MICROBIOLOGY OF SOIL

The word *soil* refers to the loose outer material of Earth's surface, a layer distinct from the bedrock that lies underneath.

It is the region supporting plant life and from which plants obtain their mechanical support and required nutrients.

Soil develops over long periods through complex interactions among the parent geological materials (rock, sand, glacial drift materials and so on), the topography, climate, and the presence and activities of living organisms.

Soils can be divided into two broad groups: *mineral soils* are derived from the weathering of rock and other inorganic materials, and *organic soils* are derived from sedimentation in bogs and marshes. Most soils are a mixture of these two basic types.

Components of Soil

The general structure of soil (Fig. 1) includes a series of layers called "horizons" that arise as a result of soil-forming factors such as rainfall, temperature variation, wind, and biological activity. Note that the soils of different habitats, such as prairie, forest, and desert, vary greatly as to the depth and quality of each layer.

The surface layer of soil we see is the organic horizon (O horizon). The organic horizon consists of dark, organic detritus, such as shreds of leaves fallen from plants. The detritus of the organic horizon is in the earliest stages of decomposition by microbes, primarily fungi and bacteria such as actinomycetes.

Early-stage decomposition is defined loosely as a state in which the origin of the detritus may be still recognizable.

Beneath the organic horizon lies the lighter-colored aerated horizon (A horizon), in which organic particles in more advanced stages of decomposition combine with minerals from rock at lower levels. In the aerated horizon, the source of the organic particles is no longer recognizable, and decomposers have broken down some of the more difficult- to-digest plant structural components, such as lignin (a complex aromatic polymer found in wood). This partly decomposed material is often sold by garden stores as peat or topsoil.

In well-drained soil, both the organic and aerated horizons are full of oxygen, as well as nutrients liberated by the decomposers and used by plants. Soil consists of a complex assemblage of organic and inorganic particles.

Between the soil particles are aeration spaces that provide access to oxygen, allowing aerobic respiration. Each particle of soil supports miniature colonies, biofilms, and filaments of bacteria and fungi that interact with each other and with the roots of plants (Fig. 2).

Below the aerated horizon, the eluviated horizon (E horizon) experiences periods of water saturation from rain.

Rainwater leaches (dissolves and removes) some of the organic and mineral nutrients from the upper layers.

Below the eluviated horizon lie increasing proportions of minerals and rock fragments broken off from bedrock below. These lower water-saturated layers form the water table. This anoxic, water-saturated region contains mainly lithotrophs and anaerobic heterotrophs.

The soil layers finally end at bedrock, a source of mineral nutrients such as carbonates and iron. Interestingly, bedrock is permeated with microbes. Core samples show that crustal rock as deep as 3 km down contains **endoliths**, bacteria growing between crystals of solid rock.

What energy source feeds microbes trapped within rock? For some endoliths, a surprising answer may be the radioactive decay of uranium. Uranium-238 decay generates hydrogen radicals that combine to form hydrogen gas. The hydrogen gas combines with CO_2 from carbonate rock, providing an electron donor and a carbon source for methanogens and other endolithic lithotrophs.

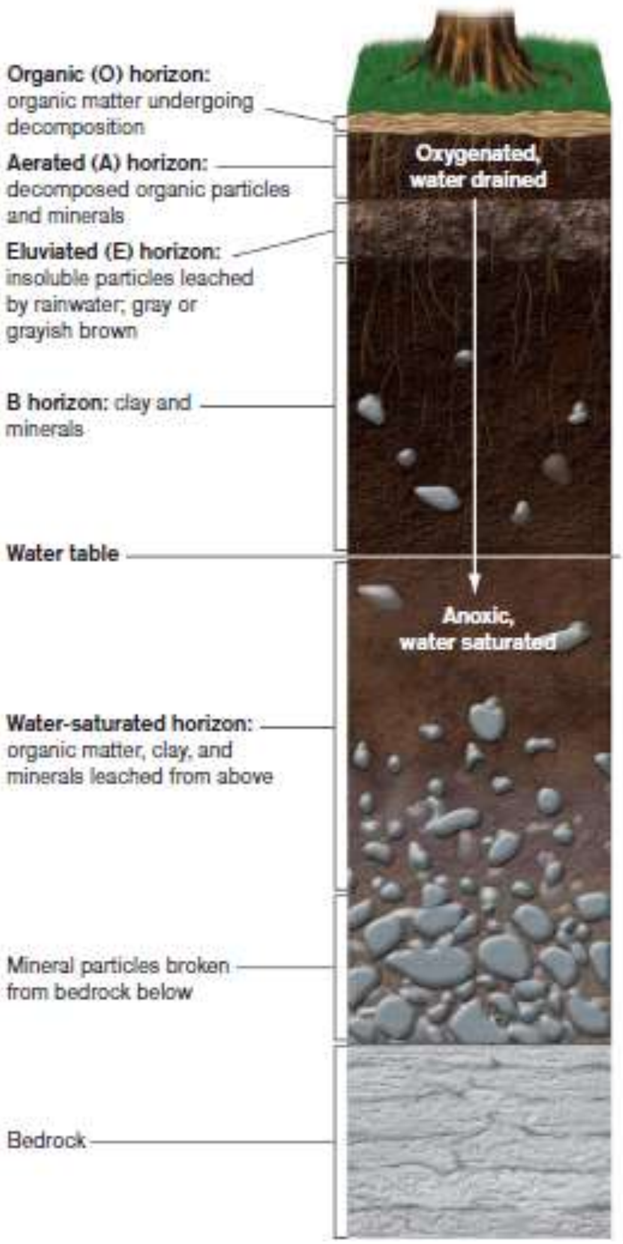

Figure 1: **The soil profile.** Soil forms layers in which decomposing organic material predominates at the top, and minerals toward the bottom, at bedrock. The top layers are aerated, providing heterotrophs with access to O_2, whereas the bottom layers are water saturated and anaerobic.

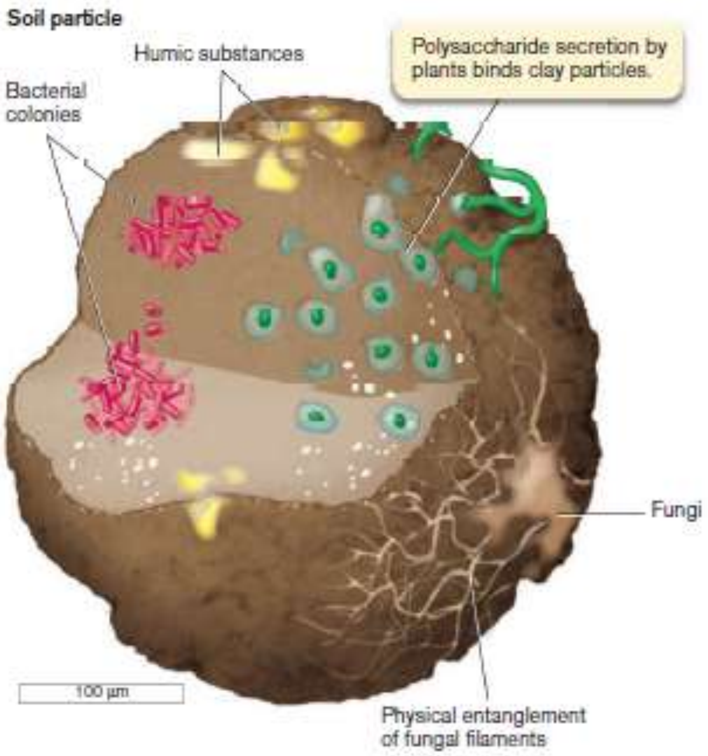

Figure 2: **Microbes in soil and rock.** Soil particles support growth of complex assemblages of microbes. A soil particle contains bacterial colonies, biofilm associations, and microbes associated with fungi and plant roots

Types of soil

❖ **Sandy Soil**: Sandy soil is light and dry in nature. It does not have moisture content and warms up quickly in the spring. Thus, it is good for the production of early crops. Sandy soil is fit for cultivation any time of the year and is comparatively easier to manage. Since it absorbs water quickly, the plants rooted in it need to be watered frequently.

❖ **Clay Soil**: Clay soil is also called late soil, because its wet nature makes it apt for planting seeds in late autumn. The soil serves as an excellent retort for the dry season, as it has a high water retention quality. It is necessary to drain clay soil frequently, for improving its texture. The soil becomes unmanageable during rainy season, as it becomes sticky. On the other hand, during draught, it becomes rock solid.

❖ **Loam Soil**: Given the tag of being the perfect soil, Loamy soil is a combination of all the three sandy soil, clay soil and silt soil, in the ratio of 40:40:20. It is suitable for any and every kind of crops. An amalgamation of three soils, loam soil has best of the characteristic of all. It has high nutrients content, warms up quickly in summers and rarely dries out in the dry weather. It has become the ideal soil for cultivation.

❖ **Peaty Soil**: Peaty soils are acidic in content, which makes them sour. This is the most exceptional feature of Peaty soils. Usually found in low-lying areas, these soils require proper drainage, as the place is accustomed to a lot of water clogging. Though peaty soils have less nutrient content, they warm up quickly in the spring, making them excellent if right amount of fertilizers are added.

❖ **Chalky Soil**: Chalky soil is alkaline in nature and usually poor in nutrients. It requires nourishment, in the form of additional nutrients and soil improvers, for better quality. The soil becomes dry in summers, making it very hard, and would require too much of watering for the plants to grow. The only advantage which such a soil has is its lime content. When deep-rooted, Chalky soil becomes excellent for plant growth and favors good growing conditions as well (Figure 3).

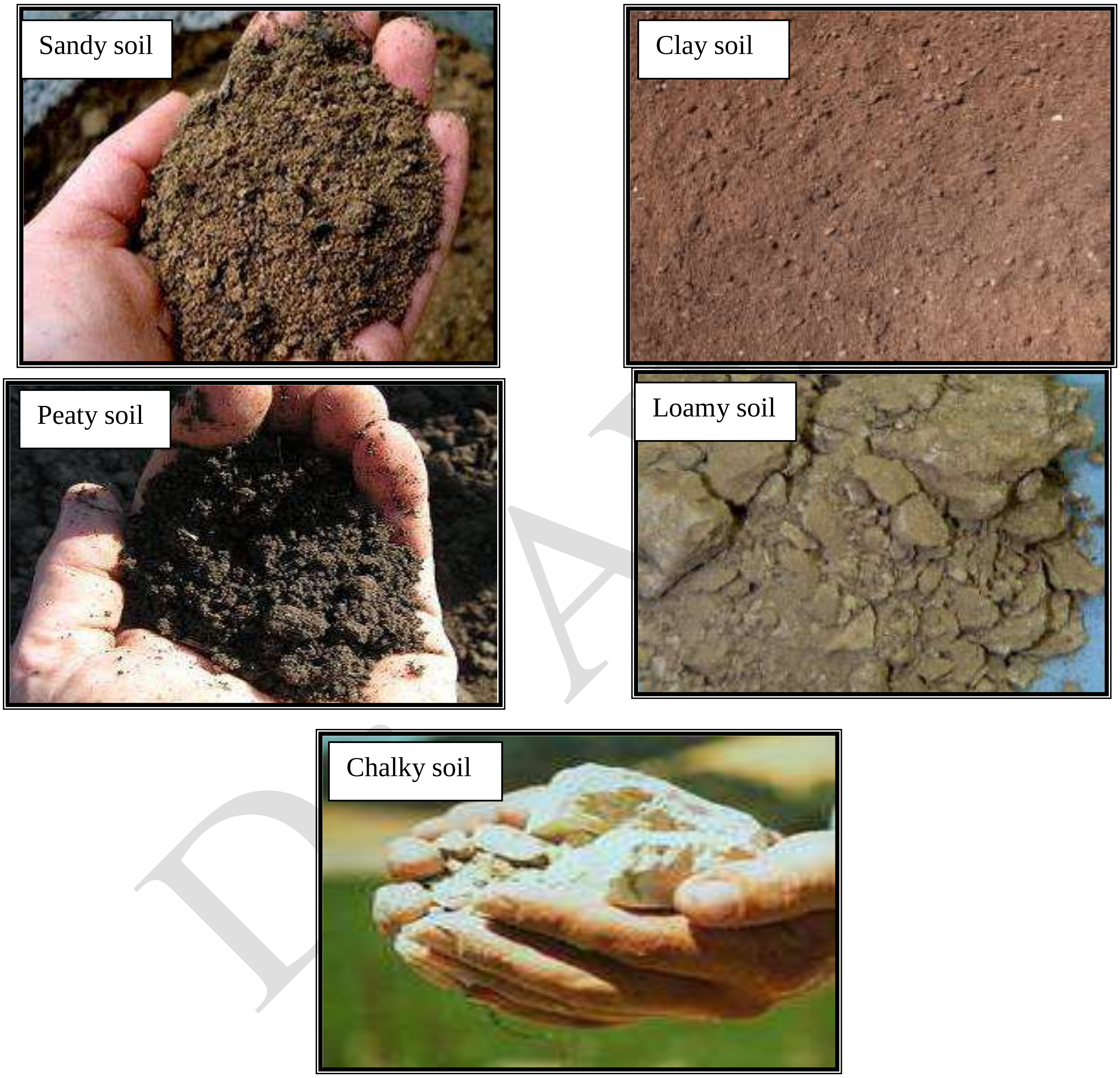

Figure 3: Type of soils.

Early Life

Microbiologists find clues to the nature of early life in a place where today only the most rudimentary life forms exist: the Dry Valleys of Antarctica.

The valleys' cold, dry winters (–40°C) preclude most multicellular life. However, in the austral summer (January), temperatures rise above zero and glacier melt trickles into streams. Long dormant cyanobacteria come to life, painting the streams orange.

They photosynthesize throughout the 24-hour daylight, fixing CO_2 and bubbling oxygen. The cyanobacteria can grow into thick masses of biofilm, called **microbial mats**; the mats support communities of protists and nematodes.

The fossils formed as silicate grains sediment in the mat and gradually replaced its organic structure. The sedimentary layers and wrinkled surface remain visible after billions of years. Remarkably, similar rock formations appear on Mars, offering evidence that our neighbor planet, too, supported ancient microbial life.

Ancient microbes formed even more complex microbial communities, called **stromatolites;** A stromatolite is a bulbous mass of layered limestone ($CaCO_3$) accreted by microbial mats. The mat layers can build over centuries, even more than a thousand years, reaching heights over 2 meters. The outermost layers of the mat contain oxygenic phototrophs, such as diatoms and filamentous cyanobacteria that exude bubbles of oxygen. A few millimeters below the surface, red light supports bacteria photolyzing H_2S to sulfate, which is then reduced by still lower layers of bacteria. This shared metabolism is another kind of cross feeding.

The Winogradsky Column

The **Winogradsky column** is an artificial microbial ecosystem and a long-term source of various bacteria for enrichment cultures.

Winogradsky columns have been used to isolate phototrophic purple and green bacteria, sulfate-reducing bacteria, and many other anaerobes. Named for the famous Russian microbiologist Sergei Winogradsky, the column was first used by Winogradsky in the late nineteenth century in his classic studies of soil microorganisms.

A Winogradsky column is prepared by filling a glass cylinder about half full with organically rich, preferably sulfide-containing mud into which carbon substrates have been mixed. The substrates determine which organisms are enriched. Fermentative substrates, such as glucose, that can lead to acidic conditions and excessive gas formation (which can create gas pockets that disrupt the enrichment and let in air).

The mud is supplemented with small amounts of calcium carbonate ($CaCO_3$) as a buffer and gypsum ($CaSO_4$) as a source of sulfate. The mud is packed tightly in the cylinder, taking care to avoid trapping air, and then covered with lake, pond, or ditch water (or seawater if it is a marine column). The top of the cylinder is covered to prevent evaporation, and the container is placed near a window that receives diffuse sunlight for a period of months.

In a typical Winogradsky column a diverse community of organisms develops.

Algae and cyanobacteria develop quickly in the upper portions of the water column; by producing O_2 these organisms help to keep this zone of the column oxic.

Decomposition processes in the mud lead to the production of organic acids, alcohols, and H_2, suitable substrates for sulfate reducing bacteria. Hydrogen sulfide (H_2S) from the sulfate reducers triggers the development of purple and green sulfur bacteria (an oxygenic phototrophs) that use sulfide as a photosynthetic electron donor.

Winogradsky columns have been used to enrich both aerobic and anaerobic prokaryotes (Fig. 4).

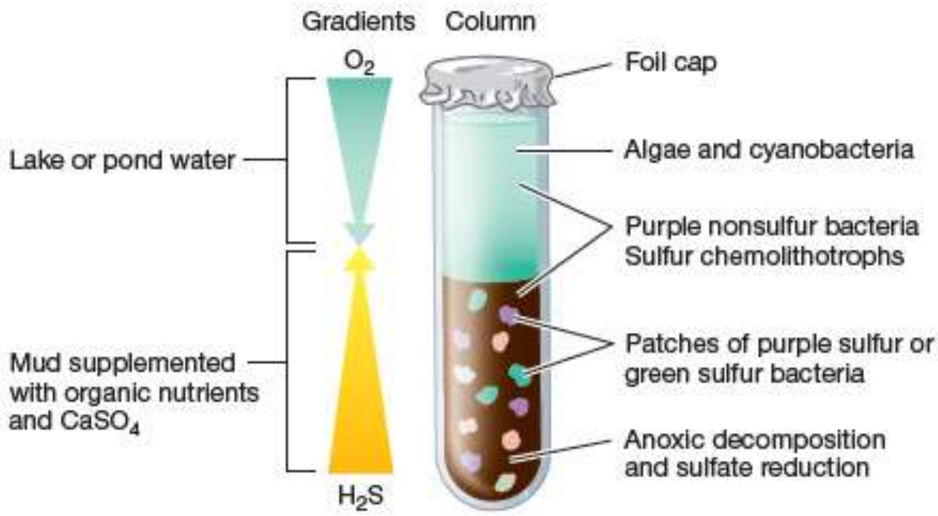

Figure 4: Winogradsky columns

A Phylogenetic Snapshot of Soil Prokaryotic Diversity

A single soil particle can contain many different microenvironments and can thus support the growth of several physiological types of microorganisms.

To examine soil particles directly for microorganisms, fluorescence microscopes are often used, the organisms in the soil having been previously stained with a fluorescent dye. To visualize a specific microorganism in a soil particle, fluorescent antibody staining or gene probes can also be used. Microorganisms can also be observed on soil surfaces directly by scanning electron microscopy.

Molecular community sampling of a typical vegetated surface has shown typically *thousands* of different species of *Bacteria* and *Archaea* in a single gram of soil, likely reflecting the numerous microenvironments present there.

Such an environmental sequence is called a *phylotype*. Besides very large species numbers, soil microbial diversity studies have also showed that diversity varies with soil type and geographical location. For example, analysis of an Alaska forest soil, an Oklahoma prairie soil, and a Minnesota farm soil (all sites in the USA) revealed approximately 5000, 3700, and 2000 different phylotypes, respectively.

The Alaska and Minnesota soils showed similar distributions at the phylum level of taxonomy (for example, *Proteobacteria*, *Acidobacteria*, *Bacteroidetes*, *Actinobacteria* and *Planctomycetes*) but shared only about 20% of their species in common.

In addition, lower bacterial diversity was observed in the farm soil than the Alaska soil, probably because modern intensive agricultural practices rely heavily on fertilization, low plant diversity, and the chemical suppression of unwanted plants and animals.

Among the bacteria in soil are autotrophs, heterotrophs, aerobes, anaerobes, and depending on the soil temperature, mesophiles and thermophiles. In addition to nitrogen-fixing, nitrifying, and denitrifying bacteria, soil contains bacteria that digest special substances such as cellulose, protein, pectin, butyric acid, and urea , many of the soil bacteria perform useful functions like decomposition of organic matter, conversion of soil constituents into useful materials, production of antibiotics in the soil, and biogeochemical cycling of elements like carbon, nitrogen, phosphorus, iron, sulfur and manganese.

Polluted soils are enriched in *Actinobacteria* and *Euryarchaeota*.

Soil fungi are mostly molds. Both mycelia and spores are present mainly in topsoil, the aerobic surface layer of the soil. Fungi serve two functions in soil: They decompose plant tissues such as cellulose and lignin, and their mycelia form networks around soil particles, giving the soil a crumbly texture. In addition to molds, yeasts are abundant in soils in which grapes and other fruits are growing.

Small numbers of cyanobacteria, algae (**found only on the soil surface, where they can carry on photosynthesis**), diatoms, protists (**mostly amoebas and flagellated protozoa they feed on bacteria and may help control bacterial populations**), and viruses are found in most soils (**infect mostly bacteria, but a few infect fungi, and quite a few infect plants and the survival of viruses in soil varies with environmental conditions and the particular virus. It ranges from hours to years**)

Viroids in soil are often spread by mechanical means and can cause serious plant diseases (Figure 5).

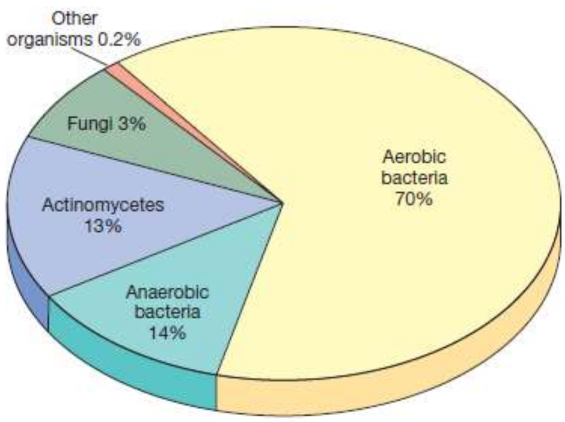

Figure 5: The relative proportions of various kinds of organisms found in soil. ‘‘Other organisms’’ include such things as algae, protists, and viruses.

FACTORS AFFECTING SOIL MICROORGANISMS

Soil microorganisms, like all other organisms, interact with their environment. Their growth is influenced both by abiotic factors and by other organisms. The microorganisms, in turn, affect the physical characteristics of soil and other organisms in the soil.

Factors that influencing microbial population include 1) Soil moisture, 2) Aeration, 3) Temperature, 4) pH and 5) Organic and inorganic nutrient supply. In addition to this, cultivation, ploughing, season and depth of soil also influence microbial population in soil.

1. **Soil moisture:** The moisture and oxygen content of soil are closely related. Spaces among soil particles ordinarily contain both water and oxygen, and aerobic organisms thrive there. Soil moisture governs microbial activity in two ways. Since water is the major component of protoplasm, an adequate supply must be available for vegetative growth and multiplication. However, where moisture becomes excessive, microbial proliferation was suppresses because the oversupply of water limits gaseous exchange and lowers the available oxygen supply, creating an anaerobic environment. Moisture is present in the form of film in soil pores. The amount of water increases with increase in porosity of soil. Soil moisture was affect through irrigation, drainage or management practices.
2. **Aeration:** The air is essential for the growth of the aerobic organisms. The water logging condition brings about a decrease in the abundance of aerobic organisms. The change from an aerobic to a largely anaerobic flora is effected by the disappearance of free oxygen because of its utilization by oxygen-requiring microorganisms, so that only microorganisms tolerant of low oxygen levels complete an aerobiosis are capable of proliferation.

3. **Temperature:** Soil temperature varies seasonally from below freezing to as high as 60°C at soil surfaces exposed to intense summer sunlight. All biological processes and temperature is thus prime factor of concern to the microorganisms. Each microorganism has an optimum temperature for growth. Most microorganisms are mesophilic that can able to grow between 25-35 °C. Certain species develop best at temperature below 20°C and they are termed as psychrophilic. Thermophilic microorganisms that grow readily at temperatures of 45°C to 65°C.

   Mesophilic and thermophilic bacteria are quite numerous in warm to hot soils, whereas cold tolerant mesophiles (and not true psychrophiles) are present in cold soils. Most soil molds are mesophilic and are found mainly in soils of moderate temperature. Surprisingly, in 2003, soil of cold high mountain ranges in the United States was found to greatly increase in number of fungi during the winter.

4. **pH:** Soil pH, which can vary from 2 to 9, is an important factor in determining which microorganisms will be present and neutral pH (6-8) is favorable for many types of microorganisms.

   Highly acidic or alkaline conditions tend to inhibit many common microbes, but some molds can grow at almost any soil pH. Molds thrive in highly acidic soils partly because of reduced competition from bacteria for available nutrients.

   Fertilizer containing ammonium salts has two effects on soil: (1) It provides a nitrogen source for plants; and (2) when it is metabolized by certain bacteria, the bacteria release nitric acid, which decreases the soil pH and increases the mold population.

5. **Organic and inorganic nutrients:** These organic and inorganic nutrients are very important for microorganisms a s these provide nutrition for growth, activity and survival of microorganisms in soil. The chemical factors are gases, acids, micro and macro elements and clay minerals etc. In the soil solution, gases and microorganisms are dissolved. However, the dissolved components are in constantly shifting equilibrium with the solid phase. The dead organic materials of plant and animal origin serve as total organic matter, which later is subjected to microbial colonization and decomposition. However, due to incorporation of green manures, crop residues etc., in soil, and the community size of microorganisms is increased. At the same time, application of these organic matters alters the composition of soil microflora, micro fauna and relative dominance of antagonistic microorganisms.

CAVES

Caves, places where soil or rock has disappeared, can be formed in four ways:

1. Slightly acid rainwater can dissolve away limestone.

2. Ocean waves pounding against cliff bottoms erode out sea caves.

3. Streams of hot lava running out of a volcano cool on their outer surfaces and harden into a tube. Inside, the lava remains liquid, running out, and leaving empty tunnels called lava tubes.

4. But strangest of all the ways is the just recently discovered process whereby microorganisms produce sulfuric acid that eats away rock. Five percent of all limestone caves are formed in this manner.

Lechuguilla Cave, the deepest cave in the continental United States, which is located in the Carlsbad Caverns National Park, New Mexico. It and Cueva de Villa Luz (Cave of the Lighted House) in Mexico are caves dissolved by microbial-produced sulfuric acid.

A film entitled ''The Mysterious Life of Caves'' was produced by NOVA, and was originally broadcast on PBS (Public Broadcasting System) on October 1, 2002. It features geo-microbiologists Penelope J. Boston and Diana Northrup visiting these and other caves, explaining the role of microbes, and some of the experiments that they have conducted in the caves. This film is now available for purchase. NOVA also has a web site describing this program, with a transcript, spectacular photos of Lechuguilla Cave, and interviews with the scientists. It also features links to many other web sites about caves. Please visit it at http://www.pbs.org/wgbh/nova/caves/ about.html.

Carlsbad and Lechuguilla caverns are no longer actively forming. However, Cueva de Villa Luz is vigorously growing. Hydrogen sulfide (H_2S) gas bubbles up from the bottom of the cave, forming sulfuric acid when it reacts with water. Microbes eating oil deposits deep below the cave release the H_2S. The walls of the cave are covered with **snottites**, mucus-like strings of bacterial colonies (Figure 6).

Figure 6: Snottites

These bacteria eat sulfur and drip sulfuric acid. The entire cave is like walking around in battery acid, so strong that it will eat away skin and clothing. Behind it, it leaves a telltale residue, beautiful crystals of gypsum (Figure 7). The bacteria living here are extremophiles, possibly much like many of the bacteria that were present when life first emerged on Earth. As we explore other planets such as Mars, and the moons of Jupiter, we are looking for similar extremophiles that may be living below their surfaces.

Figure 7: Chandelier Ballroom in Lechuguilla Cave. These gypsum chandeliers, up to 20 feet long, are thought to be the largest in the world. Note the size of the man in the red suit. *(Michael Nichols/NG Image Collection)*

Microbial interactions in soil
First: Harmful microbial interaction

The composition of the microflora micro-fauna of any habitat governed by the biological balance created through interactions and associations of all individuals present in a community. Any inhibitory effect of an organism created by any means to the other organisms known as harmful interactions or antagonistic interaction and the phenomenon of this activity called antagonism. Harmful interactions have three types. They are amensalism, competition, parasitism and predation.

1. **Amensalism**

(The Latin for *not* at the same table) Amensalism is the phenomenon where one microbial species is affected by the other species, where as other species is unaffected by first one. Amensalism accomplished by secretion of inhibitory substances such as antibiotics. Certain organisms may be of great practical importance, since they often produce antibiotics or other inhibitory substances, which affect the normal growth of other organisms. Antagonistic relationships are quite common in nature. For example, *Pseudomonas aeruginosa* is antagonistic towards *Aspergillus terreus* (Figure 8).

Figure 8: Amensalism: A Negative Microbe-Microbe Interaction. Antibiotic production and inhibition of growth of a susceptible bacterium on an agar medium.

2. Competition

A negative association may result from competition among species for essential nutrients. In such situations, the best-adapted microbial species will predominate or eliminate other species, which are dependent upon the same limited nutrient substance. This principle of competition was studied by Gause, who in 1934 described this as the competitive exclusion principle, he found that if the two competing ciliates overlapped too much in terms of their resource use, one of the two protozoan populations was excluded (Figure 9).

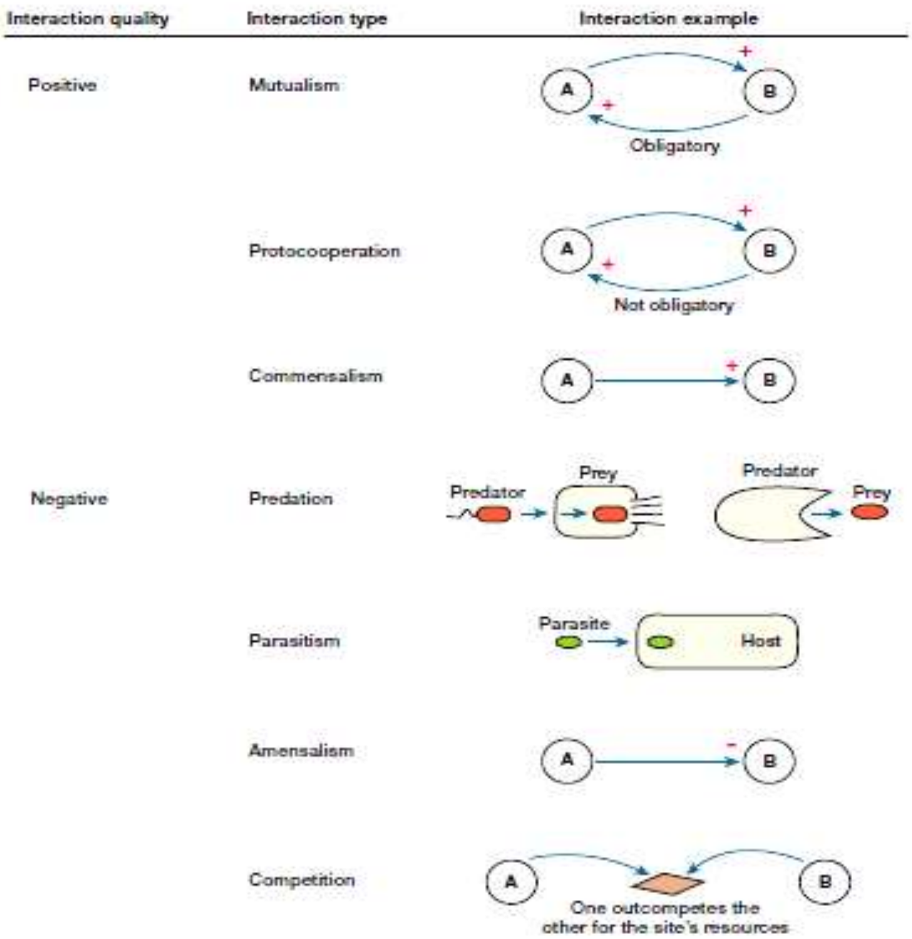

Figure 9: Microbial Interactions. Basic characteristics of positive (+) and negative (-) interactions that can occur between different organisms.

3. Parasitism

Parasitism defined as a one of the most complex microbial interactions; the line between parasitism and predation is difficult to define, this is a relationship in which one of a pair benefits from the other, and the host usually harmed.

The parasites feed on the cells, tissues or fluids of another organism, the host, which harmed. The parasite depends on the host and lives in intimate physical and metabolic contact with the host. All types of plants and animals are susceptible to attack by microbial parasites.

Some bacterial viruses can establish a lysogenic relationship with their hosts, and the viruses, in their prophage state, can confer positive new attributes on the host bacteria, as occurs with toxin production by *Corynebacterium diphtheria*.

4. Predation

A widespread phenomenon where the predator engulfs or attacks the prey. The prey can be larger or smaller than the predator, and this normally results in the death of the prey. An interesting array of predatory bacteria are active in nature. *Bdellovibrio, Vampirococcus,* and *Daptobacter*. Each of these has a unique mode of attack against a susceptible bacterium. *Bdellovibrio* penetrates the cell wall and multiplies between the wall and the plasma membrane, a periplasmic mode of attack, followed by lysis of the prey and release of progeny (Figure 5 and Figure 10).

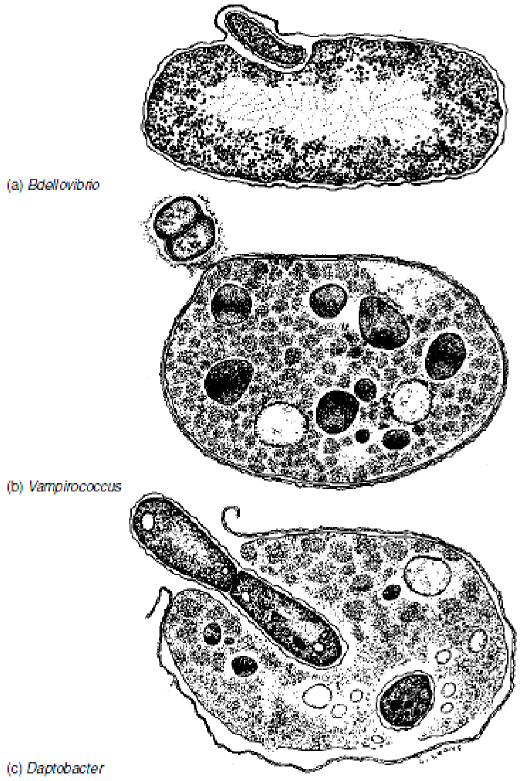

Figure 10: Examples of Predatory Bacteria Found in Nature. (a) *Bdellovibrio,* a periplasmic predator that penetrates the cell wall and grows outside the plasma membrane, (b) *Vampirococcus* with its unique epibiotic mode of attacking a prey bacterium, and (c) *Daptobacter* showing its cytoplasmic location as it attacks a susceptible bacterium.

Second: Beneficial Interactions

Microorganisms can be physically associated with other organisms in a variety of ways. One organism can be located on the surface of another, as an **ectosymbiont**. In this case, the ectosymbiont usually is a smaller organism located on the surface of a larger organism. In contrast, one organism can be located within another organism as an **endosymbiont**. There are also many cases in which microorganisms live on both the inside and the outside of another organism, a phenomenon called **ecto/ endosymbiosis**. Interesting examples of ecto/endosymbioses include fungi associated with plant roots (mycorrhizal fungi) often contain endosymbiotic bacteria, as well as having bacteria living on their surfaces.

Below is a description of beneficial interactions that include mutualism, protocooperation, and commensalism.

1. **Mutualsim**

Mutualism is an obligatory symbiotic relationship between two or more organism in which each organism benefits from the other. An example for this type of relationship is lichens. Lichens are composed of a primary producer, the algae, and a consumer, the fungus (Figure 11). The algae is a photoautotroph that synthesizes organic nutrients from light, carbon dioxide, and certain minerals. The fungus can get its organic carbon directly from the alga projections of fungal hyphae that penetrate the algal cell wall. The fungi also respires the O_2 generated during algal photophosythesis. In turn the fungus protects the algae from excess light intensities, provides water and minerals to it, and protects it from environmental stress.

Figure11: Lichens growing on a granite rock

2. Protocooperation

Protocooperation is a mutually beneficial relationship, similar to that which occurs in mutualism, but in protocooperation, the relationship is not obligatory. The organisms involved in this type of relationship can be separated, and if the required resources are provided in the environment, each microorganism will function independently. An example of this type of relationship is the symbiotic relationship between *Azotobacter* and *Cellulomonas* (Figure 12). *Azotobacter* uses glucose provided from cellulose degradation by *Cellulomonas*, which uses the nitrogen fixed by *Azotobacter*.

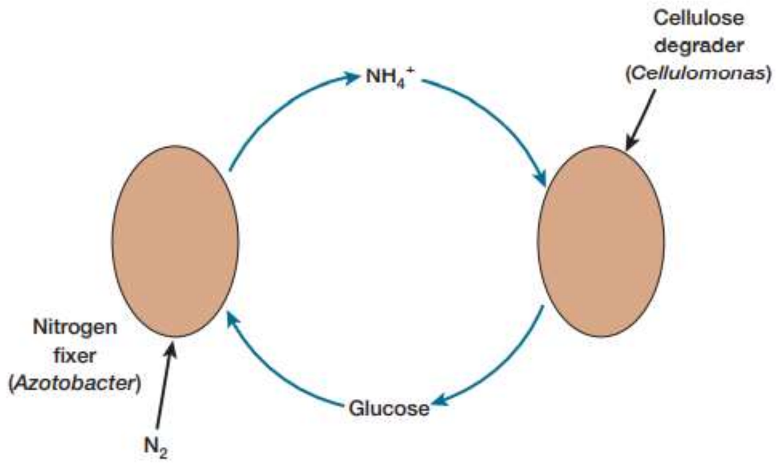

Figure 12: Protocooperative symbiotic relationship between *Cellulomonas* and *Azotobacter*.

3. Commensalism

Commensalism is a relationship in which one symbiont, the commensal, benefits while the other (sometimes called the host) is neither harmed nor helped. The commensal is not directly dependent on the host metabolically and causes it no particular harm. Commensalistic relationships between microorganisms include situations in which the waste product of one microorganism is the substrate for another species. An example is nitrification, the oxidation of ammonium ion (NH_4) to nitrite (NO_2) by microorganisms such as *Nitrosomonas*, and the subsequent oxidation of the nitrite to nitrate (NO_3) by *Nitrobacter* and similar bacteria. *Nitrobacter* benefits from its association with *Nitrosomonas* because it uses nitrite to obtain energy for growth while *Nitrosomonas* is neither harmed nor helped.

Occurrence and distribution of soil organisms

Distribution of organism within soil profile

Much time and effort have been spent in determining the kinds and members of organisms in soil and the sites colonized by them. Over geological time, micoorganisms have had great opportunity to become distributed worldwide.

Typically the abundance and biomass of most soil organisms is highest in the top 10 cm of soil and declines with depth in parallel with organic matter content and prey availability. Approximately 65% of the total microbial biomass is found in the top 25 cm of the soil profile. Below that depth, microbial densities typically decline by one to three orders of magnitude (Figure 13). Abundances of G- bacteria, fungi, and protozoa are typically highest at the soil surface, whereas G+ bacteria, Actinobacteria, and archaea tend to increase in proportional abundance with increasing depth. In forest soils, the litter layer is dominated by fungi, with fungal biomass being up to three times higher than that of bacterial biomass. Hyphal density of and root colonization by mycorrhzial fungi decrease substantially below 20 cm. Microbial grazers (e.g., protozoa, collembola) also decrease with depth, often more rapidly than either their bacterial or fungal prey.

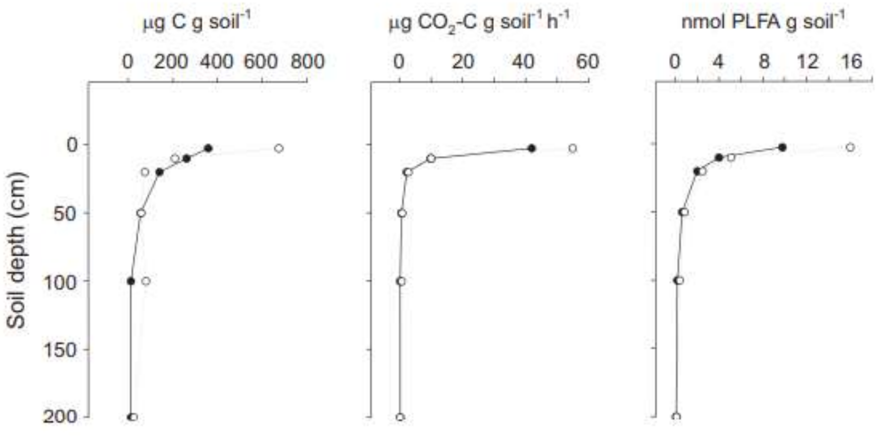

Figure 13: Microbial biomass with depth for two different soils as determined by three methods. (Left) Chloroform fumigation extraction. (Middle) Substrate-induced respiration. (Right) Phospholipid fatty acid (PLFA) analysis.

Association of organisms with plant roots

The portion of soil in which microorganism-mediated processes are under the influence of the root system is called the **rhizosphere**. The rhizosphere extends only a few millimeters from the root surface. However, it can contain up to 10^{11} microbial cells per gram of roots, with the collective microbial community being referred to as the root microbiome.

Plant roots release a wide variety of materials to their surrounding soil, including various alcohols, ethylene, sugars, amino and organic acids, vitamins, nucleotides, polysaccharides, and enzymes. These materials create unique environments for the soil microorganisms. The plant root surface, termed the **rhizoplane**, also provides a unique environment for microorganisms. As these gaseous, soluble, and particulate materials move from the plant to the soil, the rhizosphere and rhizoplane microorganisms not only increase their numbers, but their composition and function also change.

A wide range of bacteria in the rhizosphere can promote plant growth by producing complex chemical signals such as auxins, gibberellins, glycolipids, and cytokinins. Plant growth/promoting rhizobacteria include the genera *Pseudomonas* and *Achromobacter*. For this reason, total microbial count are found to be increased 10-50 fold in the rhizosphere region. A specific ratio known as R/S ratio is used to estimate microbial content of rhizosphere. R/S is the ratio of organism count in rhizosphere soil to their count in root-free soil. Structurally, the rhizosphere is known to harbor more G- bacteria (*Pseudomonas*, *Achromobacter*) and denitrifiers and fewer G+ (*Bacillus*, *Arthrobacter*). From the agronomic point of view, the abundance of nitrogen fixing and phosphate solubilizing bacteria in the rhizosphere assumes a great importance.

Association of organisms with plant herbage and litter

A wide variety of microorganisms are found on and in the aerial surfaces of plants, called the **phyllosphere**. The plant leaves and stems release organic compounds, and this can lead to massive development of microbes. High humidity and less sun exposure favors heterotrophs while both factors favor phototrophs. Plant stems and barks are often colonized by algae and lichens. Broadleaved plants support more organisms on their leaf surfaces than do grasses. Gram negative and yellow pigmented bacteria dominate the bacterial flora in the phyllosphere, while yeasts dominate the fungal flora.

The genera present on plant leaves and stems include *Sphingomonas*, which is especially equipped to survive with the high levels of UV irradiation occurring on these plant surfaces. This bacterium, also common in soils and waters, can occur at 108 cells per gram of plant tissue. *Sphingomonas* often represents a majority of the culturable species. Phyllosphere microorganisms play important roles in protection and possibly harm to the plant.

Not only leaves and stems are occupied with microorganisms but also fruits and seeds. The region, which is adjacent to the seed surface is termed as **spermosphere**. Healthy seeds carry specific bacterial flora in respect of number and species some of which are phyllosphere members. Some spermosphere members produce auxins, vitamins, and gibberellin-like substances that benefit emergent seedlings. Other organisms are known to produce substances that delay seed germination and repress plant pathogens.

As leaves and small stems fall to the ground and turn to litter, the number of bacteria thereon increases sharply. The bacterial population of moist litter exceeds that of the phyllosphere by two orders of magnitude.

Influences of agricultural managements on soil organism

Any management practice that affects soil water content, temperature, aeration, pH, and organic and nitrogen levels affects the soil biota. Some of the most important soil managements are soil tillage, addition of pesticides and chemicals, and clear-cutting and controlled burning.

1- Soil tillage

Conventional tillage stimulates microbial activity due to disruption of soil aggregates and better exposure and aeration of their degradable material.

2- Biocides and chemicals

Herbicides and insecticides when applied reach the soil in sufficient concentrations and cause injury to soil organisms. The nitrifying bacteria are the most sensitive to such chemicals. The effects are indirectly by injury of the host plant which decreases nodule formation and thus nitrogen fixation.

Fertilizers when applied to small areas may lead to local inhibition of microbial activity by causing osmotic stress. Ammonia concentrations in these fertilizers have biocidal effects on nitrifying bacteria.

3- Clear-cutting and controlled burning

Clear cutting has an impact on soil biota for several years following the year of harvesting. Most studies show marked increase in the biomass of both the microflora and fauna during the first few years. One reason is the availability of dead roots used for decomposition.

Controlled burning is used for land clearing and shifting crops. During burning both microflora and fauna are diminished but both groups recover rapidly as new plant growth and litter accumulation occur.

Nutrient cycles

The key nutrients for life are cycled by both microorganisms and macroorganisms, but for any given nutrient, it is microbial activities that dominate. Understanding how microbial nutrient cycles work is important because the cycles and their many feedback loops are essential for plant agriculture and the overall health of sustainable planet life (Figure 14).

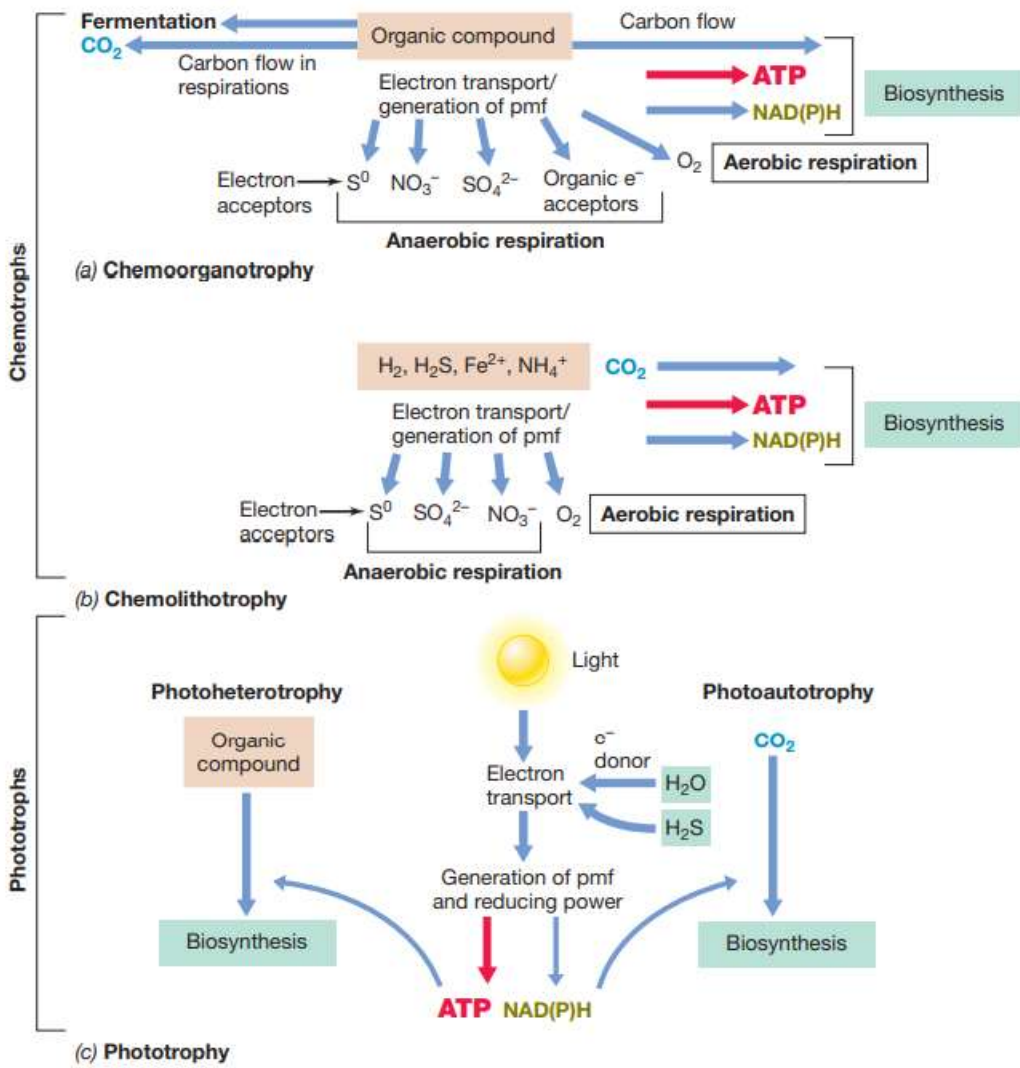

Figure 14: Catabolic diversity. (a) Chemoorganotrophs. (b) Chemolithotrophs. (c) Phototrophs. Chemoorganotrophs differ from chemolithotrophs in two important ways: (1) The nature of the electron donor (organic versus inorganic compounds, respectively), and (2) The nature of the source of cellular carbon (organic compounds versus CO_2 respectively).

The carbon cycle

On a global basis, carbon (C) cycles as CO_2 through all of Earth's major carbon reservoirs: the atmosphere, the land, the oceans, freshwaters, sediments and rocks, and biomass (Figure 15).

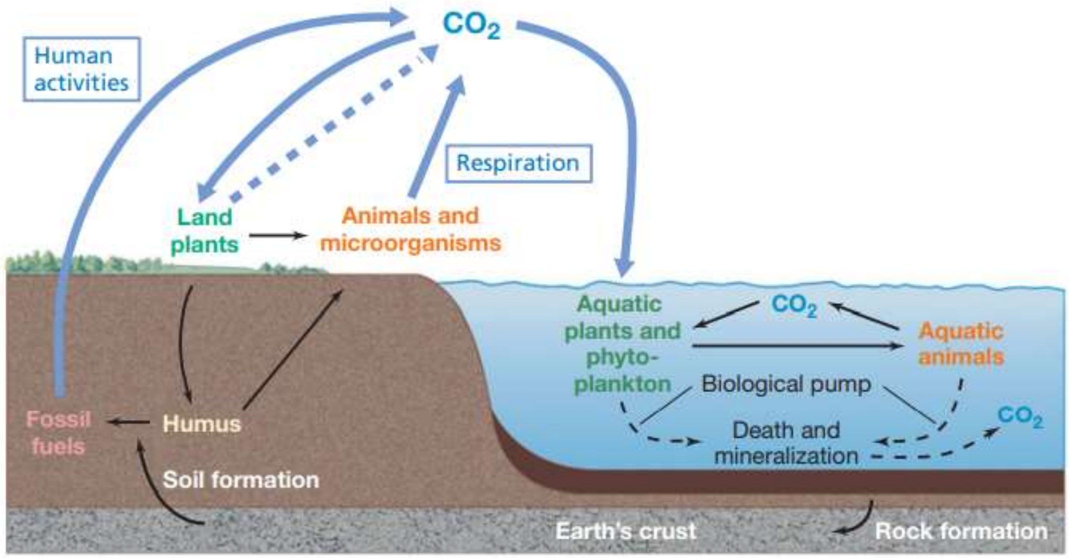

Figure (15): The carbon cycle. The carbon and oxygen cycles are closely connected, as oxygenic photosynthesis both removes CO2 and produces O2, and respiratory processes both

A large amount of C is found in land plants. This is the organic C of forests, grasslands, and agricultural crops—the major sites of phototrophic CO_2 fixation. However, more C is present in dead organic material, called **humus**, than in living organisms. Humus is a complex mixture of organic materials that have resisted rapid decomposition and is derived primarily from dead plants and microorganisms. Some humic substances are quite persistent, with a decomposition time of several decades, but certain other humic components decompose much more rapidly. The most rapid means of transfer of C is via the atmosphere. Carbon dioxide is removed from the atmosphere primarily by photosynthesis of land plants and marine microorganisms and is returned to the atmosphere by respiration of animals and chemoorganotrophic microorganisms. The single most important contribution of CO_2 to the atmosphere is by microbial decomposition of dead organic material, including humus. However, in the past 50 years human activities have increased atmospheric CO_2 levels by nearly 20%, primarily from the burning of fossil fuels. This rise in CO_2, a major greenhouse gas, has triggered a period of steadily increasing global temperatures called **global warming**.

Photosynthesis and Decomposition

New organic compounds are biologically synthesized on Earth only by CO_2 fixation by phototrophs and chemolithotrophs. However, phototrophic organisms are abundant in nature only in habitats where light is available. There are two groups of oxygenic phototrophic organisms: plants and microorganisms. Plants are the dominant phototrophic organisms of terrestrial environments, whereas phototrophic microorganisms dominate in aquatic environments.

The redox cycle for C (Figure 16) begins with photosynthetic CO_2 fixation, driven by the energy of light. Phototrophic organisms also carry out respiration, both in the light and the dark. The overall equation for respiration is the reverse of oxygenic photosynthesis: For organic compounds to accumulate, the rate of photosynthesis must exceed the rate of respiration. In this way, autotrophic organisms build biomass from CO_2, and then this biomass in one way or another supplies the carbon heterotrophic organisms need.

Organic compounds are degraded biologically to CH_4 and CO_2. Carbon dioxide, most of which is of microbial origin, is produced by aerobic and other forms of respiration. Methane is produced in anoxic environments by methanogens from the reduction of CO_2 with hydrogen (H_2) or from the splitting of acetate. Methane produced in anoxic habitats is insoluble and diffuses to oxic environments, where it is either released to the atmosphere or oxidized to CO_2 by methanotrophs. Hence, most of the carbon in organic compounds eventually returns to CO_2, and the links in the carbon cycle are closed.

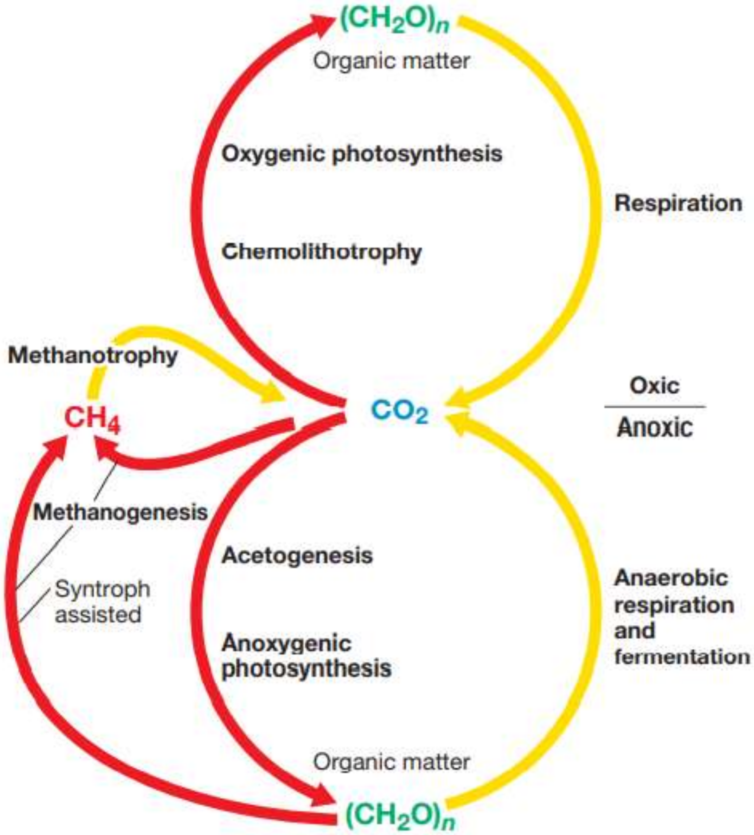

Figure (16): Redox cycle for carbon. The diagram contrasts autotrophic processes (CO_2 to organic compounds) and heterotrophic processes (organic compounds to CO_2). Yellow arrows indicate oxidations; red arrows indicate reductions.

Methanogenesis

Most organic compounds are oxidized in nature by aerobic microbial processes. However, because oxygen is a poorly soluble gas and is actively consumed when available, much organic carbon still ends up in anoxic environments. Methanogenesis, the biological production of CH_4, is a major process in anoxic habitats and is catalyzed by a large group of Archaea, the methanogens, which are strict anaerobes. Most methanogens can use CO_2 as a terminal electron acceptor in anaerobic respiration, reducing it to CH_4 with H_2 as electron donor. Only a very few other substrates, chiefly acetate, are directly converted to CH_4 by methanogens. To convert most organic compounds to CH_4, methanogens must team up with partner organisms that can supply them with precursors for methanogenesis. This is done by the syntrophs.

Anoxic Decomposition and Syntrophy

Syntrophy is a process in which two or more organisms cooperate in the anaerobic degradation of organic compounds. Polysaccharides, proteins, lipids, and nucleic acids from organic compounds find their way into anoxic habitats, where they are catabolized. After hydrolysis, these monomers become major electron donors for energy metabolism. For the breakdown of a typical polysaccharide such as cellulose, the process begins with cellulolytic bacteria. These organisms hydrolyze cellulose into glucose, which is catabolized by fermentative organisms to short-chain fatty acids (acetate, propionate, and butyrate), alcohols such as ethanol and butanol, H_2, and CO_2. H_2 and acetate are consumed by methanogens directly, but the bulk of the carbon remains in the form of fatty acids and alcohols; these cannot be directly catabolized by methanogens and require the activities of syntrophic bacteria.

The key bacteria in the conversion of organic compounds to CH_4 are the syntrophs. Syntrophs are secondary fermenters because they ferment the products of the primary fermenters.

The heart of syntrophic reactions is interspecies H_2 transfer, H_2 production by one partner linked to H_2 consumption by the other. The H_2 consumer can be any one of a number of physiologically distinct organisms: denitrifying bacteria, ferric iron–reducing bacteria, sulfate-reducing bacteria, acetogens, or methanogens.

Consider ethanol fermentation to acetate plus H_2 by a syntroph coupled to the production of methane. As can be seen in figure 17, the syntroph carries out a reaction whose standard free-energy change is positive. However, the H_2 produced by the syntroph can be used as an electron donor by a methanogen in an exergonic reaction. When the two reactions are summed, the overall reaction is exergonic, and the free energy released is shared by both organisms.

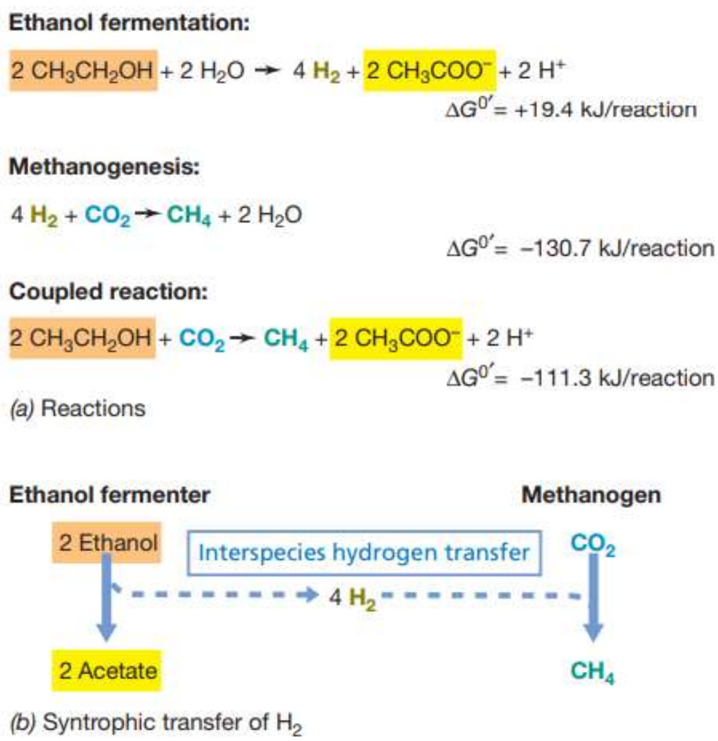

Figure 17: Syntrophy: Interspecies H_2 transfer. Shown is the fermentation of ethanol to methane and acetate by syntrophic association of an ethanol-oxidizing syntroph and a H_2-consuming partner (in this case, a methanogen). (a) Reactions involved. The two organisms share the energy released in the coupled reaction. (b) Nature of the syntrophic transfer of H_2

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butyrate to acetate plus H2 by the fatty acid–oxidizing syntroph *Syntrophomonas*:

$$\text{Butyrate}^- + 2\,H_2O \rightarrow 2\,\text{acetate}^- + H^+ + 2\,H_2 \qquad \Delta G^{0\prime} = +48.2\text{ kJ}$$

The free-energy change of this reaction is highly unfavorable, and in pure culture *Syntrophomonas* will not grow on butyrate. However, if the H_2 produced by *Syntrophomonas* is consumed by a partner organism, *Syntrophomonas* grows on butyrate in coculture with the H_2 consumer. In a syntrophic relationship, the removal of H_2 by a partner organism pulls the reaction in the direction of product formation and thereby affects the energetics of the reaction.

Carbon cycle is linked to other nutrient cycles

Although it is convenient to consider carbon cycling as a series of reactions separate from those in other nutrient cycles, in reality, all nutrient cycles are coupled cycles; major changes in one cycle affect the functioning of others. For example, consider the C and nitrogen cycles. The rate of primary productivity (CO_2 fixation) is controlled by several factors, in particular by the amount of photosynthetic biomass and by available nitrogen. Thus, large-scale reductions in biomass by, for example, widespread deforestation, reduce rates of primary productivity and increase levels of CO_2. High levels of organic carbon stimulate nitrogen fixation and this in turn adds more fixed N to the pool for primary producers; low levels of organic carbon have just the opposite effect. High levels of ammonia stimulate primary production and nitrification, but inhibit N_2 fixation due to the inhibition of nitrogenase gene expression (bacteria will not spend energy to fix N_2 and produce ammonia when ammonia is already present in the environment).

The nitrogen cycle

Nitrogen (N) is an essential element for life and exists in a number of oxidation states. Nitrogen is transformed through these states by microorganism through four major processes: nitrogen fixation, nitrification, denitrification, and anammox. These and other key N transformations are summarized in the redox cycle shown in Figure 23.

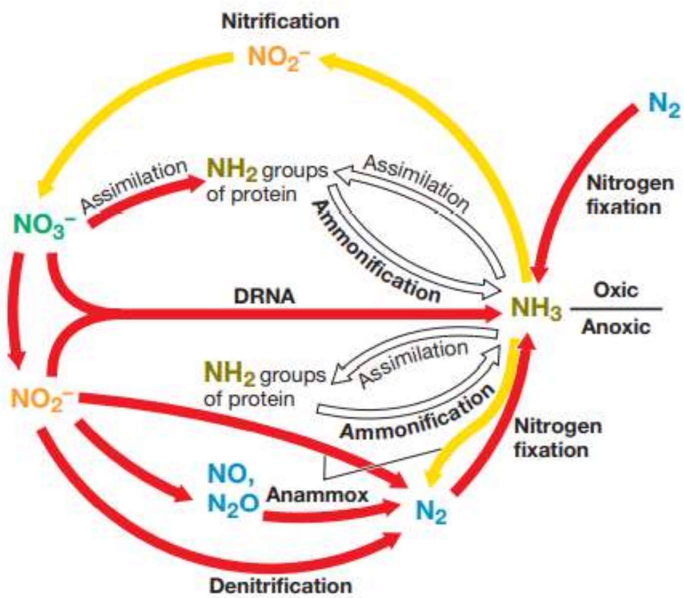

Figure 23: Redox cycle for nitrogen. Oxidation reactions are shown by yellow arrows and reductions by red arrows. Reactions without redox change are in white. DRNA, dissimilative reduction of nitrate to ammonia.

Nitrogen fixation

Nitrogen fixation is the bacterial process whereby molecular N_2 gas is converted to reactive, biologically available forms of nitrogen (NH_3) that can be assimilated into organic forms such as amino acids and nucleotides. The vast majority of nitrogen on Earth is present as molecular N_2, however, this form is not available to living organisms other than those that can fix nitrogen for growth. The process of nitrogen fixation is agriculturally important, supporting the nitrogen needs of major crops.

Only certain prokaryotes can fix nitrogen. Some nitrogen fixing bacteria are free-living and require no host in order to carry out the process. For example, under aerobic conditions a wide range of free-living microbial genera contribute to this process the best known are *Azotobacter* and *Azospirillum*. Under anaerobic conditions the most important free-living nitrogen fixers are members of the genus *Clostridium*. By contrast, others are symbiotic and can fix nitrogen only in association with certain plants. The best examples for such associations include *Rhizobium* and *Bradyrhizobium* with legumes and *Frankia* in association with many woody shrubs.

The nitrogen-fixation process involves a sequence of reduction steps that require major energy expenditures (Figure 24). Ammonia, the product of nitrogen reduction, is immediately incorporated into organic matter as an amine. Reductive processes are extremely sensitive to O_2 and must occur under anaerobic conditions even in aerobic microorganisms. Protection of the nitrogen-fixing enzyme is achieved by a variety of mechanisms, including physical barriers, as occurs with heterocysts in some cyanobacteria, O_2 scavenging molecules, and high rates of metabolic activity.

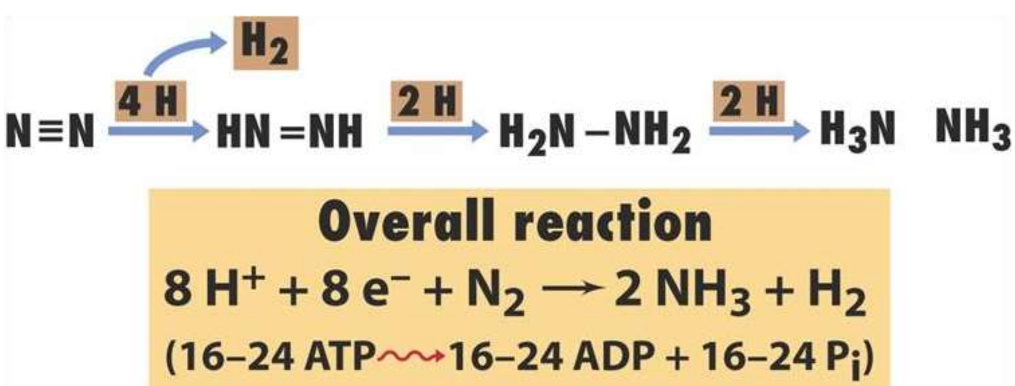

Figure 24: Energy required for nitrogen fixation

Symbiotic nitrogen fixation

Several microbial genera are able to form nitrogen-fixing nodules with legumes. These include *Bradyrhizobium*, *Sinorhizobium*, and *Rhizobium*. The mechanism of this interaction will be illustrated by work with *Rhizobium*. The genus *Rhizobium* is an important member of the rhizosphere community. This bacterium can also establish a symbiotic association with legumes and fix nitrogen for use by the plant. Infection of legume roots by rhizobia leads to the formation of **root nodules** (Figure 25) in which the bacteria fix gaseous nitrogen (N_2). Nitrogen fixation in root nodules accounts for a fourth of the N_2 fixed annually on Earth and is of enormous agricultural importance, as it increases the fixed nitrogen content of soil. Nodulated legumes can grow well on unfertilized soils that are nitrogen deficient, while other plants grow poorly on them (Figure 26). *Rhizobium* contains a large plasmid that encodes essential genes for infection and nodulation of the susceptible host plant. Plasmid-encoded genes also influence the range of host plants that *Rhizobium* can nodulate.

Figure 25 : Soybean root nodules

Figure 26 : Effect of nodulation on plant

Rhizobium can fix N_2 when grown in pure culture only under microaerophilic conditions (a low-oxygen environment is necessary because **nitrogenases** are inactivated by high levels of O_2). Inside the nodule O_2 levels are precisely controlled by the O_2-binding protein **leghemoglobin**. Production of this iron-containing protein in healthy N_2-fixing nodules is induced through the interaction of the plant and bacterial partners. Leghemoglobin functions as an “oxygen buffer” to keep unbound O_2 within the nodule low. The ratio of leghemoglobin-bound O_2 to free O_2 in the root nodule is on the order of 10,000:1. There is a marked specificity between the species of legume and rhizobium that can establish a symbiosis. A particular rhizobial species is able to infect certain species of legumes but not others. If legumes are inoculated with the correct rhizobial strain, leghemoglobin-rich, N_2-fixing nodules develop on their roots.

Steps in Root Nodule Formation (Figure 27)

1. Recognition of the correct partner by both plant and bacterium and attachment of the bacterium to the root hairs
2. Secretion of oligosaccharide signaling molecules (nod factors) by the bacterium
3. Bacterial invasion of the root hair.
4. Movement of bacteria to the main root by way of the infection thread
5. Formation of modified bacterial cells (bacteroids) within the plant cells and development of the N2-fixing state
6. Continued plant and bacterial cell division, forming the mature root nodule.

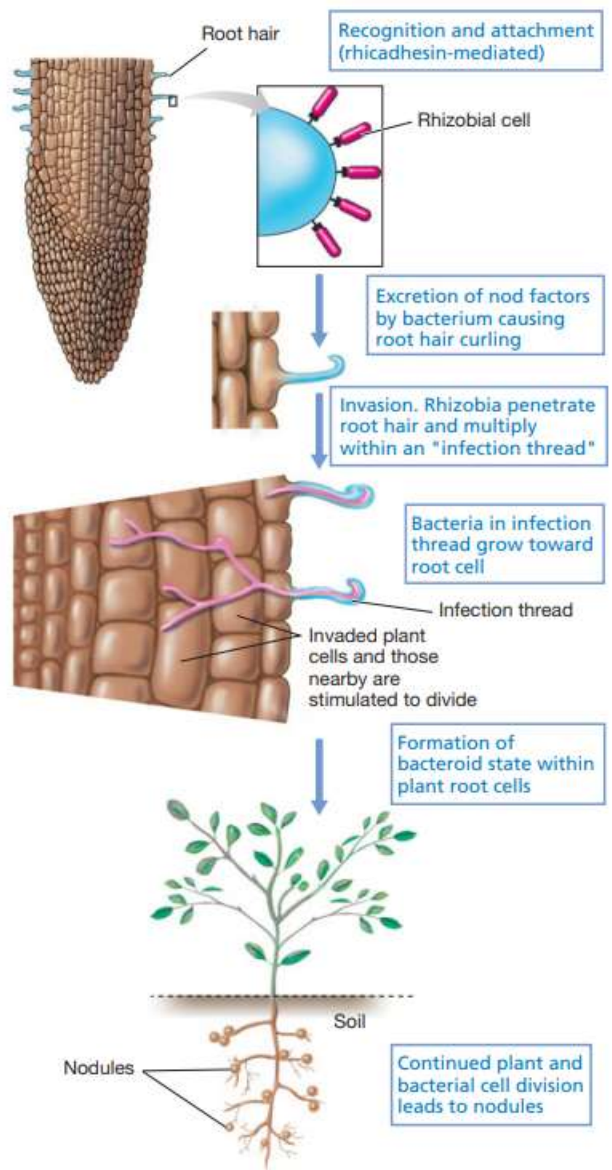

Figure 27: Steps in the formation of a root nodule in a legume infected by *Rhizobium spp.*

The nitrogen cycle (Part two)

Nitrification

The inorganic nitrogen compounds ammonia (NH_3) and nitrite (NO_2^-) are chemolithotrophic substrates and are oxidized aerobically by the "nitrifying bacteria" in the process of nitrification. Nitrifying bacteria are widely distributed in soils and water. One group (for example, *Nitrosomonas*) oxidizes NH_3 to nitrite (NO_2^-), and another group (for example, *Nitrobacter* and *Nitrospira*) oxidizes NO_2^- to NO_3^-. The complete oxidation of NH_3 to NO_3^-, an eight-electron transfer, is thus carried out by the concerted activity of two groups of organisms, a process discovered by the Russian microbiologist Winogradsky at the end of the nineteenth century. Winogradsky also showed that the nitrifying bacteria were autotrophs, obtaining all of their carbon from CO_2.

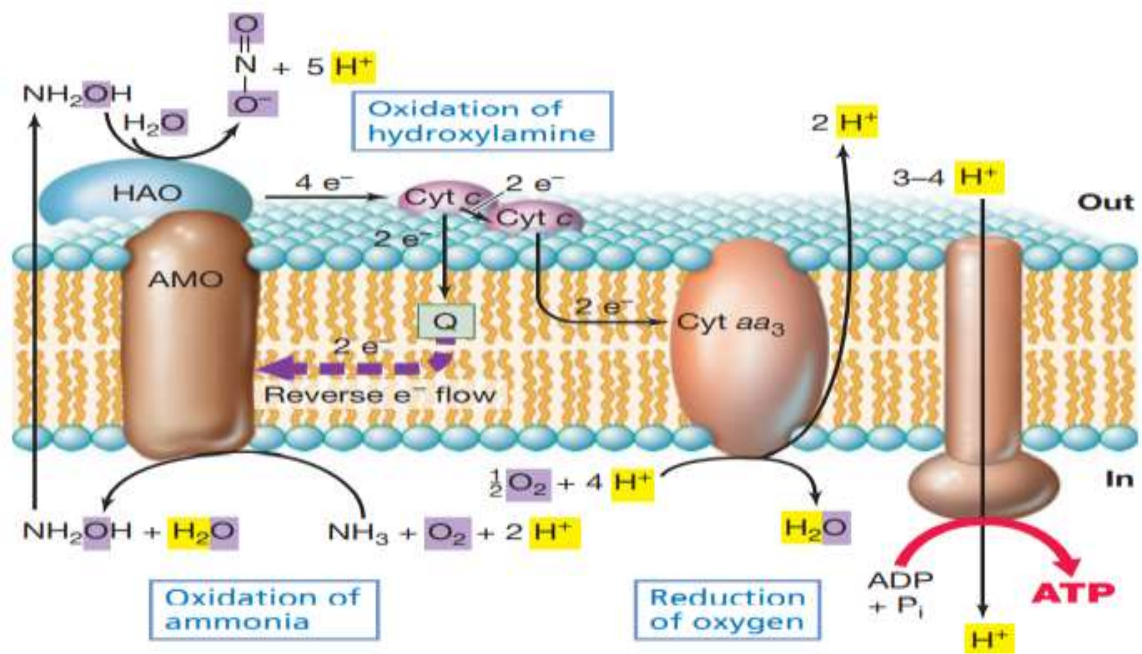

Figure 28: Oxidation of NH_3 and electron flow in ammonia oxidizing bacteria. The reactants and the products of this reaction series are highlighted. AMO, ammonia monooxygenase; HAO, hydroxylamine oxidoreductase; Q, ubiquinone.

The bioenergetics of nitrification is based on the same principles of other chemolithotrophic reactions: Electrons from reduced inorganic substrates (in this case, reduced nitrogen compounds) enter an electron transport chain, and electron flow establishes a proton motive force that drives ATP synthesis (Figure 28).

Several key enzymes participate in the oxidation of reduced nitrogen compounds. In ammonia-oxidizing bacteria, NH_3 is oxidized by ammonia monooxygenase producing hydroxylamine (NH_2OH) and H_2O. A second key enzyme, hydroxylamine oxidoreductase, then oxidizes NH_2OH to NO_2^-, removing four electrons in the process. Ammonia monooxygenase is an integral membrane protein, whereas hydroxylamine oxidoreductase is periplasmic (Figure 28). In the reaction carried out by ammonia monooxygenase, two electrons and protons are needed to reduce one atom of (O_2) to H_2O. These electrons originate from the oxidation of hydroxylamine and are supplied to ammonia monooxygenase from hydroxylamine oxidoreductase via cytochrome c and ubiquinone (Figure 28). Thus, for every four electrons generated from the oxidation of NH_3 to NO_2^-, only two actually reach cytochrome aa3, the cytochrome that interacts with O_2 to form H_2O, and yield energy.

Nitrite-oxidizing bacteria employ the enzyme nitrite oxidoreductase to oxidize NO_2^- to NO_3^-, with electrons traveling a very short electron transport chain to the terminal oxidase. Thus, growth yields of nitrifying bacteria (grams of cells produced per mole of substrate oxidized) are low (Figure 29).

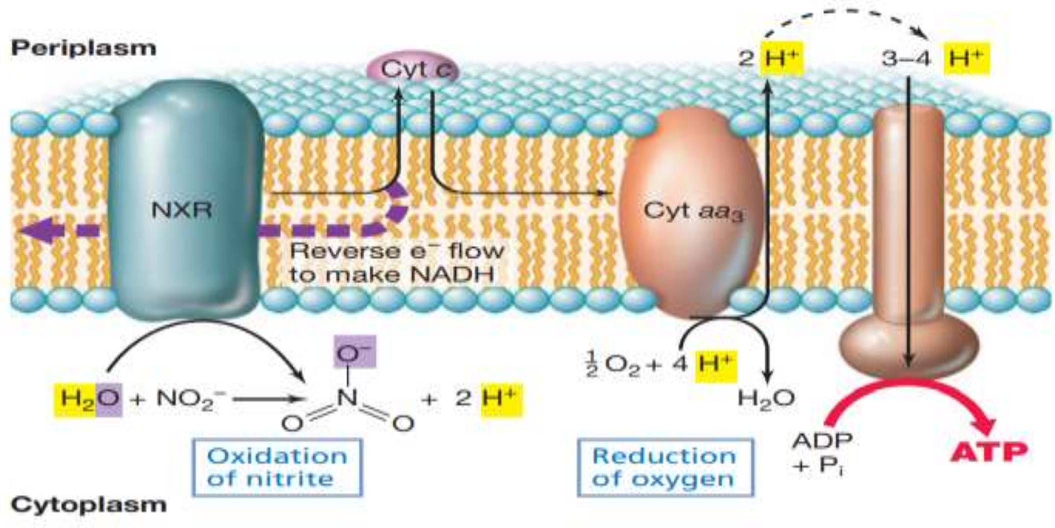

Figure 29: Oxidation of NO_2^{2-} to NO_3^{2-} by nitrifying bacteria. The reactants and products of this reaction series are highlighted to follow the reaction. NXR, nitrite oxidoreductase.

Nitrate reduction and denitrification

Inorganic nitrogen compounds are some of the most common electron acceptors in anaerobic respiration. One of the most common alternative electron acceptors is nitrate, NO_3^-, which can be reduced to nitrous oxide (N_2O), nitric oxide (NO), and dinitrogen (N_2) . This process is called denitrification which is a form of anaerobic respiration in which nitrate is reduced to nitrogen gases under anoxic conditions. Because these products of nitrate reduction are all gaseous, they can easily be lost from the environment (Figure 30). As a source of nitrogen, N_2 is much less available to plants and microorganisms than is NO_3^-, so for agricultural purposes denitrification is a detrimental process. For example, if agricultural fields fertilized with nitrate fertilizer become waterlogged following heavy rains, anoxic conditions can develop and denitrification can be extensive; this removes fixed nitrogen from the soil. However, in sewage treatment denitrification is beneficial because it converts NO_3^- to N_2. This transformation decreases the load of fixed nitrogen in the sewage treatment effluent that can stimulate algal growth in receiving waters, such as rivers and streams.

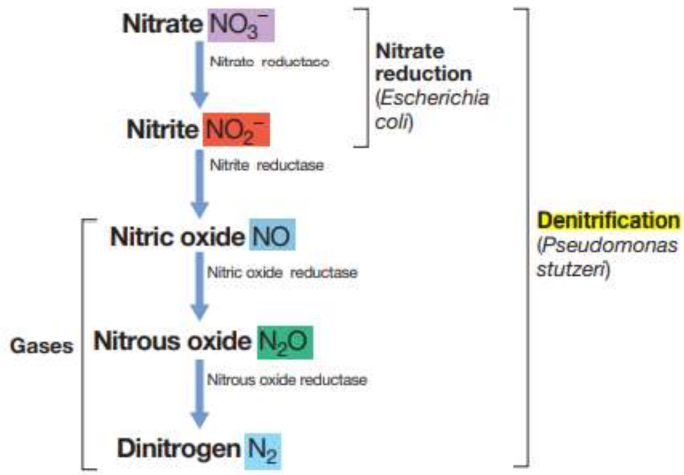

Figure 30: Steps in the dissimilative reduction of nitrate.

The enzyme that catalyzes the first step of dissimilative nitrate reduction is nitrate reductase, The first product of nitrate reduction is nitrite (NO_2^-), and the enzyme nitrite reductase reduces it to NO. The production of gaseous products by denitrification is of greatest global significance because denitrification consumes a fixed form of nitrogen (NO_3^-) and produces gaseous nitrogen compounds, some of which are of environmental significance. For example, N_2O can be converted to NO by sunlight, and NO reacts with ozone (O_3) in the upper atmosphere to form NO_2^-. When it rains, NO_2^- returns to Earth as nitrous acid (HNO_2) in so-called acid rain. The biochemistry of dissimilative nitrate reduction has been studied in detail in several organisms, including *Escherichia coli*, in which NO_3^- is reduced only to NO_2^-, and *Paracoccus denitrificans* and *Pseudomonas stutzeri*, in which denitrification occurs. In *P. denitrificans* and *P. stutzeri*, nitrogen oxides are formed from NO_2^- by the enzymes nitrite reductase, nitric oxide reductase, and nitrous oxide reductase (Figure 31). During electron transport, a proton motive force is established, and ATPase functions to produce ATP in the usual fashion.

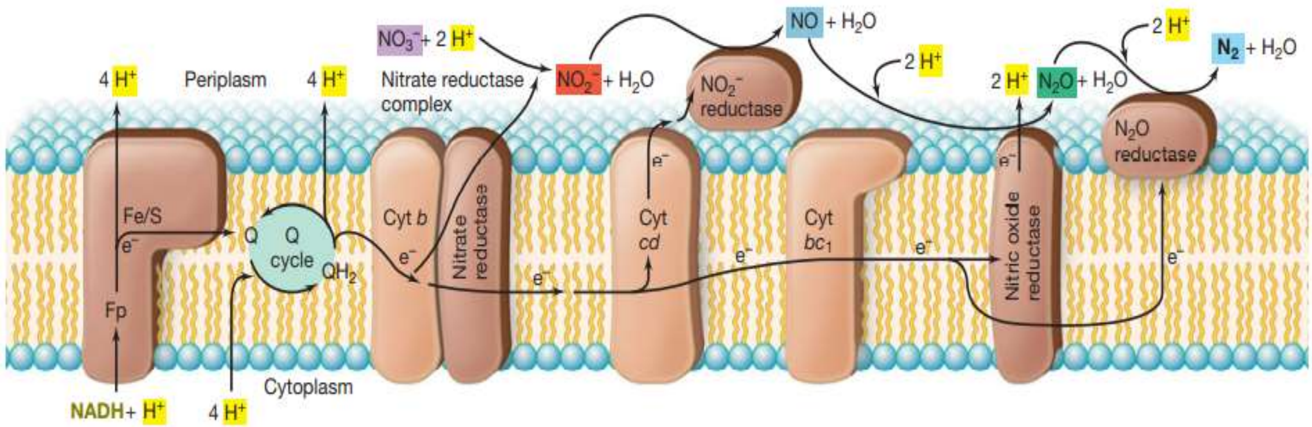

Figure 31: Scheme for electron transport in membranes of *Pseudomonas stutzeri* during denitrification. Nitrate and nitric oxide reductases are integral membrane proteins, whereas nitrite and nitrous oxide reductases are periplasmic enzymes.

Anammox

Ammonia can be oxidized under anoxic conditions by the bacterium *Brocadia* in the process called anammox (Anaerobic ammonia oxidation). In this reaction, NH_3 is oxidized anaerobically with NO_2^- as the electron acceptor, forming N_2 as the final product which is released to the atmosphere. Although anammox is a major process in sewage and marine sediments, it is not significant in well drained oxic soils.

$$NH_4^+ + NO_2^- \rightarrow N_2 + 2\,H_2O \qquad \Delta G^{0\prime} = -357\ \text{kJ}$$

The first anammox organism discovered, *Brocadia anammoxidans*, is a member of the Planctomycetes phylum of Bacteria. Planctomycetes are unusual Bacteria, lacking peptidoglycan and containing membrane-enclosed compartments of various types inside the cell. In cells of *B. anammoxidans* the compartment is the anammoxosome, and it is within this structure that the anammox reaction occurs (Figure 32).

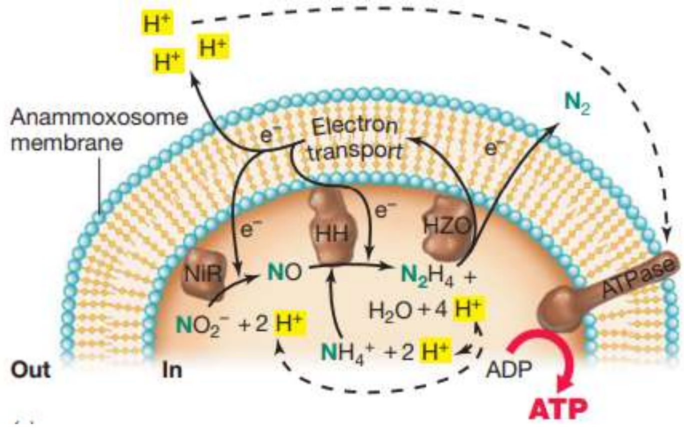

Figure 32: Reactions in the anammoxosome. NiR, nitrite reductase, HH, hydrazine hydrolase; HZO, hydrazine dehydrogenase.

The anammoxosome is surrounded by a dense membrane structure composed of untypical membrane lipids that prevent diffusion of toxic substances from the anammoxosome into the cytoplasm. These include, in particular, the compound hydrazine (N_2H_4), a very strong reductant. In the anammox reaction, NO_2^- is first reduced to nitric oxide (NO) by nitrite reductase, and then NO reacts with ammonium (NH_{4+}) to yield N_2H_4 by activity of the enzyme hydrazine hydrolase. N_2H_4 is then oxidized to N_2 plus electrons by the enzyme hydrazine dehydrogenase. Some of the electrons generated at this step enter the anammoxosome electron transport chain that yields a proton motive force and ATP by ATPase, while others feed back into the system to drive the electron-consuming earlier steps (Figure 32). Anammox bacteria are autotrophs, they grow with CO_2 as their sole carbon source and use NO_2^- as an electron donor to produce energy.

The Sulfur Cycle and Sulfur Bacteria

The **sulfur cycle** which involves the movement of sulfur through an ecosystem, resembles the nitrogen cycle in several respects. Sulfhydryl (-SH) groups in proteins of dead organisms are converted to hydrogen sulfide (H_2S) by a variety of microorganisms. This process is analogous to the release of ammonia from proteins in the nitrogen cycle. Hydrogen sulfide is toxic to living things and thus must be oxidized rapidly. Oxidation to elemental sulfur is followed by oxidation to sulfate (SO_4^{2-}), which is the form of sulfur most usable by both microorganisms and plants. This process is analogous to nitrification.

The sulfur cycle is of special importance in aquatic environments, where sulfate is a common ion, especially in ocean water.

The various sulfur bacteria can be categorized according to their roles in the sulfur cycle. These roles include sulfate reduction, sulfur reduction, and sulfur oxidation (Figure 24).

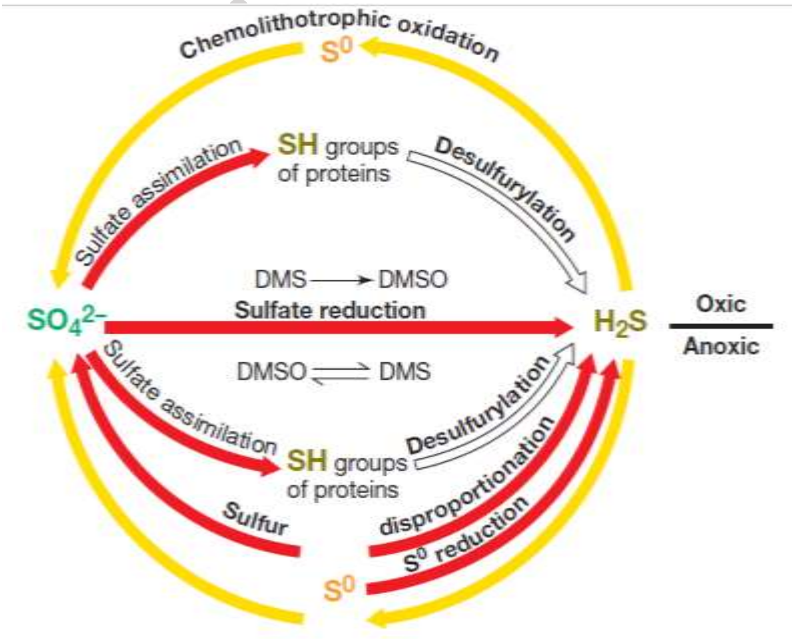

Figure 24: Redox cycle for sulfur. Oxidations are indicated by yellow arrows and reductions by red arrows. Reactions without redox changes are in white. DMS, dimethyl sulfide; DMSO, dimethyl sulfoxide.

Hydrogen Sulfide and Sulfate Reduction

A major volatile S gas is hydrogen sulfide (H_2S). Hydrogen sulfide is produced from bacterial sulfate reduction ($SO_4^{2-} + 4\ H_2 \longrightarrow H_2S + 2\ H_2O + 2\ OH^-$) (Figure 24) or is emitted from sulfide springs and volcanoes.

Although H_2S is volatile, different forms exist depending on pH: H_2S predominates below pH 7 and the nonvolatile HS^- and S^{2-} predominate above pH 7. Collectively, H_2S, HS^-, and S^{2-} are referred to as "sulfide."

Sulfate reduction is the reduction of sulfate (SO_4^{2-}) to hydrogen sulfide (H_2S). The sulfate-reducing bacteria are among the oldest life forms, probably more than 3 billion years old. They include the closely related genera *Desulfovibrio, Desulfomonas,* and *Desulfotomaculum.* In these bacteria, sulfate is the final electron acceptor in anaerobic oxidation, as oxygen is the final electron acceptor in aerobic oxidation. By reducing sulfate, these bacteria produce large amounts of hydrogen sulfide.

However, for this process to occur, energy from ATP is required to phosphorylate the sulfate and convert it to ADP-SO_4. Then ADP-SO_4 can act as an electron acceptor and compete successfully for substrates.

Sulfate-reducing bacteria are strict anaerobes. They are widely distributed and predominate in nearly all anaerobic environments sulfate-reducing bacteria can be psychrophilic, mesophilic, thermophilic, or halophilic.

Hydrogen sulfide is toxic to many plants and animals and therefore its formation is potentially detrimental (**sulfide is toxic because it combines with the iron of cytochromes and blocks respiration**).

Sulfide is commonly detoxified in nature by combination with iron, forming the insoluble minerals FeS (pyrrhotite) and FeS_2 (pyrite). The black color of sulfidic sediments or sulfate-reducing bacterial cultures is due to these metal sulfide minerals (Figure 25).

Figure 25: Enrichment culture of sulfate-reducing bacteria. Left, sterile medium; center, a positive enrichment showing black FeS; right, colonies of sulfate-reducing bacteria in a dilution tube.

Sulfur-Oxidizing Bacteria

Sulfur oxidation is the oxidation of various forms of sulfur to sulfate. *Thiobacillus* and similar bacteria oxidize hydrogen sulfide, ferrous sulfide, or elemental sulfur to sulfuric acid (H_2SO_4). When this acid ionizes, it greatly decreases the pH of the environment, sometimes lowering the pH to 1 or 2. Sulfur-oxidizing organisms are responsible for oxidizing ferrous sulfide in coal-mining wastes, and the acid they produce is extremely toxic to fish and other organisms in streams fed by such wastes.

Iron Cycle

The iron cycle includes several different genera that carry out iron oxidations, transforming ferrous ion (Fe_2^-) to ferric ion (Fe_3^-). *Thiobacillus ferrooxidans* carries out this process under acidic conditions, *Gallionella* is active under neutral pH conditions, and *Sulfolobus* functions under acidic, thermophilic conditions.

Much of the earlier literature suggested that additional genera could oxidize iron, including *Sphaerotilus* and *Leptothrix.*

Recently microbes have been found that oxidize Fe_2^- using nitrate as an electron acceptor. This process occurs in aquatic sediments with depressed levels of oxygen and may be another route by which large zones of oxidized iron have accumulated in environments with lower oxygen levels.

Iron reduction occurs under anaerobic conditions resulting in the accumulation of ferrous ion. Although many microorganisms can reduce small amounts of iron during their metabolism, most iron reduction is carried out by specialized iron-respiring microorganisms such as *Ferribacterium limneticum,* and *Shewanella putrefaciens,* which can obtain energy for growth on organic matter using ferric iron as an oxidant.

In addition to these relatively simple reductions to ferrous ion, some magnetotactic bacteria such as *Aquaspirillum magnetotacticum* transform extracellular iron to the mixed valence iron oxide mineral magnetite (Fe_3O_4) and construct intracellular magnetic compasses.

Furthermore, dissimilatory iron reducing bacteria accumulate magnetite as an extracellular product.

Magnetite has been detected in sediments, where it is present in particles similar to those found in bacteria, indicating a longer term contribution of bacteria to iron cycling processes. Genes for magnetite synthesis have been cloned into other organisms, creating new magnetically sensitive microorganisms. Magnetotactic bacteria are now described as **magneto-aerotactic bacteria,** due to their using magnetic fields to migrate to the position in a bog or swamp where the oxygen level is best suited for their functioning.

In the last decade new microorganisms have been discovered that use ferrous ion as an electron donor in an oxygenic photosynthesis. Thus, with production of ferric ion in lighted (Figure 26).

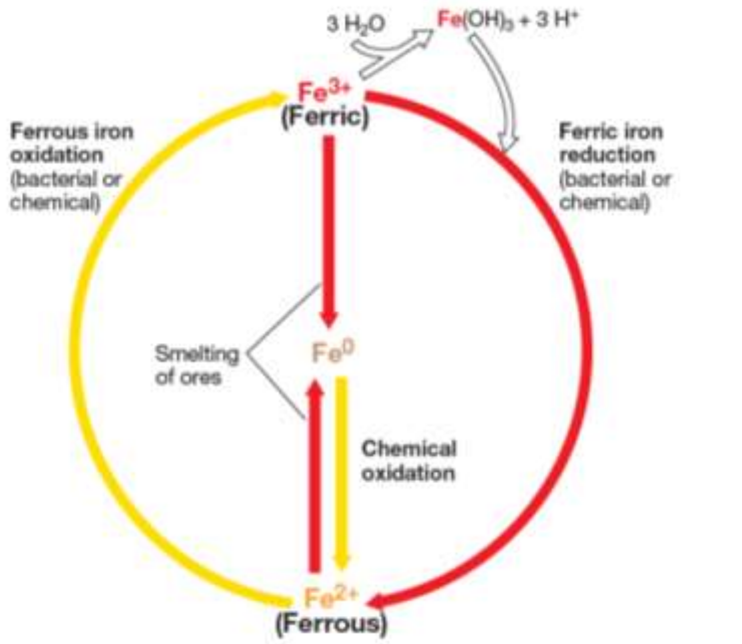

Figure 26: Redox cycle for iron. The major forms of iron in nature are Fe2+ and Fe3+. Fe0 is primarily a product of smelting of iron ores. Oxidations are shown by yellow arrows and reductions by red arrows. Fe3+ forms various minerals such as ferric hydroxide, Fe (OH) 3.

Phosphorus cycle

However, unlike the C, N, and S cycles, in the P, Ca, and Si cycles there are no redox changes or gaseous forms that can escape and alter Earth's atmospheric chemistry. Nevertheless, as we will see, keeping these cycles in balance especially that of Ca is important for maintaining sustainable life on Earth.

Phosphorus (P), involves the movement of phosphorus among inorganic and organic forms. Soil microorganisms are active in the phosphorus cycle in at least two important ways: (1) They break down organic phosphates from decomposing organisms to inorganic phosphates, and (2) they convert inorganic phosphates to orthophosphate (PO_4^{3-}), a water-soluble nutrient used by both plants and microorganisms. These functions are particularly important because phosphorus is often the limiting nutrient in many environments (Figure 27).

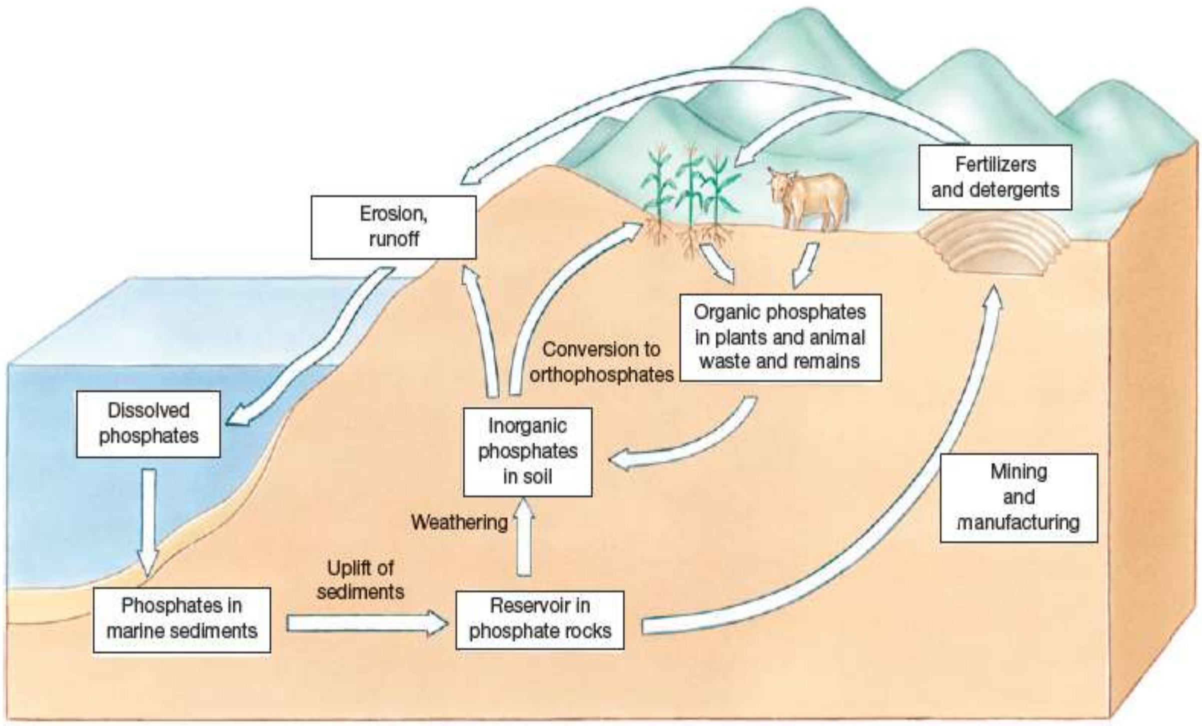

Figure 27: The phosphorus cycle

Organically bound P is not directly available to organisms because it cannot be absorbed into cells in this form. For cellular uptake to occur, P must first be released from the organic molecule through mineralization. The final stage in the conversion of organically bound P to inorganic phosphate occurs through the action of phosphatase enzymes. The phosphatase group of enzymes includes phytase enzymes that catalyze the release of phosphate from phytin and nuclease enzymes that liberate phosphate from nucleic acids. These enzymes are produced by up to 70–80% of the microbial population, including bacteria such as *Bacillus megaterium*, *Serratia* spp., *Proteus* spp., *Arthrobacter* spp., and *Streptomyces* spp. and fungi such as *Aspergillus* spp., *Penicillium* spp. and *Rhizopus* spp.

Calcium (Ca), the major global reservoirs of Ca are calcareous rocks and the oceans. In the oceans, where dissolved Ca exists as Ca^{2+}, calcium cycling is a highly dynamic process although the concentration of Ca^{2+} in seawater remains constant at about 10 mM. Several marine eukaryotic phototrophic microorganisms take up Ca^{2+} to form their calcareous exoskeletons; these include in particular the *coccolithophores* and *foraminifera* (Figure 28).

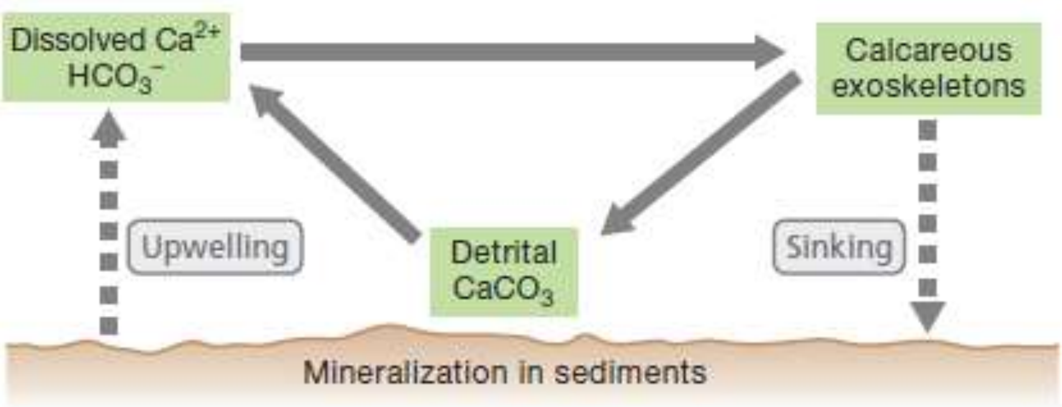

Figure 28: The Calcium cycle

The calcium-cycling activities of these planktonic phototrophs are also tightly coupled with inorganic components of the carbon cycle. The precipitation of calcium carbonate ($CaCO_3$) to form the shells of calcareous phytoplankton controls both CO_2 flux into ocean surface water and inorganic C transport into deep ocean water and the sediments.

Silica (Si), The Si cycle is controlled primarily by unicellular eukaryotes (diatoms, silico-flagellates, and radiolarians) that build ornate external cell skeletons called *frustules* (Figure 29).

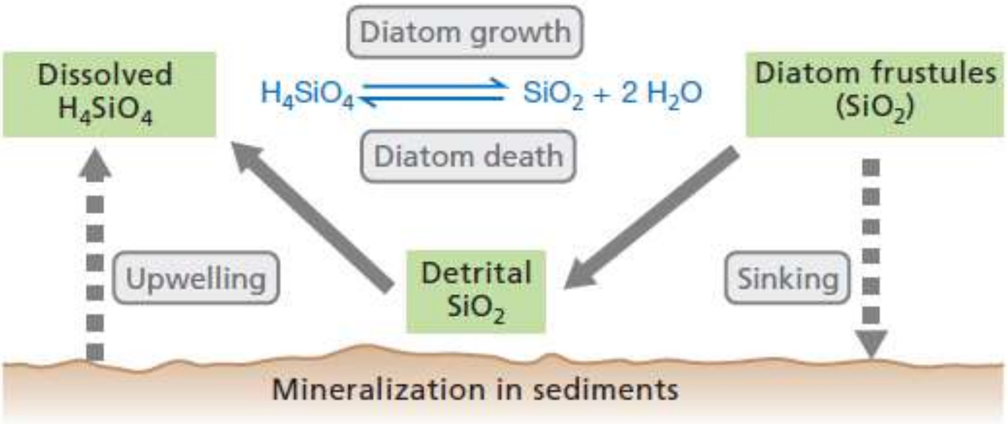

Figure 29: The Silica cycle

These structures are not constructed of $CaCO_3$ as in the coccolithophores, but of opal (SiO_2), whose formation begins with the uptake by the cell of dissolved silicic acid.

Diatoms are rapidly growing phototrophic eukaryotes and often dominate blooms of phytoplankton in coastal and open ocean waters. However, unlike other major phytoplankton groups, diatoms require Si and can become silica-limited when blooms develop. Because of their large size, diatom cells tend to sink faster than other organic particles, and in this way, they contribute significantly to the return of Si and C to deeper ocean waters.

Although Si is released fairly rapidly following cell death, during periods of high diatom production in relatively shallow waters, a significant fraction of dissolved Si can be buried in sediments and remain there for millions of years.

Soil Human Pathogens

1. Viruses:

Viruses are highly host specific and are incapable of multiplying outside of host cells. Therefore, human viruses almost certainly have no functional role within the soil system. This means that they can all be considered soil-transmitted pathogens some viruses are capable of surviving within the soil system for extended periods of time and to be able to adsorb to soil particles and so resist elution, others adsorb less strongly to the soil and so can become mobilized after rainfall and either washed deeper into the soil profile or to different areas depending on the soil hydrology, factors which most affect the survival of human pathogenic viruses in the soil are pH level, moisture content, temperature, exposure to sunlight and the presence of soil organic matter.

A. **Enteroviruses:** Human enteric viruses are a genus of picornaviruses that include (polioviruses, coxsackieviruses and echoviruses). They generally infect the gastrointestinal tract of humans but may also spread and infect tissues in other areas of the body, particularly the nervous system. They have been found to be able to survive for extended periods in some soil environments with survival times of up to 170 days reported for virus particles in loamy and sandy soils. The exception to this is poliovirus, which seems to adsorb relatively strongly to soil particles and can be able to survival within the soil environment for between 80 days and 96 days.

B. Hantaviruses: are generally considered to be zoonotic, being carried by wild rodents. Infection in humans generally occurs either from rodent bites or through coming into contact with infected rodent excreta, which may be in the soil. Hantavirus infection in humans can cause either “hantavirus pulmonary syndrome” (HPS) or “hemorrhagic fever with renal syndrome” (HFRS) The first outbreak of HFRS occurred during the Korean War and so HFRS was initially called Korean hemorrhagic fever (KHF).

C. Other viruses: Many other human viruses have either been shown or are thought to only survive relatively poorly in the soil system although studies on many viruses are still currently lacking. However, the rubella virus, mumps virus, rhinovirus and parainfluenza virus among others, have all been found to survive in the external environment, including in the soil, for only a matter of hours and occasionally for a day or two.

2. Bacteria:

Bacteria exist at relatively high levels of abundance in all ecosystems on the planet that have so far been studied and they play a vital role in global functioning through the ecosystem services and functions that they provide. However, there are several groups of bacteria, which are pathogenic in humans.

A. Actinomycetoma (Actinomycosis): caused by infectious actinomycetes species, often *Actinomyces israelii*, a soil dwelling species that is found in decaying organic matter. Actinomycetes are generally soil-inhabiting saprophytes although some species are capable of causing diseases in plants, animals or humans. They are generally anaerobic or facultative anaerobic organisms, As well as inhabiting the soil, many species of actinomycete can also be found colonizing the gut, mouth and vagina of humans although the majority of these do not cause diseases.

B. Anthrax: Anthrax is a zoonotic disease caused by bacteria of the species *Bacillus anthracis*, which are gram positive, rod shaped aerobic, spore-forming bacteria. The disease is a problem in many countries worldwide where it can affect livestock and wildlife. Animals generally get anthrax from grazing on soils that contain spores of the bacterium *B. anthracis*. Humans then may become infected through touching infected animals or animal products where the bacterial spores may enter into wounds if present, or be inhaled as is the case for “wool sorter’s diseases”; an often fatal infection resulting from the handling of infected wool. Anecdotal evidence exists that soil management practices such as tillage may increase the risk of human infection of anthrax due to inhalation of spores, when in spore form, there bacteria are highly resistant to desiccation and other environmental stresses and can remain in this inactive form in soil for many years, only becoming active once environmental conditions become favorable again.

C. Botulism: The bacterium *Clostridium botulinum* is the causative agent of botulism. *C. botulinum* is not a well-defined species of bacterium but rather refers to distinct groups of bacteria that produce seven distinct toxins (called A to G), only types A, B, E and more rarely F are capable of causing botulism in humans. Types C, D, E and G cause illness in other mammals, birds and fish. *C. botulinum* are spore forming, anaerobic bacteria whose principal habitat is the soil, although their distribution can be regional. While the bacteria are soil borne, infection generally occurs through eating contaminated food, although it can also be transmitted directly into wound infections directly from the soil. The disease can progress to weakness in the neck and arms after which respiratory muscles and muscles of the lower body become affected and this paralysis may make breathing difficult.

D. **Campylobacteriosis:** Bacteria from the genus Campylobacter, most commonly the species *Campylobacter jejuni*, are the causative agents of campylobacteriosis. It is an enteric bacterium and is one of the most common forms of bacterial infections in humans. Symptoms of campylobacteriosis usually present within 2-5 days, which is classified as inflammatory diarrhea, which may be bloody (also known as dysentery). However, *C. jejuni* has been demonstrated to survive in the soil for at least 25 days, with indications that it can survive considerably longer meaning that the soil is also a possible route of transmission.

E. **Leptospirosis:** also known as Weil's diseases in its more serious form, is caused by a species of aerobic spirochete bacteria from the genus *Leptospira interrogans* Bacteria of this genus are found in aquatic ecosystems as well as the soil, although they are generally found in higher abundances in the soil adjacent to water than the water itself. The bacteria are capable of surviving for at least 42 days in acidic soil pH 5.5 and up to 74 days in more neutral soils.

F. *Escherichia coli*: The most common form of pathogenic *E. coli* is Enterotoxigenic E. coil (ETEC), which is the most common cause of bacterial diarrhea in children in the developing world as well as among travelers to developing countries. Enteropathogenic E. coli (EPEC) which causes diarrhea but also contains virulence factors which are similar to Shigella.

Enterohaemorrhagic *E. coli* (EHEC) or Vero cytotoxigenic *E. coli* (VTEC) which causes bloody diarrhea ("Vero" indicates that this serotype produces the "Shig" toxin), the main routes of transmission of E. coli are poor hygiene or sanitation leading to contamination of food or water, E. coil can also survive in the soil for sufficient periods to lead to infection even when the infectious individual that was the source of the contamination has long left the area. The main factor affecting the length of time that E. coli can survive in soil appears to be soil moisture content, with cells surviving for 14 days in dry soils and longer in wet soils up to 6 weeks, or in the underlying soil for 8 weeks.

G. Gas Gangrene: The causative agent of gas gangrene is, in 80-90% of cases, the bacterium *Clostridium perfringens* (previously known as *Clostridium welchii*). If untreated, gas gangrene is always fatal, although with treatment this mortality rate drops to 20-30%. *C. perfringens* is bacilli, which are gram positive, anaerobic and spore forming. They are highly prevalent in soils as well as the intestinal tract of humans and animals.

H. **Listeriosis**: The causative agent of listeriosis is the gram-positive bacterium *Listeria monocytogenes*. Infection usually occurs in humans after eating food contaminated with the bacterium. While it is a generally considered to be a food borne disease, the bacterium has been demonstrated to survive for extended periods in soil, the main factor affecting the survival of *Listeria monocytogenes* in soil was found to be soil moisture content with survival averaging up to approximately 67 days in soils where the moisture content was not controlled as compared to up to 295 days in soils which were protected from evaporation.

I. **Lyme disease (Lyme borreliosis):** it is classified as a zoonosis, as it is transmitted to humans from a natural reservoir among rodents by ticks that feed on both sets of host. It is caused by at least three species of spirochete bacteria belonging to the genus Borrelia. *Borrelia burgdorferi Borrelia afzelii* and *Borrelia garinii*. Although ticks responsible for spreading Lyme disease are parasitic and hence rely on the presence of hosts for feeding, the presence and abundance of the ticks was variable even when the host population was adequate and that the presence of ticks is positively associated with deciduous, dry to mesic forests and sandy or sandy loam textured soils.

J. ***Pseudomonas aeruginosa:*** it is a gram-negative rod shaped bacterium, which is widely distributed in soil and water around the world, as well as being found on plants and humans. It is an opportunistic pathogen can cause a wide range of diseases in immuno-compromised individuals including respiratory tract infection, bacteremia (infection of the blood), endocarditis (infection of the heart), urinary tract, gastrointestinal infection, as well as infecting bones, joints and the central nervous system.

K. **Q fever:** The causative agent of Q fever is the bacterium *Coxiella burnetii,* which is a small, gram-negative obligate intracellular parasite meaning that it has to infect the cells of a host in order to complete its life cycle. It is considered a zoonotic disease with a reservoir in cattle, goats and sheep. *Coxiella burnetii* is very hardy and resistant to heat, desiccation and many common disinfectants, and so is able to persist in the environment, including soil, for up to 150 days. Infection occurs from the inhalation of the bacteria in spore form, usually from farmyard dust. The presence of vegetation and increased soil moisture.

L. **Salmonellosis:** Salmonellosis, sometimes called salmonella, is caused by a group of gram-negative motile bacteria from the genus Salmonella. The genus includes many species of bacteria and each species may contain many serotypes. Although humans generally contract salmonellosis through eating contaminated food, one of the main routes in which pathogenic Salmonella bacteria come into contact with vegetables is from the soil Once introduced into the soil, pathogenic species of Salmonella have been demonstrated to be able to persist for up to 231 days and to be capable of contaminating vegetables grown in such soils. Salmonellosis presents as one of three distinct syndromes, typhoid (enteric) fever, non-typhoidal enterocolitis, or non-typhoidal focal disease.

M. Shigellosis: Shigellosis is caused by one of four different species of bacteria from the genus Shigella: *S. flexneri; S. sonnei; S. boydii; and S. dyseneriae*. These bacteria are capable of causing an acute intestinal disease, which may present with symptoms ranging from relatively mild abdominal pains, up to stomach cramps, diarrhea, fever, vomiting and blood in stools. Transmission of shigella bacteria can occur through eating infected food, contaminated soil or water, or human to human transmission can occur through blood, saliva, sexual contact, the faecal-oral route or from the mother to fetus. Shigellae bacteria have been found to survive for up to approximately 40 days in the soil.

N. Tetanus: *Clostridium tetani* is the causative agent of the diseases tetanus. *C. tetani* bacteria, as all other species of Clostridium, are spore forming, gram positive and obligate anaerobes. The *C. tetani* bacterium, which has a global distribution, generally found in the soil as well as in animal waste. The association that many people have with tetanus and rusty metals is somewhat misconstrued. The bacterium does not have any propensity to grow on rusty metal, but rather owing to its highly resilient spores is capable of surviving on rusty metal.

O. Tularemia: Tularemia is caused by the bacterium *Francisella tularensis* which is a non-motile gram-negative coccobacillus of which several serotypes exist, The disease may also be contracted through inhalation or ingestion of infected contaminated dust or soil The bacterium is known to survive for weeks at low temperatures in water, soil and animal carcasses, and for more than a year in mud in some instances.

P. Yersiniosis: *Yersinia enterocolitica* is the causative agent of yersiniosis. It is a species of gram-negative coccobacillus bacterium, which is part of the family Enterobacteriaceae. It is a zoonotic disease, which can be transmitted from many different animal including pigs, cattle and deer. The survival of *Yersinia enterocolitica* in soil strongly affected by environmental conditions, particularly soil moisture, as the bacterium does not cope well with desiccation, infection which usually occurs through ingestion of the bacteria, usually from undercooked food, particularly pork products.

The Deep Hot Biosphere

Holds that the entire crust of the Earth, down to a depth of several miles, is inhabited by a culture of microbes. These bacteria deep inside the Earth feed on oil and methane gas deposits, which are an original part of the Earth, from the time it first coalesced out of planetary debris. Oil would no longer be viewed as a product formed by the compression and transformation of plant and animal debris. Primitive life began deep in the depths of the planet, and has only emerged onto its surfaces as the surfaces have cooled and modified. Today at the borderlands between deep and surface environments we find upwelling of chemicals and organisms, first seen in 1977 at a depth of 2.6 km, northeast of the Galapagos Islands, along the Pacific Rise.

Microorganisms Found in Air

Microorganisms do not grow in air, in part because it lacks the nutrients needed for metabolism and growth.

However, spores are carried in air, and vegetative cells can be carried on dust particles and water droplets in air. The kinds and numbers of airborne microorganisms vary tremendously in different environments. Large numbers of many different kinds of microorganisms are present in indoor air where humans are crowded together and building ventilation is poor.

Small numbers have been detected at altitudes of 3,000 m. Among the organisms found in air, mold spores are by far the most numerous, and the predominant genus is usually *Cladosporium.*

Bacteria commonly found in air include both aerobic spore formers such as *Bacillus subtilis* and non–spore formers such as *Micrococcus* and *Sarcina.* Algae, protozoa, yeasts, and viruses also have been isolated from air. While coughing, sneezing, or even talking, infected humans can expel pathogens along with water droplets. Health care workers should handle wastes from patients carefully to avoid producing aerosols (tiny droplets that remain suspended for some time) of pathogens.

Astrobiology

Astrobiology is the study of life in the universe, including its origin and possible existence beyond Earth. The discovery of life beyond Earth would arguably be the most significant advance in science since a human set foot on the moon.

If the same physical and chemical laws govern the universe everywhere, then it is hard to suppose that only one of the billions of stars would have a planet supporting life. On the other hand, we have no idea how many planets have been capable of developing and sustaining a biosphere.

If life exists elsewhere, is it built on the same fundamental elements as ours? Many lines of evidence suggest that the biochemistry of life elsewhere would resemble that of Earth. Terrestrial life is founded on macro-elements in the first two rows of the periodic table, including carbon, nitrogen, oxygen, phosphorus, and sulfur.

Thus, we suspect that the fundamental building blocks for biochemistry are universal. On the other hand, if life could indeed be founded on some other basis, how would we recognize it?

If life is found on other planets, it will almost certainly include microbes. In fact, a case can be made that the majority of biospheres in our galaxy would consist entirely of microbial life, since microbes inhabit a wider range of conditions than do multicellular plants and animals.

Even on Earth itself, the largest bulk of our biosphere including deep sediments and rock strata consists of microbial ecosystems.

Do Bio signatures Indicate Life?

If microbes do exist on Mars, they should be detectable. but the detection of unknown life, even on Earth, remains a challenge. The advent of PCR sequence detection of species based on ribosomal genes has revealed thousands of unknown species, most of which cannot be grown or recognized by other methods. Other species may well be missed if their rRNA sequences fail to amplify with our probes. On Mars, assuming life evolved independently of Earth life-forms, we would have no way to define sequence probes for detection.

Instead, researchers try to define **bio-signatures**, chemical and physical signs that could only have been formed by life. Most of the proposed bio-signatures are based on types of evidence for life on Earth, either as fossils of ancient life or as signs of current life in extreme habitats. Proposed bio-signatures include the following:

1. **Microfossils.** The mineralization of microbial cells.
2. **Isotope ratios.** Certain biochemical reactions preferentially use one isotope of an atom over another.
3. **Mineral deposits.** Certain mineral formations are observed to be caused only by microbial activity.

4. **Metabolic activity.** Samples of soil can be incubated with radioactive tracer substances such as $_{14}CO_2$.

Various kinds of evidence for life on Mars have been reported, such as possible microfossils within a Martian meteorite that landed in Antarctica. Metabolic activity was tested in samples obtained by the NASA *Viking* lander in 1975. As of this writing, however, no evidence has proved conclusive for active or past living microorganisms on Mars.