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We use this protocol and it's working

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Soil eDNA Sample Collection Protocol

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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 ten 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.

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

MATERIALS

Soil eDNA Collection Kit (13 components):

10 Marking Flags
Sampling String
Point Marker Tape
Core Tube
Rubber Mallet
Soil Pusher
Sampling Spoon
Collection Bag
Sampling Bag
Drying Trays
Sieve
Sample Tube
Bead Bag
Mailing Tube
Mailing Envelope

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 (preferably with adjustable thermostat)
Bleach
Aluminum Foil
Cooler with Ice (at least X x X)

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

Once you receive the kit

2 Join the "Fungal 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 "Fungal eDNA Initiative" is in your project list.

3 Select the general site where you will sample soil. See the Site Selection Guide for additional

information.

4 Find an ideal timeframe for sampling your soil. See the Sampling Timeline Considerations Guide for additional information.

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

6 Tie a loop on each end of the 40 meter sampling string. The #0 (origin) and unmarked sampling flags will eventually go through these loops to hold the string in place at your sampling site. Four meters (13' 1.5") from the origin, place the #1 label over the string. In four more meters, place #2. Continue until all ten included labels are used. The final point on the string should be #9.

On sampling day - In the field

7 Make sure GPS/location is enabled on your cell phone.

8 Visit the first location where you will take a soil sample. At the location where you would like your first sample, plant the #0 flag into the ground through the #0 Site/loop of the sampling string. Extend the string in the direction that you have selected to sample along. Pull the string tight and plant the unmarked flag in the final loop of the string.

8.1 At each numbered point along the string, plant the corresponding numbered flag from the kit. These will be the individual points that you will be removing soil from for your composite sample from the site.

8.2 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 "Fungal eDNA Initiative" iNaturalist project and fill in the additional required fields. Repeat this step for each of the sampling points along your string before any soil samples are taken. All of the metadata you need to obtain for this project is now recorded.

9 Put on your gloves.

10 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.

11 Remove the core tube from the packaging and hammer it into the soil at least four inches using the rubber mallet.

Note: it is possible that 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.

12 Pull the core tube out of the soil with the soil core still inside. Open the bag with the “Soil Pusher” and 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.

13 Place the collection bag on the ice in your cooler. Place the sampling tube back into it’s bag and the soil pusher back into it’s bag.

14 Repeat steps 11 – 13 for each additional numbered sampling point. You will take a total of 10 soil cores. go to step #11

15 At the end of the sampling period, you should be left with the collection bag containing 10 soil cores being stored on ice.

On sampling day - Back at base

16 Put on a fresh set of gloves.

17 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.

18 With both hands, knead the soil in the bag for about 5 minutes. You are looking to get the soil mixed together as thoroughly as is possible.

19 Open the bag and pour the soil into the drying trays. In some soil falls outside of the trays, that is fine, just discard it.

20 Place the drying trays with the soil on the dehydrator at 115-125 degrees for 24 hours. The soil should be completely dry before proceeding.

21 Pour the soil from the drying trays 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.

22 Place a piece of aluminum foil onto a table top.

23 Scoop soil out of the Sampling Bag and force it through the sieve with your gloved hand, allowing the filtered soil to fall onto the aluminum foil. The goal here is to filter out any rocks, woody debris, and/or other large chunks of material that may be present. Any material too large to pass through the screen can be thrown away. Repeat this until all of the material from the Sampling Bag has been filtered.

24 You should now be left with a pile of filtered soil on the aluminum foil. Carefully pour this soil back into the Sampling Bag.

25 Seal the Sampling Bag and gently knead/shake/caress the bag for 5 minutes to thoroughly homogenize the soil.

26 Open the 50mL “Sample Tube” by unscrewing the blue cap. Using the provided sterile spoon, scoop soil out of the Sampling Bag and into the Sampling Tube. Fill the Sampling tube with 30 – 35 mL of soil.

27 Pour the beads from the Bead Bag into the Sampling Tube with the soil and screw the cap back on tightly

28 Place the Sampling Tube in the pre-paid mailing envelope for shipment to the processing facility.