Dynamic Soil Systems Part C
Soil Ecology II

In this lab students will rotate through 4 stations designed to introduce the basic groups of soil dwelling organisms. Each student will be responsible for making a sketch of one organism of each of the major groups and reporting on that organisms' role in the soil ecosystem.

Objectives:

- To gain experience with techniques for extraction of soil organisms
- To gain the ability to recognize characteristic traits of the major groups of soil dwelling organisms
- To practice basic microscopy skills

Reading:

- Chapter 11 in Brady and Weil

Introduction:

Many of the organisms that live in the soil are visible to the naked eye; such as moles, gophers, prairie dogs, many amphibians, earthworms and even some birds (burrowing owls etc.). However, most of the organisms that live beneath the soil surface range from very small to microscopic which is usually correlated with the size of the pore spaces they inhabit.

Microarthropods:

Many arthropods live in soil environments. Larger arthropods include insects, Arachnids (spiders), Myriapods (Chilopods = centipedes, Diplopods = millipedes and Isopods = pill bugs or potato bugs). The smaller arthropods, or microarthropods, include Collembola (springtails), Acari (mites), and Pseudoscorpions. The most commonly used extraction methods involve using temperature or moisture gradients that force the microarthropods to migrate towards a collection vessel. The Berlese –

Tullgren funnel method uses both moisture and temperature gradients. Soil samples are placed in a funnel that is positioned over a small beaker of ethanol. A 60W light is placed over the funnel and over the course of a few days the soil becomes progressively dryer forcing the microarthropods to migrate downwards and eventually fall into the beaker of ethanol where they can be counted and examined.

Bacteria and Fungi:

Bacteria are prokaryotic (without a nucleus) single celled organisms that can be found in almost every habitat imaginable on this planet. There are on average 1,000,000,000 bacterial cells per gram of soil. Fungi are eukaryotic (with a nucleus) microorganisms that have visible fruiting bodies (mushrooms). Many are saprophytes, or decomposers, some are plant pathogenic and some have symbiotic associations with plants (mycorrhizal fungi). Fungi are propagated through spores that have been found to travel great distances in the upper layers of the atmosphere. Bacteria and fungi are extracted from soil by mixing the soil sample with a buffer solution, making a series of dilutions and plating these dilutions into an agar plate containing all of the necessary nutrients for survival. The plates are incubated for several days to a week and the resulting colonies are isolated and regrown on fresh plates so they can be identified based on their morphology, physiology and DNA sequence.

Nematodes:

Nematodes are microscopic animals that live in the thin films of water surrounding soil particles. They occupy almost every trophic level of the soil food web. Some are plant pathogens, others feed on fungi, and still others feed on other nematodes. The most common method of nematode extraction is the Baermann funnel assembly. Soil samples are wrapped in cheesecloth, and placed in a funnel filled with water. Because of their muscular structure nematodes can only swim downwards when they are placed in large volumes of water, so over time they accumulate at the bottom of the funnel. After a few days the funnel is opened and a couple milliliters of the water is collected. The solution is examined under

a microscope. Individual nematodes can be isolated and fixed on a slide for later classification.

Protozoa:

Protozoa are single celled soil microorganisms that also inhabit the films of water that surround soil particles. There are three main types of protozoa; amoebae, flagellates and ciliates. Amoebae can form cysts when the soil becomes too dry and then can assume their normal shape when conditions are favorable again. Protozoa are extracted from soil much as bacteria and fungi are. Soil samples are mixed with a buffered solution, dilutions are made of this solution and then plated onto agar containing a soil extract. Protozoa cultures must be fed specific amounts of bacteria in order to be stored over long periods of time.

Methods for Stations:

Notes on proper use of the microscopes:

Dissecting microscopes

- Adjust the eyepieces to fit your eyes
- Light source can be from below or from above, try them both to see which one makes your sample easier to see
- Use the large knob to focus on your sample
- For live microarthropods do your best to follow individuals by moving the Petri dish (remember that the image is inverted so it might take a while to get used to)

Compound microscopes

- Adjust the eyepieces to fit your eyes
- Place a slide with a coverslip under the objectives
- Start with the lowest objective (4x)
- While looking at the objectives, use the coarse focus knob on the right to bring the stage as high up as it will go
- While looking into the microscope, turn the coarse focus knob slowly until your sample is in focus

- Use the fine focus knob to fine tune the focus
- NEVER MOVE THE STAGE UPWARDS TOWARDS THE OBJECTIVES WHILE YOU ARE LOOKING IN THE EYEPIECES: THIS IS A GREAT WAY TO BREAK YOUR SLIDE AND DAMAGE THE MICROSCOPE
- If you want to increase your magnification, gently rotate the objective to the next highest lens and refocus
- If you are using the 100x lens, you will need to use immersion oil, please ask for help and make sure to use LENS PAPER to clean the immersion oil off of the lens before you change objectives again

Station 1. Dissecting microscopes – Microarthropods

- Fill a Petri dish with a small amount of soil or compost sample
- Observe live microarthropods (and earthworms) under the dissecting microscope
- Observe the Berlese – Tullgren extraction funnels
- Use a Pasteur pipette to transfer preserved microarthropods from the ethanol filled beaker onto a small Petri dish
- Make a detailed drawing of one of the microarthropods

Station 2. Dissecting microscopes – Bacteria and Fungi

- Observe fungal and bacterial cultures (in Petri dishes) under the dissecting microscope
- Make a sketch of one colony of each (bacteria and fungi) and describe it using the terms listed in table 1

3. Compound microscopes – Bacteria and Fungi

Bacteria

- Use a Pasteur pipette to place a drop of water onto a glass slide
- Use a sterile toothpick to transfer a tiny amount of a bacterial culture into this drop of water
- Place a coverslip over the specimen
- Observe slide under the microscope
- Sketch one particularly interesting field of view

Fungi – “squash mount”

- Use a scalpel to carefully cut out a very small section of the agar with fungi growing on it
- Place this section onto a glass slide
- Place a coverslip over the agar chunk and gently squash it down with a pencil eraser (or end of a pen)
- Observe the fungal reproductive structures under the microscope
- Sketch one particularly interesting view

4. Compound microscopes – Nematodes and Protozoa

Nematodes

- Use a Pasteur pipette to transfer a drop of the nematode sample onto a microscope slide
- Place a coverslip over the specimen
- Observe under a microscope
- If you have trouble finding a nematode – view one of the prepared slides
- Make a detailed drawing of one nematode

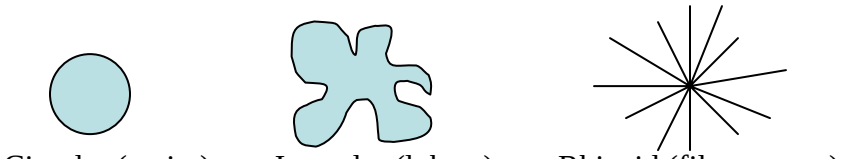
Protozoa

- Use a Pasteur pipette to transfer a drop of the protozoa sample onto a microscope slide
- Place a cover slip on the specimen
- Observe the protozoa under the microscope
- Try to find examples of amoebae, flagellates, and ciliates
- Make a detailed drawing of one protozoa

Lab report:

Hand in all of your labeled drawings making sure to include your magnification for each drawing: bacterial and fungal colonies under the dissecting scope (be sure to add colony description from table 1), individual bacteria and fungi under the compound scope, microarthropod, nematode and protozoa. Look up each type of organism in your textbook and include a brief (1 paragraph) description of this organism's role in the soil ecosystem.

Table 1. Descriptions of bacterial colonies

Term	Description
Size	Measure diameter in millimeters from the back of the plate
Shape (edges)	 Circular (entire) Irregular (lobate) Rhizoid (filamentous)
Elevation (profile)	 Flat Raised Convex Umbonate Umbilicate Fuzzy
Color	Describe color: look at both top and bottom of fuzzy colonies
Surface	*Glossy or shiny* – colonies reflect light *Matte or dull* – colonies do not reflect light Note any surface texture seen through magnification
Opacity	When you hold the plate up to the light, how much light comes through the colony? *Very opaque* – no light comes through *Very translucent* – much light comes through
Consistency	Touch the colony with a loop or a toothpick: *Soft* (butryous) – like butter at room temperature *Sticky* (viscid) – sticks to the loop like melted cheese *Hard* – impossible to pick any up with a loop or toothpick