

# FOREST DYNAMICS PLOT SOIL SAMPLING PROTOCOLS

SoilSmpl.SoilProject.doc

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## FIELD AND LABORATORY SAMPLING SCHEDULES

### I. Surface-soil samples for soil chemistry and other selected physical properties – collect and process all of these samples as a set.

A good schedule for collecting and processing samples for soil chemistry measurements would be the following (see the rest of the document for the details behind each procedure):

#### Day 1:

In the field, collect about 45 mineral-soil samples into foil packets and place PVC tubes in the ground at each of the sampling locations for *in situ* N-incubations (which will need to be collected 28 days later).

In the lab, label sample bottles and extraction bottles; weigh and label foil; prepare for 2 days of lab work by checking to make sure all equipment, supplies and chemicals are ready, including sufficient distilled water, space in drying ovens, *etc.*

#### Day 2:

First make 1 L of KCl solution for KCl extractions, so that it can temperature equilibrate.

Then, open the field-collected aluminum-foil packet for each sample and weigh:

- a. 2.50 g (two and a half grams) of soil into a 125-mL pre-labeled bottle for Mehlich III extraction. *Place the bottle with 2.50 g soil in a cold room or refrigerator to keep it cool until the next day when the extraction procedure will be run.*
- b. 3.00 g (three grams) of soil into a pre-labeled Solo<sup>®</sup> cup for pH measurement.

- c. 2.00 g (two grams) of soil into a pre-labeled Solo<sup>®</sup> cup for KCl extraction.
- d. 20 g (twenty grams) of soil onto a pre-weighed sheet of foil for determining soil moisture. *Prior to weighing the soil, form the sheet into an open-topped packet. Once the mass of the soil placed in the packet has been recorded, place the packet in a drying oven at 105° C (leave for 48 hr).*

Seal the remaining soil in its original aluminum-foil packet.

Add KCl to 2.00-g portions of soil in Solo<sup>®</sup> cups, stir, and let sit overnight.

Measure pH on 3.00-g portions of soil in Solo<sup>®</sup> cups.

**Day 3:**

Pipette KCl-extraction solution from Solo<sup>®</sup> cups that sat overnight into pre-labeled sample bottles.

Run Mehlich III extractions into pre-labeled sample bottles.

Examine soils for texture and color.

“Air dry” (drying oven at < 55° C – leave for 48 hr) the remaining soil for archiving (this step could be done up to several days later, if necessary)

**Repeat days 1 - 3 until all samples for chemistry measurements have been collected and processed.**

*Note: For each sample, use the sample i.d. assigned by Jim Dalling and Kyle Harms.*

*Note: It would be best to finish collecting all surface samples for chemistry within one month. Try to send sample bottles of extracts to Joe Yavitt at Cornell University at the earliest convenience, and do not wait more than 3 - 4 weeks from the time the samples are collected from the field and processed.*

*Note: After every batch of samples is processed, make sure to photocopy the datasheets. Keep the original datasheets in a binder in one location, and the photocopy in a binder in another location. Enter the data into a computer as soon as possible.*

*Note: Keep a daily record (on a calendar) of which procedures are done on which sets of samples. Periodically check this calendar against the datasheets to make sure every procedure is completed in a timely manner for every sample.*

*Note: For many of the procedures outlined in this document it is critical to know the date that the activity occurred, so always record dates by writing out the name of the month, as opposed to using a number to indicate the month. [In the U.S., 6/12/04 means June 12, 2004. In Panama, 6/12/04 means “6 de diciembre 2004” (i.e., December 6, 2004). The difference is between the beginning of the wet season, and the beginning of the dry season, respectively!]*

**Note:** *The target number of samples to be collected per day is an estimate based on previous work on BCI in Panama. This number may need to be adjusted to local conditions and resources.*

**II. Surface-soil samples for physical variables (texture, color, moisture) and pH from every 20x20-m quadrat, especially for Robert (Bob) Stallard *et al.*'s hydrology work – collect and process all of these samples as a set.**

A good schedule for collecting and processing the samples that come from the center of every 20x20-m quadrat (1250 of them for a 50-ha plot, or 625 of them for a 25-ha plot) would be the following (see the rest of the document for the details behind each procedure):

Collect all 1250 mineral-soil samples with a punch-corer (*e.g.*, LaMotte Soil Sampling Tube). From the corer, place each sample onto a pre-weighed and pre-labeled sheet of aluminum foil that is then folded into a sealed packet in the field.

Based on our experience on BCI, a team of two can collect all the samples in a long week if the weather cooperates. Several teams of two could probably collect all the samples in a single day.

Keep the samples sealed in their aluminum-foil packets, and keep them cool (*e.g.*, in a cold room or refrigerator) until they can be processed. If cooling facilities are not available, processing should be done immediately upon collection from the field. To process a sample, open the field-collected sample packet, and weigh out 2.00 g of soil into a Solo<sup>®</sup> cup and follow the procedure below to measure pH. Close each packet and keep it cool until all pH measurements have been made.

Open each packet again and remove enough soil to determine texture and color, but leave about 30 g of soil in the packet (to use to determine soil moisture content). Follow the procedures described below to determine texture and color.

Finally, weigh the aluminum-foil packet with its remaining fresh soil (there should be about 30 g of soil in the packet). Do not remove any further soil from the packet and dry the packet in a drying oven at 105° C (leave for 48 hr) to determine soil moisture content of the soil, according to the instructions given below.

The soils taken from the center of each 20x20-m quadrat *do not* need to be archived.

**Note:** *For each sample, use the sample i.d. assigned by Jim Dalling and Kyle Harms.*

## **FIELD SAMPLING PROTOCOLS**

### **I. a. Surface soils at 300 sampling points (for soil chemistry and other selected physical properties)**

Jim Dalling and Kyle Harms will supply a standardized set of locations and their associated sample i.d. numbers on data sheets for 300 samples that comprise an “unaligned grid sampling scheme”. Two hundred (200) of these locations will be arrayed throughout the plot in a regular grid; these 200 points will be referred to as “base points”. For every other base point, an additional sampling point will be located nearby. Each additional point will be located at a

random compass bearing away from its associated base point. One third (1/3) of the randomly selected additional points will be located at 2 m away from their associated base points, one third (1/3) will be located at 8 m away, and one third (1/3) will be located at 20 m away. This sampling scheme combines thorough spatial coverage (provided by the grid), along with a variety of inter-sample distances to allow estimation of spatial autocorrelation at a variety of spatial scales throughout the plot (short inter-sample distances are provided especially by the additional points).

Collect a composite surface-soil, sample (0-10 cm depth) from each sampling point. For a given sampling point, brush away leaf litter from three small areas within 1 m of each other and within 1 m of the exact location of the sampling point provided on the data sheet. Collect a small scoop of mineral soil (soil beneath the leaf-litter layer) from each of the 3 small cleared areas with a trowel and bulk the 3 scoopfuls in a sturdy container. Mix the composite sample well, and place about 300 g of the sample in a pre-labeled aluminum-foil packet (each about 30 x 30 cm; these *do not* need to be pre-weighed). Make sure the soil i.d. label of the aluminum-foil packet is the correct one for the soil sample (see “Equipment and supplies” below). Place a PVC tube in the ground according to the instructions in the next section (“I.b. N-incubations...”).

**Note:** *Make sure to record the EXACT date that each sample was collected.*

**To do in the lab:** (1) KCl-extraction (for N), (2) Mehlich III-extraction (for P & cations), (3) pH, (4) texture by feel, (5) color, (6) moisture, (7) archive.

### **I. b. N-incubations at 300 sampling points**

Place a PVC tube (a.k.a., “N-incubation tube”; see “Equipment and supplies” below) vertically in the ground (*i.e.*, oriented with its long axis perpendicular to the ground surface) at each of the 300 surface-soil sampling points on the same days that surface-soil samples are collected from those sampling points. It works well to place a small wooden board on top of the PVC tube and pound the PVC tube into the ground with a small sledge (or other small, but heavy) hammer. The tube should enter at least 15 cm into the ground, and there should be at least 5 cm remaining aboveground. Cover the aboveground end of each N-incubation tube with a plastic bag secured with a piece of duct tape around the outside circumference of the tube (to prevent rainwater or water from surface runoff from entering into the tube). Make one or two small holes in the bag just below the lip of the tube to allow air to move into and out of the tube, *i.e.*, to allow soil respiration to continue unimpeded.

Twenty-eight (28) days after placing an N-incubation tube in the ground (which can vary from 26 days to 32 days), pull the tube out of the ground with a vice-grip pliers (if necessary) and collect about 50 g of the soil from within the tube (0-10 cm depth). A screwdriver is useful for prying soil out of the tube. Place the sample in a pre-labeled aluminum-foil packet (each about 30 x 30 cm; these *do not* need to be pre-weighed). Make sure the soil i.d. label of the aluminum-foil packet is the correct one for the soil sample from the data sheets provided by Jim Dalling and Kyle Harms (see “Equipment and supplies” below).

**Note:** *Make sure to record the EXACT date that each tube was placed in the ground and the exact date each was removed from the ground.*

**To do in the lab:** (1) KCl-extraction (for N), (2) moisture.

### **I. c. Extra sites for chemistry to complete the hydrology portion of the project**

Collected from a select set of locations necessary for the hydrology portion of the project, and processed as in sections “I.a.” and “I.b.” above. (Bob Stallard will provide details of sampling locations to Jim Dalling and Kyle Harms, who will in turn pass the details on to other participants.)

## **II. Surface soils in each 20x20-m subplot (for physical properties and pH)**

Jim Dalling and Kyle Harms will supply sample i.d. numbers and locations on data sheets. Following the data sheets, collect one surface, mineral-soil sample (0-10 cm depth) with a punch-corer (e.g., LaMotte Soil Sampling Tube) from the center of each 20x20-m subplot (1250 total for a 50-ha plot; 625 total for a 25-ha plot). For each sample point, brush away leaf litter from a small area, then push the punch-corer into the mineral soil. Place the punch-corer sample in a pre-labeled and pre-weighed aluminum-foil packet (~ 30 x 30 cm) labeled with the correct soil i.d. Make sure the soil i.d. label of the aluminum-foil packet is the correct one for the soil sample from the data sheets provided by Jim Dalling and Kyle Harms (see “Equipment and supplies” below).

**Note:** *Keep the structure of each soil core intact, so that the deepest portion of the soil core can be examined in the lab for texture, color, and pH. Note that this means that for these particular samples the sampled depth is about 5-10 cm (which is slightly different from the 0-10 cm depth that we are sampling for surface soil chemistry (section I. above).*

**Note:** *Make sure to record the EXACT date that each sample was collected in the field.*

**To do in the lab:** (1) pH, (2) texture by feel, (3) color, (4) moisture.

### **Additional notes for all field sampling:**

**Collect mineral soil only, i.e., avoid sampling the litter layer.**

**Avoid sampling within streams, on exposed rocks, and through big trees or roots.**

For all of the above procedures, if your pre-determined sampling point is located in a small stream, take the sample (or place the incubation tube) in soil just outside of the stream itself. Similarly, if your pre-determined sampling point is located where there is a rock, large tree, or tree root, simply move to the nearest place lacking the obstruction. Make a note of this on the data sheet.

**Avoid sampling within someone else’s observation or experimental quadrats.**

For all of the above procedures, if your pre-determined sampling point is located in a seedling quadrat (*e.g.*, on BCI), or another person's marked sampling quadrat, simply move to the nearest place outside the other person's sampling quadrat. Make a note of this on the data sheet.

***Use unique i.d. numbers for each sample; Never repeat i.d. numbers.***

Each soil sample should have a unique identification number. Start labeling soil samples sequentially from 1, and never repeat numbers.

***Keep fresh soil samples cold.***

The fresh soils should be kept in a cold-storage room or refrigerator until they can be processed. Complete all fresh-soil procedures as soon as possible, and definitely within 5 days of removal from the field (even sooner if no cooling facilities are available).

***Equipment and supplies for field sampling:***

- Data Sheets for field sample collection sites and dates. Use the electronic file provided by Jim Dalling and Kyle Harms: "*FieldData.SoilProj.xls*".
- Backpacks for carrying samples.

For aluminum-foil packets:

- Aluminum foil (preferably Reynolds<sup>®</sup> aluminum foil, since many cheaper varieties break, puncture or tear too easily) – Cut squares of aluminum foil (~ 30 x 30 cm) and label each square with two pieces of labeling tape with the soil i.d. written with a Sharpie<sup>®</sup>. Place one label at the corner of the inside side of the foil and the other label in the center of the outside side of the foil. These sheets of foil can be carried to the field in the anticipated order to soil collection in a large Ziplock<sup>®</sup> bag (or other sealable plastic bag that is larger in x and y dimensions than the squares of aluminum foil). These aluminum foil sheets will then be shaped into packets in the field to carry soil in from the field. Note in the instructions above which squares of aluminum foil should be pre-weighed (if sheets need to be pre-weighed, an electronic balance will be needed). It is a good idea to carry a few extra sheets of aluminum foil into the field, since sometimes the sheets rip or tear in the field (remember to pre-weigh the sheets if necessary for the samples that are being collected).
- Labeling tape.
- Sharpie<sup>®</sup> (or other indelible, felt-tipped marking pen).
- Large Ziplock<sup>®</sup> bags (or any sealable plastic bag large enough to accommodate the flat sheets of aluminum foil).
- Electronic balance, accurate to grams (g) to 2 decimal places, *e.g.*, 0.00 g. Setra<sup>®</sup> (those used in Panama are Setra<sup>®</sup> model 5000L), Mettler<sup>®</sup>, and Ohaus<sup>®</sup> all manufacture good balances.

For surface samples:

- Trowel for composite surface-soil samples.

- Sturdy container for bulking surface-soil samples.

For N-incubation tubes:

- PVC tubes (*i.e.*, polyvinyl chloride plastic drainage pipe from construction or building suppliers; 3-inch [7 or 8-cm] diameter, thick-walled, in 25-cm lengths).
- Plastic bags (*e.g.*, small Ziplock<sup>®</sup> bags)
- Duct tape (or other strong elastic fiber-based binding tape).
- Small sledge hammer (or other small, but heavy hammer) and small wooden board.
- Vice-grip pliers and screw driver to remove incubation tubes and soil from tubes.

For surface cores from each 20x20-m subplot:

- Small punch corer (a.k.a. “plugger” or “push corer”; we have used Lamotte<sup>®</sup> Soil Sampling Tubes, 1-inch diameter x 10-inch long, available on-line through Forestry Suppliers, catalog number 76924).
- Small ruler, or marks on one of the soil-sampling implements, to gauge 10-cm depth into the soil.

## LAB PROTOCOLS

### Very important notes:

#### **Always add concentrated acid to water.**

When diluting a concentrated acid, **NEVER add water to concentrated acid solutions**. Always add concentrated acid to water.

#### **Always add water to dry chemicals.**

When mixing a solution of a dry chemical and water, first add the dry chemical to the bottle or volumetric flask, and then add the appropriate amount of water.

#### **NEVER put chemical reagents back in the bottles they came from.**

For both dry chemical reagents (*e.g.*, KCl) and solutions (*e.g.*, HNO<sub>3</sub>), when measuring out a quantity that will be used to prepare an extraction solution or *etc.*, never return remaining or extra chemicals to the reagent bottle from which they came. This will prevent contamination of the reagents.

KCl can be washed down the drain.

Acids should be diluted with large quantities of water before washing them down the drain. Make sure the drain does not lead to a human-use water supply. Where a running tap or faucet is not available, one could obtain old oil or similar 200-L drums and dilute the waste acids before putting them into the drainage.

### **Wear goggles and gloves when pouring acids.**

Wear goggles and gloves when dealing with acids, especially concentrated acids.

### **Washing glassware and 125-mL plastic bottles**

Use Alconox<sup>®</sup> or Liquinox<sup>®</sup> soap and lots of tap water. Once glassware is washed and well rinsed, rinse it again 3 times with distilled water and let it air dry.

### **NEVER touch the insides of beakers, volumetric flasks, sample bottles, *etc.* with your fingers.**

This will help avoid contamination.

### **Using graduated cylinders**

When a “TC” appears on the graduated cylinder, it stands for “to contain”. This means that the graduated cylinder is calibrated to contain the correct amount of solution. The graduated cylinder will deliver a measured amount of solution when tipped into a recipient container, *i.e.*, there is no need to rinse the contents of the graduated cylinder into the recipient container.

### **Precision of weighing soils for extractions, pH, and moisture**

Use a balance that is accurate and precise to two decimal places for grams (g), *i.e.*, 0.00 g. So, when weighing out 2.00, 2.50, or 3.00 g of soil for extractions or pH measurements, the mass should be within 0.02 g of the desired mass, *i.e.*, +/- 0.02 g.

### **Useful things for the lab that are not necessarily in the lists of “Equipment and supplies” below:**

- Alconox<sup>®</sup> or Liquinox<sup>®</sup> detergent (these are standard lab detergents for washing glassware in common use in U.S.-based labs; any similar product that has a minimal phosphate content, or that is the locally preferred laboratory soap for washing glassware should be fine; available on-line through Thomas Scientific – Alconox<sup>®</sup> 4-lb box of dry detergent, catalog number 2902G05; Liquinox<sup>®</sup> 1-quart bottle of liquid detergent, catalog number 2902N10).
- Beakers for weighing dry chemicals and *etc.*
- Calibration weights for calibrating balances.
- Distilled water – keep a good supply on hand, perhaps in a carboy dedicated to distilled water only and in squirt bottles dedicated to distilled water only.
- Funnel for pouring dry chemicals into stock-solution bottles or volumetric flasks.

- Goggles for dealing with concentrated acids.
- Gloves for minimizing contamination and for protection.
- Kimwipes<sup>®</sup> (Kimwipes<sup>®</sup> are ideal because they are lint-free, but other paper tissue could be used as a substitute).
- Paper towels (or toilet tissue if paper towels are unavailable).
- Spoons for removing soil from aluminum-foil packets.
- Thermometer for measuring the temperature of drying ovens.
- Tweezers for removing roots and stones from soil samples.
- Trays for carrying samples and supplies from place-to-place.
- Trolley or wheelbarrow for carrying supplies from place-to-place, if layout requires and allows it.



### **Extractions for Nitrogen (a.k.a. KCl-extractable Ammonium and Nitrate) – Lab Procedures**

Make the “KCl-Extraction Solution” as needed each day. Use a 2M KCl solution (2M KCl is the molarity that is conventional for soil N studies, even though 1M KCl is the norm in soil survey and pedological analyses). To make a 2M KCl solution, weigh out 149 g of dry KCl into a plastic Solo<sup>®</sup> cup or beaker. It works well to tare the balance with a beaker, so that when the balance reads “149.00 g” it means exactly 149 g of KCl. Pour the 149 g KCl through a funnel into a 1000-mL volumetric flask and add approximately 800 mL distilled water, cover the opening of the flask with Parafilm<sup>®</sup>, and swirl the flask. Let the flask nearly temperature equilibrate (it will be cold at first), swirling occasionally to dissolve all of the KCl. Once the KCl is fully dissolved (the solution will be clear), add distilled water up to within 1 cm of the line etched on the flask. Place the palm of one hand over the Parafilm<sup>®</sup> covering the opening of the flask and invert the flask three times to mix the solution well. Once the flask has completely temperature equilibrated, fill the flask to the 1000-mL line with distilled water. Label the flask with labeling tape as “KCl-Extraction Solution”.

Weigh out 2 g of fresh soil from each sample into its own, new, pre-labeled plastic Solo<sup>®</sup> cup (or equivalent) on an electronic balance (a small spatula works well for removing soil from the aluminum-foil packets). It works well to tare the balance with the empty cup, so that when the balance reads “2.00 g” it means exactly 2.00 g of fresh soil. **Note: Use soil only, i.e., do not add small stones or pieces of root to the Solo<sup>®</sup> cup.** Make sure each Solo<sup>®</sup> cup has the same i.d. number written on it as the aluminum-foil packet from which its soil comes. As soon as possible, add 20 mL of KCl-Extraction Solution to each cup (using a pipette or graduated cylinder). Use a glass stir rod to break up any soil clumps by stirring the slurry and tamping the clumps in a cup with the end of the stir rod. **Note: Wipe off the glass stir rod between sample cups with a Kimwipe<sup>®</sup>.**

Let the solution in the cups settle over night (*i.e.*, > 18 hours) in a safe place. It is best to cover the cups with paper toweling, but make certain the cups are placed on a counter where they will not be bumped, moved, or have their paper toweling blown off over night. Allow the

contents of the cups to settle for at least 18 hours. The day after the cups were stirred and left to rest (*i.e.*, after at least 18 hours), remove about 5 mL of the clear liquid from each cup (using a pipette or syringe – one without a needle) into a pre-labeled, screw-top plastic sample bottle (see “Equipment and supplies” below). After screwing each sample bottles’ lid on, tightly seal each lid by wrapping black electricians tape around the bottle and its lid.

Preserve each sample by adding 2 drops – with an eye dropper – of either 50% H<sub>2</sub>SO<sub>4</sub> solution or 50% HCl. **Note: *Either acid can be used as the preservative, but make sure to record which acid is used.***

Send the sample bottles to Joe Yavitt at Cornell University, where he will analyze them colorimetrically after adding Indophenol (which turns blue upon binding with N). Since the preservative makes the extractions acidic, there is no need to refrigerate the samples before they are mailed. However, do not keep samples more than about 1 month before mailing them to Cornell. **Note: *Be certain to work with Jim Dalling or Kyle Harms, as well as Joe Yavitt, to determined the best method to ship samples to Cornell. Joe Yavitt will need to know the details of the shipping method in advance.***

For each set of samples (where a “set” is here defined as either all the samples collected in one field day, or as all the samples extracted with a given batch of extraction solution), prepare a blank sample in exactly the same way as the soil samples, but do not add soil to the blank. Label the blank sample bottle with the name of the extraction solution and the range of samples extracted with that particular batch of extraction solution (*e.g.*, the blank for samples 1 to 60 would be labeled: “KCl blank., samples 1-60”). **Note: *Send the blank samples to Joe Yavitt along with the soil extractions. The blank samples will help calibrate values among sets of soil samples.***

Since the KCl-extraction procedure uses a non-toxic, non-corrosive salt (*i.e.*, KCl), left-over KCl-Extraction Solution can be washed down a drain with lots of water, and the Solo<sup>®</sup> cups can be thrown in the trash.

**Note:**

*Keep the samples in a cold room or refrigerator until 2 drops of acid preservative are added to the bottles and they are mailed to Joseph B. Yavitt at Cornell University.*

**Note:**

*During clean-up, wash all glassware well with Alconox<sup>®</sup> or Liquinox<sup>®</sup> detergent, rinse well with tap water, and finally rinse everything 3 times with distilled water before air-drying.*

**Equipment and supplies for KCl-extractions:**

- KCl [At least 3 kg is needed for 600 soil samples, assuming 30 samples per batch of KCl-extraction solution.]
- 50% H<sub>2</sub>SO<sub>4</sub> solution **or** 50% HCl (***Make a note of which acid is used as the preservative.***)
- Distilled H<sub>2</sub>O in large plastic container.
- Squirt bottle for distilled H<sub>2</sub>O.
- Eyedropper to add acid preservative to samples.
- 1000-mL (1-L) volumetric flask.
- 25-mL graduated cylinder or pipette to add KCl to Solo<sup>®</sup> cups.

- 5-mL pipette or 30-mL syringe (*e.g.*, VWR on-line catalog number DB301034) to remove KCl solution from Solo<sup>®</sup> cups.
- Electronic balance, accurate to grams (g) to 2 decimal places, *i.e.*, 0.00 g.
- Plastic Solo<sup>®</sup> cups (or any other brand of 50 to 100-ml disposable plastic cups), each labeled with a Sharpie<sup>®</sup> (or other indelible marking pen) with the i.d. number of the soil sample it will contain. Make sure that you obtain cups with flat bottoms. Some plastic cups have a raised edge or lip; or they are variously concave, convex, or invaginated; or they have an irregularity in the bottom that causes the sediment to collect along the edges or in the middle of the bottom of the cup. We are aiming for an even layer of fine sediment in the bottom of the cups several hours after they have been prepared, so use cups with flat bottoms. Use one cup per soil sample; discard after one use.
- Glass stir rods.
- Fine spatula.
- Labeling tape.
- Sharpie<sup>®</sup> (or other indelible, felt-tipped marking pen)
- Kimwipes<sup>®</sup> for cleaning spatula between soil samples. (Kimwipes<sup>®</sup> are ideal because they are lint-free, but other paper tissue could be used as a substitute).
- Paper towels.
- Parafilm<sup>®</sup> (clean plastic wrap or clingfilm could be used, as long as it is capable of making a good seal over the opening of a volumetric flask).
- Scissors to cut the Parafilm<sup>®</sup>.
- Sample bottles with screw-top lids (20 or 30-mL narrow-mouthed), each with a piece of tape wrapped around the circumference of the bottle and labeled on the tape as “N-xx”, where “xx” is the appropriate soil sample i.d. number. The “N” stands for “Nitrogen”. (We have found that 20-mL screw-top polyethylene scintillation vials with caps work quite well and they are the least expensive of the various sample bottles we have used; we order them from Fisher Scientific – catalog number 03-337-24B).
- Black electricians tape.

*Note: Adhesive on the outside of the sample bottles is not a problem, so write on the tape. Do not write directly on the bottles, since ink on the bottles can rub off in transit, but will not rub off the tape.*

*Note: The chemical analysis at Cornell University only requires a few mL, so we may be able to substitute snap-top sample vials or for the sample bottles.*



**Extractions for P and Cations Combined (a.k.a. Mehlich III Extraction) – Lab Procedures**

**There are two options:**

**Option A = make Mehlich III extracting solution.**

**Option B = buy Mehlich III extracting solution.**

**Option A.**

Make Mehlich III extracting solution (*Note: Do this under a fume hood or outdoors, preferably wearing dish-washing type gloves and safety glasses*):

1) First make NH<sub>4</sub>F-EDTA Stock Solution:

Add 138.9 g of NH<sub>4</sub>F and 73.5 g of EDTA to a 1-L volumetric flask. Then add about 800 mL of distilled water, place a piece of Parafilm<sup>®</sup> over the opening of the flask, and swirl the solution until the dry chemicals are dissolved and until the solution temperature equilibrates. Add enough distilled water to dilute the solution to 1 L final, total volume. Replace the Parafilm<sup>®</sup>, place the palm of one hand over the Parafilm<sup>®</sup> covering the opening of the flask, firmly hold the Parafilm<sup>®</sup> in place over the flask's opening, and invert the flask three times to mix the solution well. Pour the solution into a plastic bottle with a lid, labeled with "NH<sub>4</sub>F-EDTA Stock Solution" and the date. (*Note: Do not use a glass bottle to store this solution, since it corrodes glass.*)

2) Then make HNO<sub>3</sub> Stock Solution:

Put approximately 800 mL of distilled water in a 1-L volumetric flask. Slowly add 62.5 mL of select-grade concentrated (65%) HNO<sub>3</sub> to the water in the flask. (*Note: Always add concentrated acid to water; NEVER add water to concentrated acid.*) Place a piece of Parafilm<sup>®</sup> over the opening of the flask and swirl the flask to dissolve the HNO<sub>3</sub> and until the solution temperature equilibrates. Add distilled water to dilute the acid to 1 L final, total volume. Replace the Parafilm<sup>®</sup>, place the palm of one hand over the Parafilm<sup>®</sup> covering the opening of the flask, firmly hold the Parafilm<sup>®</sup> in place over the flask's opening, and invert the flask three times to mix the solution well. Pour the solution into a glass bottle labeled with "HNO<sub>3</sub> Stock Solution" and the date.

3) Finally, make Mehlich III Extracting Solution:

Dissolve 80 g of NH<sub>4</sub>NO<sub>3</sub> in a volumetric flask and dilute with de-ionized water to make 3 L. Add 16 mL of NH<sub>4</sub>F-EDTA solution, 46 mL of glacial acetic acid, and 13 mL of HNO<sub>3</sub> solution. Dilute to 4 L with de-ionized water. (*Note: the final dilution will require 925 mL of water, i.e., all of the other chemicals together in this step sum to 3075 mL*). Pour the solution into a plastic bottle with a lid, labeled with "Mehlich III Extracting Solution" and the date. (*Note: Do not use a glass bottle, since this solution corrodes glass.*)

**Option B.**

Buy Mehlich III extracting solution.

**The procedure:**

Set up a series of filter-lined funnels in a funnel-stand. Each piece of filter paper should be folded twice to make an inverted cone and placed in a funnel. Wet the filter paper with distilled water and let sit for 30 minutes to allow the filter paper to form to the funnel and to allow the water to evaporate (to avoid diluting the extraction solution).

Add 25 mL of Mehlich III extracting solution to 2.50 g (two and ½ g) of fresh soil placed in a 125-mL plastic bottle. Shake for 5 minutes. (Joe Yavitt suggests putting several bottles in a cardboard box and placing the box on a skateboard, and 'shaking' for 5 minutes.)

Let the samples sit for about 10 minutes and then filter each sample through a filter-lined funnel into a pre-labeled sample bottle (see “equipment and supplies” below). Pour the extracting solution into the funnels slowly in a circle and do not pour the clumps of soil from the 125-mL bottle into the filter-lined funnel. After screwing each sample bottles’ lid on, tightly seal each lid by wrapping black electricians tape around the bottle and its lid.

Send the sample bottles to Joe Yavitt at Cornell University, where he will analyze them using an inductively-coupled plasma (ICP) mass spectrometer. Since this procedure uses a dilute acid, there is no need to refrigerate the samples before they can be mailed. However, do not keep samples more than about 1 month before mailing them to Cornell. **Note: Be certain to work with Jim Dalling or Kyle Harms, as well as Joe Yavitt, to determined the best method to ship samples to Cornell. Joe Yavitt will need to know the details of the shipping method in advance.**

For each set of samples, prepare a blank sample in exactly the same way as the soil samples, but do not add soil to the blank. Label the blank sample bottle with the name of the extracting solution and the range of samples extracted with that particular batch of extracting solution. Send this blank to Joe Yavitt along with the soil extractions. This blank will help calibrate among sets of samples.

Since this procedure uses a stable, dilute acid, the stock solutions and the Mehlich III solution itself can be stored for long periods of time in their containers at room temperature. The used filter paper should be placed in a bucket of water to dilute the acid even further before the filter paper is thrown in the trash.

**Note: Always add concentrated acid to water (NEVER add water to concentrated acid).**

**Note:**

*The Mehlich III extracting solution is quite stable for several days to weeks, even at tropical outdoor temperatures. It is a bit 'smelly', with the acetic acid, so the extractions should be done in a well-ventilated area.*

**Note:**

*During clean-up, wash all glassware well with Alconox<sup>®</sup> or Liquinox<sup>®</sup> detergent, rinse well with tap water, and finally rinse everything 3 times with distilled water before air-drying.*

#### **Equipment and supplies for Mehlich III extractions:**

**Option A** [Need at least 8 L of Mehlich III for 300 soil samples]:

- NH<sub>4</sub>F [Need 140 g for 300 soil samples].
- EDTA [Need 75 g for 300 soil samples].
- Select-grade or HPLC-grade, concentrated (65%) HNO<sub>3</sub> [Need 65 mL for 300 soil samples].
- NH<sub>4</sub>NO<sub>3</sub> [Need 160 g for 300 soil samples].
- Glacial acetic acid [Need 100 mL for 300 soil samples].
- Plastic 4-L bottle labeled: “Mehlich III Extracting Solution”.

- 1000-mL (1 L) volumetric flask labeled: “NH<sub>4</sub>F-EDTA Stock Solution”.
- 1000-mL (1 L) volumetric flask labeled: “HNO<sub>3</sub> Stock Solution”.
- Fume hood (if available, for mixing solutions; otherwise mix them outdoors).

**Option B** [Need at least 8 L of Mehlich III for 300 soil samples.]:

- Buy commercially produced Mehlich III extracting solution [Need at least 8 L for 300 soil samples].

***Equipment and supplies for Mehlich III extractions (both Option A and Option B):***

- Distilled H<sub>2</sub>O in large plastic container.
- Squirt bottle for Distilled H<sub>2</sub>O.
- Electronic balance, accurate to g to 2 decimal places, *e.g.*, 0.00 g.
- 25-mL graduated cylinder.
- 50-mL graduated cylinder.
- 125-mL bottles – about 30. *Note: If 125-mL bottles are not available, 60-mL bottles can be substituted. Wide-mouth bottles work best, since small amounts of soil need to be placed into each bottle when the bottle is on a balance.*
- Fine spatula.
- Labeling tape.
- Sharpie<sup>®</sup> (or other indelible, felt-tipped marking pen).
- Kimwipes<sup>®</sup> (Kimwipes<sup>®</sup> are ideal because they are lint-free, but other paper tissue could be used as a substitute).
- Paper towels.
- Parafilm<sup>®</sup>.
- Scissors to cut the Parafilm<sup>®</sup>.
  
- Funnels (6.5-cm diameter) – about 30.
- Funnel rack – with capacity for about 30 funnels. A funnel rack can be made in the field with two parallel rods fixed at the right distance apart and with cross bars for additional stability.
- Whatman<sup>®</sup> 42 filter paper (12.5-cm diameter) – one sheet per sample.
  
- Sample bottles with screw-top lids (20 or 30-mL narrow-mouthed), each with a piece of tape wrapped around the circumference of the bottle and labeled on the tape as “C-xx”, where “xx” is the appropriate soil sample i.d. number. The “C” stands for “Cations”. (We have found that 20-mL screw-top polyethylene scintillation vials with caps work quite well and they are the least expensive of the various sample bottles we have used; we order them from Fisher Scientific – catalog number 03-337-24B).
- Black electricians tape.

***Note:*** *Adhesive on the outside of the sample bottles is not a problem, so write on the tape. Do not write directly on the bottles, since ink on the bottles can rub off in transit, but will not rub off the tape.*

*Note: The chemical analysis at Cornell University only requires a few mL, but even so we plan to use 30-mL sample bottles since they will not overflow under the filtering funnels.*

*Note: The Whatman filter paper can retain some cations, e.g., phosphate, so we need to be consistent (as in all the procedures we are using) and use the same type of filter paper at each site.*



### **Texture by feel – Lab Procedures**

Use freshly collected (“field moist”) soil. Follow the flow diagram in the guide to soil texture that is provided to you by Jim Dalling or Kyle Harms. Record the texture information in the data sheet provided to you by Jim Dalling or Kyle Harms.

**Note:**

*All the information you need to determine texture is provided in the guide provided by Jim Dalling or Kyle Harms. Nevertheless, to help interpret the texture data, you could examine the sand-silt-clay triangle that is available at the following websites:*

*<http://homepages.which.net/~fred.moor/soil/formed/f0107.htm>*

*<http://ltpwww.gsfc.nasa.gov/globe/pvg/texture2.htm>*

**Note:**

*Ian Baillie made the following comment: “The Fred Moor site is OK for the triangle but note that he uses the UK division of 60 um as the sand/silt boundary, cf the 20 um of the International Soil Science Society and the 50 um of the USDA. The different systems are well summarized on the Agvise Laboratories website, and there is a good triangle on the University of Florida soils teaching website.”*

**Equipment and supplies for determining texture by feel:**

- Data Sheets for texture [Use electronic file provided by Jim Dalling and Kyle Harms: “*pH.Color.Texture.SoilProj.xls*”].
- Guide to determining texture by feel [Use guide prepared by Nancy Hoalst Pullen and Hillary Hamann and provided by Jim Dalling and Kyle Harms].
- Squirt bottle of H<sub>2</sub>O. For determining soil texture the water does not need to be distilled.
- Paper towels.





Use freshly collected (“field moist”) soil. Weigh out 3.00 g (three g) of fresh soil into a scintillation vial or small Solo<sup>®</sup> cup. Add 9 mL of distilled water. **Note: Always use a 1:3 solution of fresh soil:water.** Use a glass stir rod to break up clumps and stir the soil and water into a slurry. Let the slurry settle for at least 30 minutes. To measure pH, swirl the container for a few seconds and immediately place the electrode in the slurry. Make sure the electrode is in the liquid and not in the soil at the bottom of the container nor touching the side of the container. Record the value on the pH data sheet (see “equipment and supplies” below).

**Note:**

*Rinse the electrode with distilled water between samples or buffers. Do not wipe the electrode with a Kimwipe<sup>®</sup>, since it is very delicate. Instead, gently dab the electrode with a Kimwipe<sup>®</sup> after rinsing it with distilled water between samples or buffers.*

**Note:**

*Even though most standard pedological or agronomic analyses use a ratio of soil to water of 1:2.5 or 1:5, we wish to maintain consistency among sites for comparative purposes, so please always use a ratio of 1:3.*

**Equipment and supplies for pH measurements:**

- Data Sheets for pH [Use electronic file provided by Jim Dalling and Kyle Harms: “pH.Color.Texture.SoilProj.xls”].
- pH meter (Checkmate<sup>®</sup> pH meters have worked well on BCI and in Yasuni).
- Standards or buffers (pH 4.0 & pH 7.0).
- Small Solo<sup>®</sup> cups or scintillation vials, each labeled for one sample.
- Squirt bottle of distilled H<sub>2</sub>O.
- Glass stir rods.
- Kimwipes<sup>®</sup> (Kimwipes<sup>®</sup> are ideal because they are lint-free, but other paper tissue could be used as a substitute).



**Soil Moisture – Lab Procedures**

For each sample, label a piece of aluminum foil with the sample’s i.d. number on both the front and back sides (a Sharpie<sup>®</sup> works well). Weigh the piece of aluminum foil, form it into a packet, and spoon about 20 g of fresh soil into the foil packet. Weigh the foil packet plus fresh soil. Dry the soil in the packet in a drying oven at 105° C for ≥ 48 hr (*i.e.*, dry these until they reach constant mass, which means further drying would not cause even more water to evaporate from the sample). **Note: Make sure the packets are open while they are drying.** Once the soil is completely dry, remove the foil packets from the oven, close them and let them cool down. **Note: Make sure the packets are closed while they are cooling.** On the same day they were removed from the drying oven, weigh each aluminum-foil packet with its dry soil. Record all





There are many additional measurements that could be made in the field, or on newly collected samples from the field. As time, resources, and researchers' interests dictate, other measurements could be included in the overall sampling design, including some of the following:

**Bulk density** – using “sand-filling” or “marble-filling” technique of Joe Yavitt and Bob Stallard. This is to be done at a subset of the 300 soil-chemistry sampling points. Dig up a batch of soil. Place a cellophane wrapper in the pit. Fill the pit with marbles of known size. Count the marbles. Weigh the soil.

## **POTENTIAL ADDITIONAL MEASUREMENTS ON ARCHIVED SAMPLES**

The “air-dried” archived soils may serve a variety of purposes in the future, depending on resources and researcher interests. Since collecting and drying soil is a time-consuming process, and since some soil properties change through time, the archived soils serve as a permanent physical record of the soil conditions at the time they were sampled. The following is a list of potential future measurements to be made on archived soil samples.

**Mineralogy**

**Particle size fractions (sand, silt, clay)**

**Total C, N, P, *etc.***

**Walkley Black titration for organic matter**

## **OTHER RESOURCES**

The Globe Program website has a variety of other protocols available for Earth Sciences monitoring: <http://www.globe.gov/fsl/welcome.html>