Calculations and Data Entry:

Dry soil weight determination for each Falcon tube

g dry wt soil = g soil material added to Falcon tube / (1+(g wet soil + can wt/ g dry soil + can wt)). These weights were recorded in the Cornell Soil Health Lab Sample Sign-In Sheet (Figure 1 in Soil Health Sample Intake 2013, CSH 01).

Time = 0

$[\text{NH}_4(\text{mg/L}) * (1000\text{ug/mg}) * (1\text{L}/1000\text{ml}) * 40] / \text{g dry wt soil (Time = 0)}$

Time = 7

$[\text{NH}_4(\text{mg/L}) * (1000\text{ug/mg}) * (1\text{L}/1000\text{ml}) * 40] / \text{g dry wt soil (Time = 7)}$

PMN Rate determination

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PMN (ugN/ g dry wt soil/ week) = ugNH₄/ g dry wt soil (Time = 7) - ugNH₄ / g dry wt soil (Time = 0)

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Add-on Root Health Bio-assay (Root Pathogen Pressure Index)

Purpose and Justification:

Root pathogen pressure is a measure of the degree to which sensitive, test-plant roots show symptoms of fungal disease pressure when grown in a soil sample for a set amount of time under controlled conditions. This assessment is qualitative and reports either the absence or presence of symptoms of damage from a variety of root fungal and oomycete pathogens which can cause major crop retardation or death if not kept in check. The apparent pathogenic pressure is given a rating from 2 to 9, with higher numbers indicating greater levels of pathogen-induced damage. This soil-indexing procedure uses a susceptible snap bean (*Phaseolus vulgaris*) variety 'Hystyle' (Harris-Moran, Modesto, CA seed source) and was developed as a simple method to determine root health or the ability of the soil to suppress root pathogens. Snap bean is known to be a good indicator of several root pathogens that are also of major importance to other vegetables, legumes and some forage crops grown in New York including *Rhizoctonia*, *Pythium*, *Thielaviopsis*, *Fusarium* and others.

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Objective:

Using ~4 cups of soil sample (as sample volume allows) prepare up to seven snap bean plantings, allow to grow 5-6 weeks in a glass house (until full flowering) rinse soil from plant roots and evaluate root health.

References:

Abawi, G.S., Ludwig J.W., Petzoldt, C.H. 2004. Assessing root health by a soil bioassay with beans as an indicator of soil health. *Phytopathology* 94:(Suppl.):S1

Materials and Equipment:

250 cc cone-tubes
Racks for cone-tubes
Cotton balls
Sterile sand
Snap bean seeds
Wash basins
Water hose
Paper towel
Indelible marker
Acetate sheet

Procedure:

1. Soil sample is stored at 4° C after sample intake and prior to planting. Generally planting occurs in the greenhouse.
2. Soil should have been well-mixed during sieving through 8mm. Space up to seven 250 cc cone-tubes at every other position in the cone-tube rack and label at front of each row. Place a cotton ball into each cone-tube and gently push to the bottom using a pencil or another cone (do not compact).
3. Use an acetate sheet to funnel ~200g sample soil into the cone-tube to ~2cm below the rim. NOTE: Be careful not to allow soil aggregates to enter other tubes.
4. Gently press snap bean seed “flat” into the center of the soil with the hypocotyl to one side and cover with soil. Cover with a ~2mm layer of sand (to prevent soil crusting and cross contamination during watering).
5. Place rack in the glass house and water. Plants are maintained in the glass house under a bank of lights (12-14 hours of light daily) and watered regularly for 4 to 6 weeks.
6. Plants are removed from cone-tubes and the roots are washed thoroughly in a tray or under running water. Plants can be rated immediately. Samples can be stored overnight at 4° C if necessary before rating. Use a labeled, wet paper towel to wrap replicate plants from a sample.
7. Plant roots are rated for root rot severity on a scale of 1 (no visible disease symptoms) to 9 (> 75% of root tissue is severely decayed). A rating between 1 and 3 indicates healthy roots, 4 to 6 indicates moderately diseased roots, and 7 to 9 indicates severely diseased and decayed roots. (See category criteria below and scale on following page).

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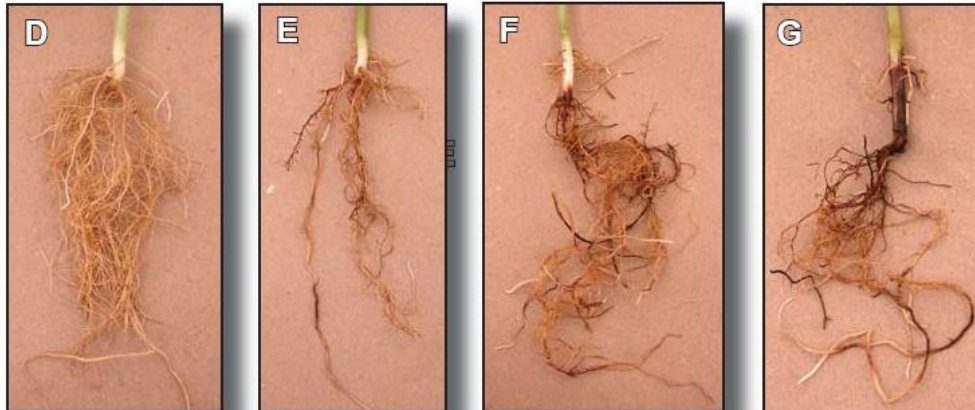
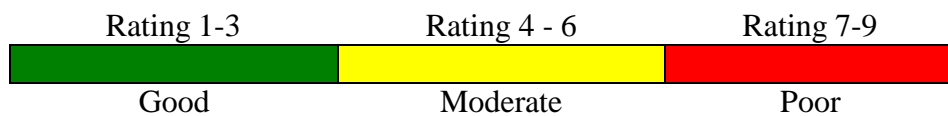
Category Criteria:

2 = white and coarse textured hypocotyl and roots; healthy (D);

4 = light discoloration and no necrotic lesions to a maximum of 10% of hypocotyl and root tissues with lesions (E);

6 = approximately 25% of hypocotyl and root tissue have lesions, but the tissues remain firm. There is little decay or damage to the root system (F);

7 to 9 = 50 to 75+% of hypocotyl and roots severely symptomatic and at advanced stages of decay (G).



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Rating scale – soil bioassay with bean

