Standard Procedures for Soil Research

in the McMurdo Dry Valleys LTER

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These methods represent those currently used by the soil biology and biogeochemistry group and have been developed throughout 20 years of working in Antarctica. Methods frequently need to be modified for different soil types. Therefore, methods included here do not precisely resemble those used for all experiments and data. Users should see the individual metadata and publications associated with each dataset for specifics regarding individual experiments.

Table of Contents

Part 1: Soil chemical and physical properties

1.1 Gravimetric soil moisture

1.2 pH

1.3 Electrical conductivity

1.4 Soluble ions (anions and cations)

1.5 Extractable inorganic nitrogen

1.6 Extractable phosphate

1.7 Total and organic carbon and nitrogen

1.8 Total and organic phosphorus

1.9 Soil texture

Part 2: Soil biology

2.1 Mesofauna extraction

2.2 Bacteria cell counts

2.3 Chloroform labile CNP

2.4 Microbial Community Analysis

Part 3: Soil organic matter

3.1 Elemental analysis for organic C and P

3.2 Density fractionation

Part 4: Incubations

4.1 Laboratory incubations

4.2 Ion exchange resins

Part 5: References

1.1 Gravimetric soil moisture

Moisture content is an important soil property that influences physical, chemical and biological characteristics and activities. It is also a necessary piece of information for determining the dry weight equivalence of a field moist soil and expressing a suite of soil properties on a gravimetric (dry weight) basis. Since all soil measurements are expressed on a dry weight basis, soil moisture content is a necessary first step for all soil analyses.

In this method the water content of soil is determined by weight loss during drying in a standard convection oven. The mass of a dried soil is subtracted from the mass of the field moist soil and this estimated mass of water is divided by the dry mass of soil. It is important to note that in this procedure, as in all gravimetric techniques, the variable of interest is expressed on a dry mass basis.

1. Pre-weigh an aluminum soil can and lid (“ointment tins”, ID 60 mm).

2. Add field moist soil to soil can and weigh (making sure that you weigh the lid as well as the can). Typically the < 2 mm size fraction is used for standard soil analysis. The standard practice for soil preparation in the Wall and Virginia lab has been to exclude larger soil particles by hand sorting. This technique is most common for soils that are being analyzed for invertebrates (the mechanical disturbance that accompanies dry sieving may kill invertebrates). Alternatively soils may be sieved through a 2 mm screen to remove the > 2 mm size fraction. If this is method is used, care must be taken to sieve the soils rapidly to minimize evaporative water loss from the soil sample.

3. Place soil can on tray and put into drying oven at 105˚C and dry for 24 hrs with the lid off.

4. Remove soil cans along with oven-dry soil and *immediately* fit the lids over the top of the can. Allow to cool for 10 – 20 min. Weigh each can plus soil. Be aware of thermal influence on the balance, vent out hot air and re-zero as necessary. Do not leave out dish with oven-dried soil too long before weighing because they will re-absorb moisture.

5. Calculate gravimetric soil moisture content.

   column A– tare mass
   column B– tare mass + field-moist mass
   column C– tare mass + oven-dry mass
   column D– H_2O g/g on an oven-dry basis = (B – C) / (C – A).

The proportion of soil moisture can be multiplied by 100 to express as a percentage gravimetric soil moisture content.

Note: The most common errors are due to inconsistencies with taring and pre-weighing of soil cans and lids. Make sure that the tare weight and final weight includes the lid.

1.2 Soil pH

Soil pH is an important chemical property that describes the hydrogen ion content of the soil. In Antarctic soils pH's are generally high (above 7) but lower pH's of 5 – 6 are occasionally reported, particularly for some of the older and perhaps more highly weathered soils. In this analysis the pH of a soil is measured in a 2:1 water:soil dilution using a standard pH electrode.

Materials:
pH meter
Buffer pH standard solutions
100-ml glass beakers, di-H_2O-rinsed (1 per sample)
Metal spatula and/or stir rod
di-H_2O in squirt bottle
di-H_2O in a 50-ml repipettor

Protocol:
1. Calibrate pH meter using standards at pH 7 and 10.
2. Add 20 g of soil to a tared beaker. Try to be as close to 20 as possible. Wipe spatula clean between samples.
3. Add 40 ml di-H_2O to each beaker using repipettor.
4. Stir until thoroughly mixed (about 5-10 sec), wiping stir rod clean between samples.
5. Let equilibrate for 10 minutes, stir again and take a reading. Rinse probe with squirt bottle between each sample.

Optional: Let equilibrate for 2 hrs, stir soil slurry and take an additional reading.

Note:
If EC is also being measured, use a 150-ml beaker and continue to step 2 of the EC protocol.

1.3 Electrical conductivity

Electrical conductivity provides an indication of the total ionic activity of a soil, i.e. salinity. Electrical conductivity varies widely in Antarctic soils depending upon the age of the soil and the proximity to marine influences. Typical ranges of electrical conductivity are in the 100's of µm Siemens cm^{-1}, but some soils (particularly those from Beacon and Arena Valley and Bull Pass may have conductivities in the mm Siemens cm^{-1} range. In this analysis the conductivity of a soil sample is measured in a 5:1 water:soil dilution

Materials:
Conductivity meter
0.01 M KCl
150-ml glass beakers, di-H_2O-rinsed (1 per sample)
Metal spatula and/or stir rod
di-H_2O in squirt bottle
di-H_2O in a 50-ml repipettor

Protocol:
1. Add 20 g of soil to tared beaker. Try to be as close to 20 as possible. Wipe spatula clean between samples.
2. Add 100 ml di-H_2O to beaker using repipettor (or add another 60 ml di-H_2O to the beaker from the pH reading).
2. Stir until thoroughly mixed (about 5-10 sec), wiping stir rod clean between samples.
3. Let equilibrate for 10 minutes, stir again and take a reading. Rinse probe with squirt bottle between each sample.

Optional: Let equilibrate for 2 hrs, stir soil slurry and take an additional reading.

Temperature corrections:
Measure the conductivity of a standard solution of 0.01 *M* KCl prior to measuring samples. Use the following equation

(1,411.8/0.01M KCl reading) * sample reading

to correct for the influence of temperature on the electrical conductivity of the soil.

1.4 Soluble Ions (anion and cation)

For estimation of soluble anions and cation concentration in Antarctic soils.

Materials:
125-ml Erlenmeyer flasks (acid-washed)
Funnels (and funnel racks) and #42 Whatman filter paper (11 cm diameter) *or*
50 ml centrifuge tubes and centrifuge
60 ml HDPE wide-mouth bottles
60 ml HDPE narrow-mouth bottles *or* 20 ml HDPE scint vials
60 ml syringe
0.45 micron syringe filters
di-H_2O in 50 ml repipettor
10 ml pipetman and tips
70% HNO_3 in 1 ml repipettor
Parafilm

Protocol:
1. Weigh 10 g of <2 mm soil into tared Erlenmeyer flask. Record weight.
2. Add 50 ml di-H_2O to each Erlenmeyer flask, including 3 blanks.
3. Cover Erlenmeyer flask with parafilm and shake on an orbital shaker for 1 hr on low speed setting.
4. Set up filter racks. Place wide-mouth bottles under funnels. Fold and place Whatman #42 filter papers in funnels. Wear gloves while folding to minimize the possibility of contamination. Carefully pour *liquid* onto appropriate filter/funnel assembly making sure not to fill the funnel past the level of the filter paper.

   —OR—

   Decant flasks into 50-ml centrifuge tubes. Centrifuge at 15,000 rpm for 15 minutes. Pour supernatant directly into syringe for step 5.
5. Filter approximately 20 ml of pre-filtered or centrifuged extracts through a 0.45 μm syringe filter pack into *another* wide-mouth bottle. (If samples were centrifuged, filtering may not be necessary, and samples can be poured directly into wide-mouth bottle). These samples will be used to measure anions via ion chromatography.
6. From this 20-ml sample, remove 9.3 ml of sample and put into *another* HDPE bottle (narrow-mouth Nalgene bottle or plastic scint vial). Add 0.7 ml 70% (stock) HNO_3. This acidified sample will be used to measure cations via ICP. Note: At least 5 ml of both acidified (at ~5% HNO_3) and unacidified are necessary for analysis, so more or less than 9.3 ml can be acidified, as long as there's 5 ml for each.
7. Freeze both acidified and non-acidified extracts until ready for analysis. Prior to analysis, prepare 1:10 dilutions of micro-filtered extracts in preparation for ion chromatography.

1.5 KCl Extraction for Inorganic Nitrogen — NH_4 and NO_2+NO_3

Materials:

2M KCl (149.1 g KCl / L di-H_2O)
125-ml Erlenmeyer flasks (acid-washed)
Funnels (and funnel racks) and #42 Whatman filter paper (11 cm diameter) *or*
50 ml centrifuge tubes and centrifuge
60 ml HDPE narrow-mouth bottles
50 ml repipettor
Parafilm

Protocol:

1. Tare an Erlenmeyer flask on scale. Weigh out 10-20 g <2 mm soil (depends on where you sampled soil from – use 10 g for upper valleys, 15-20 for Taylor, Wright and Victoria Valley). Record exact weight on data sheet.

2. Use repipettor to dispense 50 ml KCl into each Erlenmeyer flask, including 3 blanks.

3. Cover Erlenmeyer flask with parafilm and shake on an orbital shaker for 1 hr on low speed setting (or 30 minutes at 250 RPM).

4. Set up filter racks. Place 60-ml bottles under funnels. Fold and place Whatman #42 filter papers in funnels. Wear gloves while folding to minimize the possibility of contamination. Carefully pour liquid onto appropriate filter/funnel assembly making sure not to fill the funnel past the level of the filter paper.

   —OR—

   Decant flasks into 50-ml centrifuge tubes. Centrifuge at 15,000 rpm for 15 minutes. Pour supernatant into 60-ml Nalgene bottle.

6. Place samples in freezer until ready to run on Lachat using method # 12-107-06-2-A for NH_4^+ and 12-107-04-1-B for NO_2^-+NO_3^-.

NOTE: Since soil NO_3^- concentrations vary widely (from less than 1 ppm to 1000s of ppm), varying soil:extractant dilutions may be necessary. For example, in typical Taylor Valley soils 20 g of soil to 50 ml KCl is necessary to get detectable levels of NH_4^+ and moderate levels of NO_3^-. But for Beacon and Arena soils, no more than 10 g soil to 50 ml extractant should be used since NO_3^- concentrations can be quite high in these soils.

1.6 $NaHCO_3$-extractable Phosphate (ortho-PO_4^{3-})

(Modified from Olsen and Sommers, 1982)

This technique was developed for estimating available ortho-phosphate in neutral to alkaline soils to decrease the interference in the analyte solution by precipitating Ca as $CaCO_3$. Due to the high pH and calcareous nature of dry valley soils, this should be the preferred approach to estimating available PO_4^{3-}.

Materials:
0.5 M $NaHCO_3$ at pH 8.5
6 N HCl (1:1 of stock 37% HCl and di-H_2O) in 5-ml repipettor
125-ml Erlenmeyer flasks (acid washed)
Funnels (and funnel racks) and #40 Whatman filter paper (11 cm diameter) *or*
50 ml centrifuge tubes and centrifuge
60 ml HDPE narrow-mouth bottles
50 ml repipettor
Parafilm

Protocol:
Make up 0.5 M $NaHCO_3$ using 42 g $NaHCO_3$ / L di-H_2O. Adjust to pH 8.5 with 1.0 M NaOH before bringing all the way to volume. Solution only good for 2 weeks. Store in plastic in refrigerator. **Note:** Do not pipet cold. Remove from fridge the night before use.

1. Weigh 10 g of <2 mm soil into tared Erlenmeyer flask. Record weight.

2. Add 50 ml $NaHCO_3$ to each Erlenmeyer flask, including 3 blanks.

3. Cover with parafilm *tightly*. Poke a small hole in the parafilm to allow gas to escape (otherwise they'll leak). Shake for 1 hr on low speed (or 30 minutes at 250 rpm).

4. Set up filter racks. Place wide-mouth bottles or 50-ml centrifuge tubes under funnels. Fold and place Whatman #40 filter papers in funnels. Wear gloves while folding to minimize the possibility of contamination. Carefully pour *liquid* onto appropriate filter/funnel assembly making sure not to fill the funnel past the level of the filter paper.

   —OR—

   Decant flasks into 50-ml centrifuge tubes. Centrifuge at 15,000 rpm for 15 minutes. Pour supernatant directly into bottle for step 5.

   If the filtrate is not clear, add about ⅛ teaspoon of carbon black or activated charcoal that has been tested for P.

5. Pipet or pour 30 ml from each sample filtrate into an new bottle and VERY SLOWLY add 3 ml of 6N HCl. If the acid is added too quickly, the filtrate will react and foam over the edge of the vial. The resulting extract pH needs to be between 2 and 4. NOTE: Over-acidified samples will cause the autoanalyzer peaks to split, so you

should always test pH of extracts from new sample locations before adding the entire 3 ml.

6. Allow samples to sit for 1 hour uncapped in hood, and then gently swirl each sample. Allow to sit, undisturbed, for another 30 minutes and gently swirl again. Allow to sit, undisturbed, for another 30 minutes, then cap and shake.

7. Add caps to top loosely and allow to sit overnight to degas. Freeze samples until ready to run on Lachat autoanalyzer using method # 10-115-01-1-F.

8. If, after refrigeration, the samples have a dark precipitate (from incomplete precipitation), they need to be refiltered with Whatman #40 filter paper. If not removed, this precipitate will cause extreme spikes in the peaks on autoanalyzer.

9. When run on autoanalyzer, use standards in distilled water and distilled water washes. Blanks run with the samples (which may seem rather high because of the $NaHCO_3$ background) should be subtracted from values for all samples.

1.7 Total and Organic Carbon and Nitrogen

Materials:
Weigh boats (1 per sample)
Sapphire mortar and pestle
Glass scint vials with polyseal cone cap, acid washed (2 per sample)
Glass funnels, small with short, wide stem
6N HCl (1:1 ratio of stock 37% HCl to di-H_2O) in 5-ml repipettor

Protocol:
1. Put about 2 g (slightly more) of <2 mm soil into weigh boats/tins and allow to air dry.

2. Once dried, grind the soil using mortar and pestle until soil is a fine powder. Other grinding methods exist, but can contaminate the already-low nutrient levels in Antarctic soils. Between samples, wash mortar & pestle with di-H_2O and allow to dry (use lint-free cloths to dry if necessary).

3. Pour ground soil into a glass scint vial (labeled with a Sharpie directly on the glass. Don't use labels or tape as they are a source of C). Use glass funnels to transfer into scint vials (di-H_2O rinse and dry between samples). Note: if only total CN is desired, skip steps 4-7.

4. For organic CN, label a 2nd glass scint vial with Sharpie. Tare a scale, then place the vial (**including cap**) on the scale. Record weight, then tare, and add 1 g of ground soil from the original vial. Record soil weight. Cap the Total CN vial and record the weight of the vial+cap+soil together.

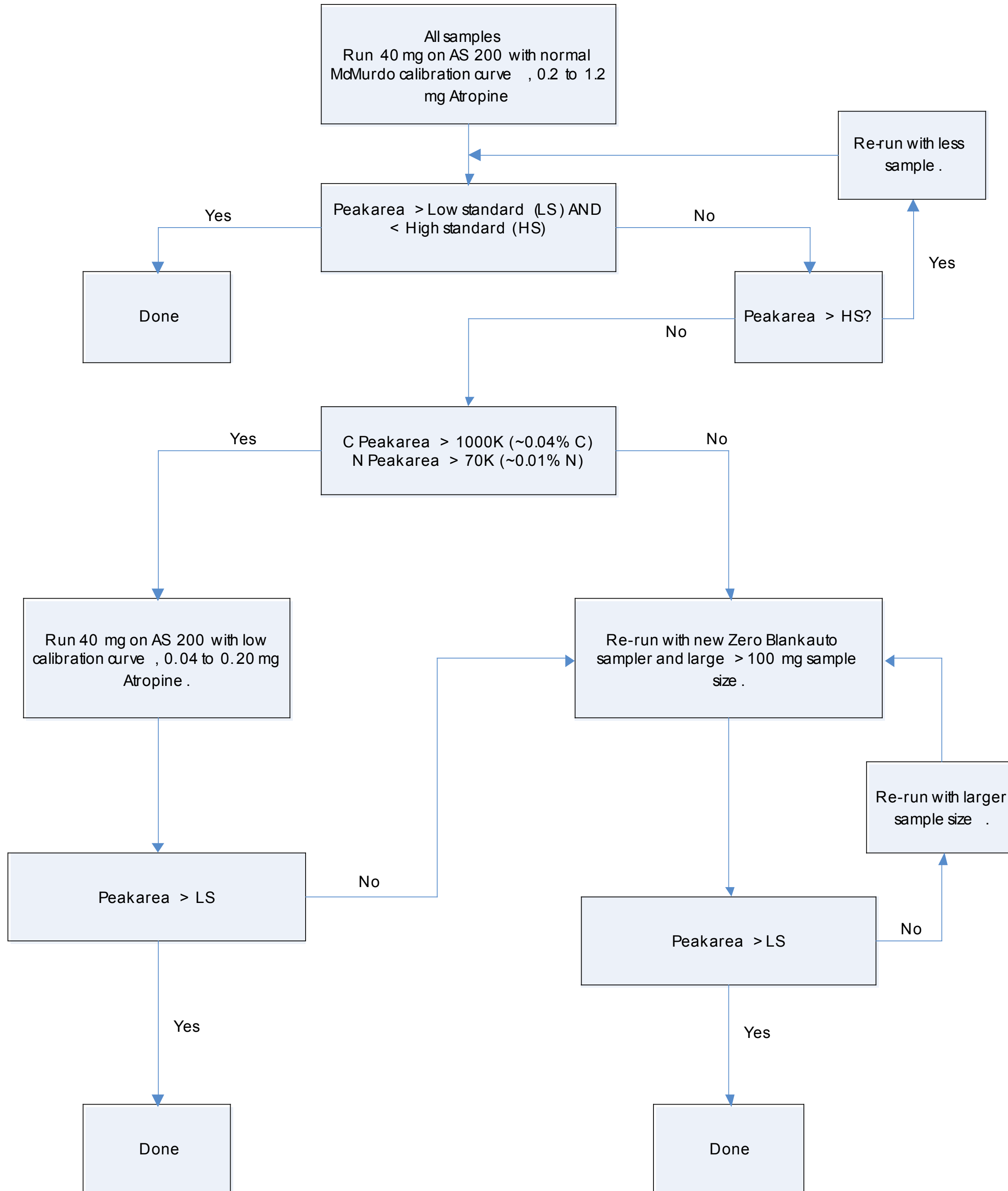
5. To the Organic CN vial, add 1 ml of the 6N HCl.

6. Allow acidified scint vials to sit in the hood until ready to put into oven (you don't want to be in the room when they're in the oven). Place scint vials in a 95°C drying oven for 24 hours. **Do not include cardboard tray or caps, but do not mix up which cap was tared with which bottle.** Unacidified vials can also be put in the 95°C oven to remain comparable, **but don't put in at the same time as the acidified vials** to prevent the volatilized HCl from acidifying the Total CN samples.

7. When remove from the oven, **immediately** tighten cap to prevent any moisture from binding to the soil. Weigh the sample+vial, and record weight. The weight of the soil is then this total weight minus the weight of the vial.

8. Place all scint vials, tightly capped, into a desiccator for storage until weighed into sample capsules for the Carlo Erba. Their shelf-life, even in a desiccator, is not very long.

9. Weigh 40 mg of each acidified and unacidified samples into tin capsules for analysis on an elemental analyzer following this protocol:


All samples
Run 40 mg on AS 200 with normal McMurdo calibration curve , 0.2 to 1.2 mg Atropine
Re-run with less sample .
Peakarea > Low standard (LS) AND < High standard (HS)
Yes
No
Done
Peakarea > HS?
Yes
No
C Peakarea > 1000K (~0.04% C)
N Peakarea > 70K (~0.01% N)
Yes
No
Run 40 mg on AS 200 with low calibration curve , 0.04 to 0.20 mg Atropine .
Re-run with new Zero Blankauto sampler and large > 100 mg sample size .
Re-run with larger sample size .
Peakarea > LS
No
Peakarea > LS
No
Yes
Yes
Done
Done

1.8 Total and Organic Phosphorus

Organic P is estimated from 1N HCl extractable P in a soil sample ignited at 550°C and an un-ignited sample (Saunders and Williams 1955 as modified by Walker and Adams 1958 & Olsen and Sommers 1982). Ignition procedures are a relatively simple approach to estimating inorganic and organic P, especially in un-weathered soils where errors due to the mobilization of Fe or Al-bound P are insignificant.

Materials:
Muffle furnace and crucibles
Clean specimen containers/caps or centrifuge tube (100 mL)
Whatman # 42's
1.0 N H_2SO_4 or 1.0 N HCl

Protocol:

1. Weigh out 1.0 g of ground soils into porcelain crucibles for ignition at 550° C in a muffle furnace.

2. Ignite soils at 550°C for 1 hour. Allow the crucible to cool and transfer the ignited soil to a clean 100 ml specimen container.

3. Weigh duplicate 1.0 g of un-ignited ground soils into clean 100-ml specimen container.

4. Extract in 50 ml 1.0 N H_2SO_4 or 1.0 N HCl and shake overnight (at least 16 h) at 150 rpm. Acid blanks should also be run.

5. Centrifuge samples at 1.5xg for 15 minutes. Decant supernatant or pour through fine filter.

6. Sample can be neutralized to pH 2 and run on an autoanalyzer or alternatively a P-reagent can be added and absorbance measured on a spectrophotometer (Olsen and Sommers 1982).

After determining the concentration of P in the extracts of the ignited and un-ignited soil samples and converting to a soil weight basis, Po is calculated as:

Po = P in ignited sample – P un-ignited sample.

1.9 Soil Texture - The Hydrometer Method

This method will determine the % sand, silt and clay fractions only (modified from Sheldrick and Wang 1993). Additional hydrometer readings may be taken to determine fine and course sand fractions if necessary. See SSSA methods manuals.

Materials:
250-ml beakers (1 per sample)
50 g/L sodium-hexametaphosphate (HMP or Calgon™)
1 L graduated cylinders (1 per sample)
Plunger the diameter of the graduated cylinders
Hydrometer with Bouyoucos scale in g/L

Protocol:

1. Weigh out 50 g air-dried <2 mm soil into 250-ml beaker.

2. Add 100 ml of HMP or Calgon™ solution to each beaker. Run a blank solution with 100 ml HMP and no soil.

3. Shake on an orbital shaker at high speed for 18 hrs.

4. Transfer to a 1-L graduated cylinder and add di-H_2O to bring volume to 1 L. Allow solution to equilibrate thermally and record temperature.

5. Insert plunger into cylinder and mix contents thoroughly. Remove plunger and record the time. Add a drop of amyl alcohol if surface tends to foam. If no plunger is available, stopper cylinder (ex. parafilm) and shake end-over-end for 60 s. Set down and begin timing.

6. Lower a standard hydrometer into sample about 10–20 s ahead of readings at 40 s and 7 hr. At appropriate time, read the hydrometer in each sample and the blank. Carefully remove after each reading.

7. To calculate, first subtract blank hydrometer readings from soil readings. The 40 s reading represents sand, so
   (corrected 40 s reading/50 g soil weight)*100 = % (silt + clay)
   % sand = 100% – %(silt and clay).

   The 7 hr reading represents clay, so
   (corrected 7 hr reading/50 g soil wt)*100 = % clay
   % (silt + clay) – % clay = % silt

2.1 Mesofauna (Nematode, Tardigrade, and Rotifer) Extraction

2.1A: ANTARCTIC SUGAR CENTRIFUGATION METHOD (Freckman 1994)
This method extracts living and dead nematodes, tardigrades, and rotifers from soils. It can be used for measuring biodiversity, species abundance, active versus dead mesofauna, population dynamics, life stage analysis and biomass calculations. It is not appropriate for measurements of mites or Collembola, although these are occasionally extracted. While this method does extract animals in anhydrobiosis, they do not remain so; the method in 2.1B should be used for enumeration of anhydrobiotic mesofauna. Extracted animals are enumerated, then reported as numbers per g dry soil to standardize across samples. Therefore, soil moisture must also be measured for each fresh sample using the method in section 1.1.

Before you begin remember to wet the screens, place two squeeze bottles near the sink (these are special and overwinter in the cage), prepare a styrofoam tube rack with holes numbered 1-4,and set a wire rack next to the microscopes to hold the vial while counting.

1. Pour 100-200 ml of soil into a beaker. Remove rocks greater than 3-4 mm in diameter.
2. Weigh remaining soil. You should have around 100 g.
3. Add tap water to approximately 650 ml line.
4. Stir carefully (star stir or figure 8) for 30 seconds.
5. Immediately pour liquid into wet screens - a stack of 40 mesh (425 μm) on top of a 400 mesh (38 μm).
6. Rinse gently with cold tap water through the top of the stack, keeping the screens at an angle, as the water filters through.
7. Remove the top screen.
8. Rinse top down, never directly on top of nematodes, but at the top of the screen and from behind. Let the water cascade down and carry the nematodes into the bottom wedge of the angled screen. Tap the side of the screen gently to filter all the water through. Rinse from the front and the back, keeping the screen at an angle and not allowing the water to overflow the edge of the screen.
9. Backwash the nematodes into a 50 ml plastic centrifuge tube, tipping the screen into the funnel above the tube. Rinse funnel gently.
10. Put in the centrifuge, making sure to balance the load, for five minutes at 1750 RPM.
11. Decant off liquid, leaving a few ml on top of the soil.
12. Fill with the 1.33 M sugar solution, which should be made up ahead of time and chilled (454 g/L tap water).
13. Stir gently with spatula until pellet is broken up and suspended.

14. Centrifuge for one minute at 1750 RPM.
15. Decant into wet 500 mesh (25 μm) screen.
16. Rinse well with tap water and backwash into a centrifuge tube. Try to keep volume below 10 ml so sample will fit in counting dish.
17. Refrigerate samples at 4-5°C until ready to count.

2.1B: ANHYDROBIOTIC NEMATODE EXTRACTION PROCEDURE

1. Prepare sugar solutions of 2 M (684.6 g/L) and 1.25 M (427.9 g/L). Make about 20 ml 2 M and 400 ml 1.25 M per sample to be processed. Always make a bit extra and make sure solutions are thoroughly chilled before starting.

2. Set up a series of centrifuge tubes on ice with 5 ml 2 M sugar in them.

3. Measure out 100 g of soil (less rocks) in a beaker.

4. Bring up to 250 ml with 1.25 M sucrose solution.

5. Star stir for 30 seconds.

6. Pour into a stack of sieves - 40 mesh (425 μm) on top of a 400 mesh (38 μm), that have been prewetted with 1.25 M sugar solution.

7. Rinse through the top screen with 1.25 M sugar.

8. Remove top screen.

9. Rinse bottom screen from the front only with 1.25 M sugar. Squirt only at an angle. Work slowly and get all the soil in the bottom wedge of the screen. Tap gently to reduce sugar volume.

10. Rinse the bottom screen into a 150 ml beaker with 1.25 M sugar, from the front, using the funnel. Rinse the funnel.

11. Using an automatic pipette, slowly pipette the sugar and sediment from the beaker into a centrifuge tube containing 2 M sugar. Pipette in slowly, at an angle in order to retain the boundary between the sugar layers. Rinse the beaker with 1.25 M sugar and pipette in the dregs. Use as many centrifuge tubes as is necessary per sample.

12. Even off tubes with 1.25 M sugar and centrifuge for five minutes at 1744 RPM.

13. Pour the liquid contents of the centrifuge tubes through a 500 mesh (25 μm) screen, stopping before the sediment can go in. Be consistent in how each sample is poured, as this will affect clarity when counting, and accuracy.

2.1C FORMALIN PRESERVATION PROCEDURE (Freckman ,1994)

Always work in the fume hood.

1. Remove a tray of centrifuge tubes containing samples to be preserved from the refrigerator extremely gently.
2. With the aspirator tip at the surface of the water, aspirate the water level in the tube down to just below 5 ml, to the point where the plastic centrifuge tube begins to taper.
3. Transfer the label (attached to each tube with a rubber band) to a glass vial and tape on with Scotch tape ensuring that the label is completely protected with tape.
4. Transfer each sample from the tube to a glass vial using two strong squirts of cold tap water into the tip of the tube. Fill the vials no more than 1/3 full. Keep the level consistent on all vials.
5. Use a hot/stir plate to heat a beaker of 10% formalin (formaldehyde). Suspend a thermometer into the formalin using a retort stand and clamp. Heat to the range of 80-90°C, but no higher or the formalin will evaporate.
6. Draw out 5 mL of hot formalin with an automatic pipetter and apply to all samples. Slant the pipette tip off the inside wall of the vial as you add to each one.
7. Screw the cap on and let samples attain room temperature before applying a parafilm seal around the cap. This way the lid can be tightened securely and ensure the nematodes a safe journey home.

10% Formalin Solution

Working in the hood, add 270 ml formalin (37% formaldehyde) to 730 ml of tap water to make 1 L of solution

2.2 Total Bacteria Cell Counts

Materials:
NaCl
KCl
Na_2HPO_4
KH_2PO_4
125-ml Erlenmeyer flasks or bottles (sterilized)
Scintillation vials (sterilized) with cone caps
0.2 µm syringe filters and syringes (sterilize)
3% formalin, 0.2 µm filtered
di-H_2O, 0.2 µm filtered
95% ethanol in squirt bottle
SYBR green *or* SYBR gold *or* Acridine orange
Sterile 15 ml centrifuge tube
25 µl Pipetman and autoclaved tips
1000 µl Pipetman and autoclaved tips
10 ml Pipetman and autoclaved tips
Aluminum foil
0.2 µm 25 mm black polycarbonate filters
25 mm membrane filters
Filter manifold and glass filter bases, towers, and clamps
Vacuum pump
Glass slides and cover slips
Immersion oil (ultra low fluorescence grade) *and/or* Glycerol (ultrapure)
Microscope with 100x objective or greater, light source epifluorescent *or* mercury lamp (100 W lamp is best) acridine orange filter set, UV filter set

Procedure:
Sample extraction

1. Prepare 1X PBS solution: 8 g NaCl + 0.2 g KCl + 1.44 g Na_2HPO_4 + 0.24 g KH_2PO_4 in 1 L di-H_2O. *Typically, PBS is adjusted to pH 7.4 with HCl, but the alkaline soils in MCM make this impractical.* Autoclave the PBS at 120°C for 30 minutes. Store at room temperature.

2. Extract fresh soil (<2 mm fraction) in PBS in 125-ml flasks covered with parafilm or bottles. The most appropriate soil:PBS ratio will vary depending on the soil type and microbial biomass. Consider 20 g soil in 50 ml PBS (1:3.5) for low biomass or 10 g soil in 90 ml PBS (1:10) for high biomass. *Record exact soil weight*. Include several blanks.

3. Shake extracts on orbital shaker at fairly low speed (to avoid breaking open bacterial cells). The appropriate length of time will depend on soil type. Consider anywhere from 1 to 60 min.

4. Remove 10 ml of the extract and put into a scint vial. Add 500 µl formalin. Store extracts at 4°C in the dark until ready to stain and count. *DO NOT FREEZE*.

Cell staining

1. Prepare a 25X solution of SYBR green or gold: *SYBR is light sensitive, so always keep wrapped in foil*. Thaw SYBR nucleic acid gel stain (10,000X in DMSO) and spin down to remove dye from cap. In a sterile 15 ml tube, add 25 µl dye to 9.975 ml sterile 1X PBS. Mix thoroughly and store at 4°C in the dark. 25X SYBR can be used for up to 5 days.

   Note: In the past, we have also used 0.1% acridine orange solution (dissolve 0.1 g of acridine orange in 100 ml of filtered di-H_2O).

2. Dilute the extract: Extracts cannot be stained and counted at the initial concentration due to high levels of sedimentation (SYBR also binds to sediment, making it difficult to distinguish between cells and sediment when counting. Also, sediment can bury cells on the slide.) Extracts must be diluted to a level that allows maximum cell visibility and minimal sedimentation. Ideally, you want 30-100 cells per microscope field for low counting error, so too dilute is also not beneficial. A total soil:liquid ratio of 1:1000 has worked well for Fryxell soils (less dilute for Bonney). Dilute extracts using serial dilutions into 0.2-µm-filtered di-H_2O.

3. Set up filtration apparatus: Filtration apparatus should be scrubbed with Alconox, soaked in ~1% HCl, rinsed with di-H_2O, and rinsed with 95% ethanol. Using ethanol-rinsed and flamed forceps, place a 0.45-µm 25-mm membrane filter on a glass fritted filter base and cover with a 0.2-µm 25-mm black polycarbonate filter (shiny side up). Clamp filtration tower to base and cap with foil.

4. Add 2 ml (or volume determined to give the appropriate number of cells per field with minimal sedimentation) of soil solution to filter tower. *Check for leaks.* Wrap in foil to maintain dark conditions. Add 500 µl of 25X SYBR (or 1 ml of acridine orange). Allow sample to incubate for 15 minutes before filtering.

5. Filter under low vacuum (~5 mm Hg). Just as a thin layer of sample remains, rinse tower with 1 ml filtered di-H_2O and continue to filter. Repeat if necessary. Turn off vacuum as soon as the last of the water has filtered.

6. Place black polycarbonate filter on an ethanol-rinsed slide used rinsed and flamed forceps. Add a drop of glycerol or immersion oil (using sterile syringe) on top of filter. (Antifade solution can be used instead, if desired.) Place an ethanol-rinsed cover slip on top of filter. Use a blunt object to push down the cover slip to form thin even film of oil between the filter and the cover slip. There should be no air bubbles and no wrinkles on the filter. Freeze upright in a slide holder until ready to count. Slides should be counted soon after staining, before the SYBR starts to fade.

7. Prepare a blank sample by following the above procedure, using di-H_2O instead of a diluted soil sample. Technically, a blank should be run with each set of filtrations.

Counting cells

1. Count bacteria using final magnification of 1000x in at least 10 different fields. Ideally, this should involve counting at least 300 cells, but this is often not possible with soil extracts, because sedimentation gets in the way.

2. For biomass measurements, categorize the cells into small or large cocci or rods. Use stage micrometer to determine the average diameter of small and large cocci and the average length and width of small and large rods.

Calculation of cell numbers is:

Cells per g soil=(S-B)*(filter area/field area)*(DF/g soil)

Where S is the average number of cells per field in the sample, B is the average number of cells per field in the blank, *filter area* is the area of the filter that has sample on it (2.54 mm^2), *field area* (7 mm^2) is the area of the microscope field that bacteria were counted in, and DF is the dilution factor (i.e. scaling up to the original volume of extract).

Biovolume is calculated using the following formulas for rods (ellipsoid) and coccoid (sphere) cells:

$$V_{Ellipsoid} = (\pi L W^2)/6 \qquad V_{Sphere} = (\pi D^3)/6$$

Where L is the length and W is the width of the ellipsoid cell, and D is the diameter of the spheroid cell.

Biovolume can be converted to g microbial C using published conversion factors.

See also Bottomley (1994), Fry (1990), and Kepner (1994).

2.3 Chloroform Labile Carbon, Nitrogen, and Phosphorus

The extremely low concentrations of organic matter in soils of the Antarctic Dry Valleys call for some special consideration in using the chloroform fumigation extraction procedure to estimate microbial biomass. For example a Taylor Valley soils on the south side of Lake Hoare typically contain from 0.01 to 0.05 % organic C by weight (or 0.1 to 0.5 mg C g $soil^{-1}$). If we assume that microbial biomass represents from 1-5% of the total C pool than we should expect to find microbial biomass C in the range of 1.0 to 25 µg CMB g $soil^{-1}$). Using standard extract dilutions of 5:1 would produce concentrations of 0.02 to 5 µg CMB ml^{-1} in sample extracts. A dilution of 2.5:1 doubles the concentration and provides a extract concentrations of 0.04 to 10 µg CMB ml^{-1}. Since even in temperate soils the CFE procedure often pushes the detection limits of commercially available TOC analyzers and wet oxidation techniques, it is advisable to exploit any analytical advantage possible. Additional ways to boost the precision of this technique might be: running the CFE on small soil particle size fractions (e.g. < 265 µm), taking advantage of long pre-incubation periods under warm wet conditions, and generally observing careful analytical protocol through all stages of analysis.

Materials:
Ethanol-free chloroform
Boiling chips
0.5 M K_2SO_4 (87.2 g K_2SO_4 per L di-H_2O)
6N HCl (1:1 ratio of stock 37% : di-H_2O) *or* 3N H_2SO_4 (1:5 ratio of stock 18N : di-H_2O)
125 ml Erlenmeyer flasks (acid washed)
100 ml glass beakers
60 ml HDPE bottles
50 ml centrifuge tubes
25 mm 0.45 µm syringe filters and 30 ml syringes
50-ml and 1-ml repipettors
Parafilm

Weighing Samples:

1. Into the “NF” Erlenmeyer flask, weigh 20 g of <2 mm soil. Record exact weight. This beaker may be labeled with Sharpie.

2. Into the “F” Erlenmeyer flask, weigh 20±0.5 grams of sieved soil. **Note: if also measuring P, include an extra 10 g, for a total of 30±0.5 g.** Record exact weight. *Label flask with pencil-on-labeling tape* (chloroform dissolves Sharpie).

Non-fumigated “Control” Extraction:

1. To the “NF” flasks, add 50 ml 0.5 M K_2SO_4. Include 3 blanks.
   (Typically a dilution of 5:1 (K_2SO_4:soil) is used for temperate soils, but for low organic C soils from the Dry Valleys, smaller volumes are suitable for the lower concentrations of chloroform labile C present.)

2. Cover with parafilm and shake on an orbital shaker at 200 rpm for 30 minutes (or low for 1 hour).

3. Decant liquid into centrifuge tubes and spin at 15,000 rpm (approx. 25,000xg) for 15 minutes. *A test showed that filtering with Whatman #42 paper filters introduces non-purgeable DOC contamination.*

4. Filter approximately 30 ml of the supernatant through a 0.45 µm syringe filter (using a 30-ml plastic syringe) into a bottle. Do not fill them too full or they will split in the freezer. **Note: If measuring N, filter 15 ml into each of two Nalgene bottles; the bottle for N should be wide-mouth.** Filtration may not be necessary if centrifugation worked really well, and samples can be poured directly into bottle.

5. Add 0.5 ml 6 M HCl to 30 ml supernatant (or 0.25 ml for 15 ml, enough to bring to pH 2) *or* 0.4 ml 3 N H_2SO_4 to 30 ml (or 0.2 ml to 15 ml) to remove inorganic forms of carbon (carbonate, e.g.). Freeze samples until analyzed on the Shimadzu. **Note: Do NOT acidify the vial to be used for N analysis.**

Fumigation:

1. Wear appropriate protective clothing and equipment: lab coat, nitrile gloves, and safety goggles.

2. Place the flasks (uncovered) into a 20 L vacuum chamber along with two beakers containing 50 ml each of ethanol-free chloroform and boiling chips. *Chloroform is highly toxic and causes fainting if inhaled. Work in a well-ventilated hood.* Cover bottom of beaker with boiling chips.

3. Place 2 pre-wetted paper towels in each chamber underneath the bottom shelf. This prevents the soil from drying out during the 5 day incubation.

4. Draw a vacuum on the chamber until the chloroform boils. Seal the chamber. After 30 minutes, vent, then draw a vacuum again until the chloroform boils. You may have to replace the boiling chips. Repeat this step for a total of three evacuations. After the last evacuation, leave the chamber sealed under vacuum for 5 days.

5. After 5 days, flush the chamber with air four to six times by evacuating and venting to remove all chloroform. Allow the chloroform to evaporate (e.g. leave open in hood overnight).

6. Add 50 ml of 0.5M K_2SO_4 and follow extraction procedure for “NF” flasks. **Note: If measuring P, first remove 10±0.5 g into a new beaker. Do not add K_2SO_4 until AFTER the P subsample has been removed! Run P subsample through the $NaHCO_3$ extraction and digest for measuring Total P. Also digest $NaHCO_3$ samples that were run for o-PO_4^{3-} (as the “NF”).**

Carbon Analysis:

1. Remove samples from freezer and thaw overnight at 4°C.

2. Once samples are completely thawed and dry, shake on reciprocal shaker for 10 minutes. Allow precipitate to settle out for approximately 30 minutes.

3. Analyze for TOC using the Shimadzu.

Nitrogen Analysis:

1. From the thawed and shaken subsample, remove two 5-ml aliquots and place into 20 ml screw cap tubes (scint vials), which gives you duplicate samples. Avoid including any of the white precipitate in the sample. Use a new pipet tip for every sample.

2. For each set of 80 tubes, include several di-H_2O blanks, and maybe 6 standards, 3 each of nicotinic acid and leucine prepared in K_2SO_4.

3. Weigh all tubes.

4. Autoclave the samples for 30 minutes on liquid cycle (121°C).

5. Reweigh tubes when cool to account for loss of water.

6. Analyze for nitrate using autoanalyzer (LACHET).

Calculations:

For determining carbon:

(Sample mg/L – Avg Blank mg/L) * (0.05 L) / (g dry wt equiv soil extracted) = mg microbial C / g dry soil

Compute for both controls and fumigated treatment.
µg Microbial C/g dry soil = fumigated - control
*Typical CFE calculations involve dividing by a factor *k* (the estimated extraction efficiency). These values are generally available in published literature for more commonly-studied ecosystems that were once determined by experimentation. There are no values for Antarctica, as we haven't determined one and the published values probably don't apply. So we tend to not use *k*'s, and refer to our results as "chloroform-extractable C" rather than "microbial C".

For determining nitrogen:
(sample Nitrogen reading *2*((10-(Initial tube weight-final tube weight))/10)

Calculate the same for all blanks. Average the blanks.
(Sample calculation - average blank calculation)*(125/g dry soil)

Subtract the controls from the fumigated samples to get µg Microbial N/g dry soil.

2.4 Biolog® (Microbial Community Analysis) Procedure

Adapted from Rillig et al. 1997

MATERIALS:

1 1 000 ml volumetric flask

100 ml centrifuge tubes (1 per sample)

100 ml volumetric flasks

Biolog® Plates (1 per sample)

NaCl

Na_2HPO_4

NaH_2PO_4

PROCEDURE:

1) Weigh 1.0 g dry weight equivalent soil into sterile beaker.
2) Suspend soil in Phosphate buffer to create a 10^{-2} solution*. Use Nanopure water throughout this procedure. *This entails adding 100ml buffer to the 1 g (DWE) soil. The phosphate buffer can be made by mixing 8.5g NaCl, 1.18g Na_2HPO_4, and 0.22g NaH_2PO_4 in 1 L Nanopure water.
3) Shake test tube in wrist action shaker for 5 minutes, make sure to tightly cap test tube and secure flasks on shaker.
4) Remove tube and hand shake for 30 seconds then let sit for 2 minutes.
5) Remove 140 µl from 1cm below the solution surface and place in all wells of a plate. Use same pipette tip for each Biolog plate, but use a new pipette tip for each new plate (sample). Make sure to use sterile techniques when plating the soil solution (keep cover over plate when).
6) Label plates and store covered (dark) at room temperature. Record room temperature.
7) Measure Average Well Color Development at 48, 72, 96, and 120 hrs. For this we will use the plate reader in Dr. McPeek's lab in Gilman Hall. We will take measurements at 595nm.

*Note: we will have to determine the appropriate dilution for our soils. This entail making sample plates with 10^{-2}, 10^{-3}, and 10^{-4} dilutions. It looks like the 10^{-3} will work well.
See also: Garland JL and Mills AL. 1991. Classification and characterization of hetertorophic microbial communities on the basis of patterns of community-level sole-carbon-source utilization. Appl Envi Microbil 57:2351-2359

3.1 Sample Preparation for Elemental Analysis

Preparation of soils for determination of organic C – see section 1.7

Preparation of soils for determination of organic P – see section 1.8.

3.2 Density Fractionation

The purpose of this technique is to separate the light fraction (LF) and heavy fraction (HF) organic matter. Heavy fraction (HF) organic matter is associated with mineral surfaces and compounds and is typically more resistant to decay than LF organic matter which is largely comprised of detritus, microbial cells and senesced biological material. The technique utilizes the different densities of organic materials to separate two fractions in a dense solution. The resulting fractions can then be analyzed for elemental and isotopic composition.

1.Dry soils and pass through 2 mm sieve.

2. Disperse ~ 100 g soil in 500 ml 1.2 g cm^{-3} NaCl solution (3.42 M or 200 g NaCl per liter H_2O). Alternatively a 1.7 g cm^{-3} of NaI can be used. Dissolve 795 g of NaI in 1.0 l H_2O.

3. Stir at 1 800 rpms for 1.0 min.

4. Cover with parafilm and let soil solutions settle for 48 hrs. at room temperature.

5. Aspirate the top few centimeters of the solution (sic Strickland and Sollins 1987) with a vacuum pump into a Bruchner receiving flask. Be sure to place a receiving flask between the pump intake and the receiving flask to avoid getting moisture into the pump. Repeat this fractionation 3 times to completely remove all LF material from the HF.

6. Treat LF with 1M HCl to remove CaCO3 and rinse. Dry at ~ 60 C, homogenize and analyze for C and N.

7. Treat HF with 1 M HCl. Dry at ~ 60 C, homogenize and analyze for C and N.

4.1 Laboratory Incubations for Antarctic Soils

Materials:
500 ml I-Chem incubation vessels
plastic scintillation vials
20 specimen cups (3 oz Dixie type cups work well)
1.0 N NaOH
1.0 M KCl
2.0 M $BaCl_2$
1.0 N HCl

Soil preparation:

1. Use "fresh" soils (soils should be at field moisture status and kept at or below 4 ºC prior to analysis. If necessary, thaw frozen soils for 48 hours at 4 ºC). Determine the gravimetric soil moisture content.

2. Weigh a 10 ml sub-sample and extract in 50 mls 1 M KCl for initial extractable N content.

3. Weigh out 50 g of fresh soil (< 2mm size fraction) into 100 ml specimen cup.

4. Add sufficient water to bring the 50 g of soil up to 10% soil moisture content.

5. Pipette 10 mls of 1.0 N NaOH into 20 ml scintillation vial.

6. Place the specimen cup of soil and the NaOH base trap into the incubation vessel.

7. Set up one blank for every four incubations (2 per box for box of twelve).

7. Pipette ca. 50 mls of water into the bottom of all incubation vessels to maintain a saturated atmosphere during incubation.

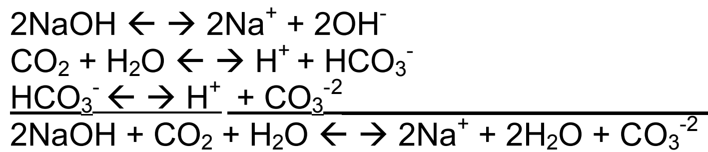
8. Tightly cap the incubation vessels, pack in cardboard box and incubate in the dark at constant temperature (~ 25 º deg).

Harvesting Incubations*

1. A the end of the incubation remove base traps and precipitate carbonates with excess 2.0 M $BaCl_2$ (0.5 ml $BaCl_2$ per ml NaOH). Cap tightly.

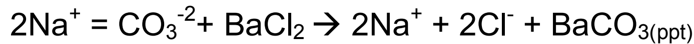
2. Extract 10 g incubated soil in 50 mls 1 M KCl for final extractable N content.

3. Titrate fixed base traps with 1.0 N HCl.
   The following equations illustrate the how NaOH captures CO2,



$$2NaOH \leftrightarrow 2Na^{+} + 2OH^{-}$$
$$CO_2 + H_2O \leftrightarrow H^{+} + HCO_3^{-}$$
$$\underline{HCO_3^{-} \leftrightarrow H^{+} + CO_3^{-2}}$$
$$2NaOH + CO_2 + H_2O \leftrightarrow 2Na^{+} + 2H_2O + CO_3^{-2}$$

Barium Chloride is added to precipitate the carbonates and prevent the reactions from reversing



$$2Na^{+} = CO_3^{-2} + BaCl_2 \rightarrow 2Na^{+} + 2Cl^{-} + BaCO_{3(ppt)}$$

After fixing with barium chloride the base traps have a moderate shelf life if tightly capped and stored at constant temperature.

4. Titrate the fixed base traps with 1 N HCl. Follow calibration directions on the auto-titrator. In addition to what the auto-titrator calls for, use several NaOH standards of known normality at the beginning and end of the titration session to test for calibration drift of the auto-titrator.

5. Use the following equation to calculate mineralizable C:

mineralizable C = (mls titrated blank - mls titrated sample) X
(0.001 mol H^{+}/ml)(0.5 mol C/mol H^{+})(12 g C/mol)(1000 mg/g)

simplified:

mg C = (blank - sample)(molarity of HCl)*6

*note: For long incubations > 60 d, on unknown soils it is advisable to check the strength of the base traps halfway through the incubation to ensure that the acid trapping capacity has not been saturated.

4.2 Ion Exchange Resin Membranes

Preparation:

Cut resin membranes into 5 X 5 cm squares for use in the field.

Anion exchange resin membranes: Before use, chloride-saturated anion exchange membranes must be converted to the bicarbonate form. Shake resin sheets for 10 mins in 3 successive solutions of 0.5 M $NaHCO_3$, rinsing with de-ionized water between each solution. Although the bicarbonate form is less stable it is preferred for determining P availability since P affinity for the resin is low relative to Cl^- and OH^-.

Cation extractions: These resins are usually supplied in the H+ saturated form. Before use, the membranes should be rinsed thoroughly with fresh 0.5 M HCl. (To prepare 0.5 M HCl, dilute 41 mls of concentrated (12.1 N) HCl in 1 liter de-ionized water.)

After resins have been cut and “charged”, 5 cation and anion resin membranes should be set aside as “batch blanks” and stored moist for later analysis. See below.

Installation and Incubation

Gloves should be worn during installation and harvesting of resins.

A cation and anion resin exchange resin should be matched for each treatment, plot, or observation.

Using a flat bladed instrument (i.e. a putty knife) insert resin membranes into the soil so that the top edge of the membrane is flush with the soil surface. The resin membranes should be placed at a slight angle from vertical (15-30°) for optimal contact with the soil medium.

Incubate for 12 to 48 hrs.

After the desired length of incubation, gently remove resin membranes from the soil. Shake loose soil particles from the resin in the field and place in a clean ziplock sample bag. Each set of resin membranes (cation and anion) should be placed in a separate, labeled bag. A small amount of soil on the resins will not create problems with the extraction. Store moist at room temperature. Avoid temperature fluctuations.

Analysis

Place each resin set (matched cation and anion resins) in a screw top specimen vial with 50 ml 0.5 M HCl.

The “batch blanks” should also be desorbed at this time.

Shake at low speed for 30 hours.

Filter through Whatman # 42 filters into clean sample vials.

Extractant blanks may be run in addition to the “batch blanks” to track down potential sources of contamination, but only the “batch blanks” are used to correct estimates of cation and anion sorption.

Extracts should be neutralized by adding an appropriate amount of concentrated NaOH prior to running on Lachat or Alpkem.

Refrigerate resin extracts prior to analysis.

5 References

Bottomley, P.J. 1994. Light microscopic methods for studying soil microorganisms. In *Methods of Soil Analysis, Part 2: Microbiological and Biochemical Properties*, eds. W.H. Mickelson, R.W. Weaver, S. Angle, P. Bottomley, D. Bezdicek, S. Smith, A. Tabatabai, A. Wollum, pp. 81-105. Soil Science Society of America, Maidson, WI.

Freckman, D.W., Kaplan, D.T., and van Gundy, S.D. 1977. A comparison of techniques for extraction and study of anhydrobiotic nematodes from dry soils. *Journal of Nematology* 9, 176-181.

Fry, J.C. (1990). Direct methods and biomass estimation. In *Methods in Microbiology, Volume 22, Techniques in Microbial Ecology*, eds. J.R. Norris & R. Grigorova, pp. 41-85. Academic Press, London.

Kepner Jr, R.L. and Pratt, J.R. 1994. Use of fluorochromes for direct enumeration of total bacteria in environmental samples: past and present. *Microbiology and Molecular Biology Reviews* 58: 603-615.

Olsen, S.R. and Sommers, L.E. 1982. Phosphorus. In *Methods of Soil Analysis, Part 2: Chemical and Microbiological Properties,* eds. A.L. Page, R.H. Miller and D.R. Keeney, pp. 403-430. American Society of Agronomy, Soil Science Society of America, Madison, WI.

Rillig, M.C., Scow K.M., Klironomos, J.N., Allen, M.F. 1997. Microbial carbon-substrate utilization in the rhizosphere of *Gutierrezia sarothrae* grown in elevated atmospheric carbon dioxide. *Soil Biology and Biochemistry* 29: 1387-1394.

Saunders, W.M.H. and Williams, E.G. 1955. Observations on the determination of total organic phosphorus in soils. *Journal of Soil Science*, 6: 254–267.

Sheldrick, B.H. and Wang, C. 1993. Particle size distribution. In *Soil Sampling and Methods of Analysis*, ed. M.R. Carter, pp. 499-511.Canadian Society of Soil Science, Lewis Publishers, Ann Arbor, MI.

Walker, T.W. and Adams, F.R. 1958. Studies on soil organic matter. I. *Soil Sci*. 85: 307-318.