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Efficient soil microbiome sampling of field crop yield trial

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Abstract

This protocol describes an efficient approach to sampling the soil microbiome in a large crop yield trial.

Multiple samples are taken near the plant stem in the middle row to a depth of ~ 15 cm, pooled, homogenized and then sub-sampled for further analysis. A 3 person approach to optimize the collection process while ensuring probe sterilization between each plot is described in detail.

This protocol results in a single pooled soil sample per plot which can then be sub-sampled into three ~ 1 mL samples to be stored at - 20 °C for further analysis. This protocol ensures consistency and reliability in the sampling while also ensuring it can be done at scale (e.g. 650 samples per site taking ~ 3 days to collect).

Image Attribution

All rights for the ACTIVATE project logo remain with the researchers who are part of the GenomeCanada ACTIVATE grant.

Materials

- **SIX** 40" soil probes with plunger set to a sample depth of 15 cm.
- 20 × 20 cm sturdy plastic zipper bag **(ONE / plot to be sampled).**
- 2 mL microcentrifuge tubes **(THREE / plot to be sampled).**
- Racks for 2 mL microcentrifuge tubes.
- Coolers with ice packs.
- Markers/pens for labelling and labels for all microcentrifuge tubes.
- Cleaning tools (brushes, scrapers, and screwdrivers).
- Disinfecting wipes.
- Thrash bags.

Safety warnings

Field sampling involves exposure to high-intensity summer heat, repetitive physical exertion, and the use of manual tools. All personnel must adhere to the following safety mandates:

- Technicians must carry a minimum of 2 L of water and take a 10-minute shade break every hour when temperatures exceed 28°C. Recognize signs of heat exhaustion (dizziness, nausea) and cease work immediately if they occur.
- Closed-toe, puncture-resistant safety boots are mandatory.
- Use a "power stance" when operating soil probes to avoid back strain; rotate physical tasks (probing vs. bagging) between team members every 60 minutes.
- Exercise caution with slide-hammer probes and augers; keep hands clear of impact zones.
- 70% Ethanol used for tool decontamination is highly flammable. Store in a cool, shaded area of the vehicle and keep away from any ignition sources or dry brush.
- In the event of a thunderstorm, follow the 30/30 Rule: if thunder is heard within 30 seconds of lightning, evacuate the field to a hard-topped vehicle immediately.
- Be vigilant for ticks - perform a tick check at the end of each sampling day.

Before start

- We recommend at least 3 people per sampling team with each person focused on a specific task as described in the protocol.
- Ensure you have a field plot layout which indicates the exact plots to be sampled.
- Plots should be pre-selected to ensure adequate biological replicates per germplasm accession combined with the distribution across the site needed for statistical analysis. This is not described here as it is dependant upon your experimental design.
- Sampling of microbiomes are laborious.
- Microbes are alive. There is a need to avoid contamination between plots and to ensure proper sample storage to prevent degradation.
- While there are built in technical replicates in this sampling protocol, each experiment will need to ensure proper biological replicates based on their own experimental design.
- When choosing plots to sample, take into account that there is a need to correct for variation in the microbiome within a single site.

Planning Stage

3m

1 3m

Based on your experimental design and the question you are looking to address, choose how many plots should be sampled, how many cores per plot and, more specifically, which plots should be sampled.

Remember that soil sampling is laborious and time consuming so you will want to find a balance between the number of plots feasible to sample within your budget and the ideal number of plots to sample for your experiment. Do not forget to take into consideration the need for multiple cores per plot. Each plot, including multiple cores sampling, takes ~ 00:03:00 to sample (it took our team of 9 people 3 days to sample 324 plots).

2 Make detailed field plot layout diagrams highlighting which plots will be sampled to provide to your sample collection team and purchase the required materials (see Materials section).

3 Collect all the materials required for the sample collection ensuring you have:

- Enough coolers to contain the samples you will be collecting in one day.
- At least 2 probes per sampling team (assuming you are taking 3 cores per plot, this ensures you have a clean probe for each sample with one extra in case of malfunction or extra sticky clay that needs more soaking to clean).
- Enough -20 °C freezer space for the microcentrifuge tubes collected (3 x number of plots to be sampled).

4 Before going out in the field, you will want to pre-label all the sample bags and tubes. Specifically,

- Label **ONE** 20 × 20 cm plastic bag per plot to be sampled. These will contain the pooled soil cores during collection. These are also where the soil from the cores are homogenized and then sub-sampled from.
- Label **THREE** 2 mL micro-centrifuge tubes per plot. These will contain ~ 1 mL homogenized soil samples sub-sampled from the bag labelled in the previous bullet point.

Note

The label should include all information needed to uniquely identify the sample throughout your experiment. I suggest including identifiers for the experiment, site-year and plot.
If using barcodes, ensure you have a barcode scanner in the field so that you can identify which bags go with which plot. Also ensure that you include the above mentioned identifiers in your barcode information file.

Site setup

5
- Label one of your 15 L pails 'dirty' and the other 'clean'.
- Put the clean soil probes in the 'clean' bucket. They will be placed in the 'dirty' bucket after a soil core is taken with them.
- Add water to your 'dirty' pail to allow for soaking of hard to remove soil debris.

6 Ensure everyone knows which job they are performing within team:
- **Person A** will be in charge of the 'clean'/'dirty' buckets and the probes.
- **Person B** will need the field plot layout and will take the soil cores
- **Person C** will be in charge of the sample bags/tubes and coolers.

7 Set your soil probes to sample to a depth of 15 cm adjusting the foot pedal. How this is done depends on your soil probe (Figure 1).

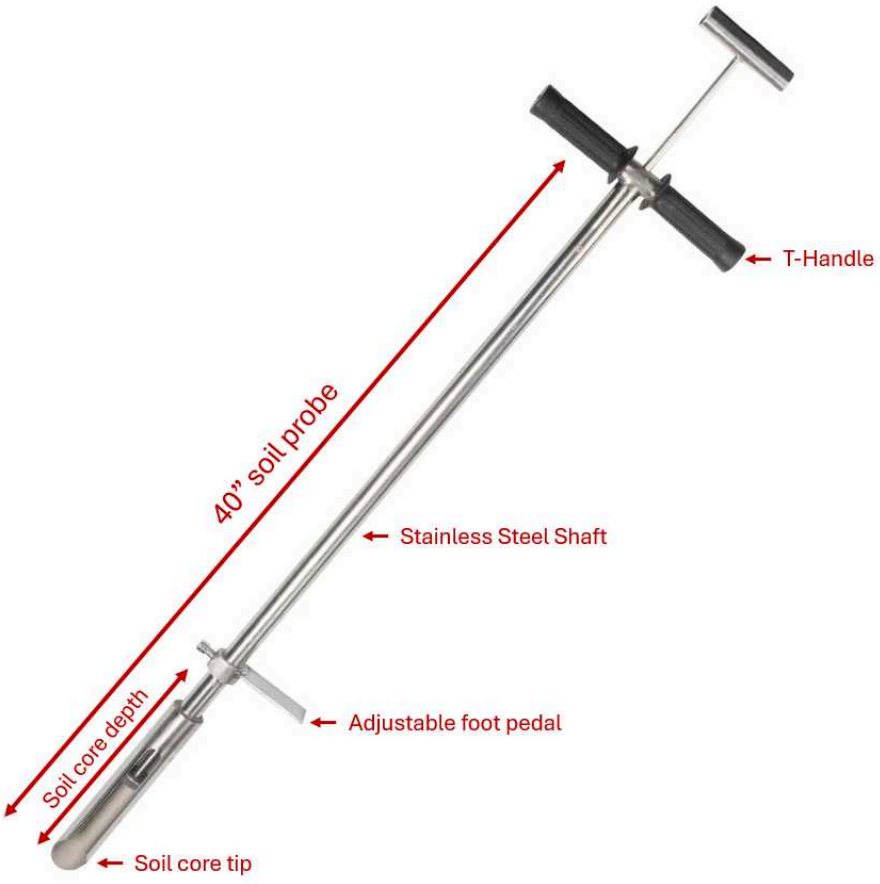

Figure 1: Example of a 40" Soil probe.

Collect Samples

1m 30s

8
- Starting at the first plot to be sampled, **Person B** will choose a location in the plot to sample, near the middle row within the first 3rd of the plot but not near the edge (see Figure 2.1 for example).
- **Person A** grabs a clean probe, wipes it with a disinfecting wipe to sterilize it, dries it with a paper towel and hands it to **Person B**. The same probe will be used for all three samples in this plot since they will be pooled anyway.

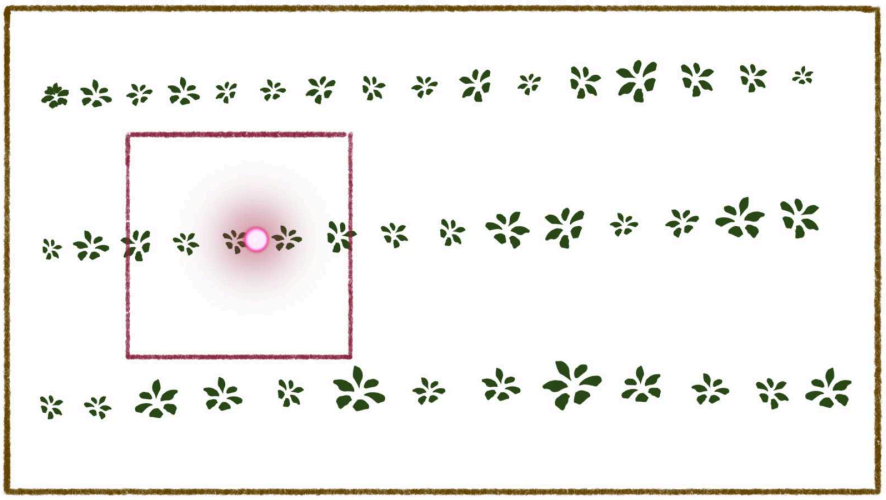

Figure 2.1: Field plot diagram showing 3 rows of plants. The pink square shows the region to target for the first soil core and the bright pink dot shows the specific one chosen.

9 The sample will be collected as follows:

20s

- **Person B** inserts the probe into the soil as close as possible to a plant stem within the row in the location chosen to a depth of 15 cm (preset on the probe) .
- **Person B** pulls the probe from the soil and discards the first 2 cm soil core (nearest probe opening) using a spoon or screwdriver in between the planting rows of the plot near where the sample was taken. This prevents plant debris enter into the soil sample.
- **Person B** uses the probe plunger to eject the remaining 13 cm soil core into the **Pooled soil sample (20 × 20 cm plastic bag labelled with plot ID)** held by **Person C**.

10
- Next, **Person B** will choose a location in the same plot, near the middle row in the centre third of the plot (see Figure 2.2 for an example). Step 9 (go to step #9)

20s

will be repeated to collect another 13 cm soil core which is added to the same plastic bag labelled with plot ID.

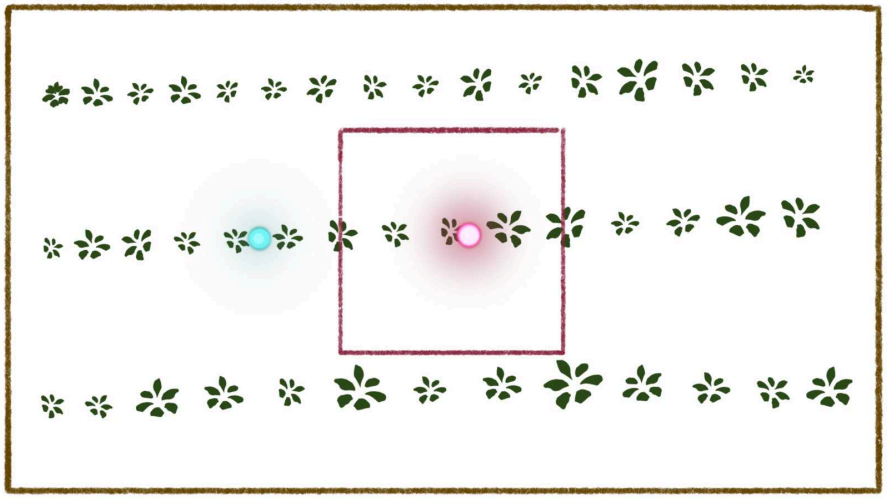

Figure 2.2: Field plot diagram showing 3 rows of plants. The pink square shows the region to target for the second soil core and the bright pink dot shows the specific one chosen. The blue dot shows the previous sample location.

11

- Finally, **Person B** will choose a location in the same plot near the middle row in the last third of the plot (see Figure 2.3 for an example). Step 9 (go to step #9) will be repeated to collect another 13 cm soil core which is also added to the same plastic bag labelled with plot ID.

20s

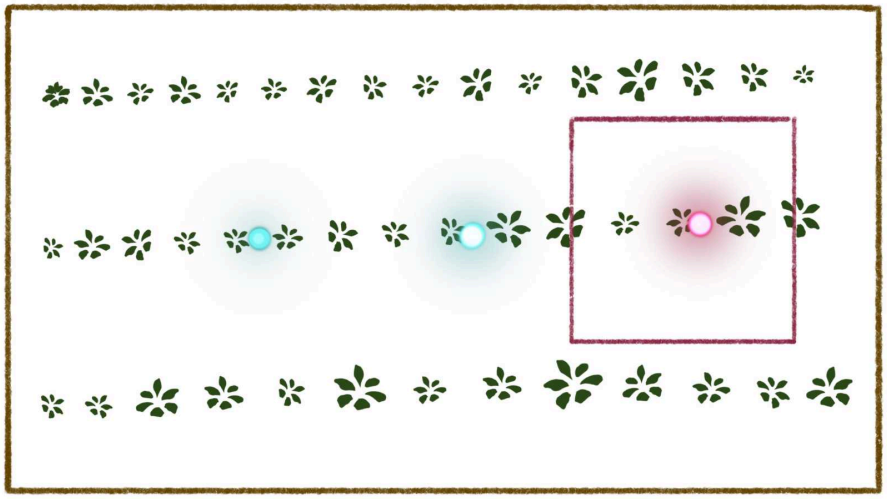

Figure 2.3: Field plot diagram showing 3 rows of plants. The pink square shows the region to target for the last soil core and the bright pink dot shows the specific one chosen. The blue dot shows the previous sample locations.

12 The following sub-steps will be occurring at the same time: 30s

12.1
- **Person A** will do a deep clean on the probe(s) used for that plot. The method depends on soil composition but will be a combination of mechanical removal with brushes and soaking in the 'dirty' pail filled with water. Once clean, the probes will be placed in the pail labelled 'clean' for use in further plots.

12.2 The plot sub-samples will be collected by **Person C** as follows:
- Close the bag containing the **Pooled soil sample (20 × 20 cm plastic bag labelled with plot ID)** once all 3 cores have been added to it.
- Once it is securely closed, homogenize the sample by breaking the soil into small particles, as fine as possible. The method will depend on the soil composition but will likely be a combination of rubbing the sample bag with hands or by hitting the sample bag with an object, being careful not to puncture the bag.
- Locate the 3 × 2 mL microcentrifuge tubes labelled for that plot, place them in the rack and open all three lids.
- Disinfect a spatula and then use it to fill each tube approximately 1/2 way resulting in 3 microcentrifuge tubes.
- Place the microcentrifuge tubes containing the soil sample in the cooler to be stored On ice .

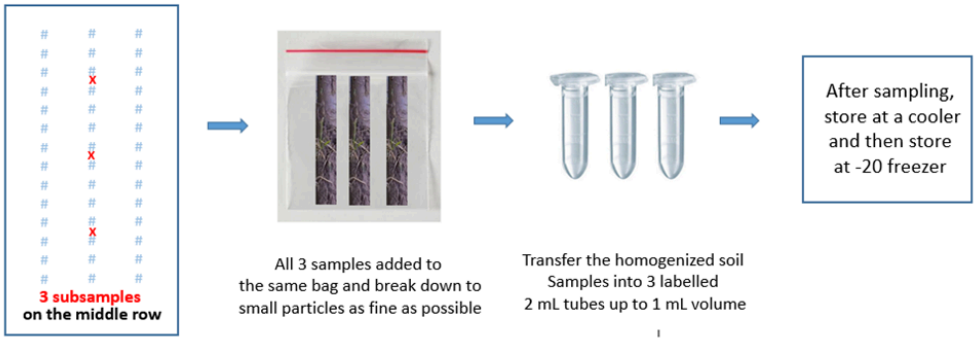

Figure 3: Shows that all three soil samples are placed into the same sample bag. The pooled sample is then homogenized and sub-sampled into 3 tubes.

Note

If you find there isn't time to do the sub-sampling in the field, you can place the homogenized pooled sample in the cooler and then complete the sub-sampling at a later time (but as soon as possible).

12.3 **Person B** will locate the next plot to be sampled using the field plot plan prepared in the planning stage. Once located the will help Person A + Person C move the equipment to the new location.

13 Steps 4 through 8 are then repeated for each plot to be sampled.

Note

Each plot should take between 60 - 90 seconds for a 3 people team. When estimating the time for the entire site, remember how laborious this process is. When we did this, it took our team ~ 3 days to collect 324 samples at a single site.

14
- Once all plots to be sampled have been sampled, the coolers are taken to the lab and all microcentrifuge tubes containing the soil samples are moved to the -20 °C Freezer for long term storage.

Note

It is ideal for the samples to be moved to - 20 °C as soon as possible at least by the end of each sampling day.

We chose not to save the **pooled soil sample (20 x 20 cm plastic bag labelled with plot ID)** and thus emptied these samples in the field once all sub-samples were taken. Make sure not to reuse these bags as that will contaminate your samples!

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