Lecture 1
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Soil Microbiology

Soil Microbiology: It is branch of science/ microbiology which deal with the study of soil microorganisms and their activities in the soil.

Soil: Is crucial for sustaining life, like water and air, because it is the source of most of our food. Soil can be defined as; the outer region of earth crust, consist of loose material formed by series of various processes called soil – forming factors (SFF), includes topography of land, the organisms present in the environment, the climate under which the soil was formed, the parent material or the original minerals that give rise to the soil, and the time that all of these processes have been occurring.

Soil Profile

Soil profile refer to the layers of soil develops over a long period of time, the soil profile consist of horizons, each with a distinctive features. The following horizons are listed by their position from the top to the bottom**:**

1. **Horizon A (Top Soil)**

Surface layer, is the top about 25cm of soil profile, with a darker in color than deeper layers and contain highest percentage of organic matter accumulation, this horizon also known as the biomantle because most biological activity occurs. The layer was likely formed from decomposing plant and mineral materials. It has a large amount of sand, and less clay.

2. **Horizon B (Subsoil)**

This horizon is found from 25 – 40cm, has a lighter color than the horizon A, with increase in clay and mineral salts such as deposits of silicates or aluminum that wash down through the top soil to create this layer, a process referred to as alleviated zone, It also contains less microbial population, but some biological activity extends into this layer

3. **Horizon C (Parent Soil)**

This third horizon has gray mottles, or patches of gray colors throughout the soil matrix. With strong increase in clay percentage, it's thickness of 45cm and more may it reach the rocky layer. The layer indicated with the absence of organic matter and microbial activities.

4. **Horizon D (Rocky bed)**

This layer is found from 75cm and beyond, represent the parent material sitting on bedrock, may be weathered to form part of soil profile.

Soil Texture and Soil Structure

Soil texture and soil structure are both unique properties of the soil that have a profound effect on the behavior of soil, such as water holding capacity, nutrient retention and supply, drainage, and nutrient leaching. The combination influence of soil texture and structure may be best described by the term "**Soil bulk density**", is a measure of percent pore space and solid in soil.

Soil texture; referred to the proportion and distribution of mineral particles, sand, silt, and clay present, the texture of soil can be determined when the percentage of these three soil constituents are known. Table.1 illustrated soil particles size,

Table (1) Soil Texture Particles size

Type of Soil Particles	Size range/mm
Sand	**2.0 – 0.05**
Silt	**0.05 – 0.002**
Clay	**Less than o.oo2**

Physicochemical feature of soil depends mainly on its texture, because it influences plants growth by its direct effects on soil, aeration, water infiltration, and cations exchange capacity (CEC).

Sand, has large particle size with small surface area in comparison with same mass of silt and clay, in accordance to this little surface area the sand proportion of any soil has an importance as organizing skeleton for other constituents, in case of reasonable sand proportion the soil has enough pores which facilitate aeration and water drainage. Sandy soils are less productive than other.

Silt, produced from fragmentation of rocks, also has small surface area, but in this character it's larger than those of sand particles, with smooth appearance look like cosmetic powders, and with low adherence capacity.

Clay, fraction made up tiny particles, despite their small size the particles have very large surface area relative to their volume, may be more than thousand times the total surface area of sand particles with same mass, resulting from the plate _ like shape of individual particles.

The increase in surface area is highly reactive and has the ability to attract and hold positively charged nutrient ions, clay particles are somewhat flexible and plastic because of their lattice – like design, this feature allow clay particles to absorb water and provides many places for soil particle to retain and supply nutrients, soil containing clay is the most productive and use fertilizers most effectively.

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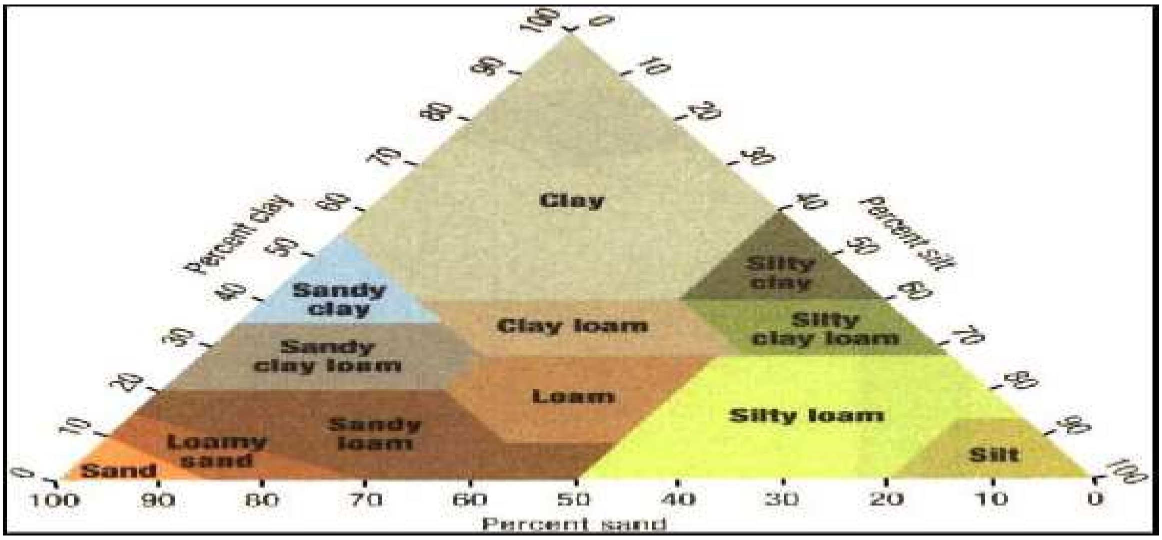
Textural class a grouping of soils based upon this relative proportion. Soil with the finest texture is called clay soil, while soil with coarsest texture is called sands. However, a soil that has a relatively even mixture of sand, silt, and clay and exhibits the properties from each separate is called loam.

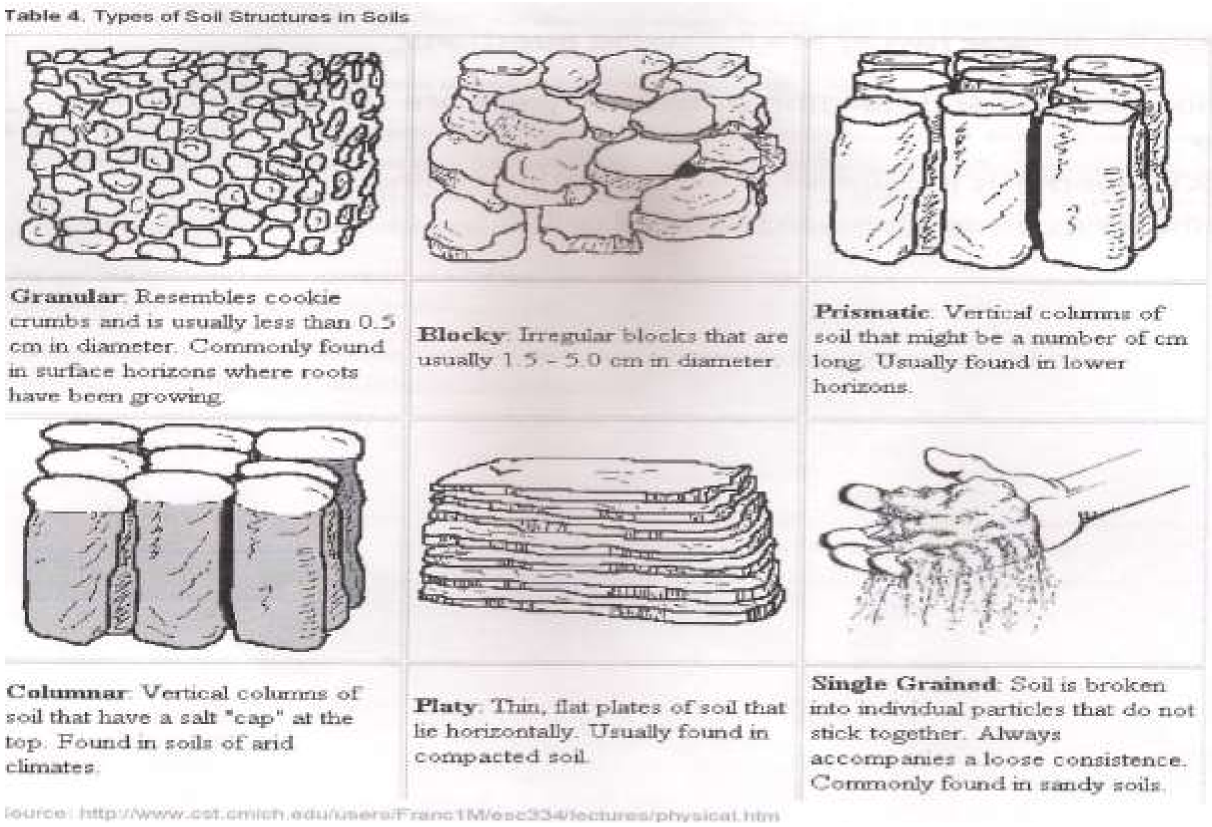
There are different types of loams, based upon which soil separate is most abundantly present. Once the sand, silt, and clay percentage of a soil are, known, the textural class can be read from the textural triangle used to determine the soil type (**Fig-1**)



(Figure – 1) Soil texture triangle for determination of soil

Soil structure; is the arrangement of soil particles into grouping. These grouping are called pads or aggregates, which often form distinctive shapes typically found within certain soil horizons. For example, granular soil particles are characteristic of surface horizon.

Soil aggregation is an important indicator of the workability of the soil. Soil that are well aggregated are said to have "god soil tilth". The various types of soil structures are provided in **Fig.2**

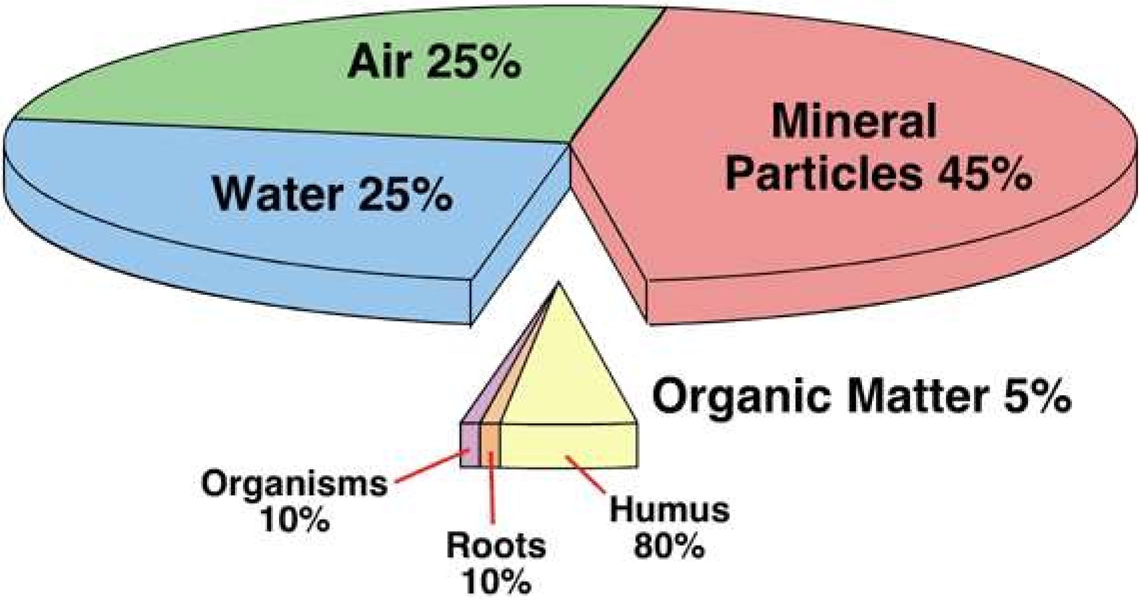


(Figure – 2)Types of soil structures

Soil Composition

Soils are made up of four basic components: mineral particles, air, water, and organic matter.

Solid materials (organic and inorganic) , which represent around half of soil content and spaces filled with air and water, each of them are important to life, four basic components: minerals, air , water, and organic matter (**Fig. 3)**



(Figure – 3)Soil composition

• Inorganic materials

In most soil types inorganic materials (mineral particles) represent about 45% of total soil volume. The mineral portion formed from the rock bed by weathering and biogeochemical factors, mineral portion consist of three distinct soil particles sand, silt, and clay Particles.

Based on chemical nature the mineral portion of soil can be divided into two groups,:

*Non – Silicate

Includes; Oxides, hydroxides, sulfates, chlorides, carbonates, and phosphates.

*Silicate group

Are very complex in structure, vary in its stability and resistance to decomposition, among these group **SiO_2** is the most abundant one. It may contain about 70% of total soil mass, except of organic soil.

Most soil influential particles are clay, it play a significant role in determining the availability of nutrient and water to different life forms

Clay particles are negatively charged, due to exchange of SiO_2 and Al^{3+}

$$Al^{3+} + SiO_2 \longrightarrow AlO^{2-} + Si^{4+}$$

Net particles charge depends on soil microorganisms' metabolic activities and pH of soil solution.

• *Water and Air*

Soil particles pack loosely, forming a soil structure filled with pore spaces, these pores contain soil solution (water) and gas (air). Water and air in soil vary significantly with soil texture, weather, and plants uptake of water, but their percentage together in most of soil types is about 50 % of total soil volume. Soil pore space doesn't change depends on soil texture and structure , but after rain the soil pore space will have a high percentage of of water in relation to air, once the

Soil water: Comes from rain, snow, dew or irrigation. Soil water serve as solvent and a carrier of nutrient for plant growth. The microorganisms inhabiting in the soil also require water for their metabolic activities. Soil water thus, indirectly affects plant growth through its effects on soil and microorganisms. Percentage of soil – water is about 25 % total volume of soil.

Soil water amount affected by many factors;

- ***Porosity:***

Soil porosity refers to the space between soil particles, which consists of various amounts of water and air, porosity depends on both soil texture and structure, for example, a fine soil has small but numerous pores than coarse soil. A coarse soil has bigger particles than a fine soil, but it has less porosity. Water can be held tighter in small pores than in large one, so fine soils can hold more water than coarse soil.

- ***Infiltration:***

Water infiltration refers to the movement of water from soil surface to the soil profile. Soil texture – structure, slope, and gravitation has the largest impact on filtration rate, water move by gravity into the open pore space in the soil, the size of soil particles and their spacing determines how much water can flow in.

- ***Permeability:***

Soil permeability refers to the movement of air and water through the soil, which is important because it affects the supply of root – zoon.

Water holding capacity is controlled permeability, by the combination effects of soil texture and organic matter, soil with smaller particle (silt and clay) have large surface area than those with large sand particles, so the first one has a high water holding capacity and allow a soil to hold more water than the second type.

Soil air*:* Apart of soil pores which not occupied with water are filled with air. Compared with atmospheric air, soil is lower in oxygen and higher in carbon dioxide, because CO_2 is continuous recycled by microorganisms during the process of decomposition of organic matter. Soil air comes from external atmosphere and contains nitrogen, oxygen, CO_2, and water vapor (CO_2> O^2).

CO_2 in soil air is (0.3 – 1.0) more than atmosphere air (0.03%). Soil aeration plays important role in plants growth, microbial population, and microbial activity in soil. A good aerated soil types lead to complete oxidation of organic matter, and characterized with high redox potential capacity, which offer e^- and H^+ donor and acceptors, results in thrive of aerobic and facultative microorganisms, but poor aerated soil types (saturated soils), which featured by low redox potential capacity cause continues release of NO^{3-} and SO_4^{2+} and accumulation of some harmfully intermediates like CH_4, that affected soil fertility and increase of anaerobic microorganisms population.

• Organic matter

Soil organic matter (SOM) is one of the most important components of soil ecosystem, in its broadest sense, and complex combination of living organisms and non – living organic matter (fresh organic residues, actively decomposing material, and humus). Generally the proportion of SOM in the soil ranging from 3 – 5 % of total soil volume.

Non – living organic matter can be considered to exist in two distinct pools:

Nonhumic Substances: Its particulate matter represents microbial metabolites products, all with identifiable structure, like polysaccharides, amino acids, organic phosphorus. This organic matter can constitute from a few percent up to 25% of total organic matter in soil.

Humic Substances: Carbon decomposition, successive decomposition of dead material, and modified organic matter results in the formation of undefined organic matter called humic substances or humus, by a process is called humification. Humus comprise both organic molecules of identifiable structure like proteins and cellulose, and molecules with no identifiable structure, like plants residues such as lignin, remains of animal carcasses (waxes, hair, nail, wool, and feather), also humin , humic acid, and fulvic acids are major components of humus. Humus is very stale, long – lived pool of organic matter in soil (with turnover rate of 100 – 500 years), which makes it a effective way to sequester excess carbon. Humus affects soil properties, as it slowly decomposes, it colors the soil darker, offer spongy appearance , encourages aggregate formation , increase water and nutrient retention and contributes to N, P, S, and other nutrients.

• Soil Living organic matter (Soil Biota)

The living part of soil organic matter includes a wide variety of organisms such as plants, insects, earthworms, animals, and microorganisms. Soil is excellent culture media for the growth and the development of various microorganisms that contains several distinct groups, and amongst them; bacteria, fungi, actinomycetes, algae, protozoa and viruses. Microorganisms form a very small fraction of soil mass and occupy a volume of less than one percent , in the upper layer of soil (top soil up to 10 – 30 cm depth i.e. Horizon A), the microbial population is very high which decreases with depth of soil. Each organism or groups of organism are responsible for a specific change/ transformation in the soil. The final effect of various activities of microorganisms in the soil is to make the soil fit for growth and development of higher plants.

Living organisms present in the soil are grouped into two categories as follow:

1. **Soil flora (micro flora) e.g. Bacteria, Fungi, Actinomycets, and Algae.**
2. **Soil fauna (micro fauna) animal like e.g. Protozoa, Nematodes, earthworms, moles, ants, rodent**

Soil Microflora

1- Bacteria

It is the most abundant groups, and usually more numerous than others. Soil bacteria numbers vary between 10^8 – 10^9 cell/ gm of soil, however, in an agriculture field their number goes about 3×10^9 cell/ gm of soil , which accounts for about 3 tones of wet weight/ acre.

Based on its regular presence soil bacteria are divided into two groups:

- Soil indigenous (true resident), or autochthonous.
- Soil invaders or allochthonous.

Bacteriai number and variety influenced by soil type, microenvironment, organic matter, cultivation processes. They are found in a high number in cultivated soil than virgin land, in a maximum in rhizospheric space than in non – rhizospheric region, possibly due to aeration and availability of nutrients. The inner region aggregates contained high level of G – ve bacteria , while the outer region contains high level of G + ve bacteria, thus may be due to ; polymer formations , motility, surface charge, and life cycle of bacteria

Bacteria do not occur freely in soil solution, but are closely attached to soil particles by motility apparatuses (flagella and pilli), or by extracellular polysaccharides , or may be embedded in organic matter.

Bacteria have some major roles in soil such as:

1. Miniralization of elements.
2. N_2 – fixation from atmosphere.
3. Stabilization of mineral ions.
4. Biotransformation of chemicals.
5. Biogas formation.

Example of some soil bacteria: ***Agarobacteriun, Arthobacter, Bacillus, Alcaligenes, Erwinia, Corynebacter, clostridium, Nitrosomonas, Nitrobacter, Rhizobium, Thiobacillus.***

Rhizosphere

It is a zone of increased microbial growth and activity in soil around of plants, it may extended several inches into soil around root system of growing plants. The m. o. growing in this zone is under the influence of roots often quantitatively and qualitatively. Therefore the rhizosephere is **a** unique substerrean habitat for m.o the microflora of one plant differ from the other plant , the rhizospheric region can be divided in to two zones;

- Inner rhizosphere , which is in close vicinity of root surface.
- Outer rhizosphere, embracing the immediate adjacent soil.

The outer epidermal walls of living roots and root hairs are covered with mucilage and cuticle, also organic and inorganic compounds , which accumulated in root cells cytoplasm and diffused out which is known as root exudation, these exudates contain carbohydrates, organic acids, enzymes, flavonones, and root hairs are continuously sloughed – off during secondary thickening, all these compounds constitute a food base for m. o.

The rhizospheric m. o. have either beneficial or harmful effects on developing of plants, the m.o. are intimately associated on rhizoplan, therefore any toxic or beneficial substances produced by them has direct effect on plants.

Some of possible effects are briefly;

- The m.o. catalyzes the reactions in rhizosphere and produces CO_2, and form organic acids that in turn solubolize the inorganic nutrients to plants.

- Aerobic bacteria utilize O_2 and produce CO_2, therefore lower O_2 and increase CO_2 tension that reduce roots elongation and nutrient and water intake.

- Some of rhizospheric m.o. produces growth – stimulating substances and release elements in organic forms through the process of mineralization.

- Some of rhizospheric m. o. secretes plant regulators such as; indole acetic acid, gibberellins, cytokinnens.

- They influence phosphorus availability through immobilization, however, when plants suffer from nutrient scarcity during summer in tropical area, the rhizospheric microflora release the immobilized nutrients, therefore they act as a sink between soil and plant roots in nutrient poor systems.

• Rhizospheric m. o. changes the availability or toxicity of sulfur in soil.

• The products of rhizosrheric zone m. o. metabolism sometimes have toxic effects on plants developing, these termed as the phytotoxins.

2- Fungi

In most aerated and cultivated soil, Fungi share a major part of total microbial biomass, because of their large diameter and extensive network of mycelia, however, population of soil fungi ranging from $2\times10^4 - 1\times10^6$ cell/ gm of dry soil. Fungi drive their growth nutrient from organic matter, lining animals (including; protozoa, arthropods, nematodes, etc.), and from living plants, establishing different types of relationships. The most important relationship between fungi and plants in soil is Mycorrhiza, which is a symbiotic relationship that occurs in plant root systems, and in two types of association;

Endotrophic; the fungi mycelia grows into the root tissue of associated plant.

Ectotrophic; the fungi mycelia ensheathes the root system, or can form like tubercles around rootlets, with limited penetration of hyphea into root tissue.

Some of soil fungi are**:** ***Alternaria, Aspergillus, cladosporium, Helimenthosporium, Humicola, Fusarium, Phytophthra, Plasmodiophora, Pythium, Rhizoctonia.***

Fungi in soil play variety roles, some of these roles are:

1- Mycorrizal fungi supplies minerals to associated plants, likewise fungi receives benefit from plants exudation, like some carbohydrates.

2- Production of fungistatic products including antibiotics.

3- Support soil microenvironments biobalance by their feeding practices on protozoa and nematodes.

4- Contributes in soil aggregation that protects soil particles from weathering effects.

5- Degrade some of tough plant residues, like lignine's.

3- Actinomycetes

Actinomycetes share the characteristics of both bacteria and fungi, they are commonly known as "ray fungi", because of their close affinity with fungi.

Actinomycetes are G+ve, and release antibiotic substances; however the earthy odor of newly wetted soil has been found to be a volatile growth products of actinomycetes. Their population remains greater in grass land and Pasteur soil than in the cultivated land. The number of actinomycetes ranging from $10^5 – 10^8$ cell/ gm of soil. The most limiting factor is the soil pH which governs their abundance in soil, its luxuriant growth favored by neutral and alkaline pH (6.0 to 8.0).

The important members of actinomycetes are:

Actinomyces, Streptomyces, Nocordia, Micromonospora, Actinoplans, Thermoactinomycetes.

Roles of soil actinomycetes

1- Contributes mainly in humus substances formation.

2- Decompose plant and animal residues that are resisting to bacterial and fungal decomposition.

3- Maintain soil biobalance for their ability to produce antibiotics, particularly *Streptomyces* spp. And the production of lysis enzymes, such as lysozyme.

4- Algae

Algae grow where adequate amount of moisture and light present due to their need of photosynthesis process, most of them prefer growth in neutral to alkaline soil (pH 7 – 10). The prominent genera in soil are; *Anabaena, Nostoc, Calothrix*, *Oscillotoria, Scytonema*.

Algae roles in soil can be listed as the follow:

1- Revolutionized the failed of agriculture due to their photosynthetic capacity, that act as a source of carbonic and nitrogenous organic matter in soil.

2- Many of algae species used commercially as biofertilizers.

3- Soil algae used in reclamation of sodas soil and alkaline soil types.

Cycles of Elements

All elements that are essential component of protoplasm undergo cyclical alteration, between an inorganic state, free in nature, and combined state in living organisms. This repeated transformation of elements from living protoplasm to Free State in nature constitutes the **cycle of elements in nature**. Among the essential elements undergoing biotransformation (recycling) is: **Carbon, Hydrogen, Nitrogen, Sulfur,** and **phosphorus**. When the above elements (except **phosphorus**) are incorporated into protoplasm, there is usually a change in the oxidation state. In protoplasm many of these elements are in reduced state, but as they are returned, they frequently are in oxidized state. Therefore these elements not only served as a source of energy (for **chemolethotrophes**) by oxidation, others may serve as e^- (H^+) acceptors in redox reactions.

The Carbon Cycle

Carbon recycling is one of several recycling processes, but it may be the most important cycle affects all life on earth, since is known to the basic building block of life. Cycling is the process in which the carbon atoms are recycled over and over, take place within Earth's biosphere and between living things and nonliving environment.

Carbon occur in nature into two main states; complex carbonated organic compounds (**C** reduced form), and inorganic carbon, most of inorganic carbon is in form of CO_2 (**C** more oxidized state). So CO_2 is the source of all carbonated compounds, both in living organism and fossil deposits.

Carbon dioxide constitutes about 0.0 45% of atmosphere gases, in this rate is more less than the requirements of photosynthetic organisms, so CO_2 release continuously from respiration and decomposition to maintain carbon balance in nature.

Biology plays important roles in the carbon cycle through three types of participation:

* **Producers** They represent the beginning of all food chains in nature, the producers fix atmospheric CO_2 and its subsequent assimilation into organic molecules through photosynthesis process.

* **Consumers** Carbon is transferred from organism to other when plants are eaten by herbivores, which are in turn eaten by carnivores along the food chain. Primary → Secondary Tertiary… , consumers get their required C either directly by eating plants or indirectly by eating animal that have eaten plants, to become their cellular materials or to release to atmosphere through respiration, in which a considerable portion of carbohydrates is oxidized to yield CO_2, H_2O, and **energy** as follows:

$$C_6H_{12}O_6 + 6O_2 \xrightarrow{\text{Respiration}} 6CO_2 + 6H_2O + \text{energy}$$

*** Decomposers** Decomposers are m.o. that lives mostly in soil, but also in water, they contribute substantially to carbon pool by their feeding and processing of rotting remains of other organisms. It is their job to consume both waste products and dead organic matter of land or ocean. Decomposers not only play key role in carbon cycle, but also break down, remove, and recycle what might be called nature's garbage.

Microorganisms play a key role in carbon recycling, they contribute to the carbon cycle by:

1. Fixation of CO_2 (Photosynthesis); An important step in carbon cycle is the fixation of atmospheric **CO_2** and its subsequent assimilation into organic molecule. Autotrophic organisms are able to covert **CO_2** into organic molecules via photosynthesis process; include plants, algae, some bacteria, and some archaea. The cyanobacteria and specific soil bacteria have the ability to conduct photosynthesis, **H_2S** and other reduced compounds serve as **e-** donor to reduce **CO_2**

Bacterial photosynthesis

$$CO_2 + 2\,H_2A \xrightarrow[\text{bactochlorophyll}]{\text{Light}} (CH_2O)_x + H_2O + 2A$$

2. Decomposition

Decomposition is a biological process that includes **physical breakdown** and **biochemical transformation** of complex organic molecules of dead materials into simpler organic and inorganic molecules. The major function of soil m.o. in the Carbon Cycle is as decomposers – degrader of complex organic matter that would otherwise permanently sequester carbon, keeping it from being useful to organisms. Each organic compound is utilize in a slightly different manner, and each yield different products when degrade, but many of these decomposers also release **CO_2**, contributing to the rising concentration in the atmosphere. Bacteria and fungi are particularly effective in breaking down of organic matter, while actinomycetes are responsible in degradation of tough remains, like lignin and chitin.

The rate of decomposition is affected by; soil temperature, moisture, aeration and food availability.

Mainly decomposition is either aerobic or anaerobic:

A) Aerobic decomposition, Most of heterotrophic microbes easily utilize aerobically soil organic compounds for both energy metabolism and as a carbon source:

$$\text{Aerobic decomposition (Mineralization)} \xrightarrow{\text{Complete oxidation}} CO_2 + H_2O + \text{energy}$$

(assimilation) → **Temporarily immobilized in form of Living matter**

(respiration) → **again recycled to atmosphere**

Mineralization: This is the process by which organic matter is decomposed to release simpler, inorganic compound (e.g. CO_2, NH_4^+, CH_4, H_2).

Immobilization: The nutrients that are converted into biomass become temporarily "tied up" from nutrient recycling, until the organism dies, at which time the **C** released back into the environment via decomposition.

B) Anaerobic decomposition, the anaerobic degradation of carbonaceous matters is a collaborative effort involving numerous bacteria; these bacteria are responsible for the bulk of Co_2 and methane that released to the atmosphere.

Anaerobic decomposition $\longrightarrow$ **H_2 + CH_4 + alcohol + organic acid**

Decomposition of organic matter under anoxic conditions such as deep compacted mud differs considerably from oxygen availability, in that reduced end products accumulated including **organic acids,** and **CH_4**. Accomplished by certain soil bacteria known as Methanogenic **bacteria,** are biologically very primitive, strict anaerobic, and sensitive to pH.

Main soil methanogens are :

Methanococcus, Methanobacteria, Methanosarcina .

To complete the recycling pattern another group of methane bacteria called **Methanotrophes** (literally methane eaters) are able to reoxidize released **CH_4** again to **CO_2**, like ***Pseudamonas*** *and* ***Methylomonas.*** This conversion also yields water and energy.

Other soil autotrophic bacteria are able to participate in the cycling of carbon by oxidizing carbon monoxide **CO.** This gas is relatively rare under ordinary condition, released from some activities, commonly from partial combustion. Exceedingly poisonous for most aerobic organisms, including man, it's relished as a source of energy and carbon by at least one bacterial species ***Carboxydomonas*** that oxidize **CO** to **CO_2**

$$CO + 1\backslash 2\ O_2 \longrightarrow CO_2$$

Because of there is an increase of **Co_2** in atmosphere about one – third, and its continue to rise. Like to **Co_2** methane concentration is likewise increasing about 1% per year, from 0.7 to 1.7 ppm. These two gases in combination with **H_2O** (water vapor), **O_3** (ozone), and **N_2O** (nitrous oxide), represents the **greenhouse gases**, the term describes the ability of these gases to trap heat within Earth's atmosphere, in that correlated with global temperature change, a phenomenon known as global warming. Soil microorganisms play a role in the generation of each of these gases.

Cellulose decomposition

Cellulose is the most abundant chemical constituent of plant cells, its polysaccharides contain glucose units linked by β-1-4 linkage, Total amount of cellulose in plants tissues varies from 15 to 60 %.

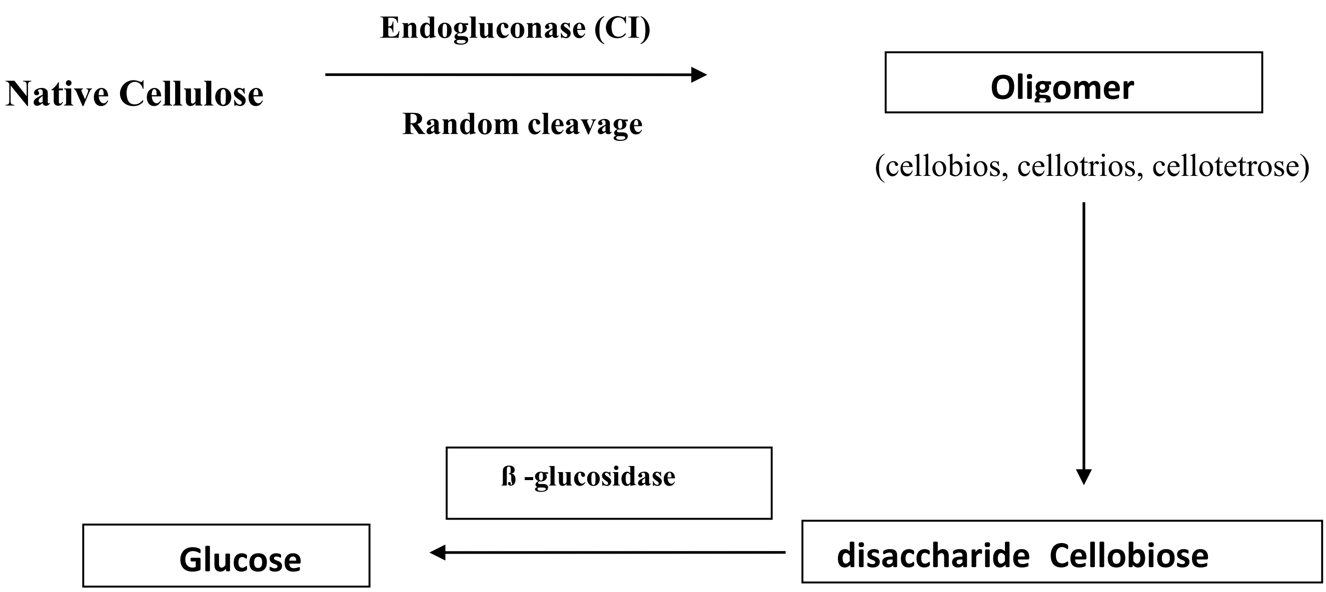
Cellulose are not tough for decomposition, variety microbes presents in millions per 1 millimeter of soil are capable to breakdown cellulose under different circumstances, oxic and anoxic , in availability of oxygen cellulose decomposes into **CO_2,** while in anoxic condition cellulose incompletely decomposes with release of many intermediates like organic acids and alcohols.

Three different enzymes involve in cellulose breakage, they collectively termed **cellulases** , each enzyme participate in certain stage of cellulose decomposition and produced by different microorganisms, m.o. that are able to biosynthesize all three enzymes called **true cellulytic microorganism**. Initial stages of cellulose decomposition take place by cleavage of cellulose by extracellular enzymes then the cleaved pieces are transported into the decomposers cell for energy generation (catabolism) or production of biomass (anabolism) and manipulated by the two rest intracellular lytic enzymes.

Bacteria e.g. (*Cytophaga, Cellulomonas, Bacillus*)

Fungi e.g (*Penicillium, Fusarium, Trichoderma*)

Actinomycetes e.g. (*Streptomyces, Nocordia*)



Nitrogen Recycling

Nitrogen is a part of vital and essential organic compounds in organisms, such as amino acids, proteins and nucleic acids the building block of life. Although the majority of air we breathe is nitrogen as it make up 78% of the atmosphere air, but most of atmospheric nitrogen is unavailable to use by organisms, this is because of the strong triple bond between the nitrogen atoms in nitrogen molecules make it relatively inert, in order for plant and animals to be able to utilize nitrogen, first $\mathbf{N_2}$ must be converted to more a chemically available forms, such as ammonium $\mathbf{NH_4^+}$, nitrate $\mathbf{NO_3}$, or organic nitrogen e.g. **urea**.

Nitrogen exists in nature in both organic and inorganic forms, as well as in many different oxidation states. The movement of $\mathbf{N_2}$ between the atmosphere, biosphere, and geosphere, in different forms is described by the **Nitrogen Cycle**, microorganisms are the key element in the cycle, provide different forms of nitrogen compounds by their metabolic activities.

The Main features of N_2 cycle are:

- **Nitrogen Fixation**
- **Nitrogen Mineralization**
- **Ammonification**
- **Nitrification and Denitrification**

Nitrogen Fixation

Nitrogen fixation is natural process, it occurs either biologically or abioticly, by which the gaseous nitrogen ($\mathbf{N_2}$) in the atmosphere is converted into combined form e.g. ammonia $\mathbf{NH_3}$ or other nitrogen organic forms, which are more reduced than when it is free, and become more available to farm corps either directly or through further microbial action .

Biological nitrogen fixation (BNF); nitrogen fixation is utilized by numerous prokaryotes, Microorganisms that fix nitrogen are called diazotrophs, are divers group of prokaryotes including free – living and symbiotic bacteria, cyanobacteria and actinomycetes. Common N_2 – fixing microorganism is given in table (1), and the phenomenon of this activity is known as **Diazotrophy,**

The biological conversion of atmospheric nitrogen taken place with the help of an enzyme called **nitrogenase,** that combines gaseous nitrogen with hydrogen to produce ammonia, which is then further converted by bacteria to make their own organic compounds. Nitrogenase in fact is a complex enzyme system, contains multiple metal – containing prosthetic groups, act in anaerobic conditions that require a reducing powers such as; **NADH** and **NADPH**, each one of these work with a certain type of bacteria, and the fixation process requires such a large amount of energy, about **5 – 6 ATP** molecules, this energy produced by substrate level phosphorylation in anaerobic microorganisms and via oxidative phosphorylation in aerobic one.

Table (1) N_2 – fixing microorganism

Free living Microorganisms	M.O Name
Aerobic	***Azotobacter* spp. ,*Beijerinckia* spp., *Trichodesmium* spp.**
Microaerophilic	***Xanthobacter* spp., *Azospirillum* spp. , *Thiobacillus ferooxidance***
Facultative anaerobic	***Klebsiella pneumonia, Erwinia* spp. *Bacillus polymyxa***
Anaerobic Bacteria	***Closteridium pasteuriunum, Propionibacter, Desulfovibrio* spp.**
Symbiotic Microorganisms	***Rhizobium spp., Burkhoderia spp. , Mesorhizobium, Frankia, Anabena***

Symbiotic N_2 – Fixation Processes

There are many symbiotic N_2 – fixation bacteria, a few actinomycetes, and cyanobacteria, but the most common symbiotic bacteria are ***Rhizobium spp***. Which live free in soil and are able to infect legume root nodules when come in contact with suitable legume. *Rhizobium* are G – ve rods, motile, non – spore forming, utilize organic salts as carbon source, and fast growing bacteria with generation time lasts about 6h.

Process of root nodules formation

The actual process of nodulation is a very coordinated effort between legume and the Rhizobium in soil. Infection typically occurs in root hair of legumes. Many rhizobia and host plant are highly specific and legumes can either attracted rhizobia to root hairs directly by excretion compounds or by induction of *nod* gene activity in bacteria.

The communication between legume and rhizobium stared by releasing of flavonoids from host root. Flavonoids are at the highest concentration at the root and bind bacterial protein product of *nod* gene, in that rhizobia colonize the soil in the vicinity of the root hair in response to the flavonoids. This binding induces encoding several enzymes that are require for nodulation of appropriate host plants.

Generally nodulation start from the following stages:

1. <u>Curling and Deformation of Root hairs</u>

Invasion of *Rhizobium* occurs through root hairs, but they are unable to hydrolyze the cellulose of plant cell walls, they appear to find non-cellulosic points at the tips of root hairs of legumes which called hyaline spots, through these points inter to root tissues. The penetrated bacteria grow in form of infection tube inside the root hair cells, this tube surrounded by a cellulose coat produced by infected plant cells, after 4h. Of infection the bacterial cells arrange themselves side by side like thread, so termed infection thread, the formation of this thread lead to the curling of root hair then deformation of infected hair.

2. Formation of nodules

The infection thread inside host cortical cells bulges and then rupture, the bacteria released into cytoplasm and after the bacteria undergoes alteration morphologically into larger forms called **bacteriods**, infected cells in turn rapidly divided to form a tumor like nodules of bacteriods –packed cells.

3. Structure of root nodules

The root nodule is formed due to tissue proliferation induced by the action of growth promoters of rhizobial in origin, probably cytokininis. The core of mature nodule constitutes the " bacteriod zone" which is surrounded by several layers of cortical cells. The bacteroids singly or in groups, surrounded by peribacteroid membranes, inhabit the cytoplasm of plant cell. The effective nodules are generally large and pink due to presence of legheamoglobine with well-developed and organized tissue. After the senescence, when the nodules dies the stationary – phase *Rhizobium* are released into the soil.

4. Function of nodules

The present evidences cited the fact the bacteroids are the site of N_2 – fixation. The isotopic (^{15}N) studies indicated that bacteroids are unable to utilize sugars, and secrete ammonia, which are apparently incorporated into organic matter as amines by glutamine synthetase enzyme present in the surrounding plant cells. This amine N – atoms are eventually introduced into protein, nucleic acids and other biomolecules.

N_2 – fixation process is an energy consuming process, for every mole of N_2 fixed about 22 moles of glucose is utilized as seen in the following equation

$$\mathbf{N_2 + 8\,H^+ + 8\,e^- \longrightarrow 2NH_3 + H_2}$$

This process indicates the involvement of true mutual symbiosis in which role played by leghaemoglobin in the fixation of nitrogen is much more significant. The formation of leghaemoglobin is specific effect of the symbiosis. The relative capacity of plant – bacterial association, once established, to assimilate molecular nitrogen is called effectiveness.

5. Leghaemoglobin

A red pigment similar to blood hemoglobin is found in the legume plant infected nodules between bacteroids and the membrane envelopes surrounding them. This pigment or protein enhance the transport of oxygen at low partial pressure to the nodules and maintains a steady supply of oxygen at low concentration of the nodule. In fact the presence of leghaemoglobin seems provide full protection to nitrogenase against oxygen damage, since nitrogenes is very sensitive to O_2 and must be protected from oxidizing conditions.

Nonsymbiotic N_2 – fixation

Many free living microorganisms are capable of converting molecular nitrogen to cellular nitrogen independently of other living organisms , they are primitive, live freely in soil and water, operate under poor aeration condition process by reduction process. ***Azotobacter*** is the most studied and best example of free living aerobic N_2 – fixers. *Azotobacter* evades the harmful effects of O_2 on its nitrogenase by having an exceedingly high rate of respiratory metabolism, thus preventing the retention of O_2 inside the cell, thus protecting enzyme complex. It produce FeS protein which complexes with two nitrogenase proteins to form a three membrane oxygen stable and inactive complex, when they do this start N_2 fixation.

Ammonification

When plant and animal dies, or animal expels wastes, the initial form of nitrogen is organic. Bacteria, or in some cases fungi convert the organic nitrogen within the remains back into **ammonium ion (NH_4^+),** and water soluble **ammonium salts**, a process called **ammonification** or **mineralization.** In fact the amino group (NH2) is split off to form ammonia (NH3) through series of enzymes reactions. Usually ammonification carries out under oxic conditions as follow:

$$NH_2-\underset{\underset{O}{\|}}{C}-NH_2 + H_2O \longrightarrow 2\,NH_3 + CO_2$$

A diver soil microflora acted the ammonification process, this includes bacteria, e.g. *Pseudomonas, Proteus, Micrococcus, Serratia, Closteridium,* etc., and Fungi, e.g. *Alternaria, Mucor, Aspergillus, Rhizopus,* etc.

The most abundant nitrogenous organic compounds in soil are proteins, which decompose by proteolytic m.o. They synthesize extracellular proteases degrade proteins to their structural subunits, amino acids through series of subsequent reactions:

$$\textbf{Protein} \xrightarrow{\textbf{Proteinase}} \textbf{Peptides} \xrightarrow{\textbf{Peptidase}} \textbf{amino acids}$$

Released amino acids are subjected to Deamination either oxidatively or reductively:

Oxidative Deamination

$$R - \underset{\underset{NH_2}{||}}{CH} - COOH + O2 \longrightarrow R - \underset{\underset{O}{||}}{C} - COOH + NH_4^+$$

Amino acid **Keto acid**

<u>Reductive Deamination</u>

$$R - \underset{\underset{NH_2}{||}}{CH} - COOH + 2\,H \longrightarrow R - CH_2 - COOH + NH4^+$$

Amino acid **Organic acid**

If the protein decomposition proceed under anaerobic conditions the process termed **"Putrefaction"** some of released amino acids converted to offensive Oder producing amines and related compounds, and in the presence of air the produced amines and related compounds are again oxidized with liberation of ammonia.

Ammonium is soluble, or capable of being dissolved, in water and often is used as fertilizer. It is <u>attracted to negatively charged surfaces of clay and organic matter in soil and therefore tends to become stuck in one place rather than moving around</u>, as nitrate does. In acidic soils, typically plants receive their needed nitrogen from ammonium, but most nonacidic soils can use only nitrate. Ammonium may be combined with nitrate to form ammonium nitrate, a powerful fertilizer and a powerful explosive. $\mathbf{NH_4}$ predominant in well – aerated soil and rich in organic matter, <u>in acidic soil types there is less ammonia production, since decomposition is carried out by fungi, and if the soil is rich by carbohydrates wastes ammonia formation also is low , since the m.o. prefer to utilize carbohydrates than nitrogenous wastes.</u>

<u>Nitrification</u>

In soil the liberated ammonia during ammonification pathway is rapidly oxidized to nitrate by some soil highly specialized bacterial groups of strictly aerobic chemolithtrophes, this oxidation process termed nitrification, which occurs into two stages:

The primary stage, involves the oxidation of ammonia ($\mathbf{NH_3}$) to nitrite ($\mathbf{NO_2^-}$), so the process called **Nitsosofication**.

$$2\,NH_4^+ + 3\,O_2 \longrightarrow 2\,NO_2^- + 2H^+ + H_2O$$

This stage performed by soil bacteria such as ***Nitrosomonas, Nitrosobacter , Nitrosovibrio***.

The secondary stage, involves the oxidation of liberated nitrite into nitrate ($\mathbf{NO_3^-}$)

$$2NO_2^- + O_2 \longrightarrow 2\,NO_3^- + \text{energy}$$

Other bacterial species are responsible for this oxidation such as ***Nitrobacter, Nitrococcus, Nitrospira***.

It is important for the nitrites to be converted to nitrates because accumulated nitrites are toxic to plant. Some plants get nitrogen from the soil, and by absorption via their root in form of either nitrate ions or ammonium ions , it is reduced to nitrite ions and then ammonium ions for incorporation into amino acids, nucleic acids, and chlorophyll, this pathway mention nitrogen assimilation process.

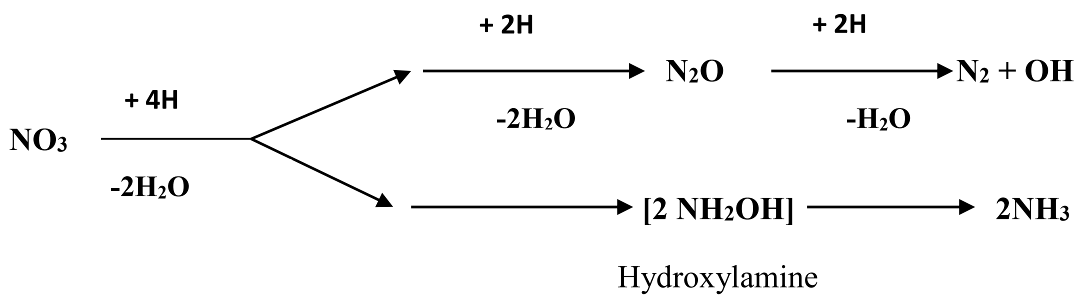
Due to their high solubility, nitrates can enter groundwater. Elevated nitrate in groundwater is a concern for drinking water use because nitrate can interfere with blood oxygen levels in infants and cause methemoglobinemia or blue – baby syndrome. Where groundwater recharges stream flow, nitrate – enriched ground water can contribute to eutrophication, a process leading to high algal, especially blue – green algal population and the death of aquatic life due to excessive demand for oxygen.

Factors affecting nitrification

1. **Acidity:** The nitrites are extremely susceptible to acidity even if they produce acids. Thus nitrification proceeds slowly in acid soil.
2. **Oxygen:** It is an obligate requirement Nitrification occurs even in submerged soils (paddy fields) in the upper few centimeters since the diffused oxygen present in waters helps nitrification in such soil.
3. **Moisture:** is needed for nitrification since the nitrifies cannot tolerate arid conditions.
4. **Temperature:** Nitrate production is high during 30° C – 35°C, since nitrifiers are mesophiles.

Denitrification

The nitrate reduction is biologically facilitated process, performed by a large group of heterotrophic facultative anaerobic bacteria. Denitrifying microbes require a very low oxygen concentration less than 10%, as well as organic C for energy. The denitrification generally proceed through a series of intermediates; nitrogen nitrite (NO_2), nitric oxide (NO), nitrous oxide (N_2O), finally resulting N_2 completing the nitrogen cycle. The products of pathway are volatile products, therefore are lost to the atmosphere and fail to enter the cell structure.



Denitrification can lower leaching of No_2 to ground water, it can be strategically used to treat swage and municipal wastewater or animal residues of high nitrogen content. Denitrification allows for the production of N_2O, which is greenhouse gas that can have a considerable influence on global warming.

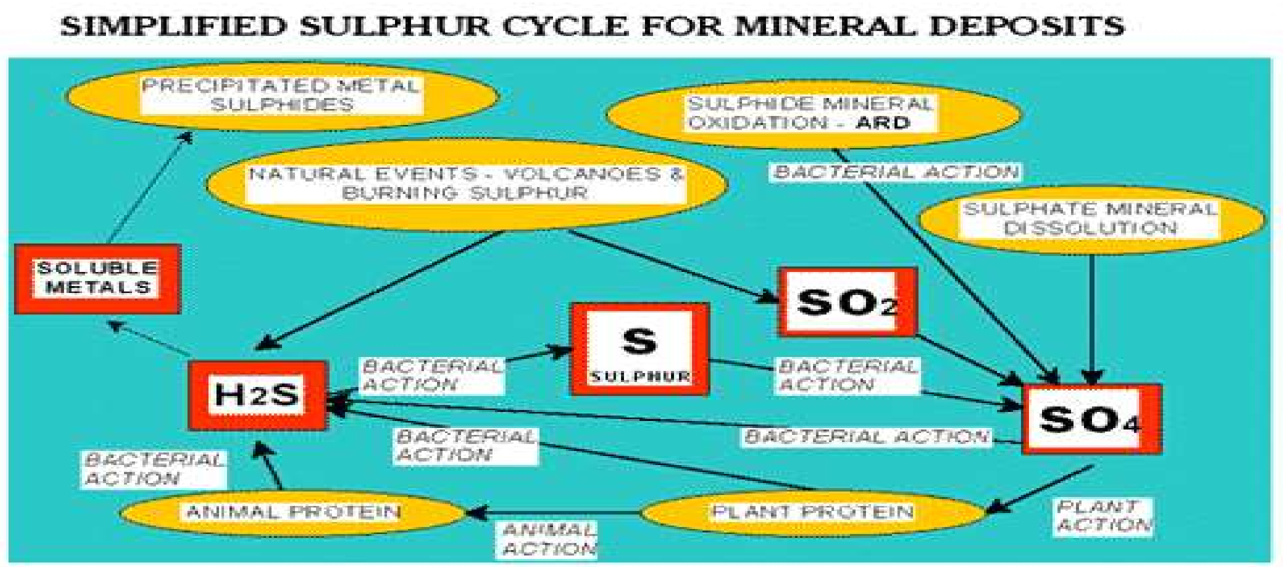
Denitrification is essentially a respiratory mechanism in which nitrate replace the molecular oxygen, therefore denitrification may be termed as nitrate respiration (dissimilatory nitrate reduction), the reduction process mediated by nitrate reductase enzyme , nitrate serve as the **e^-** acceptor, which is carried out according to the following reaction :

$$\mathbf{2NO_3^- + 10\ e^- + 12H^+ \longrightarrow N_2 + 6\ H_2O}$$

Sulfur Recycling

Sulfur is one of essential nutrient for all organisms that make up proteins and vitamins, is important element for the functioning of proteins and enzymes. Sulfur as is existing most abundantly in earth crust in low concentration, and in its native forms is unavailable to plants. occurs in nature and in soil as organic forms such as proteins of animal excretory products, and inorganic forms, such as dissolved sulfate (**SO_4^{2-}**), thiosulfate (**S_2O_3**), thiocyanate, and hydrogen sulfide gas (**H_2S**), these elements undergoes familiar alteration between organic and inorganic forms, and between oxidative and reductive states. Like nitrogen sulfur in its more oxidized form is most available to plants.

The environmental Sulfur cycle involves many physical, chemical and biological agents. A simplified schematic diagram of the cycle is shown in fig.1, which has been prepared to show the major phases of Sulfur cycling with relation to mineral deposits. As such, the figure indicates the relationships between Sulfur **(S)**, hydrogen Sulfide **(H_2S)**, Sulphur dioxide (**SO_2**) and Sulfate ion (**SO_4^{2-}**). In mineral form Sulfur may be present as sulphides e.g. pyrite (**FeS_2**), chalcopyrite, **(FeS.CuS)**, pyrrhotite (**FeS**) and/or sulphates e.g. gypsum (**$CaSO_4.2H_2O$**) and barite (**$BaSO_4$**).



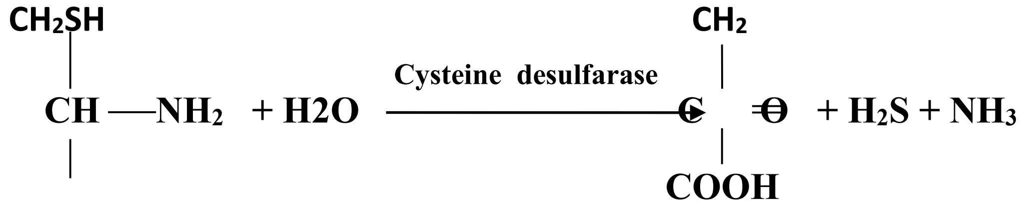
The Role of Microorganisms in the Sulfur Cycle

Microorganisms (most frequently bacteria) are often integrally involved in the chemical alteration of sulfur or intermediate products of their decomposition, may be directly or indirectly necessary to their metabolism. Sulfur is microbiologically metabolized in soil through different transformation processes:

- **Mineralization** (decomposition) of organic Sulfur to the inorganic form, hydrogen sulfide: (**H_2S**).
- **Oxidation** of sulfide and elemental sulfur (**S**) and related compounds to sulfate (**SO_4^{2-}**).
- **Reduction** of sulfate to sulfide.
- **Microbial immobilization** of the sulfur compounds and subsequent incorporation into the organic form of sulfur.

• Decomposition of organic Sulfur compounds

Many heterotrophic bacteria decompose organic compounds into smaller units and finally into inorganic compounds, (**mineralization**), as given below.



Cysteine **Pyruvic acid**

• Oxidation of inorganic Sulfur

Many bacteria are able to oxidize various forms of sulfur for energy gain, especially hydrogen sulfide (**H_2S**) to sulfate, as the major oxidation producer,

sulfur oxidizing prokaryotes are phylogenetically divers, In the domain archaea sulfur oxidation restricted to ***Sulfolobus***, and in the domain bacteria sulfur is oxidized by aerobic lithotrophes, like genera ***Thiobacillus***, ***Thiomicrospira***, (they produce sulfuric acid i.e. hydrogen ions, H+, and sulphate ions, SO_4^{2-}), or anaerobic photosynthetic bacteria belonging to the family **Chromatiaceae (**purple sulfur bacteria, used H_2S as electron donors for anoxygenic photosynthesis). Lithotrophic sulfur oxidizing bacteria are known to accelerate the generation of Acid Rock Drainage (ARD) from pyritic (FeS_2) and pyrrhotitic (FeS) rocks under suitable conditions. The bacteria catalyzed sulfide oxidation reaction may have a reaction rates six orders of magnitude (i.e. 1,000,000 times) greater than the same reactions in the absence of bacteria.

• Sulfate Reduction

As in nitrogen cycle, sulfate produced by sulfur – oxidizing bacteria may be reduced to hydrogen sulfide by a few anaerobic bacteria species. Sulfur – reducing bacteria are distributed in anoxic conditions, for example, these bacteria have been found in sewage, polluted water, sediment of lakes, see and marine mud's, and the rumen of bovine animals.

$$4\ H_2 + CaSO4 \longrightarrow H_2S + Ca(OH)_2 + 2\ H_2O$$

The direct reduction of sulfate ions to hydrogen sulfide is affected in nature by specialized strictly anaerobic bacteria of the genera:

1. ***Desulfovibirio desulfuricans***

The best known species of reducers, G – ve , pleomorphic, curved rods (vibrio – like), motile with polar flagella, anaerobic, these bacteria use molecular hydrogen in sulphate reduction.

2. ***Desulfotomaculum.***

Obligate anaerobic, sporeforming rods and heterotrophic (cell carbon from organic compounds). The bacteria utilize sulfate, thiosulfate, $S_2O_3^{2-}$, sulfite SO_3^{2-}, or other reducible sulfur-containing ions as terminal electron acceptors in their respiratory metabolism. In the process these sulfur-containing ions are reduced to hydrogen sulfide.

Due to flooding, increase in temperature and addition of organic matter when the O_2 levels decrease, so the level of sulfides considerably increases, and sometimes increase above 150 ppm. Consequently the No. of sulfate reducer also increases.

Released H_2S, S^- and S_2O_3 from sulfate reduction or amino acids decomposition are readily oxidized microbiologically aerobically by colored photosynthetic bacteria belonging to the genera ***Chlorobium*** (green sulfur bacteria) and ***Chromatium*** (purple sulfur bacteria) **.**

The oxidation process follows the equation:

$$\mathbf{CO_2 + H_2S \longrightarrow (CH2\ O)_x + H_2O + 2\ S}$$

These bacteria consume CO_2 but do not release oxygen because H_2S serves as reducing agent (electron donor), instead of water , which they give off sulfur particles inside their cells rather than oxygen in anoxygenic photosynthesis process .

Oxidation of H_2S in soil lead to pH reduction due to released H_2^+, so sulfur fertilizers added to alkaline soil types as treatment practices.

Phosphorus Cycle

Phosphorus; is an essential nutrient for all organisms in form of ions **PO_4^{3-}** and **HPO_4^{2-}** It is a part of DNA – molecules, energy storage molecules ATP and GTP, and cellular membranes. Phosphorus also a building block of certain parts of human and animal body, such as the bones and teeth. Phosphorus can be found on earth in water, soil and sediments, especially rock deposits which is the major store house of phosphorus in nature. One unique characteristic of P is its low availability due to low diffusion and high fixation in soil.

Several transformation processes recycled phosphorus in nature, m.o. integrated in these processes through different jobs:

• Inorganic phosphorus solubolization

Phosphorus is the most important key element in the nutrition of plants, next to nitrogen. It plays an important role in virtually all major metabolic processes in plant including photosynthesis, energy transfer, signal transduction, macromolecular biosynthesis and respiration. Although P is abundant in soils in both inorganic and organic forms, it is a major limiting factor for plant growth as it is in an unavailable form for root uptake. Inorganic P occurs in soil, mostly in insoluble mineral complexes, some of them appearing after frequent application of chemical fertilizers. These insoluble precipitated forms cannot be absorbed by plants.

Plants can use only a small amount of this P since 75–90% of added P is precipitated by metal–cation complexes, and rapidly becomes fixed in soils because they combined to form insoluble salts. Organic matter is also an important reservoir of immobilized P that accounts for 20–80% of P in soil. Microorganisms are an integral component of the soil P cycle and are important for the transfer of P between different pools of soil P. Phosphate Solubilzing Microorganisms (PSM) through various mechanisms of solubilization and mineralisation are able to convert inorganic and organic soil P respectively into the bioavailable form facilitating uptake by plant roots.

The main P solubilization mechanisms employed by PSM include, release of organic acids as a side products of microorganisms activities, these acids acts on conversion insoluble salts to more available to plants, such as tricalcium phosphate $[Ca_3(PO_4)_2]$. Is converted to dicalcium phosphate.

$$[Ca_3(PO4)_2] \longrightarrow Ca(H\,PO_4)_2$$

$$[Ca_3(PO_4)_2] + 2\,HNO_3 \longrightarrow 2\,Ca(HPO_4)_2 + Ca(NO_3)_2$$

$$[Ca_3(PO_4)_2] + H_2SO_4 \longrightarrow 2\,Ca(HPO_4)_2 + CaSO_4$$

Liberation of hydrogen sulfide from sulfur reduction by bacteria facilitate reaction with ferric phosphate, (other insoluble P compounds are also abundant in soil) to yield ferrous sulfide (FeS), which made phosphorus more available to plants absorption .

$$FePO_4 + 3\,H_2S \longrightarrow FeS + 2\,H_3PO_4$$

P dissolution rates, depending on size of mineral particles and soil pH, with increasing soil pH solubility of Fe and P increases but solubility of Ca phosphate decreases. PSM are abundant in root surface of plants, and a counts about $10^5 - 10^7$ cell/ gm of soil.

Many microbial species are associated with phosphorus conversion; *Pseudomonas, Bacillus, Micrococcus, Mycobacterium, Flavobacterium, Penicillium, Fusarium, Aspergillus,* etc..

• Mineralization of organic compounds

Mineralization means decomposition of organic compounds with the release of inorganic phosphate. Liberation of phosphate depends upon the rate of microbial assimilation, PH, temperature, and availability of organic phosphate.

Phosphatase enzymes catalyze split of phosphorus from organic compounds.

$$\text{Nucleic acids} \xrightarrow[H_2O]{\text{Nuclease}} \text{Nucleosides} \xrightarrow[H_2O]{\text{Nucleosidase}} \text{pentose sugars,+ PO3+hetrocyclic amines}$$

In soil 15 to 80 % of total phosphorus are in organic form, so soil rich with organic matter contains abundant of organic phosphate, therefore a good correlation exist the concentration of organic phosphorus, organic carbon, and total nitrogen.

The **C: N: P** mineralization ratio in soil in steady state is about **90: 8: 1.**

•Immobilization of Phosphorus

Conversion of inorganic, available phosphate ions into cell compounds.