



Agriculture and Agri-Food Canada

# General Field Plot Sampling Protocol for DNA-based analyses (2018-2020)

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## ABSTRACT

General field plot sampling protocol for DNA-based analyses (2018-2020)

## DOI

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## KEYWORDS

microbiology, soil, DNA, genome, microbe, microbial community

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48282

## MATERIALS TEXT

### Field sampling:

Sampling to a depth of 30cm would typically be done with a soil probe, similar to JMC's Backsaver soil probes, which can be purchased at <https://www.benmeadows.com>

- JMC Backsaver N-2 Handle: 220605
- JMC Dry Soil Tube, 15" L with 3/4"-dia. Core:PN009
- JMC Wet Soil Tube, 15" L with 11/16"-dia. Core: PN008

Sampling to greater depths would typically be done using a truck-mounted soil probe

### Soil processing:

- 70% Ethanol
- Paper towels
- 2 basins
- 4 mm sieves
- Nitrile gloves
- Small whirlpak bags (2oz)
- Small labelled 4-5b plastic bags

## BEFORE STARTING

**General sample handling considerations:** Remember that we are sampling for microbiological analyses, where for some analyses the final amount of soil that will be used is 0.5g, so care is needed to ensure that no cross-contamination occurs.

Minimize cross-contamination when sampling:

- For surface sampling, this can be achieved by 'cleaning' the soil probe out with an initial discard sample at each site. In the new plot, simply take a full depth core on the edge of the plot and discard that sample.
- For deeper sampling, ensure the liners being used are either new or have been thoroughly washed and ethanol sterilized prior to use.

Minimize microbial community turn-over. Microbial communities change rapidly in response to their environment. As soon as the soil sample is taken you have introduced changes in temperature, oxygen, light, and nutrient availability. The rate at which microbial changes occur can be minimized by:

- Keeping cores as intact as possible
  - Use soil probes instead of augers
- Placing the sample on ice in a cooler as soon as the sample has been bagged
  - When possible, keep the cooler in shade while sampling
- Placing the cooler in a 4oC walk-in upon return to the lab
- Ensuring that samples are kept cooler before and after sieving

Minimize cross-contamination when processing samples:

- Clean and ethanol-sterilize all sample processing equipment in between each composite sample
  - Bins, sieves, scoopula's etc.
  - Use 70-80% ethanol
- If samples are very wet, it may be necessary to wash each item in between samples, prior to ethanol sterilization.

## General Field Sampling Workflow

- 1 Clean the soil probe out by taking a discard sample.
  - In the new plot, take a full depth core on the edge of the plot and discard that sample.
  - If using a truck-mounted soil probe, ensure the liner is clean as per the above instructions.
- 2 The way that sampling is done depends on the questions being asked. Very generally...

## ASSESS MANAGEMENT IMPACTS ON THE SOIL MICROBIOME

When sampling to assess how management impacts the soil microbiome, sampling proceeds in a manner

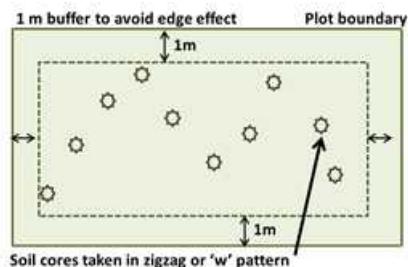
designed to capture the spatial heterogeneity of the plot.

- Stay 1 m away from the edge of the plot (avoid edge effect).

The goal is to minimize the inherent spatial variation of microbial communities associated with plot-level soil physico-chemical heterogeneity.

### Manual Probe

Sample the plot as follows:



Take 10 cores from each plot, to the determined depth, in a random W/Z pattern across the entire plot, as in the example above.

### Truck mounted Probe

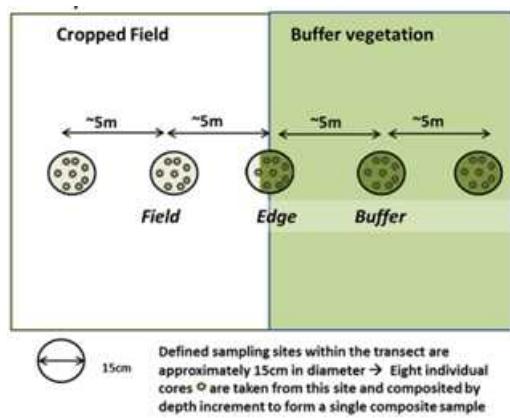
As many replicates as feasible...

## ASSESS COMMUNITIES WITHIN A SPECIFIC LOCATION

**When sampling to assess communities within a specific location** (along transects or within a specific spot in the landscape), sampling proceeds in a manner designed to capture the community within that specific location.

- Take approximately 8 cores to the required depth at each location. These cores will be tightly spaced together since we are interested in the community at that specific site. An example of one transect trial is included below.

The goal is to capture the inherent spatial variation of microbial communities associated with landscape level soil physico-chemical heterogeneity.



Larger cores or 'quadrats' of soil can also be removed at each site, if overall management allows (these cause more physical disturbance).

**3 If manual sampling**, samples can be divided into the required depth increments and then the depth-discrete composite cores from each plot can be placed into a single bag.

- Always double check to ensure the bags have been pre-labelled with the right information. If not, label with a sharpie.
- Tie the bag loosely and place in a cooler containing ice-packs.

**If using a truck-mounted soil probe**, the samples will likely be transported as intact cores, and stored at  $\text{4 }^{\circ}\text{C}$  until they can be sub-sampled and composited.

- Consistency of handling within a study is key. Don't process some samples immediately and leave the rest for weeks/months at  $\text{4 }^{\circ}\text{C}$  prior to processing.
- Process by rep and not by treatment (e.g. all treatments rep 1, all treatments rep 2, etc.)

**4** Take photographs of the plot: capture both surface soil conditions and crop stage.

Contextual information of interest includes:

- Crop stage
- Weeds present
- Soil condition
- Slope/aspect if any

**5** Ensure all samples continue to be stored appropriately in a cooler:

**If the samples are able to be sieved within 48 hours of sampling**, the cooler of soils should be stored at  $\text{4 }^{\circ}\text{C}$ .

- Once processed, the soil will then be shipped overnight in a cooler with ice-packs.

**If the samples cannot be sieved within 48 hours of sampling**, they should be frozen at  $\text{-20 }^{\circ}\text{C}$  until they are able to be processed.

Note that freezing and thawing will cause changes in overall microbial community structure.

## Soil Sampling Workflow

**6 Keep samples in the cooler until they are processed** - the goal is to minimize the amount of time each sample is at room temperature, and therefore minimize changes to microbial communities.

The objective is to homogenize the depth-discrete composite soil samples from each research plot. Processing is done in an aseptic manner to minimize cross contamination between different samples.

Open sample bag and place entire soil sample in the sieve, contained in a basin. Sieve until at least 80% of the sample has been processed.



- Note that for very clayey soils it helps to first 'grate' the soils using a cheese grater. Use the 'fine' side of the grater (pictured below).



- Any small amounts of soil remaining that are too difficult to homogenize should be discarded.

7 After each sample, wipe down sieve, grater, and basins with 70% ethanol.

Depending on how wet or muddy the soil is, the equipment may need to be washed with water prior to the ethanol wash.

8 Re-bag the sample and place back into a cooler that contains fresh icepacks.

- Double-check that the label is intact and legible. **Re-label if necessary.**



A minimum of **200 g freshly grated soil** is required.

9 Ship the cooler, with ice packs, via overnight courier to DNA processing facility.

## Standard Soil Processing Procedure (v2)

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### Homogenize Sample

The objective of this process is to homogenize composite soil samples from treatment plots or sampling points. This processing is done in an aseptic manner to minimize cross contamination between different samples. The homogenized composited sample will then be sub-sampled for different biological, chemical, and archive purposes.

Open sample bag and place entire soil sample in the sieve, contained in a basin. Quickly shake sieve to remove soil particles that are sufficiently fine to fall through the sieve.

Transfer remaining sample into the other bin, and 'grate' the soil through the finer of the two main grate sizes.

- It may be easiest to use the grater in a horizontal manner, such that the grated soil stays mainly contained within the grater itself. This soil can periodically transferred to the sieved soil basin, where gentle mixing will break up the

longer soil strings.

- Soil will need to be iteratively sieved and grated until the majority of the sample has been processed and composited.
- Any small amounts of soil remaining that are too difficult to homogenize should be discarded into a waste bucket.

Once the sample is done, wipe down sieve, grater, and basins with 70% ethanol.

Depending on how wet or muddy the soil is, the equipment may need to be washed with water prior to the ethanol wash.

## 11 Subdivide Sample

Label bags required for each of the following types of analysis:

### Molecular analysis

Use small whirlpak bags (2 oz).

- Fill approximately half full.
- Place into freezer after filling.
- Once all samples are processed, they should then be placed into a bag labelled with the trial name and date, and placed in a **↓ -80 °C freezer** for long term storage for molecular assays.

### Micronutrient Analysis

Use small labelled 4-5b plastic bags.

- Transfer approximately **◻ 125 g soil** (aim is 100 g of dry soil weight).
- Leave bags open on tray to air dry at **↓ Room temperature**.

## 12 Determine Gravimetric Moisture

: a template with sample name and relevant columns should be generated in preparation for recording data.

Record tin number for each sample.

Tare the small tin and then fill with approximately **◻ 20 g wet soil**.

- Record sample weight on template record sheet.
- Place tin on oven safe sheet (cookie sheet).

Dry samples in an oven overnight at **↓ 104-104 °C**.

Once samples are dry, place in a cooling rack which contains desiccant to minimize moisture re-absorption by the cooling soils.

Record dry weight on the same scale used to record wet weight.

- Transfer all data to the relevant spreadsheet for future analysis.

## 13 Remaining Sample

Depending on the particular trial that the sample is from, additional sub-samples may then be stored for dissolved carbon analysis and/or mineral N analysis. Any remaining soils should be air dried in appropriately sized containers for archive storage.

**If dissolved carbon analysis is being performed**, return sample to original bag, twist to close, and place back in **↓ 4 °C** fridge.

After dissolved carbon analysis sub-sample has been processed, or if dissolved carbon analysis will not be performed, transfer all but 100 g soil to pre-labeled archive containers.

- The remaining 100 g soil, stored in the original sample bag, should be frozen at -20 °C for mineral N analysis.

Archive samples should include all sample information including:

- PI name, trial name, continuous ID, relevant plot information (plot and sub-plot numbers, treatment specifics, depth, date sampled), and any other details relevant to the sample.

Store samples as appropriate for future use.

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### Overview of General Soil Processing

