Todar's Online Textbook of Bacteriology

PROCARYOTES IN THE ENVIRONMENT

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The most significant effect that procaryotes have on their environment is their underlying ability to recycle the essential elements that make up cells. The earth is a closed system with limited amounts of carbon, oxygen and nitrogen available to all forms of life. These essential elements must be converted from one form to another and shared among all living organisms. The total biomass of microbial cells in the biosphere, their metabolic diversity and their persistence in all habitats that support life, guarantee that microbes will play crucial roles in the transformations and recycling of these elements.

The table below lists the major elements that make up a typical procaryotic cell (in this case, *E. coli*). As expected, over 90 percent of the elemental analaysis consists of carbon, hydrogen, oxygen, nitrogen, phosphorus and sulfur. These are the elements that become combined to form all the biochemicals that comprise living systems. C, H, O, N, P and S are the constituents of organic material (An organic compound is a chemical that contains a carbon to hydrogen bond. Organic compounds on earth are evidence of life. Organic compounds may be symolized as CH_2O, which is the emperical formula for a sugar such as glucose.) H and O are the constituents of water (H_2O), that makes up over 95 percent of the cell composition. Calcium (Ca^{++}), iron (Fe^{++}), magnesium (Mg^{++}) and potassium (K^{+}) are present as inorganic salts in the cytoplasm of cells.

Table 1. Major elements, their sources and functions in cells.

Element	**% of dry weight**	**Source**	**Function**
Carbon	50	organic compounds or CO_2	Main constituent of cellular material

Oxygen	20	H_2O, organic compounds, CO_2, and O_2	Constituent of cell material and cell water; O_2 is electron acceptor in aerobic respiration
Nitrogen	14	NH_3, NO_3, organic compounds, N_2	Constituent of amino acids, nucleic acids nucleotides, and coenzymes
Hydrogen	8	H_2O, organic compounds, H_2	Main constituent of organic compounds and cell water
Phosphorus	3	inorganic phosphates (PO_4)	Constituent of nucleic acids, nucleotides, phospholipids, LPS, teichoic acids
Sulfur	1	SO_4, H_2S, S, organic sulfur compounds	Constituent of cysteine, methionine, glutathione, several coenzymes
Potassium	1	Potassium salts	Main cellular inorganic cation and cofactor for certain enzymes
Magnesium	0.5	Magnesium salts	Inorganic cellular cation, cofactor for certain enzymatic reactions
Calcium	0.5	Calcium salts	Inorganic cellular cation, cofactor for certain enzymes and a component of endospores
Iron	0.2	Iron salts	Component of cytochromes and certain nonheme iron-proteins and a cofactor for some enzymatic reactions

The table ignores the occurrence of "trace elements" in cells. **Trace elements** are metal ions required in cellular nutrition in such small amounts that it is difficult to determine their presence in cells. The usual metals that qualify as trace elements are Mn^{++}, Co^{++}, Zn^{++}, Cu^{++} and Mo^{++}. Trace elements are usually built into vitamins and enzymes. For example, vitamin B_{12} contains cobalt (Co^{++}) and the bacterial nitrogenase enzyme contains molybdenum (Mo^{++}).

The structure and metabolism of any organism adapts it to its environment. Thus the various groups of microbes are adapted to certain environmental niches based on their predominant type of metabolism relevant to the elemental nutrients available.

The **fungi** (molds and yeasts). The molds are aerobic organisms that utilize organic compounds for growth. They play an important role in decomposition or biodegradation of organic matter, particularly in soil.

Yeasts can grow anaerobially (without oxygen) through the process of fermentation. They play a role in fermentations in environments high in sugar. The prominant role of fungi in the environment is in the carbon cycle, during the process of decomposition, especially in the soil.

The **algae** are also an important part of the carbon cycle. They are the predominant photosyntheic organisms in many aquatic environments. The algae are **autotrophs,** which means they use carbon dioxide (CO_2) as a source of carbon for growth. Hence they convert atmospheric CO_2 into organic material (i.e., algal cells). Algae also play a role in the oxygen (O_2) cycle since their style of photosynthesis, similar to plants, produces O_2 in the atmosphere. The **cyanobacteria** are a group of procaryotic microbes, as prevalent as algae, that have this type of metabolism. Photosynthetic algae and cyanobacteria can be found in most environments where there is moisture and light.They are a major component of marine plankton which the basis of the food chain in the oceans.

Protozoans are heterotrophic organisms that have to catch or trap their own food. Therefore, they have developed elaborate mechanisms for movement and acquiring organic food which they can digest. Their food usually turns out to be bacterial cells, so one might argue that they are ecological predators that keep bacterial populations under control.....in soil, aquatic environments, intestinal tracts of animals, and many other environments.

The procaryotic **bacteria** and **archaea**, as a result of their diversity and unique types of metabolism, are involved in the cycles of virtually all essential elements. In two cases, methanogenesis (conversion of carbon dioxide into methane) and nitrogen fixation (conversion of nitrogen in the atmosphere into biological nitrogen) are unique to procaryotes and earns them their "essential role" in the carbon and nitrogen cycles.

There are other metabolic processes that are unique, or nearly so, in the procaryotes that bear significantly on the cycles of elements. For example, procaryotes called **lithotrophs** use inorganic compounds like ammonia and hydrogen sulfide as a source of energy, and others called **anaerobic respirers** use nitrate (NO_3) or sulfate (SO_4) in the place of oxygen, so they can respire without air. Most of the archaea are lithotrophs that use hydrogen (H_2) or hydrogen sulfide (H_2S) as a source of energy, while many soil bacteria are anaerobic respirers that can use their efficient respiratory metabolism in the absence of O_2.

The basic processes of heterotrophy are spread throughout the bacteria. Most of the bacteria in the soil and water, and in associations with animals and plants, are heterotrophs. **Heterotrophy** means living off of dead organic matter, usually by some means of respiration (same as animals) or fermentation (same as yeast or lactic acid bacteria). Bacterial heterotrophs in the carbon chain are important in the processes of biodegradation and decomposition under aerobic and anaerobic conditions.

In bacteria, there is a unique type of photosynthesis that does not use H_2O or produce O_2 which impacts on the carbon and sulfur cycles.

Meanwhile, the cyanobacteria (mentioned above) fix CO_2 and produce O_2 during photosynthesis, and they make a very large contribution to the carbon and oxygen cycles.

The list of examples of microbial involvement in the cycles of elements that make up living systems is endless, and probably every microbe in the web is involved in an intimate and unique way.

The Oxygen Cycle

Basically, O_2 is derived from the photolysis of H_2O during **plant (oxygenic) photosynthesis.** Two major groups of microorganisms are involved in this process, the eukaryotic algae, and the procaryotic cyanobacteria (formerly known as "blue-green algae"). The cyanobacteria and algae are the source of much of the O_2 in the earth's atmosphere. Of course, plants account for some O_2 production as well, but the microbes predominate in marine habitats which cover the majority of the planet.

Since most aerobic organisms need the O_2 that results from plant photosynthesis, this establishes a relationship between plant photosyntheisis and aerobic respiration, two prominant types of metabolism on earth. Photosynthesis produces O_2 needed for aerobic respiration. Respiration produces CO_2 needed for autotrophic growth.

$CO_2 + H_2O$-----------------> CH_2O (organic material) + O_2 plant (oxygenic) photosynthesis

$CH_2O + O_2$-----------------> $CO_2 + H_2O$ aerobic respiration

Since these photosynthetic microbes are also autotrophic (meaning they convert CO_2 to organic material during growth) they have a similar impact on the carbon cycle (below).

The Carbon Cycle

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 (no C - H bond). **Autotrophs,** which include plants, algae, photosynthetic bacteria, lithotrophs, and methanogens, use CO_2 as a sole source of carbon for growth, which reduces the molecule to organic cell material (CH_2O). **Heterotrophs** require organic carbon for growth, and ultimately convert it back to CO_2.

Thus, a relationship between autotrophs and heterotrophs is established wherein autotrophs fix carbon needed by heterotrophs, and heterotrophs produce CO_2 used by the autotrophs.

$CO_2 + H_2O$----------------> CH_2O (organic material) autotrophy

$CH_2O + O_2$----------------> $CO_2 + H_2O$ heterotrophy

Since CO_2 is the most prevalent greenhouse gas in the atmosphere, it isn't good if these two equations to get out of balance (i.e. heterotrophy predominating over autotrophy, as when rain forests are destroyed and replaced with cattle).

Autotrophs are referred to as **primary producers** at the "bottom of the food chain" because they convert carbon to a form required by heterotrophs. Among procaryotes, the cyanobacteria, the lithotrophs and the methanogens are a formidable biomass of autotrophs that account for a corresponding amount of CO_2 fixation in the global carbon cycle.

The lithotrophic bacteria and archaea that oxidize reduced N and S compounds and play important roles in the natural cycles of N and S (discussed below), are virtually all autotrophs. The prevalence of these organisms in sulfur-rich environments (marine sediments, thermal vents, hot springs, endosymbionts, etc. may indicate an unappreciated role of these procaryotes as primary producers of organic carbon on earth.

The **methanogens** play a dual role in the carbon cycle. These archaea 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. 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 digestors. Since CH_4 is the second-most prevalent of the greenhouse gases, it is best to discourage processes that lead to its accumulation in the atmosphere.

$CO_2 + H_2$ ----------------> CH_2O (cell material) + CH_4 methanogenesis

Under aerobic conditions, methane and its derivatives (methanol, formaldehyde, etc.) can be oxidized as energy sources by bacteria called

methylotrophs. Metabolically this is a version of decomposition or biodegradation during the carbon cycle which is discussed below.

Biodegradation is the process in the carbon cycle for which microbes get most credit (or blame). **Biodegradation** is the **decomposition** of organic material (CH_2O) back to CO_2 + H_2O and H_2. 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 monomeric 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 and autotrophs for recycling. Procaryotes which play an important role in biodegradation in nature include the actinomycetes, clostridia, bacilli, arthrobacters and pseudomonads.

Overall Process of Biodegradation (Decomposition)

polymers (e.g. cellulose)----------------> monomers (e.g. glucose) depolymerization

monomers----------------> fatty acids (e.g. lactic acid, acetic acid, propionic acid) + CO_2 + H_2 fermentation

monomers + O_2 ----------------> CO_2 + H_2O aerobic respiration

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 3.5 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.

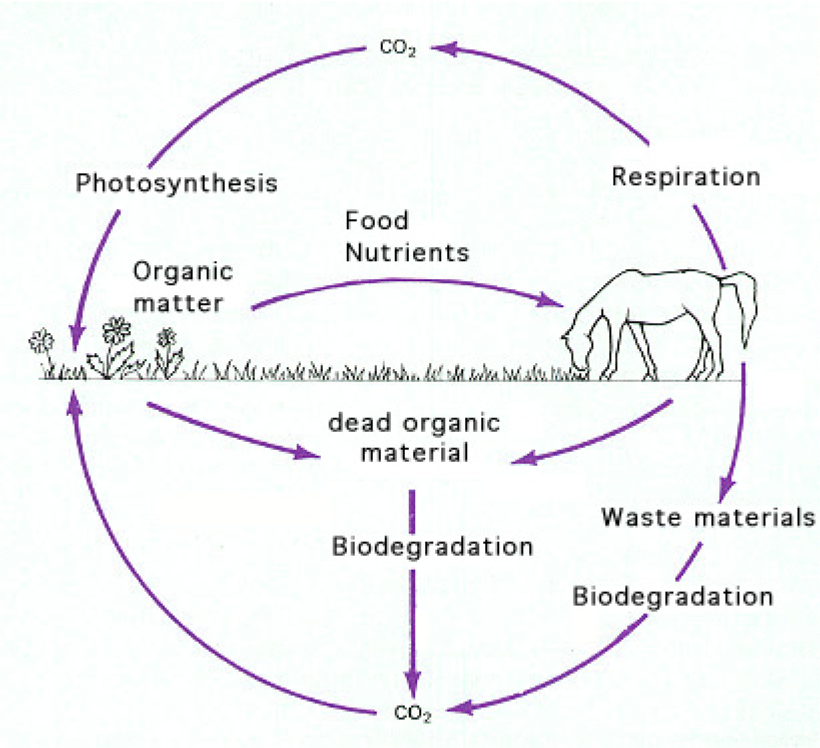

Figure 1. The Carbon Cycle. Organic matter (CH_2O) derived from photosynthesis (plants, algae and cyanobacteria) provides nutrition for heterotrophs (e.g.animals and associated bacteria), which convert it back to CO_2. Organic wastes, as well as dead organic matter in the soil and water, are ultimately broken down to CO_2 by microbial processes of biodegradation.

OFF THE WALL

The figure above mostly ignores the role of methanogenesis in the carbon cycle. Since methanogens have the potential to remove CO_2 from the atmosphere, converting it to cell material and CH_4, these procaryotes not only influence the carbon cycle, but their metabolism also affects the concentration of major greenhouse gases in earth's atmosphere.

Recently, I asked a colleague, Professor Paul Weimer of the University of Wisconsin Department of Bacteriology, whether mathanogenesis which utilizes CO_2 while producing CH_4 was better or worse on the greenhouse effect. This is his response.

"Worse. For methanogenesis by CO_2 reduction, the stoichiometry is $4H_2 + CO_2 \rightarrow CH_4 + 2\ H_2O$, so one mole of a greenhouse gas is exhanged for another. But methane is about 15 times more potent than is CO_2 in terms of heat absorption capability on a per-molecule basis, so the net effect is a functional increase in heat absorption by the atmosphere. Remember also that in most natural environments, around two-thirds of the methane is produced by aceticlastic methanogenesis ($CH_3COOH \rightarrow CH_4 + CO_2$) - an even less welcome

situation, as BOTH products are greenhouse gases.

Even though methane concentrations in the atmosphere are two orders of magnitude below those of CO_2, methane is thought to account for about 15% of the anthropogenic climate forcing, compared to about 60% from CO_2. Most of the rest of the contribution is from nitrous oxide (N_2O, a respiratory denitrification product that has something like 300 times the heat absorbing capacity as CO_2) and the old chlorofluorocarbons (CFCs), even stonger heat absorbers yet, but more famous and dangerous as stratospheric ozone-depleters."

The Nitrogen Cycle

The nitrogen cycle is the most complex of the cycles of elements that make up 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: this process converts N_2 in the atmosphere into NH_3 (ammonia), which is assimilated into amino acids and proteins. Nitrogen fixation occurs in many free-living bacteria such as clostridia, azotobacters and cyanobacteria, and in symbiotic bacteria such as *Rhizobium* and *Frankia,* which associate with plant roots to form characteristic nodules. Biological nitrogen fixation is the most important way that N_2 from the air enters into biological systems.

N_2 ----------------> 2 NH_3 nitrogen fixation

Anaerobic Respiration: 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 *Bacillus* and *Pseudomonas*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 (nitrous oxide). The process is called **denitrification**. (A related process conducted by some *Bacillus* species, called **dissimilatory nitrate reduction** reduces NO_3 to ammonia (NH_3), but this is not considered denitrification.) Denitrifying bacteria are typically facultative microbes 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. Since NO_3 is a common and expensive form of fertilizer in soils, denitrification may not be so good for agriculture, ^and one rationale for tilling the soil is to keep it aerobic, thereby preserving nitrate fertilizer in the soil.

NO_3 ----------------> NO_2 ----------------> N_2 denitrification

OFF THE WALL
The overall reactions of denitrification shown above proceed through the formation of nitrous oxide (N_2O). A recent article by Wunsch an Zumft in Journal of Bacteriology, vol. 187 (2005), sheds new light on the process of denitrification. N_2O is a bacterial metabolite in the REVERSAL of Nitrogen fixation. The anthropogenic atmospheric increase of N_2O is a cause for concern, as noted above (as a greenhouse gas, N_2O has 300 times the heat absorbing capacity as CO_2). Denitrifying bacteria respire using N_2O as an electron acceptor yielding N_2 and the thereby provide a sink for N_2O. This article provides new insight into this process by identifying a membrane-bound protein in denitrifying bacteria called NosR, that is necessary for the expression of N_2O reductase from the *nosZ* gene. The NosR protein has redox centers positioned on opposite sides of the cytoplasmic membrane, which allows it to sustain whole-cell N_2O respiration by acting on N_2O reductase.

Nitrification 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.

The Overall process of Nitrification

NH_3 ----------------> NO_2 (*Nitrosomonas*)

NO_2 ----------------> NO_3 (*Nitrobacter*)

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.

Plants, animals and protista, as well as the procaryotes, complete the nitrogen cycle during the uptake of the element for their own nutrition. Nitrogen **assimilation** is usually in the form of nitrate, an amino group, or ammonia.

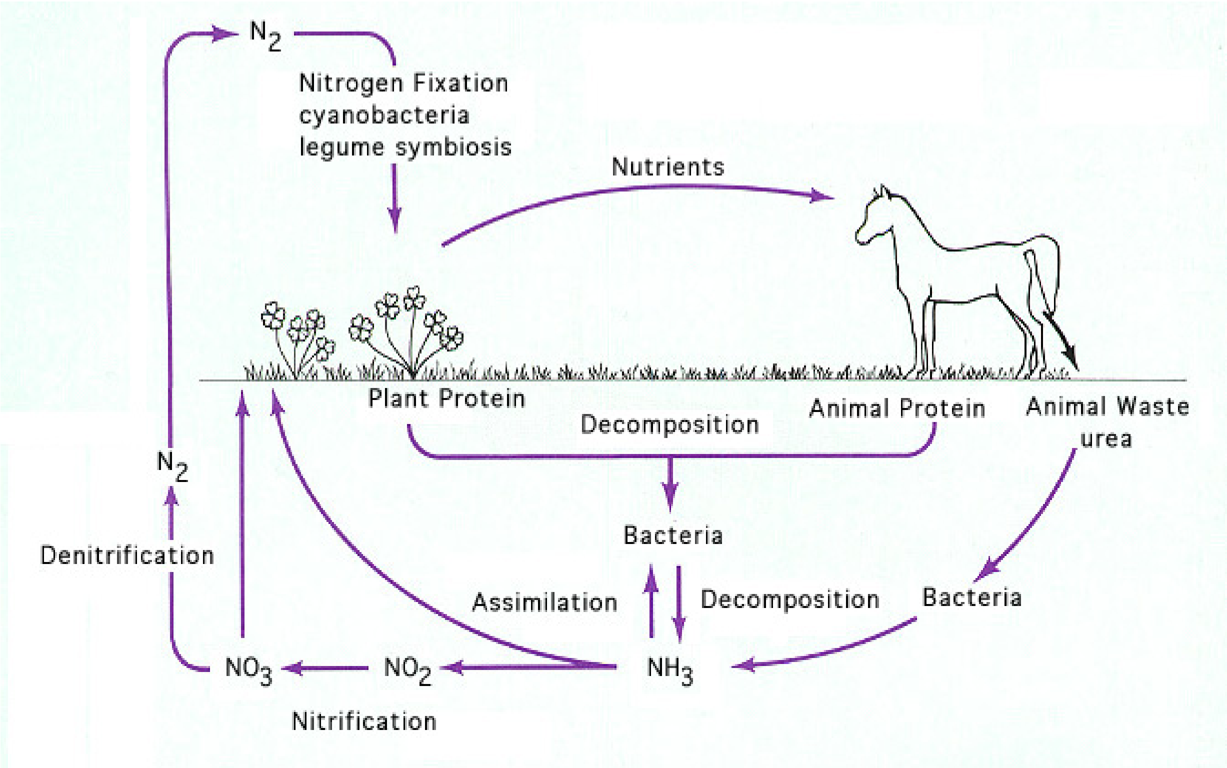

Figure 2. The Nitrogen Cycle

The Sulfur Cycle

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) to its most reduced state (sulfide or H_2S). The sulfur cycle, in particular, involves some unique groups of procaryotes and procaryotic processes. Two unrelated groups of procaryotes oxidize H_2S to S and S to SO_4. 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 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.

H_2S---------------> S ---------------> SO_4 litho or phototrophic sulfur oxidation

Sulfur-oxidizing procaryotes 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 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 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.

Sulfur is assimilated by bacteria and plants as SO_4 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.

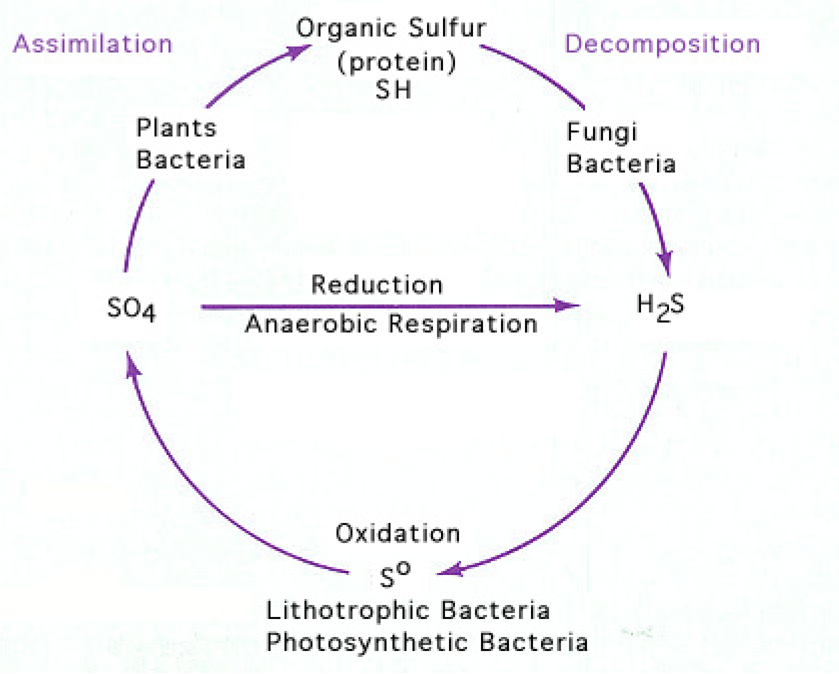

Figure 3. The Sulfur Cycle

The Phosphorus cycle

The phosphorus cycle is comparatively simple. Inorganic phosphate exists in only one form. It is interconverted from an iorganic to an organic form and back again, and there is no gaseous intermediate.

Phosphorus is an essential element in biological systems because it is a constituent of nucleic acids, (DNA and RNA) and it occurs in the phospholipids of cell membranes. Phosphate is also a constituent of ADP and ATP which are universally involved in energy exchange in biological systems.

Dissolved phosphate (PO_4) inevitably ends up in the oceans. It is returned to land by shore animals and birds that feed on phosphorus containing sea creatures and then deposit their feces on land. Dissolved PO_4 is also returned to land by a geological process, the uplift of ocean floors to form land masses, but the process is very slow. However, the figure below considers how PO_4 is recycled among land-based groups of organisms.

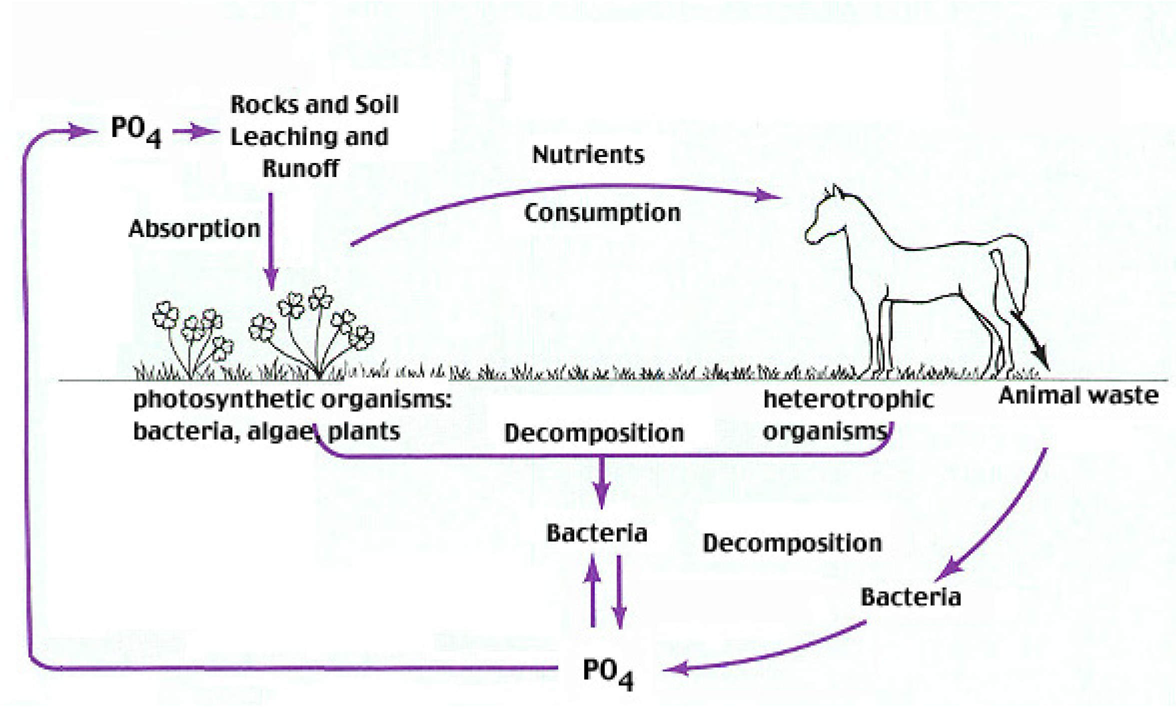

Figure 4. The Phosphorus Cycle. Plants, algae and photosynthetic bacteria can absorb phosphate (PO_4) dissolved in water, or if it washes out of rocks and soils. They incorporate the PO_4 into various organic forms, including such molecules as DNA, RNA, ATP, and phospholipid.The plants are consumed by animals wherein the organic phosphate in the plant becomes organic phosphate in the animal and in the bacteria that live with the animal. Animal waste returns inorganic PO_4 to the environment and also organic phosphate in the form of microbial cells. Dead plants and animals, as well as animal waste, are decomposed by microbes in the soil. The phosphate eventually is mineralized to the soluble PO_4 form in water and soil, to be taken up again by photosynthetic organisms.

Ecology of a Stratified Lake

The role of microbes in the global cycle of elements (described above) can be visited on a smaller scale, in a lake, for example, like Lake Mendota, which may become stratified as illustrated in Figure 5. The surface of the lake is well-lighted by the sun and is 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.

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 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 lenghts 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.

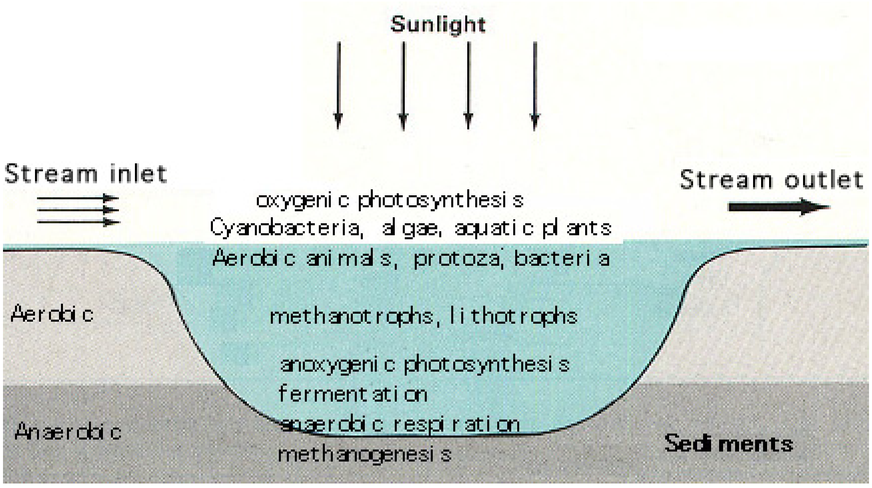

Figure 5. Ecology of a Stratified lake

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