**Soils protocol for Trelease Woods – May 2019**

Sampling grid design in file “Treleaseplotsamplingdesign”

‘Grid sampling points’ = 116 grid and off-grid sampling points for mapping

Additional 73 sampling points at Sierra’s fallen trees at Trelease

Additional 81 sampling points at Sierra’s fallen trees at Brownfields

Analytes to measure

|  |  |  |  |
| --- | --- | --- | --- |
| Analyte | Sample | Grid sampling points | Fallen tree points |
| pH in water | Fresh soil  | X | X |
| Initial inorg N | Fresh in field | X | X |
| Incubated inorg N | Fresh in lab | X | X |
| Soil moisture | Fresh in lab | X | X |
| Soil C:N | Dry in Panama | X | X |
| Bray P | Dry in Panama | X |  |
| BaCl cations | Dry in Panama | X |  |
| Soil texture | Dry in Panama | X |  |

**FIELD SAMPLING PROTOCOLS**

**Grid sampling points at 116 locations, and fallen tree sampling locations (for soil chemical and physical properties)**

The “Treleaseplotsamplingdesign” file provides the locations of 116 sampling locations that comprise an “unaligned grid sampling scheme”. 77 of these locations will be arrayed throughout the plot in a regular grid; these 77 points are 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). An additional 154 sampling points represent the locations of dead and decaying trees – 73 in Trelease and 81 in Brownfields woods.

1. 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. Use the corer to collect a core of mineral soil (soil beneath the leaf-litter layer) from each of the 3-5 small cleared areas and pool the three samples into a single large pre-marked ziplock bag. Mix the composite sample well (by massaging the soil through the bag).
2. Remove ~2 g of soil from the well mixed soil in ziplock bag and add to the labelled 50 ml centrifuge tube. Seal the tube tightly.

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

**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 pond, take the sample (or place the incubation tube) in soil just outside of the pond 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 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.

To do in the lab:

1. Sub-sample from ziplock bag for pH
2. Sub-sample from ziplock bag weighed onto Al foil for soil moisture content
3. Sub-sample from ziplock bag for in-lab incubation (a second smaller ziplock bag stored in the dark)
4. Place remaining sample from ziplock bag on plastic plate to air-dry. Place empty labelled ziplock bag beneath the plate (sample will be returned to this bag after sample has dried).

**LAB ANALYSIS PROTOCOLS**

**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).

Making the KCl Solution

1) To make a 2M KCl solution, weigh out 149 g of dry KCl into a plastic Solo® 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®, and swirl the flask. The dissolving KCl will make the flask and water cold. Swirl the flask occasionally until all of the KCl has dissolved and the solution is clear.

2) Wait until the flask temperature is the same as room temperature then add distilled water up to within 1 cm of the line etched on the flask.

3) Place the palm of one hand over the Parafilm® covering the opening of the flask and invert the flask at least three times until the solution is well mixed.

4) Once the flask has completely equilibrated to room temperature, fill the flask to the 1000-mL line with distilled water. Label the flask with labeling tape as “KCl-Extraction Solution”.

Preparation for in-field KCl extraction

1. Experiment to get a feel for how much volume of soil will give you 2 grams.
2. Number enough 50 ml centrifuge tubes for a day of soil sampling and to each one add 20 mL of KCl extraction solution. Put the lid on and record the weight of each numbered tube).
3. 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 3 blank centrifuge tube of KCl 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 labelled: “KCl blank, samples 1-60 and the date”).
4. In the field first record the tube number and the plot location on a data sheet. Add approximately 2 g of fresh soil to the centrifuge tube and secure the lid.
5. Back in the lab reweigh the centrifuge tube (with the cap on) and record the weight. This will allow you to calculate the mass of wet soil that was added to the KCl solution. Then shake the tubes vigorously and/or break up remaining soil particles using a glass stirring rod. {note: make sure that you have samples of soil collected at the same time and site to be able to calculate the dry mass of soil in each tube}
6. Let the solution in the tubes settle over night (*i.e*., > 18 hours) in a safe place. Either place caps on the tubes or cover with a paper towel. Allow the contents of the tubes to settle for at least 18 hours.
7. After the tubes have settled for at least 18 hours (i.e. next day) remove about 5 mL of the clear liquid from each tube (using a pipette or syringe – one without a needle) into a pre-labeled, screw-top plastic sample bottle scintillation tube (see “Equipment and supplies” below). Rinse the pipette/syringe in de-ionized water between samples. Preserve each sample by adding 2 drops – with an eye dropper – of either 50% H2SO4 solution or 50% HCl. ***Note*: *Either type of acid can be used as the preservative, but make sure to record which acid is used.*** After screwing each sample bottles’ lid on, tightly seal each lid by wrapping black electricians tape around the bottle and its lid.

Since the preservative makes the extractions acidic, there is no need to refrigerate the samples before they are mailed. However, samples must be analysed within a month from soil extraction.

Preparation for lab incubation for KCl samples

 When soil samples are returned to the lab we will (i) remove soil for pH measurement, (ii) remove soil for dry mass determination, (iii) remove about 5 g of soil for lab incubation.

 This last sample will be used for a KCl extraction one week after collection. Ensure that the bag is well sealed to prevent the soil sample drying out in the lab. Store at ambient temperature in the lab in a dark location.

 After one week we will follow the same procedure as used for samples collected for the initial in-field extraction: *i.e*., we will transfer soil and KCl to centrifuge tubes along with the same number of blanks as the initial sample set, then extract the supernatant the same way.

We can wash with DI water the centrifuge tubes used for the first extraction and reuse them.

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

 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.

***Note****:*

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

 KCl [1.8 kg will be needed for 540 soil samples]

 50% H2SO4 solution **or** 50% HCl (***Make a note of which acid is used as the preservative***). Use protective clothing and goggles. Remember add acid to water… slowly.

 Distilled H2O in large plastic container.

 Squirt bottle for distilled H2O.

 Eyedropper to add acid preservative to samples.

 1000-mL (1-L) volumetric flask.

 25-mL graduated cylinder or dispenser to add KCl to centrifuge tubes

 5-mL pipette or 10 - 30-mL syringe (*e.g*., VWR on-line catalog number DB301034) to remove KCl solution from 50 ml centrifuge tubes

 Electronic balance, accurate to grams (g) to 2 decimal places, *i.e*., 0.00 g.

 Enough 50 ml centrifuge tubes for all samples and blanks for each sample set, and racks to hold the tubes (these often are made of cardboard and come with the tubes when you buy them)

 Glass stir rods.

 Fine spatula.

#  Sharpie® (or other indelible, felt-tipped marking pen)

#  Kimwipes® for cleaning spatula between soil samples. (Kimwipes® are ideal because they are lint-free, but other paper tissue could be used as a substitute).

 Parafilm®

 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).

**Gravimetric soil water content – Lab Procedures**

(note: this is an urgent measurement – it is critical for the soil inorganic N measurement (based on KCl extraction). Since lab incubation will be done on soil that is sealed inside a ziplock bag in the lab we can assume that the soil moisture content in the samples used in the second extraction in the lab is the same as the soil moisture content we will measure for the initial samples)

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® 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 105º 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 masses with the corresponding i.d. numbers on the data sheet. Once the final mass is measured, discard the sample so that it does not get mixed up with the other aluminum-foil packets.

**pH Measurements – Lab Procedures**

(note: we will do this on fresh soil, however it could also be done on dry soil, so this is not the most urgent analysis to do)

Standardize/calibrate the pH meter using using pH 4.0 and pH 7.0 buffer solutions and re-calibrate every time the pH meter is turned on for its first use of the day, and at least after every 45 minutes of use.

Use freshly collected (“field moist”) soil. Weigh out 3.00 g of fresh soil into a small Solo® 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*®*, since it is very delicate. Instead, gently dab the electrode with a Kimwipe*® *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.*

**Air-dry samples**

After removing soil from the ziplock bag for the KCl incubation, pH and gravimetric moisture content empty the remaining soil onto a labelled plastic plate. Place the ziplock bag beneath the plate. Leave the plates in a safe air-conditioned space for ~1 week to air dry. After air drying, remove a small sample (~2g) into a labelled whirl-pak bag. This will be used for CHN analysis. The remaining soil will be returned to the original ziplock bag for shipping to the Turner lab in Panama.