Ammonium Acetate Method

Scope and Application

Ammonium acetate solution buffered at pH 7.0 is used to displace exchangeable cations in agricultural soils. This method gives an estimation of plant-available base cations (potassium, calcium, sodium, and magnesium). For calcareous soils (pH > 7.4 and calcium carbonate > 0.5%), this method overestimates plant available calcium, and Normandin et al. (1998) suggested using ammonium acetate solution buffered at pH 8.5 to suppress dissolution of calcium carbonates.

Summary

This is the method indexed by the OSU Extension Service Nutrient Management Guides for cations. Two grams of air-dried soil sample is shaken with 20 mL of ammonium acetate solution, filtered, and cations in the filtrate are determined by ICP-OES.

Equipment and Materials

- Balance (xx.xx g)
- 1L volumetric flask
- Weigh boat
- 50 mL polypropylene centrifuge tube with cap (Falcon tube) – 1 tube per sample
- Reciprocating shaker
- Filter papers – Whatman #1 – 1 per sample
- Collection funnels – 1 per sample

Reagents

- Ammonium acetate (mw 77.08)
- Acetic acid
- Ammonium hydroxide
- Deionized water

Procedure

1. Preparation of ammonium acetate extraction solution
    a. Add approximately 600 mL deionized water to 1L volumetric flask
    b. Measure 77.08 g of ammonium acetate into flask
    c. Bring to volume, mix well
    d. Check pH to ensure it is at 7.0. If not, adjust using either acetic acid or ammonium hydroxide
2. Weigh 2 g of air-dried soil into 50 mL tubes. Include one tube with CAL standard soil and one tube with no soil to be the method blank.

3. Add 20 mL of ammonium acetate extraction solution to each tube, including the CAL standard and method blank.
4. Place Falcon tubes on reciprocating shaker
5. Shake on low for one hour
6. Filter
7. Measure desired elements using ICP-OES

Reference

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DTPA Extraction

Summary

In this method, DTPA is used to chelate copper, zinc, iron, and manganese, and form water-soluble complexes with these micronutrients. Ten grams of soil are shaken with 20mL of DTPA solution and filtered prior to quantification using ICP-OES. Generally, reproducibility is ± 10% for copper, zinc, and manganese, and ± 15% for iron.

Equipment and Materials

- 20mL pipette
- 1L volumetric flask
- 1L graduated cylinder
- 1L beaker
- 50mL Falcon tubes
- Volumetric flasks
- Glass rod
- Plastic measuring spoons
- Shaker
- Centrifuge
- Weighing papers
- Filter papers (Whatman #1)
- Funnels
- Analytical balance
- pH meter
- ICP-OES

Reagents

- Standard calibration solution for pH meter
- Hydrochloric acid: 1.0M
- Reagent grade triethanolamine (abbreviated as TEA)
- Diethylenetriaminepentaacetic acid (abbreviated as DTPA): 99%
- Calcium chloride dihydrate: $CaCl_2 \bullet 2H_2O$
- Deionized water

Procedure

1. Prepare copper, zinc, iron, and manganese standard calibration solution
2. Prepare DTPA-TEA extraction (consists of 0.005M DTPA, 0.01M $CaCl_2$, and 0.1M TEA)
    a. Dissolve 14.92g of reagent grade TEA, 1.967g DTPA, and 1.47g of $CaCl_2 \bullet 2H_2O$ in approximately 20mL of deionized water, use glass rod to stir if needed.
    b. Dilute to approximately 900mL with deionized water when ingredients are fully dissolved
    c. Adjust pH to 7.3 ± 0.05 using 1.0M HCl and mix well
    d. Bring to 1L volume with DI water

3. Prepare filter funnels with Whatman #1 papers
4. Weigh 10g of sieved soil (<2.0mm) into a 50mL Falcon tube
5. Add 20mL of extracting solution to Falcon tube and cap
6. Shake tubes horizontally for 2 hours on the low speed setting.
7. Filter
8. Determine the concentration of copper, zinc, iron and manganese using ICP-OES

References

Gambrell, R.P., 1996. Manganese. In Sparks, D.L. (Editor). Methods of soil analysis: chemical methods, part 3. Published by Soil Science Society of America, Inc. American Socienty of Agronmy, Inc. Madison, Wisconsin, USA.

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DTPA Extraction

Summary

In this method, a DTPA-sorbitol solution is used to chelate copper, zinc, iron, manganese, and boron to form water-soluble complexes with these micronutrients. Ten grams of soil are shaken with 20mL of DTPA-sorbitol solution and filtered prior to quantification using ICP-OES.

Equipment and Materials

- 1L volumetric flask
- 1L graduated cylinder
- 1L beaker
- 50mL Falcon tubes
- Volumetric flasks
- Glass rod
- Plastic measuring spoons
- Shaker
- Centrifuge
- Weighing papers
- Filter papers (Whatman #1)
- Funnels
- Analytical balance
- pH meter
- ICP-OES

Reagents

- Standard calibration solution for pH meter
- Hydrochloric acid: 1.0M
- Reagent grade triethanolamine (abbreviated as TEA)
- Diethylenetriaminepentaacetic acid (abbreviated as DTPA): 99%
- Calcium chloride dihydrate: $CaCl_2 \bullet 2H_2O$
- Deionized water

Procedure

1. Prepare copper, zinc, iron, and manganese standard calibration solution
2. Prepare DTPA-TEA extraction (consists of 0.005M DTPA, 0.01M $CaCl_2$, 0.1M TEA, and 0.2 M sorbitol)
    a. Dissolve 14.92g of reagent grade TEA, 1.967g DTPA, 1.47g of $CaCl_2 \bullet 2H_2O$, and 36.4 g d-sorbitol in approximately 200mL of deionized water, use glass rod to stir if needed.
    b. Dilute to approximately 900mL with deionized water when ingredients are fully dissolved
    c. Adjust pH to 7.3 ± 0.05 using 1.0M HCl and mix well
    d. Bring to 1L volume with DI water

3. Prepare filter funnels with Whatman #1 papers
4. Weigh 10g of sieved soil (<2.0mm) into a 50mL Falcon tube
5. Add 20mL of extracting solution to Falcon tube and cap
6. Shake tubes horizontally for 2 hours on the low speed setting.
7. Filter
8. Determine the concentration of copper, zinc, iron and manganese using ICP-OES

References

Gambrell, R.P., 1996. Manganese. In Sparks, D.L. (Editor). Methods of soil analysis: chemical methods, part 3. Published by Soil Science Society of America, Inc. American Socienty of Agronmy, Inc. Madison, Wisconsin, USA.

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Miller, Gavlak, Horneck, 2013. Soil, Plant, and Water Reference Methods for the Western Region, 4th edition.

4.4.1 Mehlich 3 Extraction – P, K, Ca, Mg, Mn, Fe, Cu, Zn

Scope and Application

The Mehlich 3 extractant is a solution of NH_4F, EDTA, NH_4NO_3, acetic acid, nitric acid, and DI water which allows for the extraction of many nutrients from a wide variety of soil conditions while minimizing corrosive properties. For this reason, it has been nicknamed the universal extractant. The acids dissolve iron and aluminum phosphates, the fluoride ions form complexes with the aluminum, and EDTA chelates other metals. To test for plant available phosphate, the OSU Extension publications currently recommend Bray 1 for acidic soils and Olsen for alkaline soils. However, work from several other states provide evidence of high correlations between Mehlich 3 and the older extractions (Mallarino, 1999). This test can also replace the ammonium acetate (AA) extraction process for Ca, K, and Mg. Mehlich 3 demonstrated 6-8% higher extraction rates than AA while being highly correlated (Mehlich, 1984). Mehlich 3 can also be used in place of the DTPA extraction for micronutrients with good correlations for Zn, Mn, and Cu. The Mehlich 3 filtrate can be analyzed for multiple elements simultaneously using the Inductively Coupled Plasma – Optical Emission Spectrophotometer (ICP-OES)

Summary

The Mehlich 3 extractant is used to determine the amounts of micronutrients and macronutrients in a wide variety of soils. 20 mL of the extracting solution are added to 2 g of dried and ground soil and shaken for 5 minutes. The slurry is then filtered and the filtrate is analyzed using ICP-OES. One CAL standard is analyzed for every batch of samples. Generally, reproducibility is within ± 8%.

Equipment and Materials

- Weigh boats
- Balance (xx.xx g)
- Volumetric flasks (100 mL, 1L)
- Graduated cylinder (20 mL)
- Adjustable volume pipettes with tips (5 mL, <1 mL)
- Stir plate/hot plate
- Magnetic stir bar
- Scoopula
- Reciprocating shaker
- Wooden funnel frame with plastic funnels
- Lab tape
- Permanent marker
- Filter paper (Whatman #1) – 1 per sample
- 14 mL polystyrene round-bottom tube (ICP tube) – 1 per sample
- Vial holder

- 2.5L bottle with bottle-top dispenser

Reagents

- NH_4F (mw 37.04)
- Ethylenediaminetetraacetic acid (EDTA) (mw 292.24)
- Deionized (DI) water
- NH_4NO_3 (mw 80.04)
- Glacial acetic acid (>99.7%, or 17.4M)
- Concentrated HNO_3 (>68%, or 15.8M)

Procedure

1. **Solution preparation**
    a. <u>Mehlich 3 stock solution</u>
        i. Using a 100 mL volumetric flask, add:
            1. 13.89 g NH_4F
            2. 9.31 g EDTA
        ii. Bring to 100 mL volume using DI water
        iii. Add a small magnetic stir bar and put flask on a stirplate/hotplate on low heat until all reagents are dissolved
        iv. This stock solution can be stored for months in the refrigerator
    b. <u>Mehlich 3 extractant</u>
        i. Using a 1L volumetric flask, add:
            1. 20 g NH_4NO_3
            2. 4 mL of the Mehlich 3 stock solution
            3. 11.5 mL glacial acetic acid
            4. 0.82 mL HNO_3 to the flask
        ii. Bring to 1L volume using DI water
        iii. Invert flask at least three times to mix
        iv. Pour solution into bottle-top dispenser set to dispense 20 mL

2. **Extraction procedure**
    a. Weigh 2 g of dried and ground sample and place into labelled Falcon tubes
    b. Using a bottle-top dispenser, quickly add 20 mL of Mehlich 3 extractant to each tube (up to 12 at one time), including one tube with CAL standard soil and one tube with no soil (tube should be labeled as: Method Blank)
        i. Only one CAL sample and one method blank are required for each batch of samples to be run on the ICP, not necessarily one of each per ten unknown samples.
    c. Secure cap over all tubes

d. Immediately place tubes on shaker for five minutes
e. After removing samples from shaker, immediately filter
f. Once filtered, pour filtrate into ICP tube
    i. If samples are not to be analyzed immediately on ICP, pour them into labeled 15 mL polypropylene centrifuge tube and cap
g. Analyze filtrate within 24 hours on ICP-OES

Note: procedure is calibrated to 5 minutes of contact between soil and extractant. This is the reason for a max of 12 samples at one time and careful timing and preparation are necessary.

Calculation of results

$$Nutrient\ in\ soil\ = \left(Nutrient\frac{mg}{L}in\ filtrate - \ method\ blank\right) x\ \frac{extractant\ (ml)}{soil\ mass\ (g)} \quad \text{(eq. 1)}$$

Mehilch 3 soil test extractant: A modification of Mehlich 2 extractant. Communications in Soil Science and Plant Analysis Volume 15 1984 Issue 12.

http://www.lpm.iastate.edue/ipm/icm/1999/2-15-1999/mehlich3.html Antonio Mallarino John E Sawyer.

Hot Water Extraction - Boron

Summary

This is the method indexed by the OSU Extension Service Nutrient Management Guides for boron. Boron in soil is extracted using a hot 0.01M $CaCl_2$ solution. The extract can be analyzed using ICP-OES.

Equipment and Materials

- Balance
- 1L volumetric flask
- Falcon Tubes
- Glass stir rods
- Hot plate
- Large glass beaker
- Whatman #1 filter paper
- Glass or plastic funnel
- ICP sample vials
- ICP sample vial holder

Reagents

- $CaCl_2 \bullet 2H_2O$ (mw 147.01)

Procedure

1. Add 1.47 g $CaCl_2 \bullet 2H_2O$ to a 1L volumetric flask and bring to volume with DI water.
2. Weigh 5.0 g of dry soil into a labeled 50 mL Falcon tube labeled with tape.
3. Include one empty tube to be the method blank and one tube to be the CAL standard soil.
4. Dispense 20 mL of 0.01M $CaCl_2$ into each Falcon tube, including the method blank.
5. Stir solution with a glass stir rod; leave the rod in the tube.
6. Place each Falcon tube in the large glass beaker (or multiple small glass beakers).
7. Add water to the large glass beaker up to the level of the water inside the Falcon tubes.
8. Place the large beaker on the hot plate.
9. If the bottom of the beaker is very smooth, add a pinch of soil to the large beaker to provide a places for bubbles to form.
10. Turn the hot plate to high.
11. Once the water is almost boiling (95°C), turn the heat down slightly.
12. Keep the soil around 95°C for 30 minutes, stirring every 10 minutes.
13. Filter while slurry is still hot
14. Analyze the filtrate using ICP-OES

KCl extraction for NO_3-N and NH_4-N

Summary

The soil sample is shaken with 2M KCl at a 4:1 ratio (KCl to soil) for one hour to achieve equilibrium. The solution is filtered and then quantified using independent colorimetric reactions. One CAL standard and one method blank is analyzed with each group of unknowns. Generally, the reproducibility is considered to be ± 6% for NO3-N and ± 7% for NH4-N.

Equipment and Materials

- Reciprocating shaker
- 50 ml polypropylene centrifuge tube with cap (Falcon tube) – 2 per sample
- Balance
- Wooden funnel frame with plastic funnels
- Whatman #1 filter paper

Reagents

- 2M KCl (149.1 g KCl/liter)

Procedure

1. Weigh 7.5 g of soil (on dry matter basis) into a labelled 50mL tube.
2. Add 30 mL of 2M KCl into each Falcon tube.
3. Cap and shake on a reciprocal shaker on low for one hour.
4. Fold a Whatman #1 filter paper and place in a funnel tube.
5. Pour the soil slurry into the filter.
6. Once the filtration is complete, pour extracted sample into clean, labelled Falcon tube.
7. Analyze immediately or cap the tube and place in the refrigerator.
    a. See analysis procedure for Lachat Flow Injection Autoanalyzer or manual nitrate quanitification

K_2SO_4 Extraction – NO_3-N, NH_4-N, Dissolved Carbon, Dissolved Nitrogen

Summary

The soil sample is shaken with 0.05M K_2SO_4 at a 4:1 ratio (K_2SO_4 to soil) for one hour to achieve equilibrium. The solution is filtered and then quantified using independent colorimetric reactions. One blank method standard and one CAL standard is run with every set of unknown samples. Generally, the reproducibility is considered to be ± 6% for NO_3-N and ± 7% for NH_4-N.

Equipment and Materials

- Reciprocating shaker
- Plastic 50 mL tubes with caps
- Balance
- 1L volumetric flask
- Wooden funnel frame with plastic funnels
- Whatman #1 filter paper

Reagents

- K_2SO_4 (mw 174.26)

Procedure

1. Weigh 8.71 g K_2SO_4 on balance and dissolve in approximately 750 mL DI water in a 1L volumetric flask.
2. Once dissolved, bring to volume
3. Weigh 7.5 g of soil (on dry matter basis) into a labelled 50 mL tube.
4. Add 30 mL of 0.05M K_2SO_4 using a bottle-top dispenser.
5. Cover and shake on a reciprocal shaker on low for one hour.
6. Filter
7. Once the filtration is complete, pour extracted sample into clean, labelled storage tube.
8. Analyze immediately or cap the tube and place in the refrigerator.

Calcium Phosphate – SO_4^{-2}

Summary

Ground dry soil sample is shaken for half hour with $Ca(H_2PO_4)_2$ solution at a solid liquid ratio of 1:2.5. Extractant is analyzed using ICP-OES. One CAL standard and one method blank is analyzed with each group of unknowns.

Equipment and Materials

- Balance
- Reciprocating shaker
- 50 mL Falcon tubes
- Weighing paper
- Whatman #1 filter paper
- Funnels in wooden frame
- 1L volumetric flask
- 1L beaker

Reagents

- Deionized water
- Monocalcium phosphate monohydrate [$Ca(H_2PO_4)_2 \bullet H_2O$]

Procedure

1. Preparation of standard calibration solution for sulfate-sulfur from stock solution
2. Preparation of extraction solution: 0.08M $Ca(H_2PO_4)_2$
    a. Weigh 2.03 g of $Ca(H_2PO_4)_2 \bullet H_2O$
    b. Dissolve in 700 mL of deionized water
    c. Transfer to 1L volumetric flask
    d. Bring up volume to 1L
3. Extraction procedure
    a. Weigh 10.0 g ground dry soil sample (<2 mm) to 50 mL Falcon tube.
    b. Add 25 mL of calcium phosphate extraction solution.
    c. Secure in shaker and shake for half hour.
    d. Filter.
4. Analyze for S on ICP-OES

Calculation

$$\text{mg/kg sulfur in soil} = \text{mg/L sulfur in soil extract} * 0.025\text{L} / 0.01\text{kg} \qquad \text{(eq. 1)}$$

References

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Bray Dilute acid-fluoride extraction

Equipment and Materials

- 5 mL pipette
- 50 mL graduated cylinder
- 1L volumetric flask
- 500 mL volumetric flask (2)
- 100 mL volumetric flask
- Polyethylene storage bottles
- Vortex spinner
- Spectrophotometer
- Cuvettes – 1 per sample
- Filter papers (Whatman #1) – 1 per sample
- Collection funnels – 1 per sample
- 50 mL polypropylene centrifuge tube with cap (Falcon tube) – 1 tube per sample

Reagents

- Ammonium fluoride NH_4F
- Hydrochloric acid
- Phosphate stock solution : 1000 ppm P
- Ammonium paramolybdate
- Antimony Potassium Tartrate

Procedure

1. Prepare 1L extraction solution
    a. Prepare 1L 0.5N HCl
    b. Prepare 500 ml 1N NH4F
        i. 18.52 g NH4F, bring to 500 ml using DI
    c. Add 50 ml of 0.5N HCl and 30 ml of 1N NH4Fto ~900 ml DI
    d. Adjust pH to 2.60 with dilute HCl or NH4OH
    e. Bring total solution volume to 1L
2. Extract soils
    a. Weigh 2.00g soil into Falcon tube
    b. Add 14 ml Bray Extracting solution
    c. Shake for 5 minutes
    d. Filter
3. Prepare 2L Modified Reagent A
    a. Dissolve 12.0 g ammonium molybdate in ~250 ml DI
    b. Dissolve 0.291 g antimony potassium tartrate in ~100 ml DI
    c. Add 160 ml concentrated H2SO4 to ~800 ml DI, then bring to volume with DI (1L)
    d. Add all of the first two dissolved reagents to the 1L of diluted H2SO4, and bring the total volume up to 2L

    e. Modified Reagent A can be stored in a brown bottle in the fridge for up to four months.
4. Prepare Reagent B
    a. Dissolve 1.32 g ascorbic acid in 250 ml of Modified Reagent A
    b. This reagent should be prepared daily
5. Prepare standards using purchased P standard at a range of concentrations between 0.5 and 12 ppm. The matrix of the standards should be Bray extracting solution.
6. Colorimetric analysis
    a. Pipette 1 ml of extract into a 50 ml Falcon tube
    b. Add 12 ml of DI
    c. Add 2 ml of reagent B
    d. Briefly vortex on vortex mixer
    e. Pour solution from Falcon tube into cuvette
    f. Read on spectrophotometer at 880 nm ten minutes after adding reagent B
    g. Treat standards like sample extracts
    h. Include a method blank
    i. Prepare as many samples as you can in the ten minutes following the first sample, read all of those, then start a new batch
    j. Have all extracts completed before starting colorimetric analysis
    k. Create a regression using standards to calculate concentrations in unknown samples.

Olsen Extraction

Equipment and Materials

- 5 mL pipette
- 50 mL graduated cylinder
- 1L volumetric flask
- 100 mL volumetric flask
- Polyethylene storage bottles
- Vortex spinner
- Spectrophotometer
- Cuvettes – 1 per sample
- Filter papers (Whatman #1) – 1 per sample
- Collection funnels – 1 per sample
- 50 mL polypropylene centrifuge tube with cap (Falcon tube) – 1 tube per sample

Reagents

- $NaHCO_3$
- NaOH for pH adjustment
- Phosphate stock solution : 1000 ppm P
- Ammonium paramolybdate
- Antimony Potassium Tartrate

Procedure

1. Prepare 1L extraction solution
    a. Dissolve 42.01 g $NaHCO_3$ in ~900 ml DI
    b. Adjust pH to 8.5 with 2N NaOH
    c. Bring total solution volume to 1L
    d. Prepare as required
2. Extract soils
    a. Weigh 3.00g soil into Falcon tube
    b. Add 20 ml Olsen Extracting solution
    c. Shake for 1 minutes
    d. Filter
3. Prepare 2L Modified Reagent A
    a. Dissolve 12.0 g ammonium molybdate in ~250 ml DI
    b. Dissolve 0.291 g antimony potassium tartrate in ~100 ml DI
    c. Add 160 ml concentrated H_2SO_4 to ~800 ml DI, then bring to volume with DI (1L)
    d. Add all of the first two dissolved reagents to the 1L of diluted H_2SO_4, and bring the total volume up to 2L
    e. Modified Reagent A can be stored in a brown bottle in the fridge for up to four months.
4. Prepare Reagent B

    a. Dissolve 1.32 g ascorbic acid in 250 ml of Modified Reagent A
    b. This reagent should be prepared daily
5. Prepare standards using purchased P standard at concentrations of 0, 0.2, 0.5, 1, 2, 5, 10 ppm. The matrix of the standards should be Olsen extracting solution.
6. Colorimetric analysis
    a. Pipette 1 ml of extract into a 50 ml Falcon tube
    b. Add 12 ml of DI
    c. Add 2 ml of reagent B
    d. Briefly vortex on vortex mixer
    e. Pour solution from Falcon tube into cuvette
    f. Read on spectrophotometer at 880 nm ten minutes after adding reagent B
    g. Treat standards like sample extracts
    h. Include a method blank
    i. Prepare as many samples as you can in the ten minutes following the first sample, read all of those, then start a new batch
    j. Have all extracts completed before starting colorimetric analysis
    k. Create regression using standards to calculate concentrations in unknown samples.

Manual Nitrate Measurement

Summary

A solution of sulfanilamide and N-(1-naphthyl)ethylenediamine dihydrochloride is added to a solution of vanadium chloride to use as the single reagent. Reagent and sample are pipetted into a cuvette and optical density is measured spectrophotometrically after color development. Nitrate in the samples is quantified using a calibration curve from a set of N standards made fresh daily.

Equipment and Materials

- Analytical balance
- Weigh boats
- Pipettes
- Stir bar
- Stir plate
- Beakers
- Cuvettes
- Spectrophotometer

Reagents

- 1M HCl
- 400 mg vanadium(III) chloride
- 200mg sulfanilamide
- 10g N-(1-naphthyl)ethylenediamine dihydrochloride (NEDD)
- N Standards

Procedure

1. Prepare a set of N standards at 20, 15, 10, 5, 2, 1, 0.5ppm concentrations
    a. This is the general range at which this method is useful. If samples have higher concentrations, the samples should be diluted. Standards down to 0.2 or possibly 0.1 ppm may be used if concentrations are known to be very low.
2. Add 200mg sulfanilamide to 400mL DI H_2O
3. Add 10mg NEDD to sulfanilamide solution
4. Put in a beaker with a stir bar and stir until dissolved (~10 min)
5. Pipette 50ul of each standard and each samples into respective cuvettes
6. A full set of standards should be used at the beginning and end of the sample set
7. One standard should be included between every 9 samples as a check
8. Add 400mg VCl_3 to 50mL 1 M HCl in the fume hood
9. Add the VCl_3 solution to the sulfanilamide solution; stir to combine
10. Pipette 2mL of reagent into each cuvette
11. Cover box of cuvettes
12. Samples can be read 24 hours later if left at room temperature

13. Samples can be read between 2-3 hours if placed in a 60C oven
14. Create a linear regression for the calibration curve. If the points do not line up well on the curve (R2>0.995), redo the entire procedure.

Microwave Total Digestion - Soil, Plant, Sediment, Sludges

Summary

Dry sample is ground to ensure homogeneity. 500mg of a soil, sediment, or sludge sample or 250mg for plant tissues are weighed into a microwave digestion vessel. 10 mL HNO_3 is added to each sample under fume hood, making sure the sample is thoroughly wet. Acid solution is transferred to a volumetric flask and diluted to 50mL using deionized water. Elements are determined by ICP-OES.

Equipment and Materials

- Analytical balance
- Anton-Paar MultiwaveGO with digestion vessels
- 50mL volumetric flasks
- 5 mL pipette with tips

Reagents

- Concentrated nitric acid (HNO_3): 15M or 70%

Procedure

1. Weigh 500 +/- 5.0mg ground dry soil (<2mm) or 200 +/- 20 mg to vessel
2. Add 10 mL HNO_3 sample under fume hood, and make sure sample is completely wet.
3. Make sure that the slanted “lip” of the digestion vessel is clean and dry, then place conical lid, point side down, on top
4. Screw on main cap until the depressed area in the middle of the top is exactly flush with the rest of the top of the lid
5. Place vessel in the appropriate location in the sample carousel
6. The sample location in the carousel is the only way to keep track of which sample is which, so write it down and only handle samples one at a time
7. Microwave using method “EPA 3051A” on the MultiwaveGO
8. Carefully transfer acid solution to 50mL Falcon tube, and bring up to volume using deionized water
9. Measure diluted solution on Agilent 5110 ICP-OES

Dry Ash - Plant Material

Summary

Plant tissue is ground, weighed into a quartz crucible, ashed at 585C for 4 hours, and cooled down in the furnace. Ash is dissolved by using hydrochloric acid, diluted, and measured by ICP-OES. Personal protective equipment is required when working with hydrochloric acid and high temperature furnaces. One CTFS standard and one method blank is analyzed with each group of unknowns.

Equipment and Materials

- Muffle furnace
- Quartz crucibles
- Filter funnels
- Filter paper (Whatman #1)
- High temperature tongs
- Balance
- Weigh paper
- 15 mL screw-top ICP tubes

Reagents

- Hydrochloric acid: 20%
- Deionized water

Procedure

1. Weigh 0.20 g ground plant tissue into quartz tube
2. Put quartz tube in metal tube rack in the furnace and ash at 585^{o}C for 4 hours
3. Leave tubes in furnace to cool down to room temperature
4. Take tubes out, and bring them to fume hood
5. Add 5 mL of 20% (v/v) hydrochloric acid into each crucible and wait 30 minutes
6. Add 5 mL DI water to each crucible
7. Filter
8. Pour filtrate into 15 mL ICP tube
9. Run solution through ICP-OES.

Calculation

$$\text{element in plant sample (mg/kg)} = \text{element in extract (mg/L)} \times 0.01\text{L} / 0.0002\text{kg} \quad \text{(eq. 1)}$$

Reference

Jones, J.B., Jr., and Warner, M.H., 1969. Analysis of plant –ash solutions by spark-emission spectroscopy. P152-160. In Grove, E.L., and Perkins, A.J. (Eds.) Developments in applied spectroscopy. Vol. 7A, Plenum Press, New York.

Soil pH

Summary

Soil pH can be measured at either a 1:1 or 1:2 soil to water ratio. Because of the use of the log scale, the two ratios typically provide similar results. A soil with high clay or organic matter may require a 1:2 or even 1:5 ratio to allow for enough fluid to make a measurement. A sample that will also have the lime requirement (i.e. Sikora) measured will be measured with a 1:1 ratio when possible. With either soil to water ratio, the soil and water are placed in a tube and shaken for 15 minutes on a reciprocating shaker to achieve soil solution equilibrium. A calibrated probe accounting for temperature is used for the measurement. One CAL standard sample is included in each batch.

Equipment and Materials

- Hanna HI5522 with pH and temperature probe
- 50 mL polypropylene centrifuge tube with cap (Falcon tube) – 1 tube per sample
- Reciprocating shaker

Reagents

- pH calibration standards (4.01, 7.01, 10.01)
- DI water (or other solution as requested)

Procedure

1. Measure 20 g dry weight soil into the Falcon tube
2. Add 20 mL of DI water
3. Place on reciprocating shaker for 15 minutes
4. Rinse the pH probe well with DI water and gently pat dry
5. Calibrate the pH meter with a minimum of two standards that bracket the potential sample results according to the instructions on the screen
    a. The probe should be calibrated using standards in the same type of vessel as the samples will be measured in
6. Insert rinsed pH and temperature probes into each sample
    a. Be careful to keep the probes still and not against the wall or bottom of the vessels
    b. Make sure the diaphragm is submerged in suspension.
    c. Record results when the numbers stabilize.
7. Between each measurement, rinse the probe well with DI water and gently pat dry
8. **When not measuring, make sure that the pH probe is rinsed and fully submerged in 3M KCl.**

Possible Modifications

- Different solutions can be used – usually 0.01 M $CaCl_2$ for exchangeable acidity
- The total amount of soil can be changed as long as the amount of water remains at a constant ratio (e.g. 20 g soil with 20 ml water = 10 g soil with 10 ml water)
- The ratio of soil to water can be changed (e.g. 10 g soil and 20 ml water)

Electrical conductivity

Summary

The soil and water are placed in a tube and shaken for 15 minutes to achieve soil solution equilibrium. A calibrated probe accounting for temperature is used for the measurement. One standard sample is included in each batch.

Equipment

- Hanna HI5522 with EC and temperature probe
- Small beaker with glass stir rod OR Falcon tube with cap
- Reciprocating shaker

Reagents

- EC calibration standards (0.084 and 1.413 dS m^{-1})
- DI water

Procedure

1. Measure 20g dry weight soil into the beaker or Falcon tube
2. Add 20 mL of DI water
3. Place on a shaker on high for 15 minutes
4. Calibrate the EC meter with a minimum of two standards that bracket the potential sample results according to the instructions on the screen
5. Insert rinsed EC and temperature probes into each sample
    a. Be careful to keep the probes still and not against the wall or bottom of the vessels, make sure the diaphragm is submerged in suspension.
    b. The liquid needs to come up to the drain holes or the measurement will be meaningless.
    c. This may require the tipping of the vessel, using a vessel that is tall and thin, or decanting the supernatant into a test tube. Record results when the numbers stabilize.
2. Between measurements, rinse each probe well with DI water and gently pat dry.

Notes

While the ratio of soil to water should generally stay consistent at 1:1, and changes can be made, it is risky to go below 20g of soil and 20 ml of water because the total height of the slurry will frequently be too low for proper measurements.

Lime Requirement - Sikora buffer pH

Summary
pH of a 1:1 water to soil suspension is measured, the Sikora buffer solution is added, placed on a shaker for 10 minutes, then the pH is measured again. The degree of difference between the two measurements indicates the buffering capacity of the soil.

Equipment:

- Balance
- Weigh boats
- 1000mL graduated cylinder
- 200mL beakers
- 100mL sample cups
- Shaker
- pH probe

Reagents:

- Potassium chloride 149g (KCl mw=74.55)
- Glacial acetic acid 5.36g or 5.11mL (CH_3COOH, mw=60.05)
- MES (2-(N-morpholino)ethanesulfonic acid) monohydrate 6.7g ($C_6H_{13}NO_4S \cdot H_2O$ mw=213.24)
- Imidazole 0.936g ($C_3H_4N_2$, mw=68.08)
- Triethanolamine 10.38g ($(HOCH_2CH_2)_3N$, mw=149.19
- Sodium hydroxide mL (40% NaOH (w/w))

Procedure:

1. To make one liter of extracting solution
2. Mix 149g KCl into 750 mL DI water
3. Add the following, in order, making sure each is dissolved before adding the next
4. Add 5.11mL Glacial acetic acid
5. Add 6.7g MES, once that is dissolved
6. Add 0.936g Imidazole
7. Add 9.23mL Triethanolamide
8. Add 5mL 40% sodium hydroxide
9. Adjust the final volume by adding DI water to 1L
10. Adjust the solution to pH 7.70 by adding drops of 40% NaOH (w/w) or 50% HCl (v/v)
11. Transfer 50mL of buffer to beaker, measure pH to ensure 7.70
12. Transfer 50mL of water to same beaker, stir, measure pH, should be 7.53
13. Add 5mL of 0.5N HCl to the beaker, stir, measure pH, should be 5.68
14. Mix 10g of dry soil and 10g DI water, place on shaker for 15 minutes
15. Measure pH of soil slurry
16. Add 10mL of Sikora buffer
17. Place the sample on mechanical shaker for 10 minutes

18. Measure the pH of the soil buffer slurry

Notes

The soil:water:buffer ratio must be 1:1:1, but the absolute values can change as needed.

4.5.1 Ammonium Acetate pH 7 - Potential Cation Exchange Capacity

Summary

This method uses a pH 7 ammonium acetate extraction that is the standard for NRCS soil classification and the standard for agricultural applications. Soil cations are first extracted with ammonium acetate. The acetate is rinsed away with ethanol, leaving the ammonium sorbed to the exchange sites. Potassium chloride is used to exchange the ammonium and the resulting leachate is measured for total ammonium, representing the total cations the soil can hold. One CAL standard and one method blank is analyzed with each group of unknowns.

Equipment and Materials

- Balance, weigh boats
- Disposable pipette (eye dropper)
- 1L volumetric flask
- 100 mL plastic bottle
- Reciprocating shaker
- Wooden rack with plastic funnels
- Whatman #1 filter paper
- Buchner funnel
- 500 mL suction or side-arm flask (3)
- 250 mL volumetric flask
- 1L beaker
- Lachat autoanalyzer

Reagents

- Ammonium acetate 1N
- Ethanol 95% (200 mL/sample)
- Potassium chloride (1M KCl)

Procedure

1. Weigh 77.1 g of ammonium acetate, add approx. 900 mL DI in a chemical hood
2. Adjust pH to 7.0 with NH_4OH in the chemical hood on a stir plate and bring to 1L volume
3. Dilute the stock 2M KCl in a 1:1 with DI to produce 1M KCl
4. Weigh 10 g of soil into 100 mL plastic bottle
5. Add 40 mL of ammonium acetate solution
6. Shake on low speed for 30 minutes
7. Connect a 500 mL vacuum flask to a Buchner funnel
8. Place a # 1 Whatman filter flat in the bottom of the Buchner funnel. Make sure the filter is completely covering the bottom of the Buchner funnel.

9. Use a disposable pipette to add roughly 1 mL of ammonium acetate to wet the filter paper in the Buchner funnel
    a. This reduces soil loss into the extractant
10. Transfer soil suspension to Buchner funnel and leach the sample (under vacuum pressure) with 175 mL of 1 N ammonium acetate.
    a. Do not release vacuum pressure during leaching, otherwise soil will enter extract
11. Rinse ammonium acetate from soil sample in Buchner funnel by leaching with a full funnel (~100 mL) of ethanol.
12. Add the remaining ethanol (total 200 mL) – discard ethanol rinse solution
13. Transfer Buchner funnel with soil sample to another 500 mL vacuum flask
14. Leach with 200 mL of 1M KCl to exchange the NH_4 sorbed to the soil sample
15. Transfer leachate to 250 mL volumetric flask
16. Bring to 250 mL with DI
17. Measure NH_4 on the Lachat autoanalyzer
18. Make standards with 1M KCl as matrix background

Calculations

$$\text{CEC} = (ppm\ NH4 - N) * \frac{0.25}{14} * \frac{100}{sample\ weight\ (g)} \qquad \text{(eq. 1)}$$

Reference

Methods of Soil Analysis: Part 3 Chemical Methods SSSA

Dry Combustion of Solid for Total C, N, S

Summary

A dried and finely ground plant or soil sample is wrapped in tin foil. For plants, 85 mg are used. For soil, 150 mg are used. Phenylalanine (for CN) or sulfanilamide (CNS) are used to compute the daily factor that changes based on ambient temperature, pressure, and humidity. Douglas fir and NAPT standards are used as check samples for plant and soil, respectively. Check samples are used for every 8 unknown samples. Clean gloves are required for all steps. Do not touch your face with gloved hands. If the sample falls on the floor or anywhere, throw it out.

Equipment and Materials

- 35 mm tin foil square
- Metal scoopula
- Tin capsule press

Reagents

- CTFS and/or NAPT standards
- Ethanol

Procedure

1. Tare a tin square on the microbalance by pressing “T”
2. Enter the sample mass manually into the proper location in the Excel sheet
3. Remove the tin and sample from the scale to fold
4. Carefully fold corners of tin inward successively to contain all sample within tin
    a. Tin should be a small flat disk shape when folded properly
5. Place the tin with the sample into the tin capsule press
6. Lower the press with moderate force.
7. Lift the press and remove the pressed tin package. Do not let it fall out.
8. Inspect the packet for visible rips. If there are visible rips, reweigh/rewrap the sample
9. Carefully place pressed pack into the appropriate sample holder or in carousel
10. Wrap a CTFS (plant) or NAPT (soil) sample after every eleven unknown samples

Loss on Ignition (LOI)/Total Ash

Scope and Application

The Loss on Ignition method uses differences in weight before and after oxidizing the sample in a muffle furnace to determine the mass loss of organic matter. This method is commonly used because of high throughput capabilities and low cost. However, the application is limited as the precision is variable based on the mineralogy. Hydrated aluminosilicates, carbonates, and hydrated salts can also cause weight loss resulting in overestimation of total organic matter. Generally, the reproducibility is considered to be ± 20%.

Summary

Total organic matter is determined by heating dried and ground soil in a muffle furnace to a temperature where the organic matter burns off, leaving the mineral component of the soil. One check sample is included with each batch. The samples must be left in the oven overnight and cool for an hour in the morning. Approximately 5 g of dried soil are used for each sample. One CAL standard and one method blank is analyzed with set of unknowns.

Equipment

- Thermolyne F-A1730
- Ceramic crucible
- Analytical balance

Reagents

- None

Procedure

1. Soil should be dried, ground, and pass through a 2 mm sieve
2. Crucible should be placed in the 105°C oven for a minimum of 3 hours prior to weighing
3. Transfer crucible from oven immediately to desiccator for approximately 10 minutes
4. Weigh a crucible to 0.0001 g precision and record
5. Place approximately 6 g of air dried sieved soil in a metal weigh dish and place in 105°C oven for 24 hours along with empty crucibles
6. Transfer crucible with soil immediately to desiccator with drying chips
7. After 10 minutes, weigh the crucible to 0.0001g precision and record.
8. Add approximately 2-3 g of the oven dried soil and record the weight
9. Place the crucible in the furnace set to 385°C
10. Record the position of each crucible because chemical markings (Sharpie) will come off in the oven
11. Close the furnace doors using the large lever on the left side of the oven

12. Turn on the power switch and rotate the small wheel on the control box until it clicks and you hear a “thunk” from below the oven
13. Check the temperature of the muffle furnace 1 hour later to make sure the desired temperature is achieved
14. Leave overnight
15. In the morning, turn off the power and open the doors halfway. Wait 30 minutes.
16. Open the other door and wait another 30 minutes hour
17. Using crucible tongs, remove each crucible and weigh, remembering that position in the oven is how the crucibles are identified. If the crucible is still hot, allow it to cool for one minute before weighing, as heat will alter the weight reading
18. Record the new weight and calculate the percent mass loss of the soil.

    Assumption: all loss is organic matter.

$$\left[\frac{D-F}{F-C}\right] * 100 = OM\ \% \qquad \text{(eq. 1)}$$

D = oven dry weight of soil sample and crucible
F = furnace oxidized weight of sample and crucible
C= oven dry weight of crucible

Modifications

For the total ash procedure, use plant material instead of soil material. Change the oven temperature to 585C and pre-dry the samples at 65C.

Hydrometer Method

Summary

Prior to starting the particle size separation steps the sample is dried and particles greater than 2 mm are removed, the sample is precisely weighed, then organic matter and any other potential cementing-agents are removed. Sodium hexametaphosphate is added to the suspension and placed on a shaker overnight to overcome flocculation during settling. Suspension is measured using a hydrometer in a 1L graduated cylinder of DI water at multiple time points to determine the specific gravity of the suspension.

Equipment and Materials

- 1000 mL Graduated cylinder
- 500 mL HDPE container with lid
- Agitation plunger with flat plate on bottom
- Hydrometer

Reagents

- Hydrogen peroxide 30%
- Sodium Hexametaphosphate $Na_6P_6O_{18}$ (10% NaHMP in DI water)

Procedure

Sample Preparation

1. Weigh approximately 50g of dried soil into 500 ml plastic bottle with wide mouth lid
2. Add ~50 ml water and ~5 ml H2O2
3. Monitor the reaction. If there is a lot of fizzing, another addition of H2O2 will be needed after at least 12 hours. If there is not much reaction, then let the bottle sit open overnight to give the peroxide time to degrade.
4. Add 50 ml of 10% NaHMP to each bottle, screw on the lids, and shake on “low” overnight
5. Rinse all soil from plastic bottle into a 1000 mL graduated cylinder
6. Fill cylinder to 1L mark with DI water
7. Add 50 ml of NaHMP to a 1000ml graduated cylinder and fill to volume with DI water. This is the blank solution.
8. Allow the suspension to equilibrate to room temperature for at least two hours, but practically, overnight is usually better for timing the measurements.
9. Record the temperature of the texture room using the digital thermometer in the room
10. Use Table 1 to determine the time at which you should take the second hydrometer measurement based on the temperature of the room
11. Vigorously disperse sediment from bottom of column with stir rid
12. Gently place the hydrometer into the liquid 30 seconds after stirring and record the level at precisely 40 seconds

13. Record the reading as Rs
14. Set a timer for the second measurement according to temperature measurement
15. Ten seconds prior to the second reading, gently place the hydrometer into the liquid and record the level as Rc

Table 1. Settling time for clay related to temperature of suspension

Temperature (°C)	Time (hr:min)
18	8:09
19	7:57
20	7:45
21	7:35
22	7:24
23	7:13
24	7:03
25	6:53
26	6:44
27	6:35
28	6:27

Calculations:

$$Sand\ \% = \left(\frac{(T) - (Rs - B1)}{T}\right) * 100 \qquad \text{(eq. 1)}$$

T = total oven dry soil mass used
Rs = hydrometer reading at 40 seconds
B1 = hydrometer reading of blank solution at 40 seconds

$$Clay\ \% = \left(\frac{Rc - B2}{T}\right) * 100 \qquad \text{(eq. 2)}$$

T = total oven dry soil mass used
Rc = hydrometer reading of suspension at second measurement
B2 = hydrometer reading of blank solution at second measurement

$$Silt\ \% = 100 - Clay\% - Sand\% \qquad \text{(eq. 3)}$$

Sieve and Pipette Method

Scope and Application

This method is recommended for academic research that is investigating properties related specifically to texture. The method employed by CAL splits the sand sized fraction into five classes, and splits silt into two size classes. Generally, the reproducibility is considered to be ± 2%.

Summary

Prior to starting the particle size separation steps the sample is dried and particles greater than 2 mm are removed, the sample is precisely weighed, then organic matter and any other potential cementing-agents are removed. Sodium hexametaphosphate is added to the suspension and placed on a shaker overnight to overcome flocculation during settling. Sand size fractions are separated through a wet sieve, oven dried, and sieved through a series of sieves on a shaker. The silt and clay suspension is brought to a volume of 1L and specific aliquots of the fluid are removed by pipette at a specific depth at specific time points to capture the silt and clay sized particles in accordance with Stokes' Law. The fluid is dried, weighed, and the weight used in calculations to determine the final texture.

Equipment and Materials

- 2 mm sieve
- 0.05 mm sieve
- Sieve stack (US or FAO size grouping)
- Accujet pipette dispenser
- 20 mL glass pipettes
- Analytical balance
- 30 mL beakers
- 1000 mL graduated cylinders
- Pipette slide apparatus
- Stop watch
- Large funnel
- Squirt bottle
- 500 mL Erlenmeyer Flask
- Data sheet
- 105C oven
- Thermometer
- Flat bottomed stirring rod

Reagents

- Hydrogen peroxide (H_2O_2)
- Sodium Hexametaphosphate $Na_6P_6O_{18}$ (solution of 10% $Na_6P_6O_{18}$)

Procedure

A: Sample Preparation

1. Weigh approximately 50g of air dried soil
2. Sieve soil through 2 mm sieve – careful not to remove aggregates of smaller fractions
3. Remove, weigh, and record rock sized fragments
4. Remove, weigh, and record OM particles that do not pass the 2 mm sieve
5. Transfer 5-10 g of sieved soil into a weigh tin, record all weights needed for gravimetric moisture and place in 105C oven
6. After 24 hours in the oven, allow to cool in a desiccator then weigh to 0.0001g

B: Sample dispersion and removal of cementing agents

7. Assess sample for need to remove carbonates (pH greater than 7.3) or iron cementing agents (visual inspection for iron oxides). If these steps are deemed necessary, please go to Appendix A for further instructions.
8. Weigh 20 g air dried sieved soil into a 500 mL Erlenmeyer flask
9. Add 15 mL of water
10. Add 15 mL of H_2O_2
11. Let stand ~24 hrs
12. Add 15 mL of H_2O_2. Try to dispense liquid on walls of flask to rinse particles toward slurry
13. Add 2 mL of H_2O_2 when time allows through the day until bubbling stops (may be multiple days)
14. Do not let soil dry out
15. Carefully transfer all material to a high density 150 mL plastic bottle with a wide mouth rim, thoroughly rinsing all soil particles from Erlenmeyer with a squirt bottle
16. Add 20 mL of $Na_6P_6O_{18}$ solution
17. Secure cap on bottle, and place on reciprocating shaker overnight

C: Separation of fractions

18. Remove sample from reciprocating shaker
19. Set up sand sieving apparatus
    a. Oven dry and then weigh a 50µm sieve
    b. Place ring stand in the sink with funnel holder,
    c. Place large funnel in funnel holder
    d. Place a 50µm sieve above the funnel
    e. Place a 1000 mL graduated cylinder below the funnel
20. Label a 100 to 150 mL beaker with sample ID and record weight of empty beaker
    a. This beaker will be used to collected sand sized particles after silt and clay are rinsed through the sieve
21. Pour dispersed sample over the screen ensuring all smaller particles enter cylinder
22. Use water to rinse all particles smaller than the sand size, through the sieve
23. Transfer remaining sand into pre-labeled and pre-weighed beaker

24. Dry beaker with sand fraction for a minimum of 3 hours, weigh, and save for sieve stack separation
25. Stack the sieve sizes of choice with the largest on top, with decreasing sieve openings
    a. We will use 1000, 500, 250, 106, 53um sieve openings for our stack
26. Place the dried sample from step 21 on the top of the stack
27. Place the stack on a shaking apparatus and shake for 10 minutes (NOTE: our sieve shaker is old and you need to use smallest sieves available)
28. Empty each sieve into a tared weigh dish, and weigh sample retained to 0.0001g
29. If any material makes it through **ALL** of the sand sieves, it should be transferred to the graduated cylinder for the pipette determination

D: Pipette fine fractions from depth

30. Gather enough 30 mL beakers needed for taking 3 aliquots of each texture sample
31. Label each 30 mL beaker with sample ID and pipette time point (i.e. 0 min, 5 min, 5.5 hr)
32. Place all 30 mL beakers in 105C oven for at least 1 hour. After a minimum of 1 hour, place beakers in dessicator and then record the weight for each beaker.
33. In the Stokes room, fill graduated cylinder to exactly 1000 mL with DI water
34. Line up all cylinders, allowing at least 3 inches of space between cylinders so they are ready for pipetting
35. Place three oven dried, weighed, and labeled (0 min, 5 min, 5.5 hr) 30 mL beakers in front of each cylinder
36. Set a 20 mL glass pipette in the pipette slide apparatus
37. Check the temperature of the water cylinder to determine appropriate sampling depth
38. Set the pipette apparatus depth according to Table 6.1 on pg 76.
39. Rigorously stir/pump the silt and clay fraction with the flat bottomed stirring rod for 30 seconds. Do not break the surface of the water, focus on getting sediment up from the bottom of the cylinder and making sure the suspension is well distributed
40. Lower the pipette to the desired sampling depth with the apparatus.
41. Precisely 30 seconds after you finish stirring, remove 20 mL using the top button on the Accujet automatic pipetting aid
    a. This step may be skipped if only one silt size fraction is desired
42. Deposit the suspension into the 30 mL beaker labeled “0 min”
43. Proceed to stirring the next cylinder
44. Carefully follow the timing schedule in Table 6.2 **on page 4.** to efficiently perform this portion of the analysis on more than one sample in a 5-minute period
45. Precisely five minutes after you stop stirring each cylinder, pipette a second sample from the cylinder and deposit into the 30 mL beaker labeled “5 min”
46. Weigh each “0 min” and “5 min” beaker and place beakers with suspension into a 105C oven
47. Precisely 5.5 hours after the first sample is removed from the cylinder, pipette a third sample from the cylinder and deposit into the 30 mL beaker labeled “5.5 hr”
48. Weigh the beaker and place in 105C oven
49. The next day, weigh all 30 mL beakers, subtract the original weight of the empty beaker and record the sample mass into the spreadsheet

E: Blank Determination

1. Make a blank solution with 1L of DI and any other solutions used for dispersal
2. Follow pipetting steps 31-42 for this blank sample at each measurement point
3. Determine the weight of salts added to the soil suspension for calculation purposes by drying and weighing the pipetted sample

Calculations

1. A spreadsheet has been developed and provided to help make calculations
2. Enter all weights into the spreadsheet
3. Determine relative portion of fractions as follows; very course sand <2-1 mm, course sand <1-0.5 mm, medium sand <0.5-0.25 mm, fine sand <0.25-0.1 mm, and very fine sand <0.1-0.05 mm, course silt <0.05-0.02 mm, fine silt <0.02-0.002 mm, and clay <0.002 mm.

Table 6.1. Appropriate depth at which to take the sample with pipette by temperature

Temperature	Depth (cm)	Depth (cm)
°C	5 min.	5½ hrs.
19	10.5	6.9
20	10.8	7.1
21	11.0	7.2
22	11.3	7.4
23	11.6	7.6
24	11.9	7.8
25	12.1	8.0
26	12.4	8.2
27	12.7	8.4
28	13	8.6
29	13.3	8.8
30	13.6	9
31	13.9	9.1
32	14.2	9.3
33	14.4	9.5
34	14.8	9.7
35	15.1	9.9
36	15.4	10.1

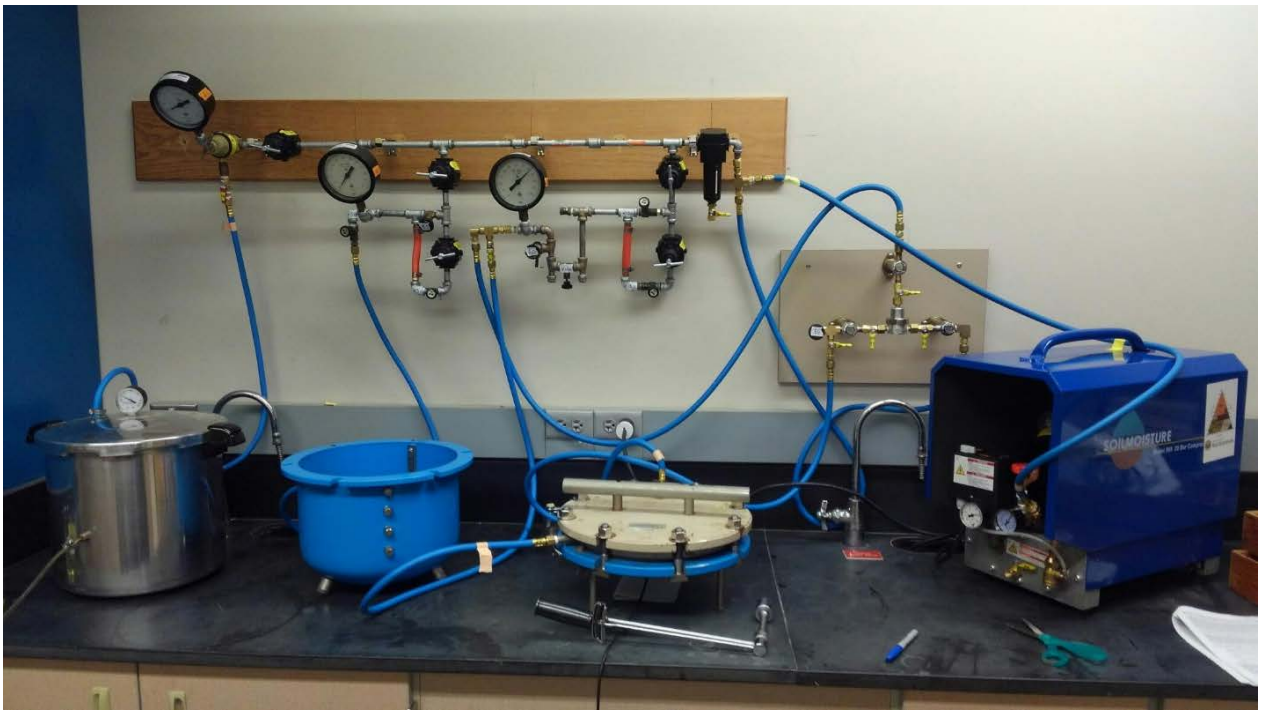
Available Water Holding Capacity Pressure Plate System

Summary

A known volume of soil is weighed, saturated, and subjected to a specific pressure until no more water leaves the soil system. At this point the sample is removed, adjustments are made to account for changes in soil volume, and the moisture content of the sample is determined. To quantify the available water holding capacity we subject the samples to 1/3 bar pressure for field capacity and 15 bar pressure for wilting point. We subtract the difference in moisture content to report

Equipment and materials

- Soil Moisture Pressure Manifold mounted on wall
    - Gauges (g), valves (v), regulators (r), hoses
- Air compressor
- 1 bar pressure cooker
- 5 bar pressure chamber (1600 SM)
- 15 bar pressure membrane extractor (PME 1000)
- 1 bar ceramic plate
- 5 bar ceramic plate
- Rings with standard height and volume for PME 1000
- Rings with standard height and volume for chambers
- Cheese cloth
- Rubber bands
- Scissors
- Spatula
- Balance
- Paper towels
- Large flat soaking containers with lids
- Cellulose membrane
- 2mm Sieved soils (may change this to ground soils)



Reagents

- Deionized (DI water)

Procedure

1. Before using for the first time, read the complete reference manual from Soilmoisture Equipment Corp available in the R drive.
2. Sample preparation (24 hr prior to pressure application)
    a. Air dry samples in drying cabinet
    b. Pass soils through 2mm sieve
    c. Place two small squares of cheese cloth at 45^{o} angles to each other
    d. Place label tape on ring with the sample identification
    e. Measure ring diameter and place on top of cheesecloth
    f. Secure with rubber band ensuring the cheese cloth is high enough to reach top of ring
    g. Trim cheese cloth that is higher than the ring height
    h. Record mass and interior volume of ring apparatus in worksheet
    i. Record start date
    j. Fill ring with sieved soil sample with a mound on top
    k. Use the edge of a flat spatula to tap the soil across the ring so that it is evenly distributed and nearly level
    l. Use the edge of a flat spatula to level the top of the sample similar to measuring flour for baking
    m. Ensure that there are not large protrusions or divots

n. Any time this apparatus is moved, hold the cheese cloth in place and support the bottom under the cheese cloth. A spatula works well for this
o. Record the mass of the ring apparatus and dry soil
p. Transfer the ring and soil to a soaking container
q. Repeat process for all samples
r. Always use a CAL standard soil for each chamber being used
s. Always use a method blank with no soil for each chamber being used
t. When a soaking container is full of samples or all samples are prepared, use a beaker to slowly pour DI water into the open space in the container between samples
u. Maintain the water level at about half of the height of the rings
v. You will need to add water for a couple of minutes while the samples pull in the water
w. When there is no more visible change in the water level, put a lid on the container and let soak for 24 hours
x. Saturate any ceramic plates that will be used for analysis of samples by submerging in water for a minimum of 24 hours

3. Sample placement in chambers
    a. Place three spacers in the bottom of the chamber to reduce plate damage
    b. Place the saturated ceramic plate of choice on the spacers
    c. Connect the outflow tube to receptor on the ceramic plate
    d. Place fresh paper towel on balance and tare
    e. Carefully remove the samples from the soaking container with a spatula
    f. Place sample and spatula on balance and record total mass
    g. Place sample on ceramic plate in chamber
    h. Record mass of wet paper towel and spatula
    i. Repeat for all samples, including the method blank
    j. When the plate is full or all samples are in place, put a damp cloth towel over the samples to minimize evaporative losses
    k. If another plate is used, repeat the processing including the placement of appropriate spacers, connections, and towels.
4. Sample placement in membrane extractor
    a. Place cellulose membrane in water to soften for 2-5 minutes
    b. Unfold the cellulose membrane once flexible
    c. Allow the flattened membrane to soak for minimum of 10 additional minutes
    d. Thoroughly clean the screen drain plate to ensure no soil particles will puncture the cellulose membrane
    e. Wet the screen with DI water squirt bottle
    f. After sufficient soaking, use the squirt bottle to rinse any particles from cellulose membrane
    g. Center the cellulose membrane carefully on the screen drain plate
    h. Remove excessive air pockets between membrane and screen
    i. Place one O-ring for the extractor cylinder on the membrane
    j. Place the extractor cylinder on the O-ring ensuring proper fit
    k. Place the second O-ring in the groove of the extractor cylinder
    l. Place fresh paper towel and spatula on balance and tare

m. Carefully remove the samples from the soaking container with the spatula
n. Place sample and spatula on balance and record total mass
o. Place sample on ceramic plate in chamber
p. Record mass of wet paper towel and spatula
q. Repeat for all samples, including the method blank
r. Slowly close the top place of the extractor
s. Place a clamping bolt and nut in each receiver (8 total) with the rectangular head securely within the grove of the bottom plate. The washer and nut should be on top of the top plate
t. Starting with the location marked 1, hand tighten the nut
u. Continue to tighten the nuts in a star pattern following the numbers on the top plate
v. Use the socket wrench to sequentially tighten the nuts in the numerical order
w. Use care not to overtighten any of the nuts, expect to use light force on each nut at least 4 or 5 times around the star pattern
x. After tightening sequentially 4 or 5 times, check the tightness with the torque wrench
y. Each nut should carry 3-5 foot-pounds of torque
z. Adjust each nut in sequence making sure that position 1 is still at the appropriate pressure after the full sequence has been checked
aa. Place the outflow tubes into a volumetric container for water collection

5. Applying pressure
    a. Wear protective goggles and ear plugs when operating the pressure plate system.
    b. Ensure that all valves (labeled with yellow V1, V2… V7) are in the closed position
        i. Valves are closed when they are perpendicular to the piece they are attached to
        ii. Valves are open when they are in line
    c. Ensure that all regulators (labeled R15 high and low, R5 high and low, R1 high and low) are not allowing air through. When the regulator screw is all of the way open or turned counter clockwise the air cannot get through. When the metal crank is turned clockwise it pushes the membrane in allowing air to pass through.
        i. Regulators are closed by turning counter clockwise = disengaged membrane
        ii. Regulators are opened by turning clockwise = engaged membrane
    d. Ensure all exhaust valves (labeled with E15, E5, ED) are closed.
        i. Exhaust valves are closed when they are fully turned clockwise
        ii. Exhaust valves are open when they are turned counterclockwise
    e. Valves labeled in white; V15a, V15b, V15c, V5a, and V5b stay in position during shutdown and do not need to be changed during start up
    f. The red star shaped knob in the compressor does not need to be adjusted during start up or shut down unless an experimental change is needed
    g. Turn the white nob in the compressors to the on position
    h. You should hear the compressor kick on
    i. The gauges on the compressor should both start to move up.
    j. Gauge 1, on the left should go to roughly 20 bars
    k. Gauge 2, on the right should stop at 15 bars or 220 psi

l. When adjusting the regulators ALWAYS make sure that you are not directly in line with the metal crank. These regulators can fail and the crank can become a projectile. These are close to eye level so you should stand to the side. While this position is awkward, it is a necessary safety precaution
m. Once gauges are stabilized you can start to adjust the regulators on each plate
n. Starting with the R1 high turn the metal crank on the regulator clockwise about half way
o. Move to R1 low and turn the metal crank until you see the pressure register on the gauge
p. Move crank on R1 high to adjust to nearly the pressure you want
q. Use R1 low to fine tune the pressure to precisely the pressure you want
r. Once the pressure is stabilized where you want it (5psi = ~1/3bar) you can turn V7 to the on position
s. The pressure will likely fall because the chamber is large and filling with air

t. You may need to adjust both the low and high regulators to allow for more air flow

u. Once the chamber is pressurized the small exhaust plug on the pressure chamber lid will be pushed up. If this is depressed, you may need to turn the R1 low clockwise more until the pressure gauge registers pressure
v. While making adjustments the compressor will likely kick on again
w. Be ready for a somewhat loud bursting noise when it kicks off, ear protection is provided and encouraged
x. Once the gauge is steady at the desired pressure you can move onto the next chamber
y. Repeat the process for the 5 bar chamber, starting with the high, then low pressure regulator adjustment.
z. The valve on the 5 bar chamber is a turn nob (V5b for now) and does need to be opened
aa. Then fine tune with the low pressure regulator
bb. Repeat the process for the 15 bar membrane extractor, starting with the high, then low pressure regulator adjustment, then open V6 valve
cc. Water should come out of the outflow tubes when system is working
dd. It may bubble a bit from the 15 bar extractor, but should not bubble from either the 1 or the 5 bar.
ee. After 2-3 hours the compressing diaphragm on the top of the 15 bar extractor should be engaged with the differential system (Fig 3)

ff. First turn on V1, then V2, V3, and V4 with about 20 seconds between each valve
gg. This should apply pressure to the top of the samples even after soil volume loss

hh. Samples have been equilibrated when there is no further change in water volume
ii. This normally takes several days but is highly dependent on the soil texture

6. Need to detail the sand adjustment for shrinkage and all of the calculations.

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.

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.

6.4.1 Water Stable Aggregates (Cornell)

Scope and Application

Soil aggregate stability is an indicator of soil health. The formation of stable soil aggregates is often caused by increased soil organic matter content, resulting in improved infiltration and aeration of soils, and decreased soil erosion caused by wind or water. Unstable soil aggregates fall apart when hit by rain drops or irrigation water. The dispersed soil particles can block soil pores and form surface crusts, which reduce air exchange and water infiltration. The method described here is modified from the Cornell Soil Health Manual-Wet Aggregate Stability (Gugino et al., 2009), which simulates an impactful natural phenomenon: a heavy rainstorm when the surface soil is dry.

Summary

Soil is air dried at 30^{o}C, soil that passes through a sieve with 2 mm openings, but not through a 0.25 mm sieve openings are evenly spread on a sieve with 0.25mm openings. A rain simulator is placed 50cm above the sieve, and 1.25cm of rain is dropped by the simulator over a 5 minute period. Wet stable aggregates are the aggregates that remain in the sieve after test. The mass of sand sized particles is subtracted from the total mass of particles that remain on the screen.

Materials

- 2.0mm sieve
- 0.25mm sieve
- Rain simulator
- Balance
- Stop watch
- Oven

Reagents

- Deionized water

Procedure

1. Record the weight of 0.25mm sieve (W_{sieve})
2. Stack 2.0mm sieve on top of 0.25mm sieve
3. Scoop soil sample onto 2.0mm sieve
4. Collect ~20g of 0.25-2.0mm aggregates in 0.25mm sieve
5. Record the weight of 0.25mm sieve with soil aggregates ($W_{initial}$)
6. Adjust the rain simulator so the diameter of water drops is about 4.0mm, and the amount of water delivered within 5 minutes is about 1.25cm depth
7. Place the sieve 50cm underneath the rain simulator
8. Run the rain simulator for 5 minutes, use stop watch to monitor time
9. Wait until all the water have been drained
10. Dry remaining soil aggregates in 0.25mm sieve at 105^{o}C

11. Weigh total particles remaining on sieve
12. Transfer materials to a sieve with 0.05mm openings
13. Wash all particles remaining on sieve to break up any aggregates, allowing them through the sieve
14. The remaining sand sized particles are oven dried for 24 hours
15. The sand particles are then weighed to subtract from the stable aggregates
16. Calculate the wet aggregate stability using the equation below

Calculations

$$
\begin{aligned}
total\ soil &= (sieve\ and\ soil) - sieve\ wt \\
sand &= (dry\ sieve\ and\ sand) - sieve\ wt \\
unstable\ aggregates &= (dry\ filter\ and\ soil) - filter \\
stable\ aggregates &= total\ soil - unstable\ aggregates - sand \\
total\ aggregates &= unstable\ ag + stable\ ag \\
water\ stable\ ag\ as\ percent &= \left(\frac{stable\ ag}{total\ ag}\right) * 100
\end{aligned}
\qquad \text{(eq. 1)}
$$

Reference

Gugino, B.K., Idowu, O.J., Schindelbeck, R.R., van Es, H.M., Wolfe, D.W., Moebius-Clune, B.N., Thies, J.E. and Abawi, G.S. 2009. Cornell Soil Health Assessment Training Manual, Edition 2.0, Cornell University, Geneva, NY.

Gravimetric Moisture Content

Summary

The net weights of wet and dry soil are needed for calculation. Soil core sample is stored in ice cooler to maintain field condition during transportation. Wet weight of soil core is determined immediately in lab after arrival. Soil is air-dried or oven-dried at 105^{o}C.

Equipment and Materials

- Balance
- Soil core rings with known volume and mass
- Oven
- Desiccator
- Aluminum drying tins

Procedure

1. Record the weight of the aluminum drying tin
2. Record the weight of soil core ring, if present
3. Dry entire setup at 105C for 24 hours
4. Cool down soil sample in desiccator for 2 hours
5. Record the weight of dry soil with soil core ring
6. Subtract weight of drying dish and core to get the total mass of dry soil
7. Use known volume to calculate bulk density (dry soil/total volume = bulk density)

Potentially Mineralizable Nitrogen 28 day Aerobic Incubation

Summary
Samples should be sent to the lab cold and/or as fresh as possible to minimize error for the NO_3-N an NH4-N measurement from time 0. Samples are sieved to two millimeters and air dried for 3 - 5 days. 7.5 grams of sample is extracted with 2M KCl for quantification of NO3 and NH4. One hundred grams of air dry soil is weighed and placed in a glass jar. The bulk density of the sample is calculated and water is added with a spray bottle to reach 50% water filled pore space. Samples are covered with a polyethylene film and secured. This allows for gas movement, but limits moisture loss. Samples are weighed every seven days to adjust for any moisture loss. On day 28 the sample is thoroughly mixed and extracted for NO_3-N and NH4-N again. These two time points allow for the calculation of the average rate of N mineralization per day.

Equipment and Materials

- Quart sized Mason jars (extra headspace is good for gas exchange)
- Screw top band of a Mason jar lid
- Spray bottle
- Polyethylene film
- Balance
- Drying oven (105^{o}C)
- NO_3-N and NH4-N extraction and measurement materials

Reagents

- Water
- NO_3-N and NH4-N extraction and measurement reagents listed on separate SOP

Procedure

1. Sieve sample to 2 mm and air dry for 3-5 days
2. Use 7.5 g air dry sample to extract NO3-N and NH4-N with 30 mL 2M KCl (follow SOP)
3. Weigh 100 g air dry, sieved sample in plastic beaker
4. Transfer soil to a pint sized glass jar with volumetric marks
5. Record the volume the soil fills
6. Calculate the bulk density, % porosity, and volume of pore space of the sample in order to determine the water needed to add to the sample

$$Bulk\ density = \frac{mass\ soil\ (g)}{volume\ soil\ (mL)}$$

$$\%Pore\ space = 1 - \left(\frac{Bulk\ density}{Particle\ density}\right)$$

$$Volume\ pore\ space = \%\ pore\ space * volume\ soil\ (mL)$$

$$Water\ to\ add\ (mL) = Volume\ pore\ space(mL) * 0.5$$

7. Place jar and soil on balance and use a spray bottle to add water until the desired water volume (1 mL= 1 g water) has been achieved.
8. Secure double layer of polyethylene film over jar with rubber band
9. Record the mass of the total incubation set-up
10. Check the weight of the incubation set up each week.
    a. When the weight falls >1 g below the starting point, use a gentle mist spray bottle to add water until it reaches the weight recorded in step 9.
11. On day 28, empty the sample into a Tupperware container and mix thoroughly
12. Follow the SOP for KCl extraction and quantification of NH4-N and NO_3-N
13. Final data should be reported as the µg NO_3-N g dry $soil^{-1}$ day^{-1}
    a. NO_3-N for each time point is reported in ppm or mg N kg^{-1} dry soil
    b. The rate is calculated with the difference of the two measurements divided by the number of days

$$((NO3 + NH4\ day\ 28) - (NO3 + NH4\ day\ 0))/28\ days \qquad \text{(eq. 1)}$$

Scope and Application

OSU Extension publication EM 9020 describes the work done over several decades to correlate a laboratory test to the nitrogen available to soft white winter wheat grown in Western Oregon. Our laboratory method follows their prescription as closely as possible which is a slight modification of the anaerobic incubation described by Keeney (1982) in the SSSA Methods of Soil Analysis. In order for these results to be used to predict N mineralization, please follow the guidelines in EM 9020 (also listed below in procedure).

Summary

Fresh soil is sieved and immediately extracted using 2M KCl, the NH4-N in the extractant is then quantified using the Lachat colorimetric autoanalyzer. A separate portion of the sample is placed in a sealable container, saturating water is added, container is sealed, and incubated for 7 days at 40°C. After incubation the sample is again extracted for NH4 and quantified.

Equipment and Materials

- 125 mL sealable plastic containers
- Balance
- Scoopula
- Reciprocating shaker
- Filter rack
- Whatman #1 Filter papers
- Incubation chamber
- Graduated cylinder
- Parafilm

Reagents

- 2M KCl solution
- DI water

Procedure

1. Samples should be kept cold immediately after sampling and prior to analysis
    a. If samples cannot be kept cold, they should be air dried immediately
2. Once at the lab, sieve fresh cold sample to 2 mm
3. Determine gravimetric moisture content of fresh soil
4. Weigh 20 g of well mixed fresh soil into 125 mL bottle
5. Add 100 mL of 2M KCl and seal
6. Put sample on reciprocating shaker for 1 hour

7. Filter
8. Quantify the NO3-N and NH4-N of solution following the appropriate SOPs
9. Weigh 20 g of well mixed soil into a 125 mL bottle
10. Add 25 mL of DI water and stir well with a glass stir rod
11. Add another 25 mL of distilled water ensuring to rinse the stir rod and the sides of the 125 mL bottle
12. Ensure that the bottle is tightly sealed. Use a layer of Parafilm under the lid if seal is questionable
13. Place the sample in the incubator at 40^{o}C
14. Enter a reminder in the Google calendar to remove the sample after 7 days
15. Remove the sample from the incubator
16. Add 50 mL of 2M KCl
17. Replace the lid and tighten securely
18. Shake by hand to ensure sample is free from the bottom, then shake for 1 hr on shaker
19. Filter
20. Quantify the NH4-N of solution

Carbon Dioxide Burst Test - Activity

Summary

Soil is sieved to 2 mm and air dried prior to starting this procedure. The soil is added to a sample cup with volumetric markers used to calculate experimental bulk density and the amount of water to use for rewetting. Immediately prior to the baseline reading a volume of water sufficient to fill 50% of the pore volume with water is added. The sample is placed inside of a quart sized jar and a baseline CO_2 measurement is made. The soil is incubated with an air tight seal for 24 hours, in the dark, at 23°C and the CO_2 accumulation in the headspace is measured again after 24 hours. It is very simple to add an additional 96 hour measurement if desired. One CAL standard and one method blank is analyzed with set of unknowns.

Equipment and Materials

- Quart sized canning jar
- Lid fitted with a gas sampling septum
- 100 mL sample cup
- Transfer Pipette
- 25 mL gradated cylinder
- Deionized (DI) water
- Incubation chamber to maintain 23°C
- Picarro isotopic CO_2 analyzer

Procedure

1. Prepared soil sample is air dried (~35°C, 48-72 h) and sieved to 2.00 mm
2. Weigh 40 g[1] of air dried soil sieved soil into a 100 mL sample cup
3. Record the volume of soil
4. Use table x.x to determine the water to use and indicate on data sheet
5. Place the sample cup with soil inside the glass canning jar
6. Gather sample apparatus, the graduated cylinder, a DI water squeeze bottle, transfer pipette, and data sheet and bring to ALS 3011 where the Picarro is located
7. Set the Picarro to measure 12CO2_dry, 13CO2_dry, and the outlet valve pressure
8. Set the Picarro to measure each jar for 2 minutes until port 16, which is set to 30 minutes
9. Press “restart user log” to enable easy data retrieval from Picarro datalogger
10. Record the baseline CO2 values in the room prior to measuring samples
11. Measure desired water volume and pour into the first soil sample
12. Place lid with septa and canning seal on the jar
13. Place the needle from port 1 into the first jar and press “apply”
14. Record time that the sample is started
15. When 1 minute of time has passed add water to the next sample, seal jar, and insert needle from port 2
16. At the 2 minute mark, record the 12CO2, 13CO2, and outlet valve on data sheet from the first sample

17. Continue with up to 15 samples
18. Allow the machine to read the room baseline while at port 16 while you remove the needles, place the samples into the 23^{o}C incubation cupboard, and arrange your next set of samples
19. Assess the outlet valve readings, if there is a significant drop an outlet valve value check the integrity of the needles and the seal of the respective jars. Measure again if needed
20. Prior to reading another set, press "restart user log" again
21. Incubate for 24 hours (record time from initial water addition)
22. Once samples are done, select "Turn Off Machine in Current State" in Picarro software
23. After 24 hour incubation period, turn on the Picarro at least 35 minutes before samples need to be measured
24. Set the machine up following steps 7-9 and set up to 15 jars on the ports
25. Restart User Log
26. Set a timer for 25 minutes
27. Allow the machine to read the baseline while you change out the next set of samples
28. If measuring more samples, Restart the user log and press apply when new samples are ready
29. Check the work order sheet to see if customer has requested the optional CO_2 measurement again after 72 or 96 hours
30. Re-incubate samples or bring back to 3079 for cleaning
31. Data can be consolidated from the computer system using the R code saved in the "Protocols and Procedures" folder in the R drive
32. Enter data into the spreadsheet from all time points to calculate the CO2 rate

[1]Smaller volumes can be used if sample is limited, though results are less robust.

***CO_2 concentration measurement**

A Picarro CO_2 Isotope Analyzer with a 16-port interface will be used to measure the concentration of CO_2. Although intended to measure the isotopic composition of CO_2, it is also convenient for measuring its concentration, which is measured based on the absorption of infrared light by CO_2. The Picarro is turned on at least 30 minutes before measurements begin to allow it to warm up and stabilize. The jars are attached to the 16-port multisampler via hypodermic needles. The sampling program is opened and launched. Each port will draw gas for 2 min from the headspace of one jar and then switch to the next jar in the designated sequence. The CO_2 concentration is stabilized by the end of the 2-min sampling period and the average is calculated for the final 30 s. The final reading can be recorded manually by reading the value off of the monitor or an R program can be used to extract the data from a file that is saved to the instrument.

References

Franzluebbers AJ. 2016. Should soil testing services measure soil biological activity? *Agricultural and Environmental Letters* doi:10.2134/ael2015.011.0009.

Franzluebbers AJ, Haney RL, Hons FM, Zuberer DA. 1996. Determination of microbial biomass and nitrogen mineralization following rewetting of dried soil. *Soil Science Society of America Journal* 60, 1133-1139.

Active Carbon

Summary

Soil is weighed and shaken for two minutes in a solution of a known concentration of potassium permanganate. The soil is allowed to settle for eight minutes and the supernatant is diluted and measured colorimetrically to determine the amount of permanganate that was reduced during the oxidation of carbon. One CAL standard and one method blank is analyzed with each group of unknowns.

Materials

- Balance
- Falcon tubes
- Shaker
- Pipettes
- Spectrophotometer
- Stopwatch

Reagents

- 0.02M Potassium permanganate $KMnO_4$
- DI water

Procedure

1. Prepare a set of $KMnO_4$ standards at 0, 0.005, 0.01, 0.015, 0.02 M
2. Weigh 2.50 g of air-dried soil into a 50 mL Falcon tube
3. Measure 49.5 mL of water into separate Falcon tubes for the dilution so that proper timing can be maintained
4. Pipette 0.5 mL of standard into falcon tube containing water. Cap and shake by hand. Pour into cuvette. Measure standards on spectrophotometer at 550 nm
5. Add 20 mL of 0.02M $KMnO_4$ to the first Falcon tube with soil. Samples should be started one at a time to maintain timing.
6. Shake for exactly 2 minutes
7. Allow to settle for 8 minutes. Pipette 0.5 mL of supernatant of sample solution with $KMnO_4$ into Falcon tube with 49.5 mL of water
8. Give one good shake to mix solutions
9. Pipette 2 mL of solution water into a 4 mL cuvette
10. Measure on spectrophotometer at 550 nm
11. Measure standards after each run of samples

$$AC\ \left(\frac{mg}{kg}\right) = \left(0.02\ \left(\frac{mol}{L}\right) - y\right) * \left(\frac{9000\ mg\ C}{mol}\right) * \left(\frac{0.02\ L\ solution}{0.0025\ kg\ soil}\right)$$

Where y = mx+b using the standard concentrations to calculate the slope and intercept of the standard and x is the spectrophotometer reading of the unknown sample.

Weil, R., K.R. Islam, M.A. Stine, J.B. Gruver and S.E. Samson-Liebig. 2003. Estimating active carbon for soil quality assessment: A simplified method for laboratory and field use. American Journal of Alternative Agriculture. Vol 18, No. 1 pg 3-17.

Chloroform Fumigation Extraction Procedure - Microbial Biomass

Summary
Two 10 g soil samples are weighed into Falcon tubes. One is placed inside of a desiccation chamber with chloroform. Vacuum pressure is applied to volatilize the chloroform, allowing it to permeate the soil samples. After 24 hours the pressure is released and the chloroform gas is evacuated from the chamber. Both samples are then extracted with 0.05M K_2SO_4 and the extracted solution is diluted and measured on the Shimadzu TOC in order to quantify the dissolved C and N. The difference between the two extractions is assumed to be the C and N from inside of the microbial cells that were lysed through the extraction process. One CAL standard and one method blank is analyzed with each group of unknowns.

Equipment and Materials

- Fume hood
- Desiccator
- Vacuum pump
- Small sized glass canning jar
- 50 mL specimen cups
- Funnels
- Whatman #1 filter paper
- Silicone sealing grease

Reagents

- Chloroform (ethanol-free)
- 0.05 M K_2SO_4 (8.713 g/L)

Procedure

1. Weigh two 10 g dry weight samples into 50 mL sample tubes
2. Add 30 mL 0.05 M K_2SO_4 to the sample that will not be fumigated, cap the tube
3. Shake for 1 hr on low speed.
4. Filter
5. The extract is stored in a fridge for analysis
6. Write a sample label with pencil on paper and place it in the sample tubes to be fumigated (if chloroform splashes from the boiling, it will remove ink)
7. Place all samples to be fumigated into a glass vacuum desiccator inside of a fume hood
8. Place a small glass jar containing desiccation chips in the middle of the samples
9. Fill the jar with chloroform, do not fill past the area where the jar lid threading starts
10. Use about 1-inch worth of silicon grease to thoroughly lubricate the rim of the desiccation chamber
11. Carefully place the lid on the chamber. (**NOTE: do not only hold by the knob on top, it is removable and may slip off breaking the chamber**)
12. Fit the black tube to the chamber valve and flip the switch to turn on the vacuum knob to the left of the fume hood

13. Carefully listen for a change in gurgling noises to indicate that all of the air has been removed (NOTE: at this point you will also see the chloroform start to boil due to the reduced pressure)
14. Turn the knob sleeve ¼ of the way around
15. Place a dark bag over the desiccator and allow to incubate for a minimum of 24 hours
16. After incubation, rotate the nob sleeve and allow air flow
17. Open the desiccation chamber lid and remove the jar with the chloroform
18. Replace the lid
19. Attach the vacuum tube to the vent and evacuate all gas
20. Remove the tube, allow the chamber to vent and repeat for a total of three ventilation cycles
21. Remove samples from the desiccation chamber and extract following steps 2-5
22. Store extractant until results are validated
23. Non-fumigated and fumigated extracts are analyzed for solution organic C and N using a Shimadzu TOC/TN Analyzer (Shimadzu Corp, Kyoto, Japan) (dilution may be needed)

Calculations

The difference in the solution organic C (or N) between the fumigated and non-fumigated samples represents the C (or N) in the microbial biomass.

$$[CFs]-[CNFs] = [C] \text{ in the microbial biomass}$$

Where [CFs] represents concentration of carbon in fumigated sample and
[CNFs] represents concentration of carbon in non-fumigated sample

This is converted into µg C (or N) by multiplying the corresponding values obtained from the Shimadzu TOC/TN Analyzer by the volume of the extractant (30 mL plus the mL of water in the soil that was extracted) and dividing by the dry weight of the soil that was extracted. Division of the C (or N) flush values by the correction factors of 0.45 for C or 0.56 for N will yield the microbial biomass C (or N).

$$([C]*(30mL+\text{soil water})/\text{dry soil}))/0.45 = \mu g \text{ C in microbial biomass } g^{-1} \text{ dry soil}$$

$$([N]*(30mL+\text{soil water})/\text{dry soil}))/0.56 = \mu g \text{ N in microbial biomass } g^{-1} \text{ dry soil}$$

References

Brookes, P. C., A. Landman, G. Pruden, and D. S. Jenkinson. 1985. Chloroform fumigation and the release of soil nitrogen: a rapid direct extraction method to measure microbial biomass nitrogen in soil. Soil Biol. Biochem. 17:837-842.

Vance, E.D., P.C. Brookes, and D.S. Jenkinson. 1987. Soil Biology and Biogeochemistry 19:703-707.

Inorganic C correction

Supplies:
Falcon tubes 50 ml – one per sample plus one for water evap, one for pure $CaCO_3$, one for soil check
Falcon tube cap with three small holes (all equal sizes, consistent between caps)
Calcium carbonate
3M HCl
Balance that goes up to 30 g with 0.1 mg precision (our yellow or white balances)

Record tare weight of tube, cap, and 10 ml of 3.0M HCl
Weigh soil on weigh paper and transfer in increments to acid
Put lid on tube
Do for nine unknown samples, plus one water sample, plus one $CaCO_3$ sample, plus one soil std
Shake batch on orbital shaker for fifteen minutes
Let sit for one hour and forty-five minutes
Weigh to get final weight and record
Calculate mass loss
Determine if water loss is significant (>0.003g): if so, add that mass back into the calculations for the unknown samples
Convert to calculate total C lost (inorganic C)
Confirm that total C lost from $CaCO_3$ check and soil check are within tolerable limits (+/- 10%)
Subtract inorganic C from total C to get organic C

After: Western States Methods Manual 2013