

Mar 07, 2025

Soil Microbiome Sample Collection Protocol (adapted from NEON)

DOI

<https://dx.doi.org/10.17504/protocols.io.yxmvmekz9g3p/v1>

Ellen Dow¹, Anna McLoon², Carlos Goller³, Elisha Wood-Charlson⁴, Benjamin Allen⁵, Aaron Schirmer⁶, Courtney Meier⁷

¹Berkeley Lab - KBase; ²Siena College; ³Biotechnology Teaching Program (BIT), North Carolina State University;

⁴Berkeley Lab; ⁵KBase, Oak Ridge National Laboratory; ⁶Biology Department, Northeastern Illinois University;

⁷National Ecological Observatory Network (NEON)

MICRONet



Ellen Dow

Berkeley Lab - KBase

Create & collaborate more with a free account

Edit and publish protocols, collaborate in communities, share insights through comments, and track progress with run records.

Create free account

OPEN  ACCESS



DOI: <https://dx.doi.org/10.17504/protocols.io.yxmvmekz9g3p/v1>

Protocol Citation: Ellen Dow, Anna McLoon, Carlos Goller, Elisha Wood-Charlson, Benjamin Allen, Aaron Schirmer, Courtney Meier 2025. Soil Microbiome Sample Collection Protocol (adapted from NEON). **protocols.io**

<https://dx.doi.org/10.17504/protocols.io.yxmvmekz9g3p/v1>



License: This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working

We use this protocol and it's working

Created: July 31, 2024

Last Modified: March 07, 2025

Protocol Integer ID: 104336

Keywords: soil microbiome sample collection protocol, soil sample, soil collection, specific site for soil collection, national ecological observatory network, metadata about the sample, metagenomic analysis, soil ph, soil, standard soil, soil core, soil temperature, sample collection, sample, sampling protocol, sampling device, leaf litter depth, sampling, mineral horizon sample, protocols for student use, combined sample, student use, metadata, approximate location of all sample

Funders Acknowledgements:

NSF

Grant ID: Research Coordination Network for Undergraduate Biology Education Incubator: KBase Educators: Program for Microbiome Workforce Development [# 2316244]

NSF

Grant ID: National Science Foundation Research Coordination Network for Undergraduate Biology Education: Microbiomes in Computational Research Opportunities Network (MICROnet) [# 2418285]

Disclaimer

Adapted from the National Ecological Observatory Network's publicly available protocol.

Abstract

Following this protocol, students will identify a specific site for soil collection, test it for suitability, and collect metadata about the sample including location coordinates, leaf litter depth, and soil temperature. Students will then collect soil cores (separated organic and mineral horizon samples or combined samples) for metagenomic analysis and to measure soil pH and moisture. Before sampling, have a plan for the type, number, and approximate location of all samples to collect, gather and prepare necessary supplies, and practice using the corer or other sampling devices. After completing this protocol, students should return to the lab for downstream analyses on the soil sample(s). This protocol adapts the National Ecological Observatory Network's standard soil sampling protocols for student use in a wider variety of settings and contexts.

Guidelines

Sampling/Field Advice:

- Practice using your soil corer before class. To remove the soil from the corer, you might need to push the soil out "upwards" to not compress the core.

Materials

- Logsheet per sampling area, clipboard, pen or pencil
- GPS or phone for determining site coordinates
- Camera for documenting site (recommended)
- Tarp or other clean work surface
- 70% ethanol in spray bottle
- 70% ethanol wipes
- Gloves for each person handling sample or cleaning equipment (nitrile recommended)
- skewer or thin metal probe (to test for obstructions and to make a guide hole for the temperature probe)
- Temperature probe
- Corer or auger as appropriate for your soil
- hori-hori or soil knife (optional)
- tablespoon, coffee scoop, or scoopula(s)
- ruler
- plastic zip-top bag(s) for bulk soil and/or mineral horizon and/or organic horizon
- Plastic zip-top bag(s) or whirlpak bag(s) or 50 ml conical tube for aliquot for microbiome analysis
- Cooler filled with dry ice (optional if collection site is near your -80 degree freezer)
- -80 degree freezer

Before start

- Logsheet per sampling area, clipboard, pen or pencil
- GPS or phone for determining site coordinates
- Camera for documenting site (recommended)
- Tarp or other clean work surface
- 70% ethanol in spray bottle
- 70% ethanol wipes
- Gloves for each person handling sample or cleaning equipment (nitrile recommended)
- skewer or thin metal probe (to test for obstructions and to make a guide hole for the temperature probe)
- Temperature probe
- Corer or auger as appropriate for your soil
- hori-hori or soil knife (optional)
- tablespoon, coffee scoop, or scoopula(s)
- ruler
- plastic zip-top bag(s) for bulk soil and/or mineral horizon and/or organic horizon
- Plastic zip-top bag(s) or whirlpak bag(s) or 50 ml conical tube for aliquot for microbiome analysis
- Cooler filled with dry ice (optional if collection site is near your -80 degree freezer)
- -80 degree freezer

Assess sampling location

- 1 Check safety of site (ice, unstable rocks, flood conditions, animals, noxious plants like poison ivy, traffic) and relocate if needed.
- 2 Assess specific sample location for use as a suitable sampling site - for instance, it is very difficult sampling from an area compacted by foot or vehicle traffic, and you need sufficient working area for team members and sample equipment.
- 3 Set up equipment on clean tarp (e.g., plastic table cloth or trash bag)

Identify site to sample and collect initial sample metadata

- 4 Within your sampling location, identify the specific soil site that you will sample.
- 5 Record collection date (YYYY-MM-DD), collection time (HH:MM(:SS) in GMT), and geographic location (Country: State, City) on your logsheet

 Soil sampling log sheet.docx 15.9KB

- 6 Record a description of the biome of your sample site. While not required, we recommend photographing your sample location from some distance away from your sample site. You will need to describe the area using environmental ontology terms, which you can do now or you can determine the appropriate terms later using the photographs.

Prepare to collect soil samples

- 7 Put on gloves and clean with ethanol and allow to air dry.
- 8 Sweep aside the litter layer of recognizable decaying leaf biomass from a small area after measuring and recording its depth. Use a probe near but not directly where you plan to sample to check the site for impediments like rocks or roots. If your probe encounters something hard, clear another spot within the area you selected for sampling, and probe near the location until you locate a spot without obstructions. Mark this spot while you record other measurements.
- 9 Create pilot hole. At a spot adjacent to but not directly on top of the marked sampling location, push aside the litter layer and create a pilot hole with the pilot hole tool. Try



multiple locations until little resistance is found. Insert pilot hole tool to the bottom of electrical tape and spin.

- 10 Once you have confirmed that the site will work (probe depth and pilot hole were successful), use a GPS or a map application on your phone to identify GPS coordinates in decimal degrees then record in the metadata log sheet using this format: 50.586825 6.408977.

Measure soil temperature

- 11 Sample Metadata Collection: Soil temperature.
 - 11.1 Measure soil temperature. Insert temperature probe into the pilot hole to a depth of 10 cm, which is indicated by the bottom of the electrical tape on the probe stem.
 - 11.2 When handling the probe, do not push or pull on the digital display as this may cause the unit to break. Instead, handle at the top of the metal stem under the digital display, at or near the electrical tape marking. Do not touch the tip of the probe.
 - 11.3 Allow probe to equilibrate for about 2 minutes, then record soil temperature in degrees C. Important: Do not make measurements with the sun directly on the probe, if needed, shade it with your body or a piece of field equipment (clipboard, backpack, etc.)

Measure litter depth

- 12 In the area around the marked sampling location, use a ruler to measure the depth of the undisturbed litter layer in cm (litterDepth, ± 0.1 cm) by gently wiggling the ruler through the layer to the ground surface, minimizing disturbance to the litter profile to minimize compaction.
- 12.1 Take and record 4-6 readings (this parameter is likely to be spatially variable at a fine scale), then calculate the average value for your official metadata value.

Reminder: The litter layer is dead and detached but recognizable, intact plant material (i.e., leaves, wood, etc) that is not consolidated, whereas an organic horizon will contain friable (easily crumbled) organic material in various states of decomposition that is consolidated as a layer. If your sample site has large leaves or other bulky materials, expect your individual measurements to vary greatly!

Collect soil core



- 13 To collect the sample, return to the original site you cleared of litter. The person ready to collect the soil sample should still be wearing gloves sterilized with 70% ethanol.
- 13.1 Sterilize tools with 70% ethanol using a wipe.
- 13.2 Carefully push your corer down to the appropriate depth (should be marked with tape on your device; the NEON standard is 30 cm). Achieving a standard depth of 30 cm is important to enable comparability across sites and with other datasets. Twist gently and pull up to pull the core out of the ground.
- 13.3 Record core depths in your metadata sheet as follows:
- 13.4 Record total depth of core (this should be the full depth of your corer unless your total soil depth is shallower). Note: If you hit a root or rock that is NOT bedrock/saprolite, then replace that core into its hole and move the corer to a new spot within 0.5 meters of the location you selected, clearing off the litter layer. You do not need to re-sterilize the corer if this happens.
- 13.5 Record depth of the organic horizon. The organic horizon is typically darker than the mineral layer and has a different texture. You can probably see bits of decaying plant matter in this layer as well.
- 13.6 Depending on your specific experimental design, move the organic and mineral soil horizons from your corer into separate pre-labeled zip-top plastic bags for processing, or combine into one bag.
- 13.7 Once enough soil has been collected from the sampling location, clean equipment thoroughly between sampling locations: Rinse or wipe down reusable sampling equipment (corer, thermometer, hori hori, etc) with deionized water. For very dirty equipment, use a scrub brush or wipe. Then sterilize using 70% ethanol wipes or an ethanol squirt bottle. Allow to dry before collecting another sample.

Collect additional Organic (O) Horizon samples (if necessary)

- 14 You need approximately 40 g of soil for downstream analyses, so depending on the depth of your organic horizon, you may need to collect additional samples.
- 14.1 Push the litter layer away from where you are going to core into the soil surface of the additional sampling area.
- 14.2 Cut out an organic horizon "brownie" using a frame cutter tool and/or soil knife (hori-hori) and/or using your corer. With deep organic horizons, only 1 "brownie" may be

needed; from many sites, two or three will be needed. At those sites, select multiple locations within 0.5 m of the original sampling location.

- 15 Record the number and depth of additional organic horizon samples that you collect. If the depth of the organic horizon layer is variable, record all measurements, and average them for your official metadata value.
- 16 Record any additional notes about the O horizon. For instance, if it is thin, woody, or has any other notable characteristics.
- 17 Place all brownies collected at one location into a pre-labelled, zip-top plastic bag.
- 17.1 Break up and mix the samples and then with a pre-sterilized, gloved hand, remove rocks, insects, recognizable and undecomposed plant material, and other non-soil debris, and homogenize. Shake large root masses to dislodge soil, then discard. Break up large clods to facilitate subsampling.
- 17.2 Note: If it is difficult to pick the entire bag of homogenized soil in the field, plan to use a pair of pre-sterilized tweezers to pick rocks, roots, and non-soil debris from the microbial subsamples while generating them. Re-sterilize tweezers with ethanol and a sterile wipe in between samples. Regardless of approach, it is imperative that field subsamples are picked clean of non-soil material: failure to do so can render the samples useless.

Pack samples for transport and create sub-samples

- 18 Ensure that you have sufficient soil for all subsampling and analyses: In general, using the methods described above should provide plenty of soil for all subsampling and lab processing. However, if concerned, aim for a minimum 40 g of organic soil in each bag. If you need to take additional mineral, organic, or combined samples from one location, you may use the same tools and gloves.
- 19 Using a pre-labeled 50 ml conical tube or whirlpack bag, separate out approximately 10-20 g of the sample for microbial sampling. You can do this with a disinfected tablespoon, coffee scoop, or lab scoopula. Ensure that this sample is free of rocks, roots, insects, or other large identifiable components.
- 20 To maintain sample integrity, place microbial samples on dry ice or move to a -80 degree freezer as quickly as possible after verifying labels are correct and complete. Ensure all newly added samples are in contact with dry ice so that they freeze quickly.
Note: if using dry ice, move to a -80 degree freezer within 24 hours for longer term storage.
- 21 The remainder of your soil sample should be left in the zip-top bag and will be used to measure pH and moisture. It can be stored at ambient temperature while you are transporting it and gathering remaining samples.



Decontamination between sample plots

- 22 Between plots, thoroughly rinse or wipe down reusable sampling equipment (corer, thermometer, hori hori, etc) with deionized water. For very dirty equipment, use a scrub brush or wipe. Then sterilize using 70% ethanol wipes or an ethanol squirt bottle. Allow to dry before collecting sample.
- 23 Discard gloves between plots.

Protocol references

Stanish, L. (2024). TOS Protocol and Procedure: SLS – Soil Biogeochemical and Microbial Sampling. NEON.DOC.014048 version P. NEON (National Ecological Observatory Network).