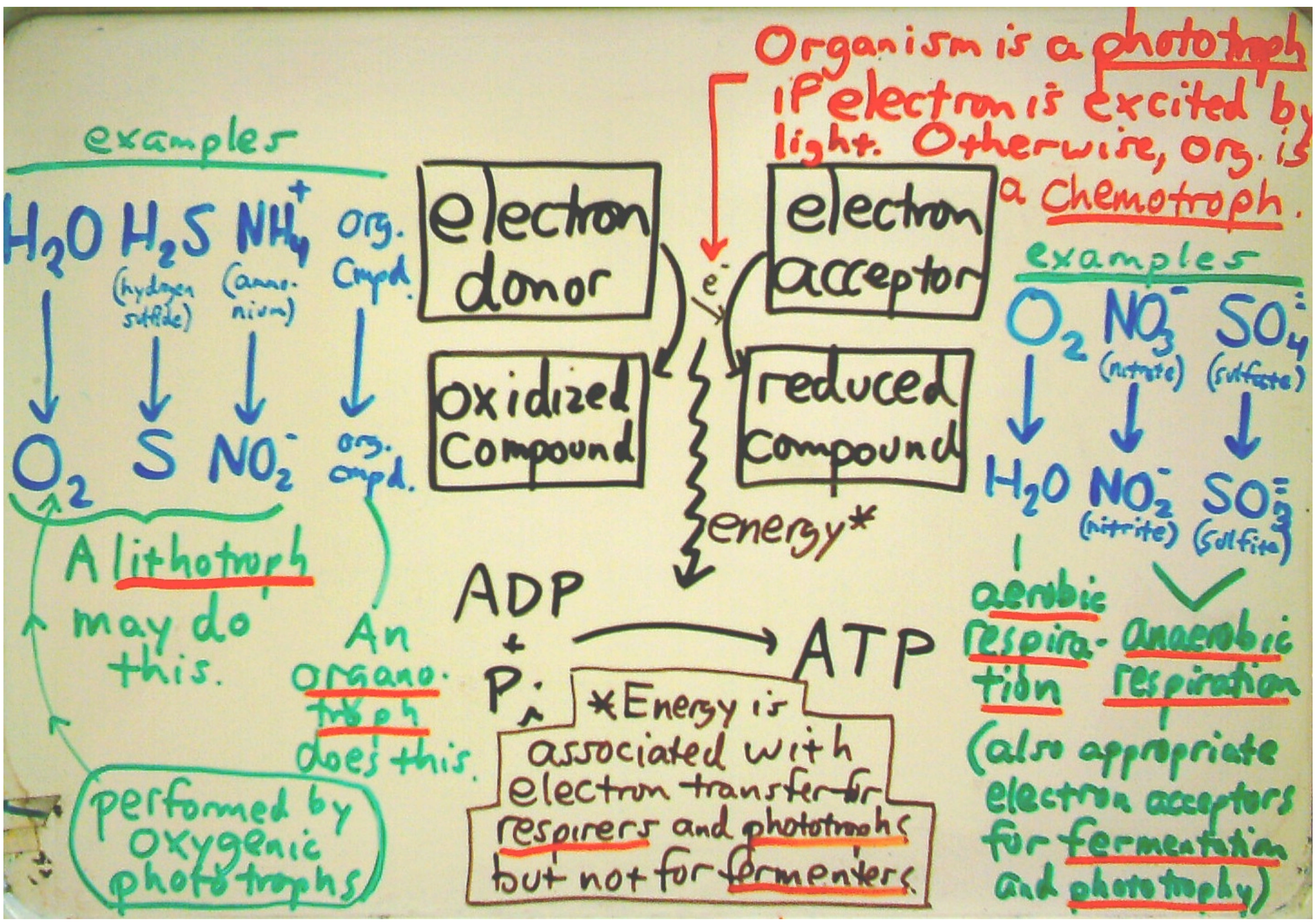
FARM MICROBIOLOGY 2008
PART 5: SOIL MICROBIOLOGY, CYCLING OF ELEMENTS AND BIODEGRADATION

I. A GENERAL INTRODUCTION AND REVIEW.

A. Effect of microorganisms on the global cycles of elements. The most significant effect that the microbes have on their environment is their underlying ability to recycle the essential elements needed to build biological systems. Earth is a closed system with limited amounts of carbon, oxygen, and nitrogen to support life. These essential elements of living systems must be converted from one form to another and shared among all living organisms. In the global environment, procaryotes are absolutely essential to drive the cycles of elements that make up living systems, i.e., the **carbon, oxygen, nitrogen and sulfur cycles.**

B. Catabolism (extremely simplified). Some of the examples given in our discussion of the various cycles in soil are indicated below:

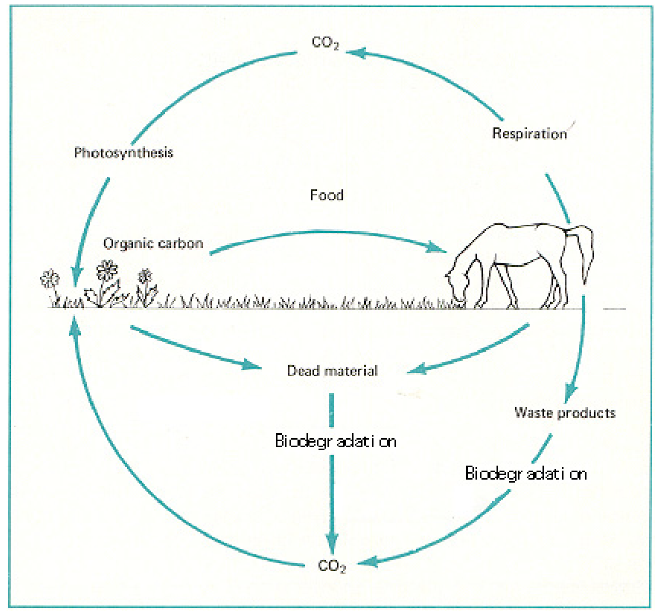


C. Anabolism. Some of the chemical compounds involved in catabolic reactions are also directly assimilated and used in biosynthetic reactions by plants, animals and microorganisms. Examples include ammonium, nitrate, sulfate and (of course) water.

II. The Carbon Cycle.

A. General Summaries.

Carbon is the backbone of all organic molecules and is the most prevalent element in cellular (organic) material. In its most oxidized form, CO_2, it can be viewed as an "inorganic" molecule. **The essence of an organic molecule is the C-H bond**, so organic forms of carbon, with the empirical formula of CH_2O, are reduced forms of carbon.

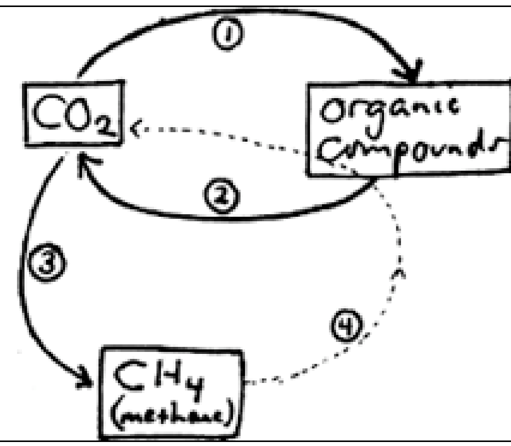


A GENERAL VIEW OF THE CARBON CYCLE

Note: As is also the case for the **Nitrogen and Sulfur Cycles (below),** organic compounds are converted from one to another within plants, animals and microorganisms – also animals eating plants, microorganisms decomposing plants and animals, microorganisms undergoing fermentation, etc.

A MORE DETAILED VIEW OF THE CARBON CYCLE:

1. Use of CO_2 as carbon source of **AUTOTROPHS** which then build up organic compounds that can be used as carbon source by **HETEROTROPHS**.
2. Depolymerization and release of CO_2 as an **end product** in **respiration** (and some **fermentation**) by CHEMO**ORGANOTROPHS**.
3. Methanogenesis: Usually thought of as use of CO_2 as **electron acceptor** being reduced to methane (CH_4).
4. Methane oxidation: Methane as **electron donor** is oxidized to CO_2.



B. Exchange/Interaction between Primary Producers and Biodegraders.

1. In general: Autotrophs (by definition) **use CO_2 as the sole source of carbon** for growth, thereby reducing it to cell material, and their form of catabolism is generally **lithotrophic**. Autotrophs include plants, algae, photosynthetic bacteria, methanogens and many kinds of lithotrophic bacteria. **Heterotrophs** require organic carbon for growth, and if their metabolism (which is generally **organotrophic**) proceeds far enough, they eventually convert the fixed carbon back to CO_2.

2. **An example often given of a balance between two sets of organisms:**

Primary producers: **Oxygenic phototrophs** (such as cyanobacteria which are autotrophic – i.e., use CO_2 as source of carbon).

$$CO_2 + H_2O \text{ ---> } CH_2O\text{(organic cmpds. in cell material)} + O_2$$

Biodegraders: **Aerobically-respiring chemotrophs** (for example, many bacteria that are organotrophic – i.e., oxidize organic compounds as source of energy).

$$CH_2O \text{ (organic cmpds.)} + O_2 \text{ ---> } CO_2 + H_2O$$

(Note how the oxygenic phototrophs are not only autotrophic but also lithotrophic. What is the electron donor for oxygenic phototrophs?)

C. Biodegradation (decomposition). This is where the microorganisms get most of their credit for participation in the carbon cycle. Biodegradation is the **decomposition** (by organotrophs/heterotrophs) of organic material (CH_2O) back to CO_2 and H_2O. In soil habitats, the fungi play a significant role in biodegradation, but the procaryotes are equally important. The typical decomposition scenario involves the initial degradation of biopolymers (cellulose, lignin, proteins, polysaccharides) by extracellular enzymes, followed by oxidation (fermentation or respiration) of the polymer subunits. The ultimate end products are CO_2, H_2O and H_2, perhaps some NH_3 (ammonia) and sulfide (H_2S), depending on how one views the overall process. These products are scarfed up by lithotrophs/autotrophs for recycling. Procaryotes which play an important role in biodegradation in nature include the Actinomycetes (including *Streptomyces*), *Pseudomonas* (and other pseudomonads), *Clostridium, Bacillus* and *Arthrobacter*.

The importance of microbes in biodegradation is embodied in the adage that "there is no known natural compound that cannot be degraded by some microorganism." The proof of the adage is that we aren't up to our ears in whatever it is that couldn't be degraded in the last 4 billion years. Actually, we are up to our ears in cellulose and lignin, which is better than concrete, and some places are getting up to their ears in Teflon, plastic, styrofoam, insecticides, pesticides and poisons that are degraded slowly by microbes, or not at all.

1. Overall Process.

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polymers---------->monomers    (depolymerization)
monomers---->fatty acids + CO2 + H2   (fermenters)
monomers + O2 ---->CO2 + H2O  (aerobic respirers)
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2. The Compost Pile. This is constructed with layers of organic material (plant matter, garbage, manure), soil and some inorganic materials (such as rock phosphate and granite dust) and is covered with soil or straw. This mixture is kept moist and is mixed on a regular basis to allow air to get in and to mix parts of the pile that have developed differing temperatures. Microbial activity degrades the material and builds up considerable heat (up to around 80°C). Fibers, cellulose and other polymers are effectively degraded to the more easily-utilized fats, sugars and proteins. Organisms that participate in the overall process include bacteria & fungi, respirers & fermenters, thermophiles & mesophiles (i.e., organisms that prefer high and moderate temperatures, respectively).

3. The Rumen. In the anaerobic environment of the rumen, polymers such as cellulose are broken down to sugars which can be assimilated or fermented by microorganisms. Anaerobic bacteria and protozoa participate in the process and can themselves undergo decomposition and serve as nutrients (amino acids, vitamins, etc.) for other microorganisms and also the animal itself. Products of fermentation (organic acids and carbon dioxide) pass into the animal's blood stream and are oxidized in the tissues. Gases expelled by the animal include carbon dioxide and methane (see "methanogens" below).

4. Importance of *Pseudomonas*. The above adage regarding the degredation of natural compounds must have been coined to apply to members of the genus *Pseudomonas*, known for their ability to degrade hundreds of different organic compounds including insecticides, pesticides, herbicides, plastics, petroleum substances, hydrocarbons and other of the most refractory molecules in nature. However, they are usually unable to degrade biopolymers in their environment, such as cellulose and lignin, and their role in anaerobic decomposition is minimal.

5. Importance of *Streptomyces*. Species of this genus have a world-wide distribution in soils. They are important in aerobic decomposition of organic compounds and have an important role in biodegradation and the carbon cycle. Also, products of metabolism called **geosmins** impart a characteristic earthy odor to soils. Certain species are major producers of commercially-available **antibiotics** such as tetracyclines, erythromycin, streptomycin, gentamicin, etc.

D. The Methanogens.

1. Dual role: As autotrophs (utilizers of CO_2 as carbon source) and as reducers of CO_2 to CH_4 (methane). Methanogens are inhabitants of virtually all anaerobic environments in nature where CO_2 and H_2 (hydrogen gas) occur. They use CO_2 in their metabolism in two distinct ways. About 5 percent of CO_2 taken up is reduced to cell material during autotrophic growth; the remaining 95 percent is reduced to CH_4 (methane gas) during **a unique process of generating cellular energy**. (Generally, for convenience, we have included the methanogens among the **anaerobic respirers**.) Hence, methane accumulates in rocks as fossil fuel ("natural gas"), in the rumen of cows and guts of termites, in sediments, swamps, landfills and sewage digesters. Since CH_4 is the second-most prevalent of the so-called Greenhouse gases, it may be a good idea to discourage those processes that lead to its accumulation in the atmosphere.

2. Rough summary:

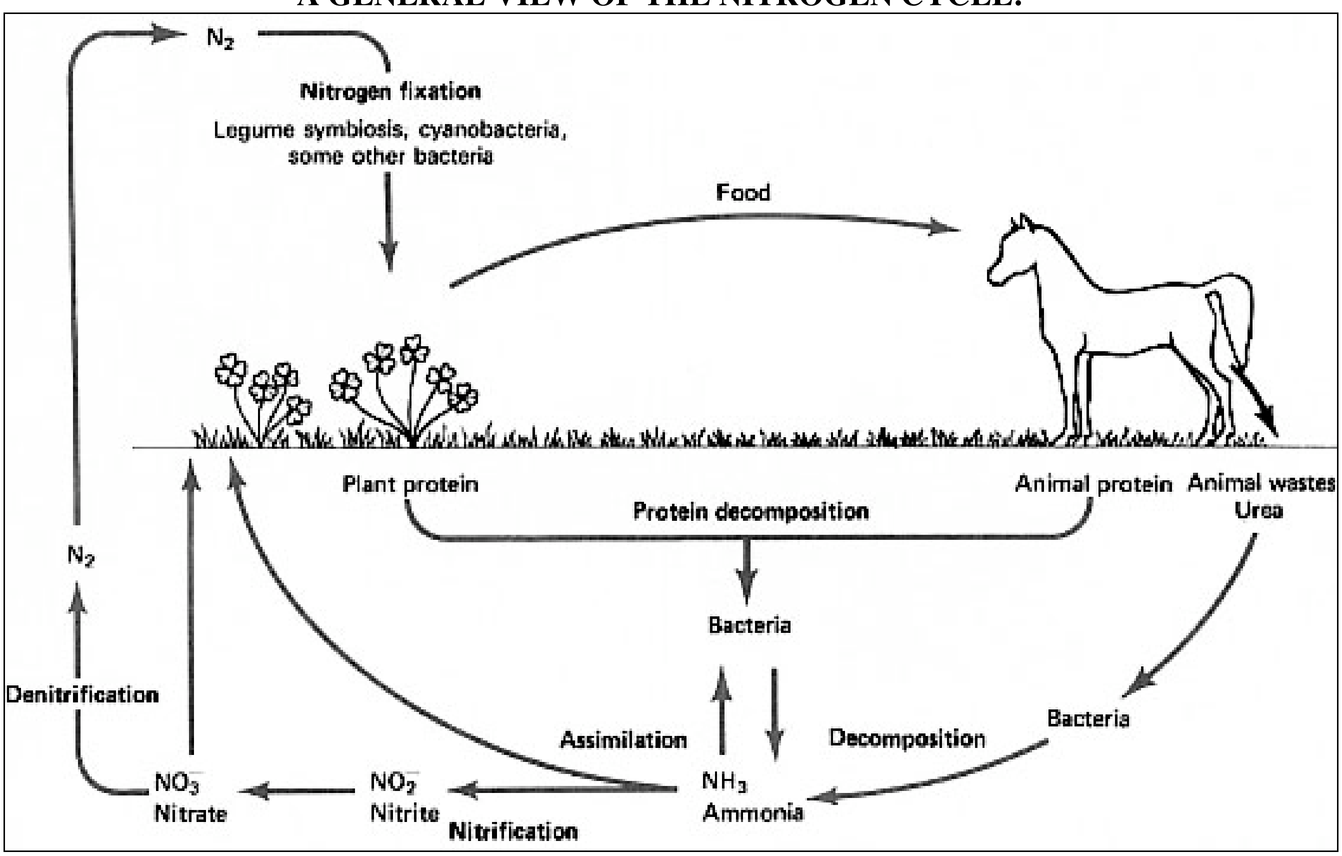
$$CO_2 + H_2 \rightarrow CH_2O \text{ (cell material)} + CH_4 \text{ (methane)}$$

E. Methane Oxidizers. These bacteria have a unique metabolism that converts methane to carbon dioxide.

III. THE NITROGEN CYCLE.

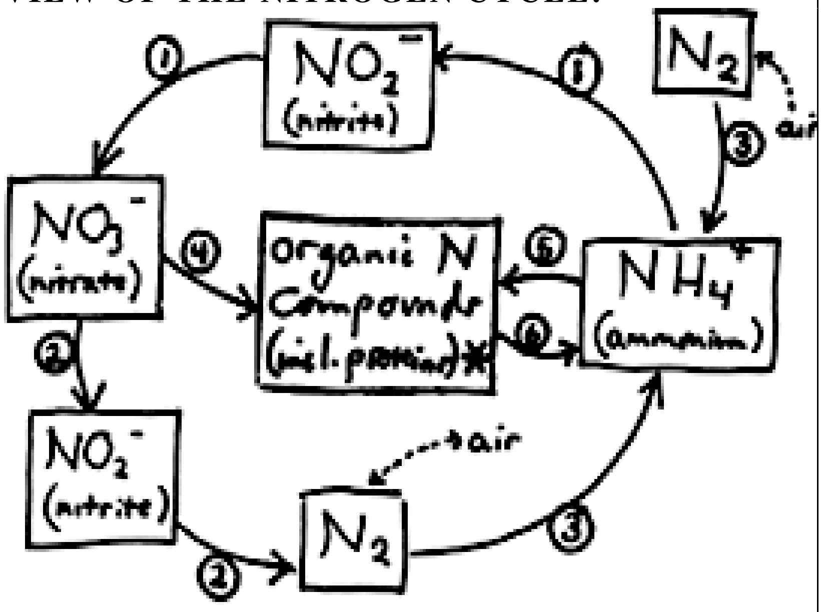
A. General Summaries.

A GENERAL VIEW OF THE NITROGEN CYCLE:



A MORE DETAILED VIEW OF THE NITROGEN CYCLE:

1. **Respiration** of ammonium & nitrite by **CHEMOLITHOTROPHS**. This process is called **nitrification**.
2. **Dissimilatory nitrate reduction** – a form of **anaerobic respiration**. Process is called **denitrification** when nitrate is reduced all the way to N_2.
3. **Nitrogen-fixation** by certain bacteria under aerobic (upper) and anaerobic (lower) conditions.
4. **Assimilatory nitrate reduction** performed by plants and micro-organisms.
5. **Assimilatory ammonium uptake** performed by plants and micro-organisms.
6. **Ammonification** performed by microorganisms.



B. Complexity. The Nitrogen cycle is the most complex of the cycles of elements in biological systems. This is due to the importance and prevalence of N in cellular metabolism, the diversity of types of nitrogen metabolism, and the existence of the element in so many forms. Procaryotes are essentially involved in the biological nitrogen cycle in three unique processes: nitrogen-fixation, nitrification and anaerobic respiration.

C. Nitrogen-Fixation.

1. Process.

N_2----->$2NH_3$ (**nitrogen fixation**)

2. Symbiotic Nitrogen-Fixing Bacteria – main example: *Rhizobium*.

Rhizobium lives in nodules on the roots of legumes (peas, beans, soybeans, trefoil, alfalfa, etc.) and fix atmospheric nitrogen. The plants benefit from the nitrogen compounds, and the bacteria benefit from the specialized environment provided by the plant.

3. Non-Symbiotic (Free-Living) Nitrogen-Fixing Bacteria: *Azotobacter*, most Cyanobacteria, and many strains of *Klebsiella, Bacillus* and *Clostridium*. In the laboratory, one can add soil to a medium containing all of the required nutrients for growth of most bacteria – minus any nitrogen compounds – and the first organisms to start growing will be the nitrogen-fixers which are able to utilize the atmospheric nitrogen dissolved in the water.

D. Nitrification.

NH_3----->NO_2^- (*Nitrosomonas*)
NO_2----->NO_3^- (*Nitrobacter*)

This process is a form of lithotrophic metabolism that is chemically the opposite of denitrification. Nitrifying bacteria such as *Nitrosomonas* utilize NH_3 as an energy source, oxidizing it to NO_2^-, while *Nitrobacter* will oxidize NO_2^- to NO_3^-. Nitrifying bacteria generally occur in aquatic environments and their significance in soil fertility and the global nitrogen cycle is not well understood.

E. Anaerobic Respiration of Nitrate. This relates to the use of oxidized forms of nitrogen (NO_3^- and NO_2^-) as final electron acceptors for respiration. Anaerobic respirers such as certain species of *Pseudomonas* and *Bacillus* are common soil inhabitants that will use nitrate (NO_3^-) as an electron acceptor. NO_3^- is reduced to NO_2^- (nitrite) and then to a gaseous form of nitrogen such as N_2 or N_2O or NH_3. The process is called **denitrification**. Denitrifying bacteria are typical aerobes that respire whenever oxygen is available by aerobic respiration. If O_2 is unavailable for respiration, they will turn to the alternative anaerobic respiration which uses NO_3^-.

1. Process.

NO_3^------>NO_2^------>N_2 **(denitrification)**

2. Problem in anaerobic soil with *Pseudomonas*. Some *Pseudomonas* species are **anaerobic respirers**, using nitrate in place of oxygen, and can deplete soil of expensive nitrate fertilizer (converting nitrate to nitrogen gas) if the soil is allowed to become anaerobic. One rationale for tilling the soil is to keep it aerobic, thereby preserving nitrate fertilizer in the soil. Effective drainage can prevent waterlogging of the soil – a cause of anaerobic conditions.

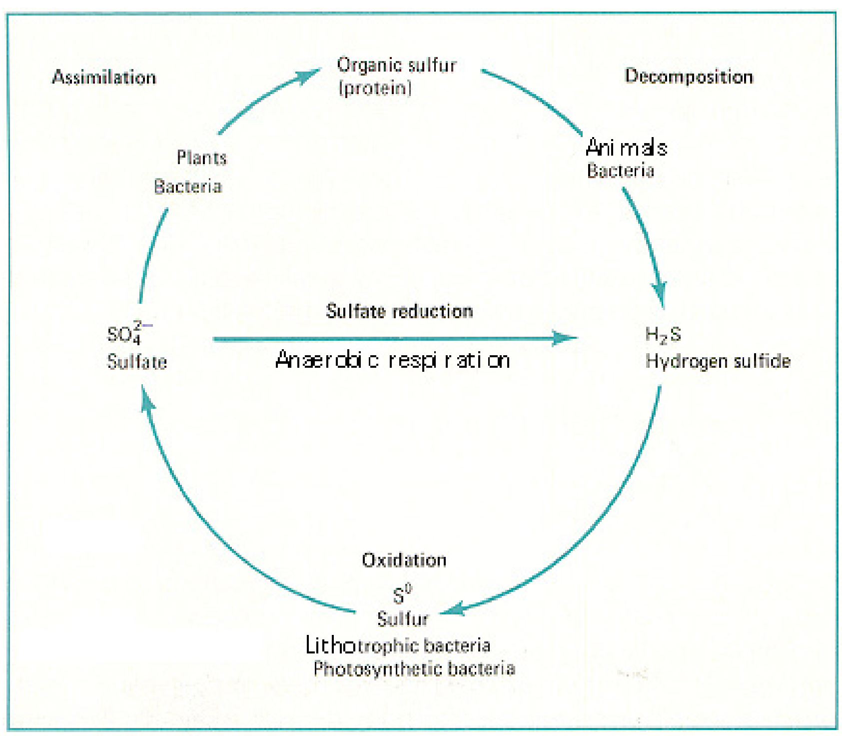
F. Assimilation of Nitrate and Ammonium. Eucaryotes and procaryotes of all kinds take up the element for their own nutrition. Nitrogen assimilation is usually in the form of NO_3^-, an amino group, or ammonia.

G. Ammonification. A final important aspect of the nitrogen cycle that involves procaryotes, though not exclusively, is **decomposition** of nitrogen-containing compounds. Most organic nitrogen (in protein, for example) yields ammonia (NH_3) during the process of **deamination**. Fungi are involved in decomposition as well.

IV. THE SULFUR CYCLE.

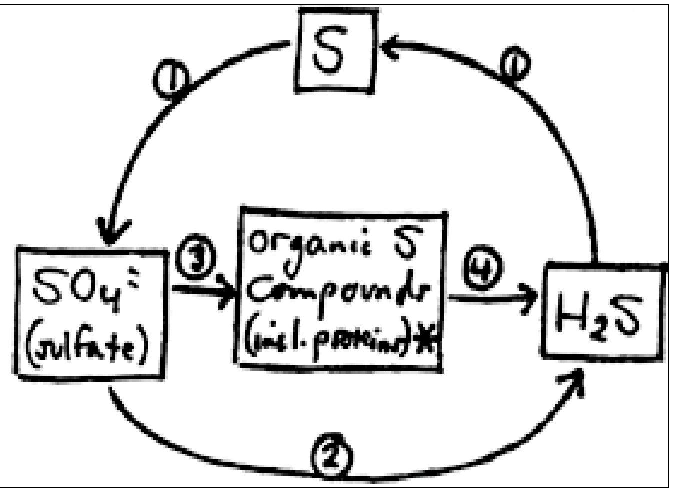
A. General Summaries: Sulfur is a component of a couple of vitamins and essential metabolites and it occurs in two amino acids, cysteine and methionine. In spite of its paucity in cells, it is an absolutely essential element for living systems. Like nitrogen and carbon, the microbes can transform sulfur from its most oxidized form (sulfate or SO_4^{2-}) to its most reduced state (sulfide or H_2S). The sulfur cycle, in particular, involves some unique groups of procaryotes and procaryotic processes.

A GENERAL VIEW OF THE SULFUR CYCLE:



A MORE DETAILED VIEW OF THE SULFUR CYCLE:

1. Oxidation of sulfide and sulfur by **CHEMO-LITHOTROPHS** (by **respiration**) and **PHOTOLITHOTROPHS**.
2. **Dissimilatory sulfate reduction** – a form of **anaerobic respiration**.
3. **Assimilatory sulfate reduction** performed by plants and microorganisms.
4. **Desulfurylation** performed by animals and microorganisms.



B. Exchange between Lithotrophs and Anaerobic Respirers.

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H2S----->S----->SO4 2−   (sulfur oxidation)
SO4 2−--------->H2S     (sulfate reduction)
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Two unrelated groups of procaryotes oxidize H_2S to S and S to SO_4^{2-}. The first is the anoxygenic photosynthetic **purple and green sulfur bacteria** that oxidize H_2S as a source of electrons for cyclic photophosphorylation. The second is the "**colorless sulfur bacteria**" (now a misnomer because the group contains so many **Archaea**) which oxidize H_2S and S as sources of energy. In either case, the organisms can usually mediate the complete oxidation of H_2S to SO_4^{2-}.

Sulfur-oxidizing bacteria are frequently thermophiles found in hot (volcanic) springs and near deep sea thermal vents that are rich in H_2S. They may be acidophiles, as well, since they acidify their own environment by the production of sulfuric acid.

Since SO_4^{2-} and S may be used as electron acceptors for respiration, sulfate reducing bacteria produce H_2S during a process of anaerobic respiration analogous to denitrification. The use of SO_4^{2-} as an electron acceptor is an obligatory process that takes place only in anaerobic environments. The process results in the distinctive odor of H_2S in anaerobic bogs, soils and sediments where it occurs.

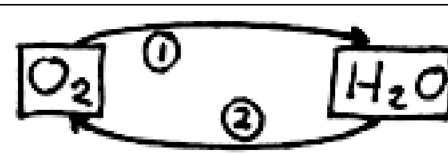
C. Assimilation and Desulfurylation. Sulfur is assimilated by bacteria and plants as SO_4^{2-} for use and reduction to sulfide. Animals and bacteria can remove the sulfide group from proteins as a source of S during decomposition. These processes complete the sulfur cycle.

V. THE OXYGEN CYCLE.

A. General Summary:

A VIEW OF THE OXYGEN CYCLE:

1. **Respiration** by **CHEMOTROPHS**.
2. O_2 production by **OXYGENIC PHOTOTROPHS**.



B. Interaction between oxygenic phototrophs (such as cyanobacteria) and aerobically-respiring chemotrophs (both organotrophs and lithotrophs).

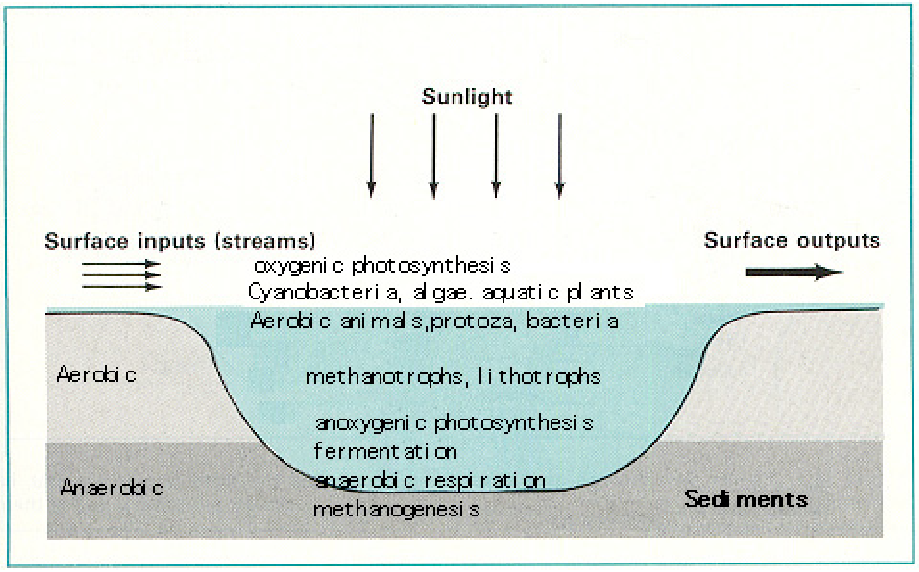
This is basically the same overall process as noted in the example given above in the carbon cycle (II. B. 2).

Basically, O_2 is derived from the photolysis of H_2O during **plant-type (oxygenic) photosynthesis**, and it is converted back to H_2O during **aerobic respiration**. Given that the procaryotic cyanobacteria existed for millions of years before the evolution of eukaryotic algae and plants, and that contemporary cyanobacteria are the photo-synthetic "grass of the sea", it is inferred that these procaryotes are the source of a lot of the O_2 in the earth's atmosphere that is required by aerobic organisms from all domains of life. This establishes a relationship between **autotrophs** and **heterotrophs** that has a counterpart in the carbon cycle wherein autotrophs fix carbon needed by heterotrophs, and heterotrophs produce CO_2 used by the autotrophs. Of course, plants and algae conduct a significant amount of CO_2 fixation and O_2 production in this metabolic relationship between O_2-producing autotrophs and O_2-consuming heterotrophs. CO_2 is the most prevalent Greenhouse gas in the air, it isn't good if these two equations to get out of balance by natural means or otherwise.

C. Association of Oxygen with Other Elements. Our simplified view of the O_2-H_2O cycle disregards the occurrence of radical forms of the oxygen molecule, and the persistent association of the oxygen atom with the other elements and molecules in living systems.

VI. ELEMENTAL CYCLES IN A TYPICAL LAKE. (Extra Added Attraction)

The role of procaryotes in the global cycle (described above) can be visited on a smaller scale, in a lake, for example, like Lake Mendota, which may become stratified as illustrated in the figure below. The surface of the lake is well-lighted by the sun and aerobic. The bottom of the lake and its sediments are dark and anaerobic. Generally there is less O_2 and less light as the water column is penetrated from the surface. Assuming that the nutrient supply is stable and there is no mixing between layers of lake water, we should, for the time being, have a stable ecosystem with recycling of essential elements among the living systems. Here is how it would work.



Ecology of a Stratified Lake.

At the surface, light and O_2 are plentiful, CO_2 is fixed and O_2 is produced. Photosynthetic plants, algae and cyanobacteria produce O_2, cyanobacteria can even fix N_2; aerobic bacteria, insects, animals and plants live here.

At the bottom of the lake and in the sediments, conditions are dark and anaerobic. Fermentative bacteria produce fatty acids, H_2 and CO_2, which are used by methanogens to produce CH_4. Anaerobic respiring bacteria use NO_3^- and SO_4^{2-} as electron acceptors, producing NH_3 and H_2S. Several soluble gases are in the water: H_2, CO_2, CH_4, NH_3 and H_2S.

The biological activity at the surface of the lake and at the bottom of the lake may have a lot to do with what will be going on in the middle of the water column, especially near the interface of the aerobic and anaerobic zones. This area, called the **thermocline**, is biologically very active. Bacterial photosynthesis, which is anaerobic, occurs here, using longer wave lengths of light that will penetrate the water column and are not absorbed by all the plant chlorophyll above. The methanotrophs will stay just within the aerobic area taking up the CH_4 from the sediments as a carbon source, and returning it as CO_2. Lithotrophic nitrogen- and sulfur-utilizing bacteria do something analogous: they are aerobes that use NH_3 and H_2S from the sediments, returning them to NO_3^- and SO_4^{2-}.

Note: The essay on this page and much of the explanatory material on previous pages are from Farm Microbiology lecture notes handed down from the previous instructor, Dr. Ken Todar.