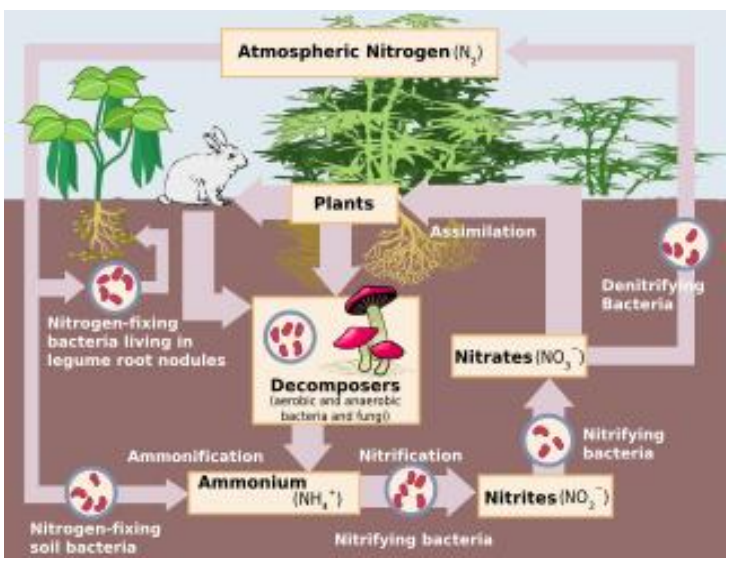
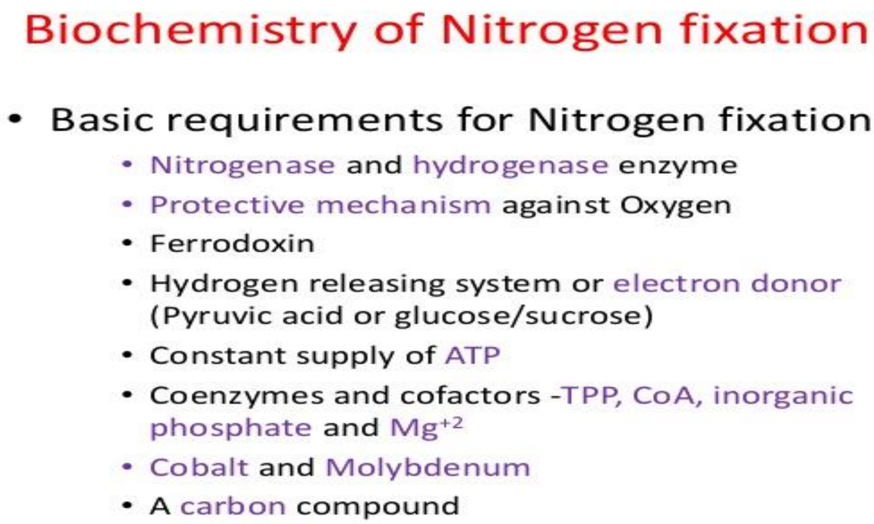
# UNIT- 4.1

## Biological Nitrogen Fixation- Symbiotic and Asymbiotic

**Nitrogen** is essential for all living things because it is a major part of amino acids, which are the building blocks of proteins and of nucleic acids such as DNA, which transfers genetic information to subsequent generations of organisms. Our atmosphere has 78% **nitrogen** gas and this atmospheric nitrogen is molecular dinitrogen, a relatively nonreactive molecule that is metabolically useless as it cannot be utilized directly by the plants or animals. The living organisms especially bacteria, convert **nitrogen** gas of the atmosphere or air into compounds of **nitrogen**, which can be used by the plants The process of converting atmospheric nitrogen into ammonia or related nitrogenous compounds in soil, which plants require, is known as Nitrogen fixation.

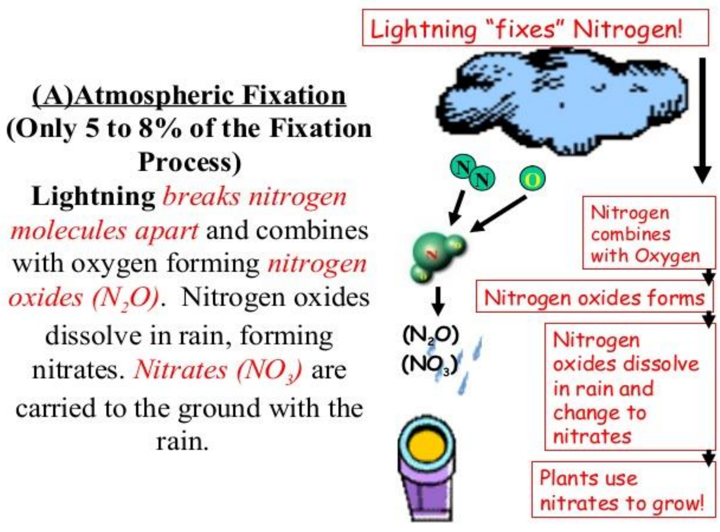
Nitrogen fixation is essential to life because fixed inorganic nitrogen compounds are required for the biosynthesis of all nitrogen-containing organic compounds, such as amino acids and proteins, nucleoside triphosphates and nucleic acids.






# Nitrogen Fixation:

## A. Non-biological




Nitrogen can be fixed by lightning that converts nitrogen and oxygen into nitrogen oxides, may react with water/rain water to make nitrous acid or nitric acid, which seeps into the soil, where it makes nitrates, which is of use to plants. Nitrogen in the atmosphere is highly stable and nonreactive due to the triple bond between atoms in the $N_2$ molecule. Lightning produces enough energy and heat to break this bond allowing nitrogen atoms to react with oxygen, forming $NO_x$. These compounds cannot be used by plants, but as this molecule cools, it reacts with oxygen to form $NO_2$ This molecule in turn reacts with water to produce $HNO_3$ (nitric acid) or its ion $NO^-_3$(nitrate),which is usable by plants.

## B. Biological

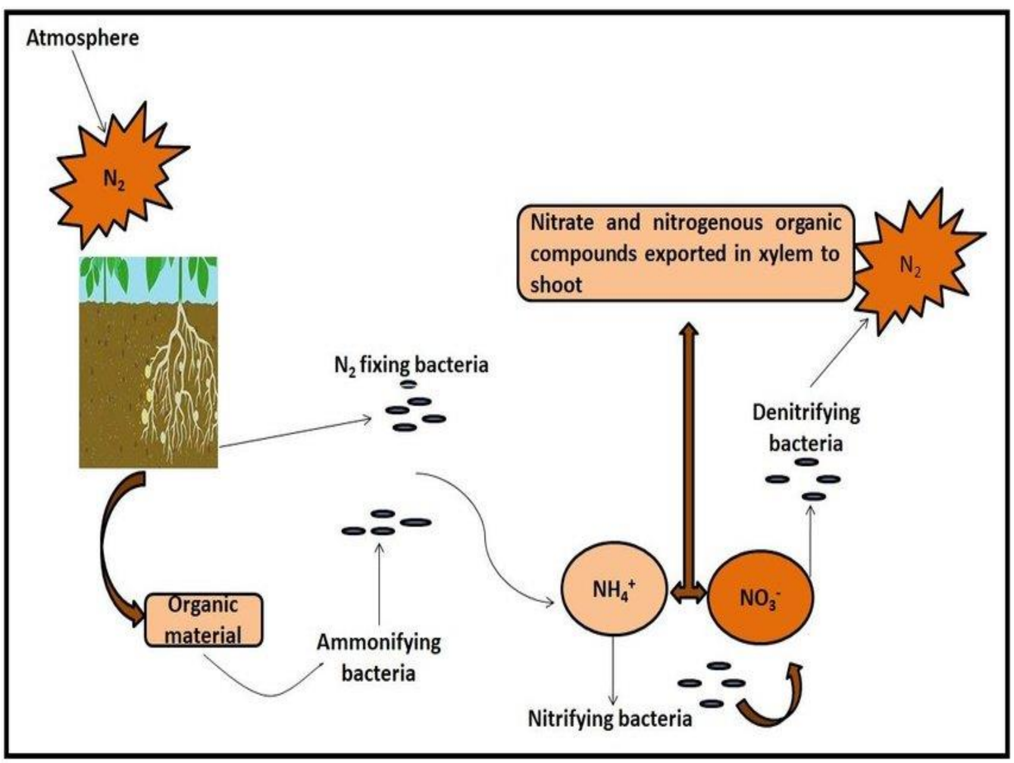
Biological nitrogen fixation was discovered by German agronomist Hermann Hellriegel and Dutch microbiologist Martinus Beijerinck. Biological nitrogen fixation (BNF) occurs when atmospheric nitrogen is converted to ammonia by a nitrogenase enzyme.[1] Biological nitrogen fixation is a very valuable alternative to nitrogen fertilizer. Biological Nitrogen fixation is carried out naturally in soil by micro-organisms termed diazotrophs that include bacteria such as Azotobacter and archaea. All biological nitrogen fixation is affected by enzymes called nitrogenases. These enzymes contain iron, often with a second metal, usually molybdenum but sometimes vanadium.

The overall reaction for Biological Nitrogen Fixation is:

The process is coupled to the hydrolysis of 16 equivalents of ATP and is accompanied by the co-formation of one equivalent of $H_2$ The conversion of $N_2$ into ammonia occurs at a metel cluster called FeMoco, an abbreviation for the iron-molybdenum cofactor. The mechanism proceeds via a series of protonation and reduction steps wherein the FeMoco active site hydrogenates the $N_2$ substrate. In free-living diazotrophs, nitrogenase-generated ammonia is assimilated into glutamate through the glutamine synthetase /glutamate synthase pathway. The microbial nif genes required for nitrogen fixation are widely distributed in diverse environments. Nitrogenase are rapidly degraded by oxygen. For this reason, many bacteria cease production of the enzyme in the presence of oxygen. Many nitrogen-fixing organisms exist only in anaerobic conditions, respiring to draw down oxygen levels, or binding the oxygen with a protein such as leghaemoglobin.

# Biological Nitrogen Fixation

- Biological nitrogen fixation was discovered by the German agronomist Hermann Hellriegel and Dutch microbiologist Martinus Beijerinck.
- Biological nitrogen fixation (**BNF**) occurs when atmospheric nitrogen is converted to ammonia by an enzyme called nitrogenase
- The reaction for BNF is:
- $N_2 + 16ATP + 8H^+ + 8\ e^- \rightarrow 2\ NH_3 + H_2 + 16ADP + 16Pi$



Biological Nitrogen fixation may be categorized into following types:

**A. Non- Symbiotic/ asymbiotic Biological Nitrogen Fixation.**

**B. Associative Biological Nitrogen Fixation.**

**C. Symbiotic Biological Nitrogen Fixation.**

## A. Non- Symbiotic/ asymbiotic Biological Nitrogen Fixation:

Soil contains a number of free living nitrogen fixing organisms. These include a number of aerobic and anaerobic bacteria and blue green algae. Biological nitrogen fixation by micro-organisms living freely or staying out of plant cell is called **non-symbiotic Biological Nitrogen Fixation** The asymbiotic nitrogen fixers can be classified as follows:

**1) Free living aerobic nitrogen fixing bacteria:**

- Photosynthetic: *Chlorobium*, *Chromatium*
- Non-Photosynthetic: *Azotobacter*, *Azomonas*, *Derxia*, *Beijerinckia*

**2) Free living anaerobic nitrogen fixing bacteria:**

- Photosynthetic: *Rhodospirillum*
- Non-Photosynthetic: *Clostridium*

**3) Free living chemosynthetic bacteria:**

- Heterotrophic: *Desulfovibro*

**4) Cyanobacteria or Blue green algae:**

- Heterocyst bearing: *Nostoc, Anabaena, Rivularia, Calothrix.*
- Non-Heterocyst bearing: *Oscillatoria, Gloeocapsa, Lyngbya, Plectonema.*

**5) Free living Fungi:**

- Yeasts and *Pullularia*

The asymbiotic free living nitrogen fixers are quite primitive. The fixation is a reduction process independent of respiration. These organisms fix nitrogen more actively under poor aeration, provided no hydrogen gas is being produced

## B. Associative Symbiotic Nitrogen Fixation:

Certain bacteria, living in close contact with the roots of cereal and grasses, fix nitrogen. This association is a loose mutualism, Called associative Symbiosis. The bacteria reside in the transition zone between soil and root (the rhizosphere) and sometimes enter the roots. Some of the fixed nitrogen is absorbed by the roots and in turn the bacteria get nourishment from the carbohydrates released by the roots. Some of the examples are:

1. *Azospirillum brasilense* in association with cereal roots.
2. *Beijerinckia* in association with the roots of Sugarcane.
3. *Azotobacter paspali* in association with roots of tropical grass- *Paspalum notatum*.

## C. Symbiotic Biological Nitrogen Fixation:

**Symbiotic nitrogen fixation** is part of a mutualistic relationship in which plants provide a niche and fixed carbon to bacteria in exchange for fixed **nitrogen**. Such an association between bacteria and host is ecological, long term and mutually beneficial to both, microbial partner fixes atmospheric nitrogen. The various examples of Symbiotic biological nitrogen fixation can be grouped under the following three categories:

### 1. Nitrogen Fixation through nodule formation in leguminous plants:

Symbiotic nitrogen fixers in large number of legume plants include genus *Rhizobium* mainly. They established themselves inside specialized structures on the roots called root nodules. The bacteria fix nitrogen only when they are present inside the nodules. The association is regarded as symbiotic because the host plant supplies the nodule bacteria the required organic carbon (carbohydrates). In return micro-organism supply fixed nitrogen to the host plant.
*Bradyrhizobium japonicum* is a slow growing symbiont of Soybeans.
*Azorhizobium caulinodans* is a stem nodule forming symbiont in Sesbania species.

### 2. Nitrogen Fixation through nodule formation in non-leguminous plants:

Many plants belonging to *families* other than Leguminosae are known to produce root nodules. The important among them are primarily trees and shrubs. The important examples of non-leguminous plants that produce root nodules and fix nitrogen are:

1. Genus *Frankia* forms root nodules in association with *Alnus* sp., *Casuarina equisetifolia, Myrica gale, etc.*
2. *Rhizobium* also root nodules in genus *Parasponia* **.**

3. Leaf nodules are formed by bacteria *Klebsiella* in genus *Psychotria* and by bacteria *Burkholderia* in genus *Pavetta zimermanniana.*

**3. Nitrogen Fixation through Non-nodulation:**

In some plants symbiotic nitrogen fixation occurs but nodules are not formed. Such associations are Pseudo symbiotic (Pseudo symbiosis). Some of the examples are:

1. Lichens, an association with fungi and algae(cyanobacteria or green algae
2. *Anthoceros*, a Bryophyte, associated with *Nostoc*.
3. Azolla, a fern, in association withAnabaena.
4. *Cycas*, a Gymnosperm, in association with *Anabaena* or *Nostoc*, blue green algae in its coralloid roots.
5. *Gunnera macrophylla*, an angiospermin association with *Nostoc* in its stem.
6. Roots of *Digitari*a, *Sorgham* and Maize associated with *Spirillum*.

**Symbiotic diazotrophs are more advanced and efficient (100-200 times) than the asymbiotic diazotrophs.**

**REFERENCE/ SYLLABUS BOOK ( For material & diagrams)**

1. Plant Physiology by H.S Srivastava ( Rastogi Publications)

2. A text book of Plant Physiology by S.K. Verma ( S. Chand& Company Ltd.)

3. Plant Physiology and Metabolism by Dr. H.N. Srivastava (Pradeep Publications)

4. Plant Physiology and Metabolism by Dr. Kamaljit & co-workers ( S. Vinesh &Co.)