Biodiversity Laboratory

Measuring Impacts on Soil Biodiversity in Agroecosystems

Part Two: The Soil Invertebrate and Soil Seed Bank Communities

Objectives:

1) To become familiar with methods of ecological sampling.
2) To become familiar with the soil invertebrate community.
3) To compare soil invertebrate and soil seed bank diversity among differing agroecosystems.
4) To calculate two different measures of biodiversity.

Introduction:

Sampling and Experimental Design
During our field trip five weeks ago, we obtained replicate soil samples from the following natural ecosystem and three agroecosystems: 1) a climax forest dominated by white ash, hop hornbeam, and sugar maple; 2) an organically managed mixed-crop vegetable garden; 3) an organically managed permanent pasture hay field dominated by timothy-grass, red clover, and wild carrot; and 4) a monoculture stand of silage corn grown with the use of herbicides. The soil samples you collected were first placed in Berlese-Tullgren funnels and then allowed to slowly dry for three weeks, during which the invertebrates in the soil gradually migrated to the collection vials beneath the funnels. Following that step, the dried soil was placed in the greenhouse and watered so that seeds contained in the soil (a.k.a., the soil seed bank) could germinate.

Over the next two laboratory sessions we will compare the diversity among the four different ecosystems by counting the number of soil invertebrate species and the number of soil seed bank species in each sample. Each replicate sample will be processed separately and the data recorded into a class data file. The use of replicate samples will allow us to measure how thorough our sampling procedure was with respect to the actual diversity that exists at the sites we sampled.

The most challenging part of our work will be identifying the species we isolate from the soil so that we can make comparisons across replicate samples and across ecosystems.

Soil Invertebrates
The Berlese-Tullgren technique for isolating soil invertebrates involves slowly drying out the soil from the surface downwards. As the soil dries out, invertebrates seek out the moister conditions that exist lower in the funnel. Eventually as they go lower and lower, the invertebrates fall out of the funnel into a collection vial filled with a preservative solution of 70% ethanol.

This week in lab you will be sorting through your collection of invertebrates to 1) count the number of different species it contains, 2) count the number of individuals for each species, and 3) identify each species membership to a particular invertebrate taxon.

We will be using a ‘morphotype’ approach for designating species. This means that if organisms differ from one another morphologically, we will designate them as different species. The advantage of the morphotype approach is that it allows those of us who do not have an extensive knowledge of invertebrate lifecycles to conduct a diversity study. The disadvantage of the approach is that it can lead to designating different-appearing stages of a single species as separate species.

Soil Seed Bank
The term ‘soil seed bank’ refers to the quantity of seeds that remain in the soil and that could potentially germinate in the future. The most accurate measure of the soil seed bank is to pass the soil through screens of progressively smaller and smaller mesh size openings, very carefully inspecting the screenings for seeds. Since many seeds are very tiny, this can be a time consuming and painstaking process. A simpler, though less accurate, measure of the soil seed bank can be obtained by placing the soil under moisture and temperature...
conditions favorable to seed germination, and then counting the seedlings that have germinated after two weeks time. We will employ the latter technique for our study.

**Procedure:**

**Soil Invertebrates**

1) Carefully detach the plastic vial containing invertebrates and preservative from the bottom of the Berlese funnel. Avoid vibrating the funnel during the procedure to prevent soil particles from falling into the vial. Also try not to agitate the vial too much. There should be a ‘raft’ of tiny invertebrates floating on the surface of the preservative. It would be ideal to prevent this raft from sinking. Set the vial aside in a place where it won’t spill while you prepare the soil in the Berlese funnel for the soil seed bank study.

2) Remove the lamp from the funnel, and transfer the soil from the funnel to a plant-growing tray. Remove the label tape from the funnel and attach it to the plant-growing tray. Smooth out the soil and remove any stones or other debris that would interfere with germination of seeds.

3) Place your plant-growing tray on the misting bench in the Olin greenhouse.

4) Obtain your sample vial and a few small plastic Petri dishes. Carefully pour off the portion of the preservative containing the raft of tiny invertebrates into a single dish. Pour off the remaining contents into a second dish. Use a squirt bottle of preservative to wash out any other invertebrates remaining in the vial into a third and fourth dish depending on the volume of preservative required to rinse the vial.

5) Using forceps, dissecting needles, and squirt bottles, gently sort the invertebrates by morphotype. With the aid of a dissecting microscope, segregate invertebrates by size and appearance into separate Petri dishes, each dish containing individuals of a single morphotype.

6) Once the invertebrates have been sorted, the class needs to decide as a group what we are calling the various species that are found in the samples. We will prepare a class voucher specimen for each of these species. A projecting microscope will be available in the lab so that we can project an image of each species for everyone to see. To name the species we will use the name of the taxon to which it belongs followed by a unique letter for each species, e.g., Collembola A, Collembola B, Acarina A, Acarina B, etc.

7) Once we have the species named, count the number of individuals for each species. Record the information on the data sheets provided.

8) Transfer the information on the data sheets into the appropriate computer spreadsheet for your replicate sample.

9) If time allows this week, we will pool the replicate data for each ecosystem to calculate two common measures of biodiversity for each of the systems. The two measures of biodiversity we will calculate are species richness and species diversity. Species richness is simply a function of the number of species present in a sample, whereas species diversity takes into account both the number of species present and the dominance and evenness of species in relation to one another. For more details on the derivation of these diversity indices, refer to the Calculating Biodiversity handout.

If you have already read the Calculating Biodiversity handout you may be a little intimidated by the math involved in computing the diversity indices. Fear not, for we will make this a relatively painless process by setting up a computer spreadsheet to do all those nasty calculations for us. Once the computer has done the math, we will compare invertebrate diversity among the four ecosystems involved in our study.

**Soil Seed Bank**

10) Following the two-week germination period, we will retrieve the plant-growing trays from the greenhouse and perform, as time allows, an abbreviated version of the methods used above to measure the diversity of the soil seed bank.