Time to Get a Little Dirty…
Soil Microbiology and Biogeochemical Cycles

Lots and Lots of Stuff

- Bacteria are ubiquitous.
- Found in organisms, surfaces, air.
- Soil contains an abundance of microorganisms.
- As many as 1.2 x 10^7 bacterial organisms are found in the soil.
- Of these, about 2.0 x 10^6 are filamentous.
- There are also fungal and algal organisms, totaling 1.2 x 10^5 and 2.5 x 10^4, respectively.

A Microcosm Right in Your Backyard!

- Not just microbes in the soil.
- Plants, insects and worms, and other animals also inhabit this environment.
- There are several different interactions that are occurring between these organisms.
- One very important "service" that microbes provide are that of decomposers.
- Microbes play a very important role in the recycling of organic material.

The Circles of Life?

- Yes!
- A biogeochemical cycle can be defined as the recycling of chemical elements by microorganisms for use by other organisms.
- Microbes, bacteria specifically, utilize cycles dealing with Carbon, Nitrogen and Sulfur.
- If these cycles never existed, neither would we!

The Three Big Ones

- Carbon Cycle
- Nitrogen Cycle
- Sulfur Cycle

Carbon is the Fundamental Atom in All Biomolecules

- Accounts for at least ½ of the dry weight of biomass
- Exists predominantly in the mineral state and as an organic reservoir in the bodies of organisms
- Recycled through ecosystems via carbon fixation, respiration, or fermentation of organic molecules, limestone decomposition, and methane production

Carbon cycle

- Organic reservoir are organisms
- Recycle via carbon fixation, respiration, and fermentation, limestone decomposition, and methane production
- Carbon dioxide is central

Methane CH_4

- Methane gas plays a secondary part in the carbon cycle
  - Methanogens: methane producers that live in anaerobic ecosystems

- More potent greenhouse gas than CO_2:
  - Can trap nearly 20 times more heat in the atmosphere
  - Methane released from the GI tracts of ruminant animals accounts for 20% of global methane production

The Nitrogen Cycle

- All life requires nitrogen compounds, e.g., proteins and nucleic acids.
- Air, which is 78% nitrogen gas (N_2), is the major reservoir of nitrogen, but most organisms cannot use nitrogen in this form.
- Plants must secure their nitrogen in "fixed" form, i.e., incorporated in compounds such as: nitrate ions (NO_3^-), ammonia (NH_3), and urea $(NH_2)_2CO$
- Animals secure their nitrogen (and all other) compounds from plants (or animals that have fed on plants).

The Path of Nitrogen

- Decomposition
- Deamination
- Ammonification
- Nitrification
- Denitrification
- Nitrogen Fixation

Decomposition

- Almost all of the nitrogen in the soil is in the form of organic molecules (proteins, to be specific).
- When an animal dies and undergoes microbial decomposition, protein breakdown occurs.

Deamination

- The amino groups in the amino acids are then removed and converted to ammonia in a process called deamination.

Ammonification

- The ammonia is released and this process is called ammonification.
- Because ammonia is an a gaseous state, it would dissipate in dry soil.
- In moist soil, however, the ammonia will solubilize in water and form ammonium ions, which can be used in the amino acid synthesis by plants and bacteria.

Nitrification

- Nitrification is a biological process during which nitrifying bacteria convert toxic ammonia to less harmful nitrate.
- There two bacterial species involved.
- *Nitrosomonas* sp. bacteria which oxidize ammonia to nitrite, while *Nitrobacter* bacteria convert nitrite to nitrate, with both species utilizing the energy released by the reactions.
- For *Nitrosomonas:* $55NH_4^+ + 76\ O_2 + 109HCO_3^- \rightarrow C_5H_7O_2N + 54NO_2^- + 57H_2O + 104H_2CO_3$
- For *Nitrobacter*: $400NO_2^- + NH_4+ + 4H_2CO_3 + HCO_3^- + 195\ O_2 \rightarrow C_5H_7O_2N + 3H_2O + 400$

NO_3^-

- What these reactions tell us in plain language is that…
  - In equation (1), ammonium ion (NH_4^+) is combined with oxygen and hydrogen carbonate to produce bacterial cell mass, nitrite (NO_2^-) , water and carbonic acid.
  - In equation (2), nitrite is combined with ammonia, carbonic acid, hydrogen carbonate and oxygen to produce bacterial cell mass, water and lots of nitrate (NO_3^-).

- Plants will use the nitrate present as their source of nitrogen for protein synthesis because it is highly mobile in the soil and it is more likely that the plant will encounter this as opposed to ammonia.
- Ammonium ions would actually be a better source of nitrogen because it would require less energy to make it into proteins.
- However, because of the positive charge that ammonium carries, it makes it more likely to bind to negatively charged particles found in the soil.

Denitrification

- Conversion of NO_3^- through intermediate steps to atmospheric nitrogen
- The result of nitrification (nitrate) is fully oxidized, meaning it can be used as an electron acceptor for microbes that are metabolizing other organic energy sources in anaerobic conditions.
- Carried out by hundreds of different species
  - Genera: *Bacillus*, *Pseudomonas*, *Spirillum*, *Thiobacillus*
- Usually, denitrification will occur in areas of water-logged soils, where the level of oxygen is low.
- In some cases, the bacteria present will use the nitrate present as an electron acceptor, thus, converting it to nitrogen gas.
- This can prove to be a financial loss to the agricultural community.

Nitrogen Fixation

- The only organisms on Earth that can use atmospheric nitrogen as a nitrogen source are some bacteria and cyanobacteria.
- They do this by a process called nitrogen fixation.
- In this process, the nitrogen gas is converted to ammonia, with the aid of an enzyme that these organisms have.
- This enzyme is called nitrogenase.

- The activity of the enzyme is done under anaerobic conditions.
- The presence of oxygen will inactivate the enzyme and nitrogen fixation will not occur.
- There are two groups of bacteria that fix nitrogen: Free-living nitrogen fixing bacteria and symbiotic nitrogen fixing bacteria.
- The free-living bacteria can protect the nitrogenase enzyme in a few different ways.
- Some aerobic N_2-fixers, like *Azotobacter*, use up so much of their oxygen intake, that minimal amounts (if any) make it to the interior of the cell, where the enzyme is located.
- Other bacteria are anaerobic and do not have to do anything special to protect the enzyme.

- Cyanobacteria have specialized structures called heterocysts, where the enzyme is found.

- These heterocysts are formed during nitrogen starvation.
- Heterocysts:
  - produce three additional cell walls, including one of glycolipid that forms a hydrophobic barrier to oxygen
  - produce nitrogenase and other proteins involved in nitrogen fixation
  - degrade photosystem II, which produces oxygen
  - up regulate glycolytic enzymes, which use up oxygen and provide energy for nitrogenase
  - produce proteins that scavenge any remaining oxygen

- Symbiotic nitrogen-fixing bacteria will fix nitrogen and use this nitrogen for the synthesis of amino acids for the plant that it has a symbiosis with.
- In the case of some leguminous plants, bacteria (*Rhizobia*) will accumulate on root hairs.
- The plant will form an infection thread, which will allow the bacteria to enter the root cells.
- Once inside, the bacteria change into bacteroids and pack themselves into the root cells.
- The enlarged root cells form a nodule.
- What does it provide?
  - 1.
  - 2.
- Other examples of symbiotic nitrogen-fixing bacteria include *Frankia*, *Anabaena*, and lichens.

The Sulfur Cycle

- Similar to the nitrogen cycle.
- The most reduced form, hydrogen sulfide, generally forms under anaerobic conditions.
- This form can also provide a source of energy for some autotrophs.
- These autotrophic bacteria can convert the H_2S into elemental sulfur and sulfates.
- In the 1900s, Winogradsky observed that *Beggiatoa* were using both H_2S and elemental sulfur as inorganic energy sources.
- Sulfates are assimilated by plants and bacteria to form sulfur-containing amino acids that animals (and humans) can use.
- The resulting proteins can later be broken down (when an animal is decomposed) and the sulfur re-enters the cycle.

Phosphorus cycle

- Phosphorous is part of DNA, RNA and ATP
- Inorganic phosphate is central
- Phosphate rock is phosphatized by sulfuric acid (*Thiobacillus* - sulfur cycle) into soluble phosphate
- Soluble phosphate is source for autotrophs
- Organic phosphate is source for heterotrophs
- Decomposers return to soluble phosphate
- Mineral reservoir – sedimentation (rock)

Other Forms of Cycling

- Involvement of microbes in cycling elements and compounds is escalated by the introduction of toxic substances in the environment
  - Some are converted into less harmful substances by microbial actions, but others, such as PCB and heavy metals, persist and flow along with nutrients into all levels of the biosphere
  - Bioaccumulation: accumulation of pollutants in higher concentrations as they move up the food chain

Soil Microbiology

- Soil is a dynamic ecosystem that supports complex interactions between numerous geologic, chemical, and biological factors
  - Antibiotic-producing and antibiotic-degrading microbes are present in the soil
  - Porous nature of the soil provides numerous microhabitats:
    - Aerobic and facultative organisms occupy looser, drier soils
    - Anaerobes are adapted to waterlogged, poorly aerated soils

Humus

- Slowly decaying organic litter from plant and animal tissues
- Holds water like a sponge
- Important habitat for microbes to decompose the litter and recycle nutrients
- Varies with climate, temperature, moisture, mineral content, and microbial action

Living Activities in Soil

- A gram of moist loam soil with high humus content can have a microbe count as high as 10 billion

- Rhizosphere
  - Zone of soil surrounding the roots of plants
  - Contains associated bacteria, fungi, and protozoa
  - Plants interact with microbes in a synergistic fashion

Deep Subsurface Microbiology

- Sampling of the deep subsurface: 2 miles and more below the surface
  - Bacteria found 30 meters below the seafloor in clay deposited 86 million years ago
  - Scientists are discovering new metabolic capabilities in deep subsurface microbes that may provide clues to the origin of life

Aquatic Microbiology

- Surface water is evaporated through exposure to the sun and wind
- Enters the vapor phase of the atmosphere
- Most water that falls on land is first returned to the atmosphere by plants
- Water returned to the earth through condensation or precipitation

Marine Environments: The Ocean

- Extreme variations in salinity, depth, temperature, hydrostatic pressure, and mixing
- Supports a great abundance of bacteria and viruses

- J. Craig Venter expedition sampling the oceans:
    - 6 million new genes
    - Thousands of new proteins
- Estimated 10 million viruses per milliliter
    - Most are bacteriophages and therefore pose no danger to humans

Bacteriophage in the Ocean

- Natural control mechanism for bacteria
- Lysis of bacteria plays an important role in nutrient turnover
- Bacteriophages can swap and exchange microbial genes, playing a role in their biology and evolution
- Cyanophages contain genes that can alter the light-harvesting abilities of cyanobacterial hosts

Red Tides

- Harmful algal blooms (HABs)
- Environmental factors cause an increase in the number of algae, leading to an increase in food for organisms up the food chain
- Produce toxins that harm fish, shellfish, and even humans
- Potent muscle toxin concentrated by shellfish through filtration feeding

Freshwater Communities

- Microbial distribution associated with sunlight, temperature, oxygen levels, and nutrient availability
- Plankton
    - A floating community that drifts with wave action and currents
    - Phytoplankton: photosynthetic algae and cyanobacteria
    - Zooplankton: microscopic consumers such as protozoa and invertebrates

Nutrient Levels of Water

- Oligotrophic
    - Nutrient-deficient aquatic ecosystems
    - *Hyphomicrobium* and *Caulobacter* capture miniscule amounts of hydrocarbons in these environments

Eutrophication

- Addition of excess nutrients to aquatic ecosystems
- Causes heavy surface growth of cyanobacteria and algae that shuts off the oxygen supply to the lake
- Aerobic heterotrophs deplete oxygen further by decomposing organic matter
- Causes massive die-offs of strict aerobes (fish, invertebrates)
- Only anaerobic or facultative microbes will survive
- Triggered by the addition of industrial wastes, detergents in household wastewater, or runoff from manure and fertilizer-rich fields
- Can be remediated over long periods of time

The Concept of "One Health"

- Microorganisms circulate among human hosts, animal hosts, and environmental reservoirs
- Changes in the environment can lead to transmission of pathogens to animals and humans that previously were not exposed to them

Pathogen Evolution

- Mixing of microbes in different animal hosts and under different environmental conditions can lead to the evolution of new and potentially dangerous pathogens
  - Infectious diseases emerge from ecological disturbances and the movement of animals, both human activities
  - Results in acceleration of newly emerging diseases as well as the reemergence of diseases previously under control

Increase in Disease Incidence

- Acute respiratory distress disease due to hantavirus because of population growth of the deer mouse following El Niño-associated heavy rainfall in 1993
  - Decreased the number of deer mouse predators
  - Increased growth of the pinon nut, a favorite food source
- Increase in malaria cases in East Malaysia as deforestation causes the reservoir of *Plasmodium knowlesi*, a monkey, and the mosquitos that transmit it to live closer together

Climate Change Affects Disease Location

- Changes in the environment, such as a warming climate, alter the habitats of disease-carrying animals (insects, for example) and lead to changes in who is at risk
- Examples: Zika virus and Dengue virus are moving north with their host, the *Aedes aegypticus* mosquito, as temperatures increase even slightly there

Pathogens Newly Recognized in Recent Decades

- Acanthamebiasis
- Australian bat lyssavirus
- Babesia, atypical
- *Bartonella henselae*
- Ehrlichiosis
- *Encephalitozoon cuniculi*
- *Encepalitozoon hellem*
- *Enterocytozoon bieneusi*
- Hendra virus
- Human herpesvirus 6
- Human herpesvirus 8
- Lyme disease
- Parvovirus B19
- Ebola
- Marburg
- Lassa fever
- MERS
- SARS

- Nipah
- Rift Valley fever

Reemerging Pathogens

- *Clostridium difficile*
- Enterovirus 71
- Mumps virus
- *Staphylococcus aureus*
- *Streptococcus,* group A
- Crimean Congo hemorrhagic fever
- Zika virus

2016 WHO List of Most Concerning Emerging Pathogens

- Crimean Congo hemorrhagic fever
- Ebola virus disease
- Marburg
- Lassa fever
- SARS
- Nipah
- Rift Valley fever
- Zika virus