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Plant-associated microbiome sampling protocol for field work

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EisenLab Plant microbiomes

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ABSTRACT

This protocol details sampling of plant-associated microbiomes from plant communities in field plots.

ATTACHMENTS

Grassland Microbiome Sampling Protocol.docx

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MATERIALS TEXT

Supplies needed:

- **50 mL** conicals – prefilled with autoclaved nanopure water (or PBS)
- Ziplock bags
- **1.5 mL** or **2 mL** tubes
- Scoopulas
- Scissors
- Notebooks or note pages
- Bulb planters (or corer of choice, bulb planters are nice as they are inexpensive and usually ~10 cm deep allowing for standardized cores)
- 70% EtOH in squirt bottles
- Paper towels
- Alcohol wipes
- Gloves

Samples collecting:

- Rhizosphere - **50 mL** conicals
- Roots - tube of roots (note: could alternatively collect all root tissues for biomass)
- Leaves – tube of leaves (note: could alternatively collect all leaf tissues for biomass)
- Soil - tube of bulk soil
- Biogeochemistry - bag of bulk soil

Microbiome Sampling Protocol 30s

1 Take a photo of the subplot plot or subplot to be sampled

a. Make sure the photo is GPS tagged!.
b. From the photo, we can later get time of sampling and approximate GPS location.

2 Take any environmental measurements at this time (e.g. temperature, soil moisture).

3 Put on new gloves.

Sampling Plots 2m

4 Using a bulb planter, core the subplot.

a.Try to get a representative sample that includes all species of interest.
b. If plant community composition is important, make sure to note which species were included in the core and which were missing

Push core out into new labeled ziplock bag using a sterile scoopula or gloved hands (replace gloves after sampling if

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5 hands are used)

5.1 Spray the inside of the bulb planter with EtOH and wipe down with paper towels (or alcohol wipes) between uses.

5.2 Can stop here and place bags **On ice** for transport back to lab for processing or can immediately proceed and process in field.

Processing Samples 5m

6 Take plant species out of bag and shake bulk soil off of roots, what soil remains on the plant constitutes as the rhizosphere soil

If individual plant species is important to research questions and identification and separation of individuals is possible, then separate individual plant species and sample each species individually for next steps.

Rhizosphere Sample 30s

7 Swirl and shake roots in **50 mL** conical (prefilled with autoclaved sterile water) to collect soil associated with roots.

30s

8 Swirl/shake for ~ **00:00:30** .

Note: post processing of this sample to get a rhizosphere pellet will be needed later in lab. This involves centrifuging **50 mL** conical tubes (e.g. at 4000 g for **00:01:00**), decanting excess water and then using a flame sterilized scoopula to take ~250 mg of the soil pellet for DNA extraction. Alternatively - one can take subsample(s) from the **50 mL** conical tubes by first shaking/vortexing tubes and then transferring **1.5 mL** into **2 mL** eppendorf tube to use with a benchtop centrifuge (e.g. at 10,000 g for **00:00:30**). This procedure is modified from Edwards et al (2015).

Root Sample 30s

9 Using sterilized scissors, cut roots off of aboveground biomass and place in a labelled **1.5 mL** tube.

If tons of roots or large roots, only use 1-3 from each species in core.

Leaf Sample 30s

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10 Using sterilized scissors, cut leaves off of specimens using sterilized scissors and place in labelled **1.5 mL** tube.

a. Standardize sampling of leaf age and position between plants depending on research question.
b. Only need ~1-3 cm section of leaf tissue.

Bulk Soil Sample 30s

11 Shake remaining soil in bag to homogenize.

12 Using sterilized scoopula, put soil in a labelled **1.5 mL** tube.

12.1 Fill soil as much as possible, but at least halfway.

Transport and clean up 30s

13 Place all samples (**50 mL** conical, tube of roots, tube of leaves, tube of soil, bag of soil) **On ice** for transport or directly into **-20 °C** or **-80 °C** freezer in processing in lab.

14 Replace gloves and make sure to clean bulb planter/scoopulas with EtOH.

15 **go to step #1** and repeat for all plots sampling

Example Note Pages 30s

16

Example note pages:

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A	B	C	D	E	F	G
Plot	Suplot	Photo	Species Cored	Collector	Date	Notes
92	a	X		Cassie		Gopher destroyed plot
	b	X		Marina		
	c	X				
	d	X				
	e	X				
	control	X	none			

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