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Soil eDNA Sample Collection Protocol V.3

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Stephen Douglas Russell[1,2]

[1]Biodiverse; [2]Mycota Lab

Stephen Douglas Russell
Biodiverse, Mycota Lab

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

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Abstract

This protocol represents an initial working draft for community-science-based collection and processing of soil samples for eventual eDNA analysis. This notice will be removed once the protocol has been fully 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.

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 (in the future)

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 a front-loading dehydrator that has trays with adjustable levels, has trays larger than 9" on each side, and that have an adjustable thermostat. We have created a page fully outlining **recommended dehydrators** for this project.

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

5 Prepare your negative control sample.

In order to give the results from your sampling more scientific credibility, an empty sample without any soil, known as a negative control, should be taken. Preparing our negative control sample follows four steps.

1. Wearing gloves at your home base, open one of your sieves and pour the sterile media from the 50mL negative control tube through the sieve into the tray.
2. Place the tray with the media into your dehydrator for 24 hours. You can put your sieve back into the autoclave bag until final use for a sample.
3. Spoon the media back into the negative control sample tube.
4. Place the 50mL negative control tube back into the box to return with the remainder of your samples.

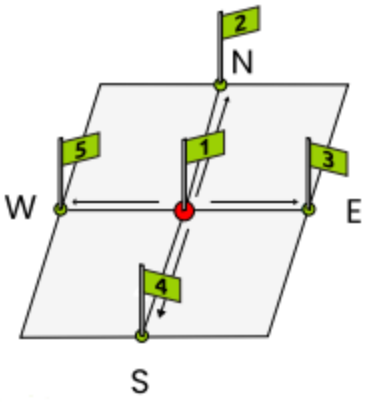
On sampling day - In the field

6 Equipment Needed:
If you are planning to take five samples during this outing, you will need five collection bags (gallon Ziplocks), five coring syringes, a rubber mallet, marking flags, 3 backup sampling spoons, sampling measurement string, compass, at least five pairs of nitrile/latex gloves (not included in kit), and a cooler with ice (not included in kit). Keeping your samples on ice will help to maintain the community structure that was present when the sampling occured until the soil can be dried back at your home base.

The backup sampling spoons can be used in place of the syringes in case one of them breaks or if you have particular soil type, such as sand, that does not stay in the syringe when pulled from the ground.

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 point where you would like to take your first sample, plant one of the flags into the ground. Extend the string from this central flag in the each of the cardinal directions - N, S, E, and W, planting your four remaining flags at each of these points. Each should be ~3 meters from the central flag. These five flags will be the individual points that you will be removing soil from for your composite sample.



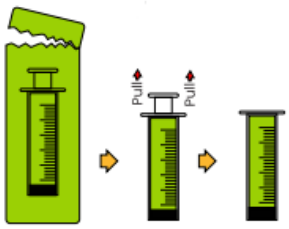
8.1 Open the iNaturalist app and create a new observation. Standing above the origin flag, take a picture from above the flag looking down with the iNaturalist app. This will record your GPS position into the observation. Take some additional photographs at the origin flag location. North, East, South, 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.

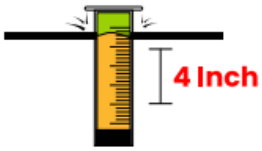
9 Put on your first pair of gloves.

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 coring syringe tube from the packaging and pull out the plunger. Place the plunger 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 deep. 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.

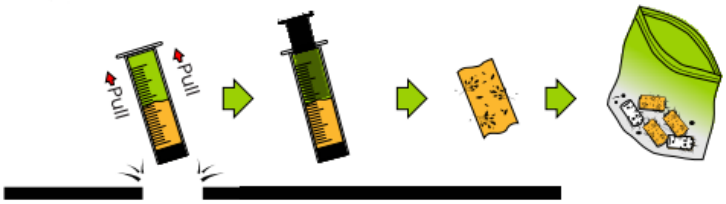
Note: it is possible that for the general area, the soil may contain too much clay or be too dry to hammer in the tube or that the soil may be too rocky for this process. In this case, open the sampling spoon from the kit and manually spoon material out of the sampling site and into the Collection Bag. There is no need to transfer rocks or any woody debris into the Collection Bag as a part of this process.





12 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” with the number corresponding to the number you associated with the iNaturalist observation for this site.

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 containing the five composite cores on the ice in your cooler.

14 Repeat steps 8 – 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 #8

At each new sampling site, you can reuse the flags and sampling string. You will want to use a new pair of gloves, open a new coring syringe, and a new sample collection bag. Make sure that the numbers on the collection bag from a given site are documented properly on the corresponding iNaturalist observation.

15 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.

16 Once you arrive home, It would be preferably to continue

On sampling day - Back at base

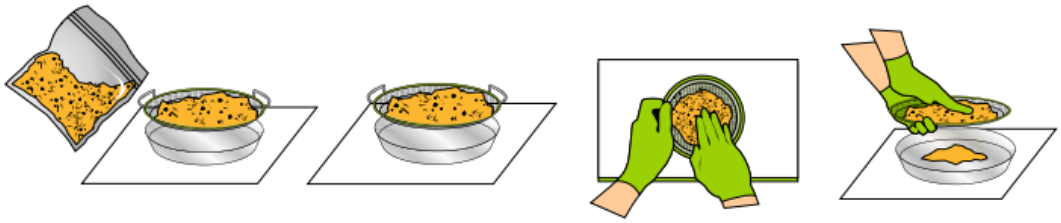
17 Put on a fresh set of gloves.

18 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.

19 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.

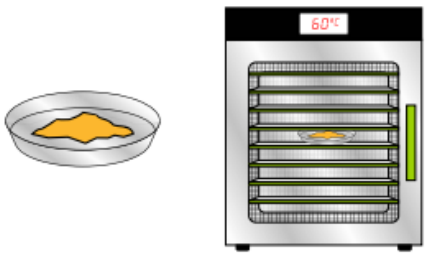
20 Remove the soil sieve and drying tray from the autovlave bag. On a hard, clean surface, place the sieve on top of the tray.

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.

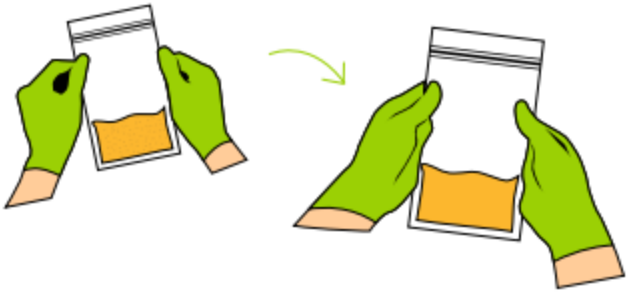


21 Place the drying tray with the sieved soil on the dehydrator at 140 degrees F (60 °C) for 24 hours. The soil should be completely dry before proceeding.

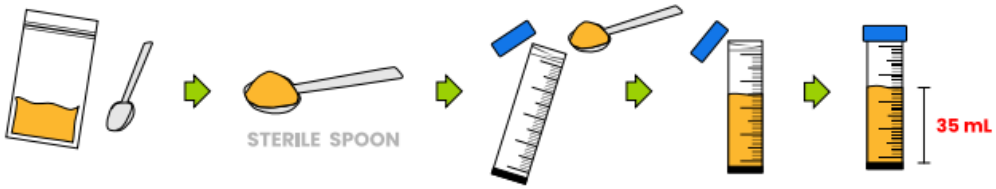
Remove the sticker from the Collection bag and place it on the metal tray with the soil.



22 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.



23 Open the 50mL “Sample Tube” by unscrewing the 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 up to the 35 mL line with the dried and homogenized soil. Screw the cap on tightly. Seal the Sampling Bag. These are the final samples that you will mail to the sequencing facility.



24 Place the Sampling Bag and Sample Tube into the mailing bag (gallon Ziplock) and place the mailing bag in the return box for shipment to our lab. Each kit should have a prepaid return label included for shipment via USPS.