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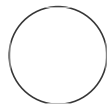
Protocol status: In development
We are still developing and optimizing this protocol

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Soil eDNA Sample Collection Protocol V.2

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ABSTRACT

****This protocol represents an initial working draft for citizen-science-based collecting and processing of soil samples for eventual eDNA analysis. This notice will be removed once the protocol has been tested and revised.****

This protocol outlines the steps that participants will follow to collect and mail environmental samples of soil for eDNA analysis - from ordering the kit to mailing the processed composite soil sample. This protocol is a part of the "Soil eDNA Initiative" and uses a Soil eDNA Sample Collection Kit available from Mycota Lab.

After some initial at-home preparatory work when the kit is received, participants will begin by selecting the site of their choosing for the soil sampling. Sampling at each location will involve taking five soil cores and mixing them together into a "composite sample." This composite sample is mixed well, dried, and then subsampled before it is mailed to the laboratory for DNA sequencing.

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89588

Keywords: eDNA, soil, soil sampling, citizen science, mycology, fungi, DNA, metabarcoding, DNA barcoding

MATERIALS

Soil eDNA Collection Kit (13 components):

For the field:

5 Marking Flags

Compass

Sampling Grid String

Core Tube & Plunger (Sterile)

Rubber Mallet

Sampling Spoon (Sterile)

Collection Bag

Back at home:

Sieve & Drying Tray (Sterile)

Sample Bag

Sampling Spoon (Sterile)

Sample Tube w/ Beads (Sterile)

Mailing Bag, Box, & Label

Things you will need that are not in the kit:

Nitrile or latex gloves. Not powdered. (not included in kit due to latex allergies and sizing)

Cell phone with iNaturalist app

Dehydrator (w/ adjustable thermostat)

Bleach

Cooler with Ice (at least 12" x 8" x 8")

Ordering the kit

- 1 This protocol requires that you have a Soil eDNA Sample Collection Kit from Mycota Lab. They can be ordered online here:

<http://www.mycota.com/shop>

Before ordering the kit, be sure your intended sampling location is not within one of the [USDA's APHIS Quarantine Areas](#). We are not able to accept soils from quarantined areas into the lab.

Once you receive the kit

- 2 Join the "[eDNA Initiative](#)" iNaturalist Project. With Android, it is sometimes easiest to search and join the project on the iNaturalist website first, and then join within the Android app. To test whether you have successfully joined the project, open a new test observation, select projects, and ensure "[eDNA Initiative](#)" is in your project list.
- 3 Select the general site and ideal timeframe for sampling your soil. See the [eDNA Site Selection &](#)

[Sampling Timeline Guide](#) for additional information.

- 4 Ensure you have an appropriate dehydrator. A standard dehydrator with vertical stacking trays will not work with this kit. We suggest an [Excalibur-style dehydrator](#) for this project. We have created a page fully outlining [recommended dehydrators](#) for this project.

Clean your dehydrator and racks with a 10:1 water:bleach solution.

On sampling day - In the field

- 5 Make sure GPS/location is enabled on your cell phone.
- 6 Visit the first location where you will take a soil sample. At the point where you would like to take your first sample, plant one of the flags into the ground. Extend the string in the each of the cardinal directions - N, S, E, and W - with the red marker at the central flag. Pull the string tight in each direction and plant the final four flags in the end loops of the string. The five flags will be the individual points that you will be removing soil from for your composite sample.
 - 6.1 Open the iNaturalist app and create a new observation. Standing above the origin flag, take a picture from above the flag looking down with the iNaturalist app. This will record your GPS position into the observation. Take some additional photographs at the origin flag location. North, East, South, and West. Add a new observation to the "[eDNA Initiative](#)" iNaturalist project and fill in the additional required fields. All of the metadata you need to obtain for this project is now recorded.
- 7 Put on your gloves.
- 8 Next to the origin flag, with your hands, remove any leaf litter or other organic debris, such as wood or needle duff. You want the underlying soil layer to be exposed. If you notice any large rocks, you can either sample next to them or remove the rocks from your sampling area.
- 9 Remove the core syringe tube from the packaging and pull out the plunger. Place it back into the bag for the moment. Using the mallet, hammer the syringe into the soil until the handles are near ground level, or at least four inches. If you hit a rock, roots, or some other obstruction, it is fine to pull the syringe out and try again at a slightly different point next to the original core attempt.

Note: it is possible that for the general area, the soil may contain too much clay or be too dry to hammer in the tube or that the soil may be too rocky for this process. In this case, open the sampling spoon from

the kit and manually spoon material out of the sampling site and into the Collection Bag. There is no need to transfer rocks or any woody debris into the Collection Bag as a part of this process.

- 10** Pull the core tube out of the soil with the soil core still inside. Using the plunger, push the soil out of the core tube and into the "Collection Bag."

Note: In certain environments, such as with sandy soils, it is possible that the soil will not remain in the tube when you attempt to pull it out. In this case, open the sampling spoon from the kit and manually spoon material out of the sampling site and into the Collection Bag.

- 11** Place the collection bag on the ice in your cooler.

- 12** Repeat steps 10 – 13 for each of the four additional sampling points - N, S, E, and W. You will take a total of five soil cores. [➡ go to step #9](#)

- 13** At the end of the sampling period at your site, you should be left with the collection bag containing five soil cores. Ensure the bag is sealed properly and leave it on ice until you arrive back at base.


- 14** Once you arrive home, place the soil in the freezer until you are ready to continue on to future steps.

On sampling day - Back at base

- 15** Put on a fresh set of gloves.

- 16** Open your collection bag fully and then seal it closed, trapping some air inside. You will be homogenizing this soil by hand, and a little bit of air in the bag will make this process easier.

- 17** With both hands, knead the soil in the bag for about 2-3 minutes. You are looking to get the soil mixed together as thoroughly as is possible.

- 18 Open the soil sieve and drying tray. On a hard, clean surface, place the sieve on top of the tray. Pour the composite soil sample onto the sieve. Using your gloved hands, gently caress the soil through the holes in the sieve so it lands in the drying tray. If some soil falls outside of the tray, that is fine, just discard it at the end of this process (Don't scoop it back into the drying tray). The goal here is to filter out any rocks, woody debris, and/or other large chunks of material that may be present. Once all of the soil has passed through the screen, examine the screen for any fine/small roots. Add them to the sieved soil tray. Any remaining material too large to pass through the screen can be thrown away.
- 19 Place the drying tray with the sieved soil on the dehydrator at 140 degrees F ( 60 °C) for 24 hours. The soil should be completely dry before proceeding.
- 20 Pour the soil from the drying tray into the "Sampling Bag." Once again, knead the bag for 5 minutes to break up any clumps and to homogenize the soil as much as possible.
- 21 Open the 50mL "Sample Tube" by unscrewing the blue cap. Be sure to not let any of the beads fall out of the tube. Using the provided sterile spoon, scoop soil out of the Sampling Bag and into the Sample Tube. Fill the Sample Tube with 35 mL of soil. Screw the cap on tightly. Seal the Sampling Bag. These are the final samples that you will mail to the sequencing facility.
- 22 Place the Sampling Bag and Sample Tube into the mailing bag and place the mailing bag in the pre-paid mailing box for shipment to the processing facility.