Exploring Native Microbiota Lab Manual

BIO398, Winter 2024

Lab 3 : Self-Directed Sampling

Overview:

In this week's lab, you will go out into the field and take the environmental samples you are going to further analyze with metabarcoding. You will also have to bring these samples back to the lab & process them the same day, so it's critical you have a clear sampling plan so that you can accomplish everything within the allotted time.

To make sure everyone is ready, we will spend time during Monday's lecture to discuss & critique each other's sampling plans, with the goal of anticipating as many possible complications as possible so you don't get caught off guard in the field. We will also use Tuesday's lecture period to go up to the lab and get materials ready for sampling.

Lab 3 Preparatory Activity - Coming Up With a Concrete Plan:

1. What environment are you going to sample? Where, specifically will you get samples (GPS, google maps pin)? How will you access the site? Do you need transportation to the site?

2. What is your research question (e.g. how does microbial diversity vary across chemical, depth, spatial, temporal scale, or a specific type of organism, interactions)? *Try to write out a succinct summary sentence, but it's OK if it's not hypothesis-driven.* What is your sampling matrix (soil, sediment, water, porewater, plant/animal tissue, etc)? What volume of sample will you need to answer your research question?

3. What chemical, physical, or other data/observations would be needed alongside your microbial samples in order for your microbial community composition to be interpretable? What equipment would you need in order to make these measurements?

4. Propose a detailed procedure. Try to write out all the steps (even if it seems redundant), and prepare it to present to your peers during class, using the chalkboard or a laptop for visuals. *Include how to process your samples back in the lab. Don't worry if you have questions - we can discuss as a class how to best approach this on Tuesday's class (especially true for sediment samples).*

5. What tools will you need for your sampling? Think both big (e.g. shovels, coring device, hip waders) as well as small (scalpels, spoons, filtration devices, etc). Are there any special requirements you need for your sampling? A DIY coring device? Scalpels/razor blades? Something else? Make a complete list for yourself for sampling this week:

Example packing list:

Sampling tools:

☐ Disposable gloves
☐ Syringes of various sizes
☐ Clean sampling tools, wrapped in tinfoil
☐ Kimwipes or paper towel or Kleenex
☐ 70% ethanol spray bottle
☐ 50mL centrifuge tubes, and a rack
☐ 15mL centrifuge tubes, and a rack
☐ Cryovials with 100µL glycerol, and a cryobox
☐ Labelling tape
☐ Pens, pencils, and permanent markers
☐ Notebook
☐
☐
☐

Larger equipment / other:

☐ Large cooler or box for storing supplies
☐ A tray to use as a clean surface
☐ A small cooler with -80°C ice packs
☐ A small cooler with regular ice
☐ YSI or other probes for water chemistry measurements (and a thermometer)
☐ Secchi disk
☐ Buckets and rope
☐ Meter sticks / rulers
☐ Snacks and a drink
☐
☐
☐
☐

To prepare for return to lab:

☐ Filtration rigs pre-loaded with 0.2 µm filters
☐ Tubes ready to collect 0.2 µm filtrate
☐
☐

6. Practice sampling sheet for field sampling

Fill this out for practice so you know what you will record in the field. You can also pre-label your tubes so you don't need to freeze your fingers off!

Fill in all sections with a "*", and as many others as possible, using the following naming scheme: YY(MMDD)-Initials-S(ite)##-L(ocation)##-analysis.

For example, "240111-JM-S01-L01" would be the first site I sampled on Jan 11th 2024, and the first location at that site. You would append the analysis type (see below) at the end for each separate tube, and you can shorten the date to YY(season) if you don't have enough space on the tube. e.g. "24w-JM-S01-L01-DNA". This may seem a bit overkill, but it will allow someone to know what your sample is when they pull it out of the freezer 20 years later.

Site*:_____ Date / time*:_____

Sample name*: _____ Location (GPS or lat/long)*:_____

Elevation* :_____

SampleID *	Temp (°C) *	Salinity (ppt)	Notes *

Basic analysis types (you can define others, just make sure to record what they mean):

DNA (required) = Unpreserved filter, sediment, biomass, for subsequent DNA extraction. Store on ice in the field, process in lab ASAP, or freeze at -80°C prior to extraction.

cells (required) = Whole, unfiltered liquid from site preserved with glycerol or DMSO for future cultivation attempts. Frozen on site or kept on ice until returning to lab.

FISH (not required) = Whole, unfiltered liquid from site, preserved with formaldehyde, and kept frozen at -20°C for possible future fluorescence *in-situ* hybridization analysis

chem (required) = 0.2µm-filtered liquid from site, kept at -20°C until analysis. If analysis is to be done within a few days (e.g. pH reading, refractometer reading), it's OK to keep at 4°C.