The Study

Critters beneath our feet: Diversity of soil organisms

Nathan Basiliko and Tara Sackett

Learning objectives

- Learn about the important role animals play in soil ecosystems
- Define "disturbed" and "undisturbed" environments
- Collect and observe animals that live in soil and litter
- Measure the number and kinds of animals living in soil and litter
- Document how this diversity changes in disturbed and undisturbed environments.

The story behind the study

If it wasn't for **organisms** living in and on the soil, no dead **organic matter** (i.e. matter that was once part of living things, such as leaves, wood, even dead animals) would ever break down, or "decompose". This **decomposition** not only prevents these materials from building up in huge quantities, it also allows nutrients trapped in the organic matter to be released and reabsorbed by plants. In this exercise, you will learn more about the diversity of organisms that decompose this organic matter in soils.

The organic layer of soil (called either the **O, L, F or H horizon** depending on its level of decomposition) tends to sit on the surface of the soil. Soil also consists of: **mineral particles** which were at one point rocks, but have been broken down into smaller pieces; air held in **pore spaces** in the soil; and water. If you examine some of this soil organic material you will see that, with the exception of the freshest **litter** (freshly fallen leaves and vegetation) sitting on the surface, it has been transformed by soil organisms into material that is quite different than the original plant tissues. The organic matter is usually darker than the underlying mineral soil, and if you rub it between your fingers, it feels less gritty than the mineral soil.

In many soils, most of the living creatures are microscopic organisms – or **micro-organisms** (a.k.a. microbes) – which are primarily **bacteria** and **fungi**. Soil microbes, like humans, need to consume organic matter to build their bodies and generate energy. To consume the organic matter they break it down into a partially decomposed and reworked material called **humus** (pronounced "hue- muss" and not to be confused with the delicious Mediterranean chickpea dish hummus which is pronounced "hum-us"). By decomposing this organic matter, the microbes get the energy and nutrients they need to grow, and also release enough nutrients into the soil for plants to use for their growth. Nearly all (90%) of all nutrients that plants need to grow comes from the decomposition of the organic matter from the previous generation of plants.

In addition to microbes, many animals live in the soil. These animals range in size from tiny (either microscopic, or nearly microscopic), to larger animals you may already recognize, such as earthworms, millipedes, or centipedes. These soil fauna play a very important role in structuring the soil as they burrow and move through the soil, but also altering the makeup of the microbial community by selectively feeding on bacteria and fungi.

Sandy's Notes – Critters Beneath our Feet

Earthworms are an important group of "macro-fauna" (i.e. big animals) in soils. Although they are traditionally thought of as being universally "good" in gardens and agricultural fields, the same is not necessarily true in forests. In most of Canada earthworms are actually **non-native** (i.e. were brought here from other countries by humans). In all soils, earthworms ingest plant litter and soil organic matter and stimulate decomposition by soil micro-organisms. In the short-term, this results in release of important nutrients required by plants that are tied up in the organic matter – this is why earthworms are good for the fast-growing plants in agricultural or garden soils. However, in temperate forests, this rapid removal of the litter and organic matter means that forest plants, accustomed to a thick layer of slowly decomposing leaf litter, may have trouble re-establishing or growing. Because earthworms are non-native in temperate forest ecosystems, and their dispersal is associated with humans, more disturbed forests tend to have more earthworms.

See photographs at the end of this document.

Research Summary

Although earthworms are traditionally thought of as being universally "good" in gardens and agricultural fields, the same is not necessarily true in forests. Most forests in Canada did not have earthworms until the European colonists came, and earthworms are now readily spread even to remote areas by humans along forest roads and through discarded fishing bait. [Do you want to learn more about how earthworms alter forest soils? **Click here**. http://greatlakeswormwatch.org/forest/soil.html]

Dr. Nathan Basiliko's laboratory group, at the University of Toronto Mississauga, studies soils and the organisms that live within it. In one study, they are researching non-native earthworms in forest soils. The main goal of this study is to determine how long term forest sustainability (i.e. healthy growth of trees and other forest plants) will be affected by the presence of non-native earthworms in the forest soils. To accomplish this goal they need to observe the effect of earthworms on all the important aspects of the forest ecosystem. These include experiments testing how earthworms alter the micro-organisms in the soil – remember that these microbes are the most important players in regulating the decomposition of organic matter. They are also studying how earthworms affect the availability of nutrients in the forest soil (required for plant growth), and how earthworm burrowing affects the distribution of organic matter and nutrients in the soil.

See photographs at the end of this document.

Definitions

Bacteria (singular bacterium): a diverse biological domain of microscopic, single-cellular organisms that lack organelles. The typical diameter of a soil bacterium is about 2 micrometers (that's 2-one millionths of a meter!). Most soil bacteria are "chemoheterotrophs" meaning that they rely on dead organic matter to gain energy and build their biomass.

Decomposition: the breakdown of organic matter by heterotrophic organisms, predominately fungi and bacteria in soil. In soil, decomposition includes both the initial formation of soil humus and final breakdown back in to mineral products, including carbon dioxide.

Fungi (singular, fungus) A diverse group of heterotrophic organisms that carry out decomposition in soil. Unlike bacteria, fungi contain cellular organelles (e.g. a nucleus) and can exist as either single cellular or multi-cellular organisms. Although considered to be microbes, many fungi are actually macroscopic (think of mushrooms, the fruiting body of certain fungi).

Humus: The partially decomposed plant matter in soils produced by fungi, bacteria, and soil fauna (e.g. earthworms)

Microbes: Organisms that are typically too small to be seen with the naked eye. Common soil microorganisms include the "microflora" made up of fungi and bacteria and the "microfauna" made up of protozoa, mites, and nemotodes.

Organic matter: Technically, organic matter consists of any molecule containing both hydrogen and carbon; however more simply organic matter is produced by living things (however bones, teeth, and exoskeletons of insects are the exceptions to this as they don't contain carbon and hydrogen strung together). In soil, organic matter is mainly made up of plant litter and humus.

Student Activities

- *Exercise 1: Extraction and examination of soil and plant litter fauna*

In this exercise you will construct a collection apparatus (Berlese funnel) to collect and observe the small fauna that live in leaf litter and the organic horizon of the soil in a forest ecosystem. See Exercise 1 at the end of this document.

For a video of how a Berlese tunnel is constructed, watch this one developed by the Wildlands school: http://www.youtube.com/user/wildlandschoolvideo#p/u/4/yWFyA2H9jos

- *Exercise 2: Measuring earthworm populations in disturbed and undisturbed ecosystems*

In this exercise you will compare the abundance of earthworms in disturbed and natural areas, and use these numbers to extrapolate to the numbers of earthworms found in larger areas. See Exercise 2 at the end of this document.

- *Canada Worm Watch*

The goal of Canada Worm Watch is to survey earthworms and the habitats in which they live. The site is full of great scientific information on earthworms and also has some great activities and resources, including a detailed taxonomic key.
http://www.naturewatch.ca/english/wormwatch/about/key/taxonomic.html

- *Great Lakes Worm Watch*

This site provides tools and resources that will help participants document the distribution of exotic earthworms and their impacts across the region. There are also resources for educators to help build understanding of the methods and results of scientific research about exotic earthworms and forest ecosystems ecology. http://greatlakeswormwatch.org/identification/index.html

Sandy's Notes – Critters Beneath our Feet

- *Detailed lesson plans for three soil biology activities produced by the Natural Resources Conservation Service (NRCS):*
  - Earthworm Farm
  - How Fast Does it Rot?
  - What Lives in Your Soil?

These exercises will allow students to observe soil fauna and assess how organic matter decays. http://urbanext.illinois.edu/soil/sb_class/activity.pdf

- *NRCS Soil Biology Primer*

This document provides more detailed information on soil biology including the many different fauna that make up the soil foodweb. http://soils.usda.gov/sqi/concepts/soil_biology/biology.html

Headlines

Canadian Geographic: How Avatar got it right (2010)
[http://www.canadiangeographic.ca/magazine/jf11/fungal_systems.asp]
Learn about how "mother trees" use an extensive underground fungal systems to feed the forest.

Smithsonian.com: Foreign Worm Alert (2000)
[http://www.smithsonianmag.com/science-nature/phenom_aug00.html?c=y&page=1]
An article that addresses what it means to be a non-native worm and what the downside may be for northern ecosystems.

Explorers

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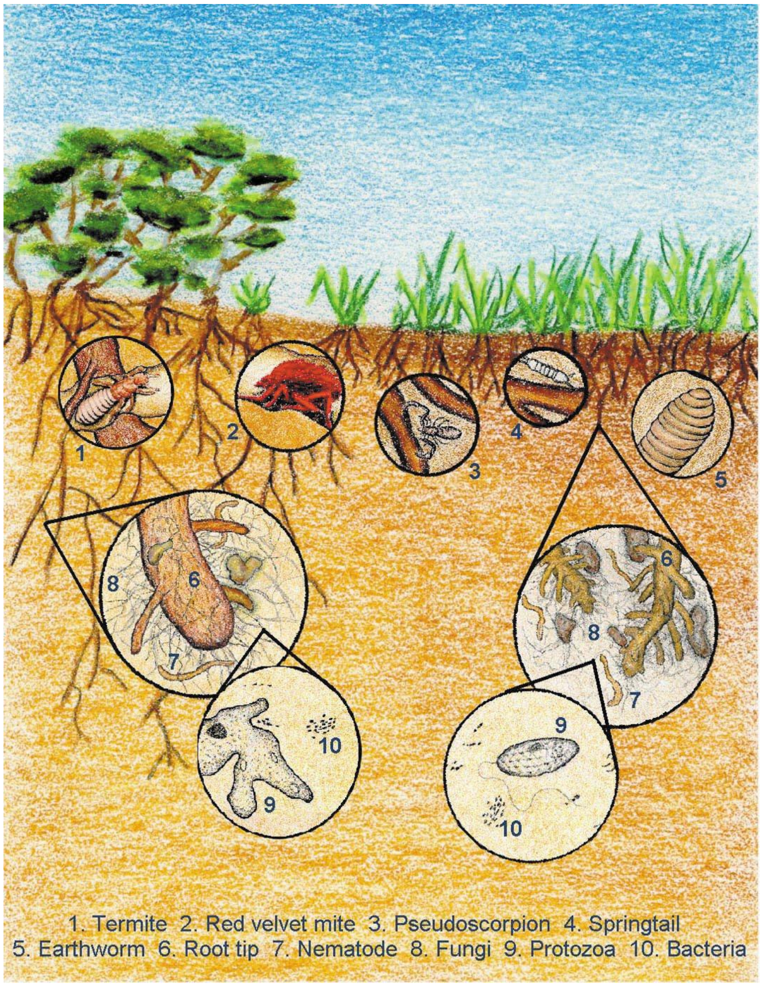
Photographs

Photographs: Story Behind the Study



Soil Biota

Organisms that live in the soil range from very small microbes such as bacteria, to large macro-organisms like ants and earthworms. A diverse community of soil biota helps create a healthy soil environment.

Image from U. S. Department of Agriculture http://soils.usda.gov/sqi/management/files/RSQIS8.pdf

Fungal hyphae

Hyphae are below ground branching filaments of fungi and are collectively called mycelia. Mycelia are often connected to plants through their roots – the mycelia help the plants by providing greater access to nutrients and water.

Photo from Wikimedia Commons by Lex vB.

Fungal fruiting body

Fungi are more commonly recognized by their above-ground fruiting bodies, typically called mushrooms.

Photo from Wikimedia Commons by Tomas Čekanavičius

Macro-fauna – Ants

Ants are an important component of the biological soil community, and play many different roles. They break organic matter down into smaller components that can be consumed by smaller organisms, hunt and consume other arthropods, and some species burrow and live in the soil.

Photo from Wikimedia Commons by Sanja565658

Macro-fauna - Earthworms

Earthworms are decomposers that help cycle nutrients throughout the soil.

Tara Sackett, U of T (Missisauga)

Agroecosystem

Soil biota are an important component of agroecosystems. Healthy populations keep agricultural systems well balanced.

Photo courtesy of Lynn Betts USDA Natural Resources Conservation Service

Forest Ecosystem

A healthy forest ecosystem is made up of diverse flora and fauna both above and below ground. Without the activities of soil organisms, organic materials would accumulate on the soil surface, and would not be available for uptake by plants.

Photo by Nathan Basiliko, U of T (Mississauga)

Photographs: Research Summary

Forestry Equipment

Large equipment moving across a forested landscape can lead to disturbance, such as soil compaction. In addition, earthworms and other biota can be transported between sites where equipment is operating.

Photo from Wikimedia Commons by Rvannatta

A Disturbed Ecosystem

This photo of a clear cut forest shows an example of a disturbed ecosystem.

Photo by Nathan Basiliko, U of T (Mississauga)

Collecting Earthworms

Earthworms can be collected from the soil by applying a liquid mustard solution. The earthworms come to the surface of the soil to avoid the solution.

Tara Sackett, U of T (Mississauga)

Counting Earthworms

Earthworms slowly emerge from the ground after applying the liquid mustard solution and can be counted for research purposes.

Tara Sackett, U of T (Mississauga)

Earthworm Burrow

Earthworm burrows are often visible due to the middens (earthworm casts (i.e. poop) around the burrow.

Tara Sackett, U of T (Mississauga)

Molecular Biology Lab

Technology in the lab is used to help classify the organisms collected from the field.

Photo by Nathan Basiliko, U of T (Mississauga)

Exercise 1

Critters Beneath our Feet – Exploring Earthworms

Measuring earthworm populations in contrasting disturbed and undisturbed ecosystems

In this exercise you will compare the abundance of earthworms in disturbed and natural areas, and use these numbers to extrapolate to the numbers of earthworms found in larger areas.

Worms are from a group of animals called "annelilds" (from the Latin "annelus", meaning little ring). These rings can be easily seen on an earthworm's body. On some earthworms you can see a thicker ring around the body, called the clitellum (refer to drawing of earthworm). If it has a clitellum, this means the earthworm is reproductively mature – it can mate and produce offspring – the clitellum is the structure that produces the cocoon that protects the earthworm eggs in the soil until they hatch. Earthworms are hermaphrodites, meaning they have male and female genitalia in one individual! The clitellum also makes the earthworms easier to identify if you are interested in what species it is.

Earthworms either eat leaf litter or soil – it depends on the kind of earthworm. But most earthworms eat a lot! When you collect your earthworms, you may be able to see their digestive tube through their skin – this tube will be filled with soil or litter, and appear dark.

For older students who are identifying earthworms they collect:

The non-native earthworms found in Canada come from three different functional groups – in other words, they have three different ways of feeding and burrowing (important functions for earthworms!) The first type of earthworm is called "anecic", and it digs semi-permanent vertical burrows that can go quite deep into the soil (even up to a metre). These worms eat litter, not soil, so they come to the top of their burrow and drag leaf litter down into the burrow to feed upon it. The burrows can be easily spotted because the worms also cast (poop) outside the entrance of their burrow. These worms are also pigmented – they are often a reddish or brown colour. We have one kind of anecic earthworm in Canada; its scientific name is *Lumbricus terrestris*, but it is also known as a nightcrawler. It is our biggest worm, and the one you usually see crawling on the soil (or sidewalk) after a rain (why might the worms come to the surface when it rains?).

The second type of earthworm is called "epigeic" (epi = on; geic = earth). These worms are much smaller than *L. terrestris* (about 1-7 cm) and we have a few different species. These worms don't actually make burrows – they live on the surface of the soil, and eat the leaf litter above them. These worms are also pigmented – being red, brown, or sometimes pink in colour.

The third type of earthworm is called "endogeic" (endo=in; geic=earth). These earthworms are variable in size (from 2-12 cm), and they are the only type of worm that eats soil, not litter. These worms are not pigmented, unlike the epigeic and anecic species. Sometimes they do appear dark because of the soil in their digestive tube, though!

In the field:

1) Chose 2 locations, one in a more natural forest or grassland setting and one that is more disturbed such as an agricultural field, a garden, or a heavily landscaped area.
2) Draw a 25×25 cm square in the soil in each field site. Try to remove any leaf litter or overlying grass from your quadrat.

3) Mix up dry mustard powder (available at bulk food stores): 80ml into 4l of water. This solution will be used to collect the earthworms! The mustard is mildly irritating to earthworms (but does not permanently harm them) and when poured on the soil earthworms will quickly come to the surface.

4) Pour 2 L of the mustard solution over each delineated square in the soil.

5) Over the next 5 minutes, collect the earthworms emerging from the soil. After 5 minutes, use a trowel to dig through the moist soil to find any earthworms that haven't yet come to the surface. Count the earthworms, taking note of any mature earthworms – the ones with a clitellum. Release them after counting them.

   (Option for older students: they can bring the mature earthworms back to the lab to try and identify them to species.)

6) Which site had the largest number of earthworms over the same 625cm^2 area? Convert to a total population of earthworms per m^2 (10,000 cm^2), per km^2 (1000m^2). Did you find any mature earthworms? Did these mature earthworms look similar to each other, or might they be different species?

Back at the lab (older student option):

7) Use the key provided to try and identify your mature earthworms to species. Are there any differences in the number of species at disturbed and natural sites? Is this what you would expect? What functional groups do your worms belong to?

Note: keys to worm identification for school activities can be found at the following sites:

http://www.naturewatch.ca/english/wormwatch/about/key/taxonomic.html

http://greatlakeswormwatch.org/identification/index.html

These sites also have more great background information on earthworms.

Exercise 2

Critters Beneath our Feet – Critter Catcher

Extraction and examination of soil and plant litter fauna

In this exercise you will construct a collection apparatus (Berlese funnel) to collect and compare the small fauna that live in leaf litter and the organic horizon of the soil in a forest ecosystem.

The smaller soil fauna (smaller than earthworms or millipedes, for example) are not well known, but they are very diverse, and very important. They are usually around 1-2 mm in size. There are thousands of species of these smaller animals, some of them not yet named by scientists (or probably even collected), but many of them fall into two groups: (a) mites or (b) springtails.

Mites are actually relatives of spiders – they have eight legs. They have a variety of roles in the soil and litter, from chewing up the litter into smaller pieces, thereby making it more available to microbes for decomposition, to grazing (eating) the fungi that grows on leaf litter. Some of the bigger mites are predators, and will eat any smaller animal they come across (refer to picture of mite – need to insert into document)

Springtails are closely related to insects – they have six legs. Like many mites, they chew up leaf litter into smaller pieces, and eat the fungi that grow on the leaf litter. The reason they are called springtails is because they have a tail-like appendage, called a furcula, which is held beneath the body and flips outwards, projecting them high into the air. They usually jump when they feel threatened (such as by a predatory mite!) (refer to picture of springtail).

Laboratory preparation:

1) Construct a Berlese (pronounced bur-lee-zee; named after Italian scientist Antonio Berlese) funnel trap using a glass jar, thick paper, some small-pore size plastic window mesh, a moist paper towel, and a desk lamp.
    a. Fold the moist paper towel into a square that fits flat against the bottom of the glass jar.
    b. Role the heavy paper into a funnel in a shape that allows it to rest on the edge of the jar and so the end of the funnel is above, but not touching, the moist paper towel on the bottom of the jar
    c. Cut and place the mesh into the funnel such that soil or plant litter can be placed in the funnel without falling through
    d. Position the lamp as close to the top of the funnel as possible, but taking note that the paper does not catch fire.

In the field:

2) Collect decomposing leaf litter and O horizon soil from a forest ecosystem: In a small area (10 x 10 cm), using your hands or a small trowel, collect the litter layer from the soil surface. Then collect a sample of the surface O horizon from the same area. This should be distinguishable from the lower layers/horizons because it is dark brown or black coloured and feels soft (has little or no 'grittiness' when rubbed between your finger and thumb that would indicate the presence of mineral sand particles). Note that in some ecosystems the O horizon of soils can be very thin (less than 1cm thick). Does the plant litter that is the primary parent material for the O horizon of each soil actually resemble the O horizon?

3) Place your samples in ziplock bags, seal them, and label the outside with a sharpie with your name and which layer of soil is in the bag.

Back at the lab:

4) Place a thin layer of the soil on the mesh in the Berlese funnel and turn on the light. The heat from the lamp is uncomfortable for the soil animals, so they burrow downwards and fall through the funnel onto the paper towel.
5) After 30 minutes, carefully remove the moist paper towel and examine and count the soil insects that are stuck to the towel with your naked eye, and with a 5× hand lens or dissecting microscope, if available.
6) Repeat the procedure with the leaf litter.
7) What differences do you observe in terms of total numbers and types (e.g. different sizes, shapes, colours) of soil fauna between the leaf litter and the soil organic matter?
8) Can you spot any mites or springtails in your soil or litter samples? What traits did you use to help you find them?