LAB 10
SOIL ORGANISMS AND BIOLOGICAL PROCESSES

Learning outcomes

Student will be able to

1. identify common soil organisms
2. describe some biological processes, particularly with respect to N, related to soil organisms.

Introduction

The soil is home to a wide range of plant and animal life. Roots are the largest form of plant life and certain rodents, insects and earthworms, the largest animals. Though higher plants are the primary producers of chemical energy that sustains the terrestrial ecosystem, plants are dependent on the invisible (other than certain fungal structures) community of soil microorganisms for continued supply of many essential nutrients. Nutrient elements bound in organic combinations would be inaccessible to plant without microbial decomposition of organic matter and mineralization of these elements. Not only would nutrient cycling stop but also biological N-fixation. Soil microorganisms are indispensable to life on earth.

Healthy soil teems with an immense community of living organisms. In fact, a hectare supports about 20,000 kilograms of soil organisms, equivalent to the weight of 40 horses. Although they make up only about 5% of soil organic matter, these organisms are vital to many soil processes.

Through their roles in the decomposition cycle, they regulate the flow of energy through the soil, the cycling of nutrients, and the productivity of agro-ecosystems. Soil organisms span a wide range in size, from microscopic forms, such as bacteria, fungi, and protozoa, to large animals, such as insects, worms, and burrowing mammals.

The larger organisms assist in decomposition by ingesting plant residues, breaking them into finer particles, and mixing them as waste throughout the moist soil environment. These wastes become food for the microorganisms, which digest the organic matter, releasing plant nutrients and gases, and producing glues that stick the soil mineral particles together to form aggregates.
Macro soil organisms influence soils mainly by being mixers of soil materials. Ground squirrels, badgers, gophers and crawfish are some animals that mix soil horizons with their digging.

**Materials**

1. Video clips on various soil organisms
2. Demonstrations of some biological process in soil

**Activities**

1. Watch some of the movies on the soil organisms.
2. Make notes on some of the specimens on the display.

**Questions**

1. Name the soil organisms that can fix nitrogen gas from atmosphere.
2. How important is nitrogen fixation to the world of plant and life in general?
3. Name the group of organisms that are called saprophytes.
4. How important is this saprophytic organisms to the soil realm?

**Appendix**

1. **SOIL ORGANISMS**

   **A. Worms**

   Earthworms were described by Aristotle as 'the intestines of the earth'. Numerous soil scientists have been equally fascinated by the amount of work done by them, Charles Darwin said of them "It may be doubted whether there many other animals which have played so important a part in the history of the world as these lowly organized creatures".
Earthworms are miniature topsoil factories. The surface soil will eventually pass through an earthworm. Earth worms are amazingly strong, and can easily shift stones 60 times their own weight.

Worms may deposit 10 to 15 tons of castings per hectare on the surface of the soil during a year. Research on the beneficial effects of worms generally does not show they will increase yields. However, we do know that the casts they leave behind are high in bacteria, organic matter, and plant nutrients. Castings have an NPK (nitrogen, phosphorus, potassium) ratio of 0.5-0.5-0.3 and are 50% organic matter and 11 trace minerals. Worm castings work like time-released fertilizer.

Worms prefer a moist non-acid environment in which to live. They also need organic matter, which they use as a food source, and high amounts of available calcium. Worms also leave numerous channels in the soil which may result in pesticides and nutrients entering the subsoils at faster rates. These channels allow preferential flow of water, rather than the water moving only through the soil pores.

The non-native species have been introduced via bait containers and horticultural activities. Earthworms prefer the basic pH of the maple-basswood forest and are able to successfully exploit the food resources contained in the duff layers (O horizons). Normally a tree leaf may take three to five years to decompose and be incorporated into the soil humus. In forests infested with night crawlers, this process can take as little as four weeks. By accelerating the breakdown of plant material, earthworms change the way nutrients are recycled back to the plants. They may also be changing the ecology of the soil microorganism community be reducing the food for fungi and bacteria which rely on the duff layer as a main food source.

Grasses and sedges tend to succeed on forest floors after the invasion of earth worms and some of the more common woodland plants tend to disappear like wild ginger and yellow violet. Further study on this problem will help us to better understand the problem caused by setting free all those night crawlers that did not catch fish.

**B. Roots**

Roots absorb the water and nutrients that are needed by the plants for photosynthesis and respiration.

Roots in the soil play an important role in the activity of organisms. Roots are often a little leaky, and the material that they leak is referred to as root exudates. The area immediately around the root is known as the rhizosphere. The rhizosphere environment has a lower pH, and the soil atmosphere has lower O₂ and higher CO₂ concentrations. The rhizosphere is higher in soil organism activity due to increased food supply leaked by the root for the organisms to use. This includes: amino acids, organic acids, carbohydrates, nucleic acids, growth factors, enzymes, and souged-off tissue. Benefits for the plant of having a rhizosphere include enhanced N mineralization, enhanced N₂ fixation, and nutrient solubilization.
The rhizosphere is defined as an intense zone of stimulated microbial activity around the root. Within the rhizosphere microbial numbers are much greater than in the bulk soil.

Microbes in the rhizosphere can be arbitrarily subdivided into the following groups: A. Pathogenic (invades and kills plants) B. Beneficial (often symbiotic with plants) C. Harmful (normally non-pathogenic opportunists on plants) D. Saprophytic (live on dead plants) E. Neutral (no effect on plants)

The microbes listed above are all competing for the same resources (space, nutrients and carbon) in the rhizosphere. The rhizosphere is a battlefield between pathogenic and non-pathogenic microorganisms. While we are mostly concerned with deleterious and pathogenic bacteria in the rhizosphere, there are some microorganisms present in the rhizosphere which are good for roots. An example of this is the actinomycete Streptomyces which secretes antibiotics and toxins into the soil which then inhibits the growth of other rhizosphere microorganisms. e.g. It prevents the spread of the pathogenic "damping-off" fungus.

C. Nematodes

Another worm-like organism is the nematode. Nematodes are microscopic worms that feed on organic matter and other soil animals or infect plant roots. Under a 10x hand lens, nematodes appear as transparent, thread-like worms. Parasitic nematodes are the most important from an agricultural standpoint. Many plants are affected, such as tomatoes, carrots, potatoes, peas, alfalfa, turfgrass, and fruit trees. Nematodes can parasitize virtually all crops and ornamental plants and can cause significant economic damage by reducing both yield and quality. Properly taken samples from small field units can reduce production costs by allowing the grower to eliminate nematodes

Lance nematodes, Hoplolaimus spp., are large nematodes which are highly resistant to effects of temperature extremes and dry soil conditions. One species, H. columbus, causes severe damage to soybeans and cotton. Another more widely distributed species, H. galeatus, is primarily a pathogen on grasses. Lance nematodes feed externally along root surfaces but may also feed with at least part of the body embedded in the root. Larvae look similar to adults except that they are smaller. This group of nematodes is easily detected with soil sampling. The life cycle of this nematode takes about 30 days under ideal conditions, and females lay eggs singly rather than in a mass. Though a female may lay as many as 100 eggs in a lifetime, that lifetime may last an entire growing season.

Root-knot nematodes, Meloidogyne spp., are one of the important plant-parasitic nematodes because of their wide host range and widespread distribution. Root-knot larvae enter roots of host plants near root tips and remain inside the root at one location throughout their life. As larvae feed, the root cells divide rapidly near the nematode’s head. This rapid cell division and enlargement cause the swelling or knots on roots.
D. Fungi

Fungi are not plants. Living things are organized for study into large, basic groups called kingdoms. Fungi were listed in the Plant Kingdom for many years. Then scientists learned that fungi show a closer relation to animals, but are unique and separate life forms. Now, Fungi are placed in their own Kingdom. It is a hidden kingdom. The part of the fungus that we see is only the "fruit" of the organism. The living body of the fungus is a mycelium made out of a web of tiny filaments called hyphae. The mycelium is usually hidden in the soil, in wood, or another food source. A mycelium may fill a single ant, or cover many acres. The branching hyphae can add over a half mile (1 km) of total length to the mycelium each day. These webs live unseen until they develop mushrooms, puffballs, truffles, brackets, cups, "birds nests," "corals" or other fruiting bodies. If the mycelium produces microscopic fruiting bodies, people may never notice the fungus.

The most active decomposers of organic materials in a forested soil are the soil fungi. This is mainly because they are tolerant of acid soil conditions. All of us have seen fungi. Their size varies from single-cell yeasts to molds and mushrooms. The woody residue of the forest floor provides an abundance of food for certain fungi that are effective decomposers of lignin.

Most fungi build their cell walls out of chitin. This is the same material as the hard outer shells of insects and other arthropods. Plants do not make chitin. Fungi feed by absorbing nutrients from the organic material in which they live. Fungi do not have stomachs. They must digest their food before it can pass through the cell wall into the hyphae. Hyphae secrete acids and enzymes that break the surrounding organic material down into simple molecules they can easily absorb.

Fungi have evolved to use a lot of different items for food. Some are decomposers living on dead organic material like leaves. Some fungi cause diseases by using living organisms for food. These fungi infect plants, animals and even other fungi.

Athlete's foot and ringworm are two fungal diseases in humans. The mycorrhizal fungi live as partners with plants. They provide mineral nutrients to the plant in exchange for carbohydrates or other chemicals fungi cannot manufacture. You probably use fungal products every day without being aware of it. People eat mushrooms of all shapes, sizes and colors. Yeasts are used in making bread, wine, beer and solvents. Drugs made from fungi cure diseases and stop the rejection of transplanted hearts and other organs.

Mycorrhizae are fungi associated with the fine roots of most plants. The term itself means "fungus root". There are hundreds species of fungi which function as mycorrhiza; most are basidiomycetes, the class of fungi which form mushrooms. An individual plant may have several different mycorrhizae associated with its roots, and some mycorrhizae may be limited to only a few species of plant. These fungi can benefit plants by enhancing the nutrient absorbing ability of roots.

Mycorrhizae are especially important in facilitating uptake of phosphorous. This enhancement of nutrient uptake is a result of the extensive system of hyphae and
mycelia (thread-like filaments of the mycorrhizal fungus) that pervade soils. They function like root hairs but are much more far reaching.

The relationship of this fungus with plants is a mutually beneficial one, with the fungi receiving energy in the form of carbohydrates from the host plant. There are two types of mycorrhizae. Filaments of the first type, called ectomycorrhizae, penetrate between cells of roots, but not into root cells, and also form a thick cylindrical sheath around young lateral roots. The affected roots become short and thickened, and are deficient in root hairs. It is believed that this sheath may protect roots from invasion by plant pathogens. Most trees have this type of mycorrhizae.

A second type, called endomycorrhizae, actually penetrate into root cells and extend out from roots like root hairs to absorb nutrients from the soil solution. They do not form a sheath around roots nor do they alter the structure of roots. Endomycorrhizae are found on a greater range of plants than are ectomycorrhizae. For drawings of mycorrhiza see ectomycorrhizae or VA mycorrhizae

Studies have repeatedly shown that plant growth is enhanced by the presence of mycorrhizae. Mycorrhizae are particularly abundant in forest soils but are found in almost all soils, with the possible exception of grasslands where no trees have previously grown. Growth enhancement is especially significant for plants growing on infertile soils and dry soils. Interestingly, mycorrhizae development decreases following heavy fertilization of soil. The reduced growth of the pine seedlings in the middle of this photo was because of the lack of mycorrhizae on the roots. These seedlings were planted in an old limestone rock roadbed. The soil has a pH greater than 8, which the fungus could not tolerate.

While further studies are needed, it seems that mycorrhizal inoculations may indeed benefit tree and shrub plantings, especially on sites where exposure to stresses are commonplace.

E. Bacteria

The most abundant organisms in the soil are bacteria. Bacteria are minuscule, one-celled organisms that can only be seen with a powerful light (100X) or electron microscope. They can be so numerous that a pinch of soil can contain millions of organisms. Soils often have between 1,000,000 to 10,000,000 bacteria per gram. Bacteria are tough, they occur everywhere on earth and have even been found over a mile down into the core of the earth.

Bacteria have an extremely varied metabolism. Bacteria can use reduced inorganics, the sun, or organics as an energy source. Some bacteria can live without free molecular oxygen.

Bacteria are common throughout the soil, but tend to be most abundant in or adjacent to plant roots, an important food source. Actinomycetes are a broad group of bacteria that form thread-like filaments in the soil. They are responsible for the distinctive scent of freshly exposed, moist soil. Actinomycetes are particularly effective at breaking down tough substances like cellulose (which makes up the cell
walls of plants) and chitin (which makes up the cell walls of fungi) even under harsh conditions, such as high soil pH.

Free-living bacteria fix atmospheric nitrogen, adding it to the soil nitrogen pool. Other nitrogen-fixing bacteria form associations with the roots of leguminous plants such as lupine, clover, alfalfa, and milk vetch. Actinomycetes form associations with some non-leguminous plants (important species are bitterbrush, mountain mahogany, cliff rose, and ceanothus) and fix nitrogen, which is then available to both the host and other plants in the near vicinity.

Some bacteria exude a sticky substance that helps bind soil particles into small aggregates. So despite their small size, they help improve water infiltration, water-holding capacity, soil stability, and aeration.

Bacteria are becoming increasingly important in bioremediation, meaning that we (people) can use bacteria to help us clean up our messes. Bacteria are capable of filtering and degrading a large variety of human-made pollutants in the soil and groundwater so that they are no longer toxic. The list of materials they can detoxify includes herbicides, heavy metals, and petroleum products.

Bacteria can be divided into 2 large groups based on their carbon source. Autotrophic bacteria are independent of any carbon in the soil since they fix atmospheric CO₂; and obtain energy from the reactions of nitrogen and sulfur compounds in the soil. Heterotrophic bacteria require carbon compounds as a food source. They are extremely important in decomposing organic matter. In the carbon cycle, CO₂; is absorbed by plants during photosynthesis. As these plants die and are incorporated into the soil, heterotrophic bacteria and other soil organisms decompose this organic matter and release CO₂; into the soil atmosphere. This movement of CO₂ into the atmosphere completes the carbon cycle.

2. NITROGEN CYCLE

This section is about the important role soil bacteria play in providing nitrogen for plant growth via the nitrogen cycle. The nitrogen cycle moves atmospheric nitrogen into organic N, next it is converted into ammonia N, and next into nitrate N, and finally back to atmospheric N.

Nitrogen is the most abundant element in our atmosphere. It is a vital element since compounds essential to living systems are nitrogen-containing compounds (a necessary element in the composition of proteins, nucleic acids and other major cellular components). Nitrogen is a primary nutrient for all green plants, but it must be modified before it can be readily utilized by most living systems.

It is one of nature’s great ironies, however, that most life forms, including all plants and animals, are unable to enlist dinitrogen gas (N₂), which comprises 80 percent of the atmosphere, in their life-sustaining biochemical processes. Plants are able to use nitrogen in the form of nitrate (NO₃⁻) or ammonia (NH₄⁺), but these compounds are present in limited supply in the soil and are easily lost by leaching and by biological reduction of NO₃ (denitrification). Because crop plants generally require relatively
large amounts of nitrogen for growth, it frequently becomes the limiting soil nutrient for plant growth.

Where is all the soil nitrogen?

<table>
<thead>
<tr>
<th>Nitrogen in the Soil</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.5</td>
</tr>
</tbody>
</table>

Most of the N is in the soil organic matter

Diagram of N Cycle

Nitrogen Fixation - first step in the N Cycle - conversion of nitrogen gas (N₂) into NH₃ or organic nitrogen.

Nitrogen fixation is defined as the reduction of atmospheric nitrogen gas (N₂) to ammonia (NH₃) and it can only be done biologically by a small and highly specialized group of microorganisms in the presence of the enzyme nitrogenase which catalyzes the reduction of dinitrogen gas (in the atmosphere) to ammonia. ---
The ammonia is now combined with organic acids to form amino acids and proteins.

**Non Biological Fixation**

In the atmosphere molecular nitrogen is modified via the discharge of lightning. The energy released by the electrical discharges breaks the rather strong bonds between nitrogen atoms, causing them to react with oxygen. In this process, nitrogen is oxidized and oxygen is reduced. This process is similar to the nitrous oxides produced in the burning of fossil fuels.

- lightning + N₂ + O₂ ------------> 2 NO (nitric oxide)
- The nitric oxide formed combines with oxygen to form nitrogen dioxide.
  - 2NO + O₂ ------------> 2NO₂

- Nitrogen dioxide readily dissolves in water to produce nitric and nitrous acids;
  - 2NO₂ + H₂O --------> HNO₃ + HNO₂
- These acids readily release the hydrogen, forming nitrate and nitrite ions. The nitrate can be readily utilized by plants and micro-organisms.
  - HNO₃ --------> H⁺ + NO₃⁻ (nitrate ions)
  - HNO₂ --------> H⁺ + NO₂⁻ (nitrite ions)

**Biological Fixation**

**Nitrogen Fixing organisms:** Cynobacteria -- formerly blue-green algae. These organisms used to be called blue green algae because it was thought they were more akin to algae than any other organism. It is now known that they are bacteria-like in cell structure and definitely prokaryotic organisms (algae are eukaryotic). No eukaryote fixes nitrogen. Cyanobacteria include nostoc and anabaena and both fix N both non-symbiotically (for example in a rice paddy) and symbiotically in water ferns and other plants.

**Carbon source** (energy) for nitrogen fixation

1. **Heterotrophic** (need to assimilate pre-formed organic carbon) example is the bacteria, Rhizobium.
2. **Autotrophic** (make their own C by fixing CO₂ in photosynthesis) example is anabaena.

**Nitrogen Fixing Associations**

1. Free living N fixers --(they fix N₂ on their own). Free living nitrogen fixers that generate ammonia for their own use (e.g. bacteria living in soil but not associated with a root) include the bacteria, Azospirillum, Azotobacter spp. and Clostridium spp. (30 % of all N₂ fixed in world)
2. Symbiotic N fixers --example is bacteria (Rhizobium) and plant (soybean) (70 % of all N2 fixed in world). Symbiotic nitrogen fixers are associated with plants
and provide the plant with nitrogen in exchange for the plant's carbon and a protected home.

**Rhizobium-legume symbiosis**

The gram negative bacteria Rhizobium, Bradyrhizobium and Azorhizobium associate with leguminous plants (members of the bean family), the gram positive bacteria Frankia associate with certain fast growing trees, and cyanobacteria associate with some aquatic ferns.

In the Rhizobium-legume symbiotic process, the bacteria infect the roots of the plant and a structure known as a nodule is formed. Once the nodule is established, the differentiated bacteria (they become non-motile bacteroids) living in the infected plant cells, reduce atmospheric nitrogen to ammonia which is excreted to the plant cell and is, in turn, assimilated to organic nitrogen (proteins and amino acids) by the plant. The plant provides the bacteroid with carbon skeletons (photosynthate) which are required by Rhizobium, a strict aerobe, to provide the energy that is needed for nitrogen fixation.

This symbiosis is a specific process, a certain species of Rhizobium can only nodulate a certain type of legume, for example: R. etli nodulates beans (Phaseolus), R. meliloti nodulates alfalfa (Medicago). The bacterial enzyme responsible for the reduction of gaseous $\text{N}_2$ to ammonia is the nitrogenase enzyme complex which is formed from the joining together of three different polypeptides. Different nitrogenase enzyme systems have been found in different microorganisms.

**Soil N**

When soil nitrogen ($\text{NO}_3^-$ or $\text{NH}_4^+$) levels are high, the formation of nodules is inhibited. Also, anything that impacts the carbohydrate production will effect the amount of N fixed. In order for the nitrogen to be used by succeeding crops, the nodules and plant must be incorporated into the soil, or no nitrogen will be gained. Harvesting the alfalfa for animal feed reduces the chances for a net nitrogen gain, unless the manure is returned to the soil.

Nitrogen fixation research will undoubtedly make important contributions to agriculture by substituting traditional fertilizer N inputs (which are costly, polluting and time consuming), with a cheap natural biological alternative. Indeed, inoculation of crops with Rhizobium strains (coat the seeds with Rhizobium) can induce nodule formation and $\text{N}_2$ fixation in legume crops such as soybean. Future goals of the field include the manipulation of the genome of both bacteria and plants to improve existing symbiosis with legumes, the extension of symbiotic nitrogen fixation to other crops and, eventually, the production of plants able to fix nitrogen themselves.

Besides the knowledge of the molecular basis of biological nitrogen fixation and the technology to manipulate the genome, a deep understanding of the ecology of nitrogen fixing organisms and of the fate of introduced new genetic information into the soil will be necessary to achieve these goals.
**Ammonification** - Second step in N cycle

The biochemical process whereby ammoniacal nitrogen is released from nitrogen-containing organic compounds. Soil bacteria decompose organic nitrogen forms in soil to the ammonium form. This process is referred to as ammonification. The amount of nitrogen released for plant uptake by this process is most directly related to the organic matter content. The initial breakdown of a urea fertilizer may also be termed as an ammonification process.

In the plant, fixed nitrogen that is locked up in the protoplasm (organic nitrogen) of \( \text{N}_2 \) fixing microbes has to be released for other cells. This is done by the process of ammonification with the assistance of deaminating enzymes.

In the plant = Alanine (an amino acid) + deaminating enzyme \( \rightarrow \) ammonia + pyruvic acid,

or in the soil = \( \text{RNH}_2 \) (Organic N) + heterotrophic (ammonifying) bacteria \( \rightarrow \) \( \text{NH}_3 \) (ammonia) + R.

In soils \( \text{NH}_3 \) is rapidly converted to \( \text{NH}_4^+ \) when hydrogen ions are plentiful (pH < 7.5).

When microbes have too much N for their own requirements they excrete the excess as \( \text{NH}_4^+ \) into the soil. This happens mainly when the microbes are degrading crop residues with low C:N ratios. In high pH soils \( \text{NH}_4^+ \) (ion in solution) is unstable and changes to \( \text{NH}_3 \) (gas) which can be lost via volatilization. Volatilization is prevalent in soils to which farmyard manures or urea have been added (you can smell the \( \text{NH}_3 \) coming off chicken manure for example). \( \text{NH}_4^+ \) is held by the soils cation exchange capacity (negative charge sites) and thus will not leach, but can be lost when soil erosion occurs.

**Nitrification** - Third step in the N Cycle

Nitrification is the conversion of \( \text{NH}_4^+ \) to \( \text{NO}_3^- \). This aerobic reaction is carried out by autotrophic bacteria. Maximum nitrification rates occur at neutral pH and high temperatures (factors that favor the bacteria involved in this process - Nitrosomonas and Nitrobacter).

**Denitrification** - Fourth and last step of N Cycle

Involves conversion of \( \text{NO}_3^- \) to \( \text{N}_2 \) gas in the presence of low oxygen levels.

\[
\text{C}_6\text{H}_{12}\text{O}_6 + 4\text{NO}_3^- \rightarrow 6\text{CO}_2 + 6\text{H}_2\text{O} + 2\text{N}_2(\text{gas}) + \text{NO} + \text{NO}_2
\]

Bacterial denitrification is the microbial reduction of \( \text{NO}_3^- \) to \( \text{NO}_2^- \) or N. For example Pseudomonas use \( \text{NO}_3^- \) instead of \( \text{O}_2 \) as a terminal electron acceptor.

Denitrification is accelerated under anaerobic (flooded or compacted) conditions and high nitrogen inputs. Denitrification results in environmental pollution (destroys ozone) and also contributes to global warming since nitrous oxides do have a minor effect as a greenhouse gas.
Through nitrification and denitrification 10 - 20 % of the applied N is lost.

**Nitrogen Management**

N Gains=$N_2$ fixation, rainwater (dissolved $NH_4^+$ and $NO_3^-$), animal manure, and plant residues (indirect).

N Losses=crop removal, leaching, gaseous losses (volatilization), soil water erosion and runoff.

Do these inputs provide more N than what is lost or is the soil N sustainable? In some cases excess N is lost from leaching or erosion due to inadequate crop cover in winter, or N is lost from improper application of animal manure as $NH_3$. Generally there is insufficient N in the soil for maximum crop yields, and N fertilizer is needed. Using N fertilizers efficiently is an important for crop management and environmental protection.

The use of N fixing plants (i.e. beans) can reduce the use of N fertilizers, however not all management plans will allow for this. The need to optimize gains (enhance N fixation) and minimize losses by reducing $NO_3^-$ leaching will aid all aspects of nitrogen management and environmental protection. Understanding the N Cycle is important in managing the nitrogen needs of the future. See *Nitrogen Cycle* for a review of this process.