Centurion
UNIVERSITY
Shaping Lives...
Empowering Communities...

Module-8

Nitrogen fixation

Centurion University
Shaping Lives...
Empowering Communities...

Nitrogen fixation is a process by which nitrogen (N_2) in the Earth's atmosphere is converted into ammonia (NH_3).

- Atmospheric nitrogen or molecular dinitrogen (N_2) is relatively inert: it does not easily react with other chemicals to form new compounds.
- The fixation process frees nitrogen atoms from their triply bonded diatomic form, N≡N, to be used in other ways.
- Nitrogen fixation, natural and synthetic, is essential for all forms of life because nitrogen is required to biosynthesize basic building blocks of plants, animals and other life forms, e.g., nucleotides for DNA and RNA, the coenzyme nicotinamide adenine dinucleotide for its role in metabolism (transferring electrons between molecules), and amino acids for proteins.

Centurion UNIVERSITY
Shaping Lives...
Empowering Communities...

- Therefore, as part of the nitrogen cycle, it is essential for agriculture and the manufacture of fertilizer.
- It is also, indirectly, relevant to the manufacture of all chemical compounds that contain nitrogen, which includes explosives, most pharmaceuticals, dyes, etc.
- Nitrogen fixation occurs naturally in the soil by nitrogen fixing bacteria (for example, *Azotobacter* affiliated with some plants, especially legumes).
- Some nitrogen-fixing bacteria have very close relationships with plants, referred to as symbiotic nitrogen fixation.
- Looser relationships between nitrogen-fixing bacteria and plants are often referred to as associative or non-symbiotic, as seen in nitrogen fixation occurring on rice roots.
- It also occurs naturally in the air by means of lightning

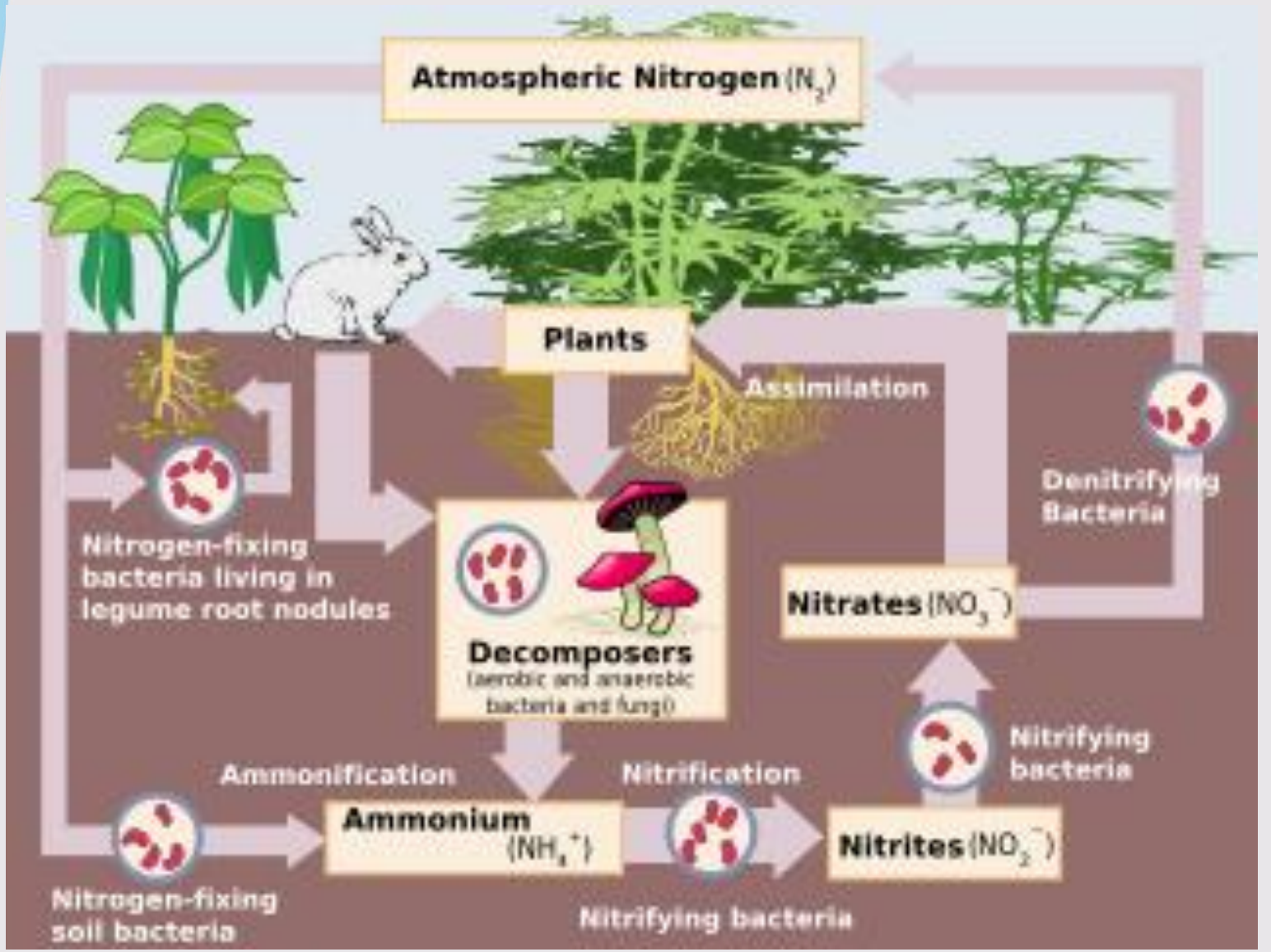
Centurion UNIVERSITY
Shaping Lives...
Empowering Communities...

All biological nitrogen fixation is done by way of metalloenzymes called nitrogenases.

- These enzymes contain iron, often with a second metal, usually molybdenum but sometimes vanadium.
- Microorganisms that can fix nitrogen are prokaryotes (both bacteria and archaea, distributed throughout their respective kingdoms) called diazotrophs.
- Some higher plants, and some animals (termites), have formed associations (symbiosis) with diazotrophs

Centurion
UNIVERSITY
Shaping Lives...
Empowering Communities...

Biological Nitrogen Fixation



Centurion UNIVERSITY
Shaping Lives...
Empowering Communities...

Schematic representation of the nitrogen cycle.

Biological nitrogen fixation was discovered by the German agronomist Hermann Hellriegel and Dutch microbiologist Martinus Beijerinck.Biologica nitrogen fixation (**BNF**) occurs when atmospheric nitrogen is converted to ammonia by an enzyme called a nitrogenase. The overall reaction for BNF is:

$$N_2 + 8\ H^+ + 8\ e^- \rightarrow 2\ NH_3 + H_2$$

The process is coupled to the hydrolysis of 16 equivalents of ATP and is accompanied by the co-formation of one molecule of H_2.The conversion of N_2 into ammonia occurs at a 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.

Centurion University
Shaping Lives...
Empowering Communities...

- In free-living diazotrophs, the nitrogenase-generated ammonium is assimilated into glutamate through the glutamine synthetase/glutamate synthase pathway.
- The microbial genes required for nitrogen fixation are widely distributed in diverse environments.
- Enzymes responsible for nitrogenase action are very susceptible to destruction 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 leghemoglobin.

Centurion UNIVERSITY
Shaping Lives...
Empowering Communities...

Microorganisms that fix nitrogen

Diazotrophs are a diverse group of prokaryotes that includes cyanobacteria (e.g. the highly significant *Trichodesmium* and *Cyanothece*), green sulfur bacteria, along with Azotobacteraceae, rhizobia and *Frankia*.

- Cyanobacteria inhabit nearly all illuminated environments on Earth and play key roles in the carbon and nitrogen cycle of the biosphere.
- In general, cyanobacteria are able to utilize a variety of inorganic and organic sources of combined nitrogen, like nitrate, nitrite, ammonium, urea, or some amino acids.
- Several cyanobacterial strains are also capable of diazotrophic growth, an ability that may have been present in their last common ancestor in the Archean eon.
- Nitrogen fixation by cyanobacteria in coral reefs can fix twice the amount of nitrogen as on land—around 1.8 kg of nitrogen is fixed per hectare per day (around 660 kg/ha/year).
- The colonial marine cyanobacterium *Trichodesmium* is thought to fix nitrogen on such a scale that it accounts for almost half of the nitrogen fixation in marine systems on a global scale.

Centurion UNIVERSITY
Shaping Lives...
Empowering Communities...

Root nodule symbioses: *Legume family*

Plants that contribute to nitrogen fixation include the legume family – Fabaceae – with taxa such as kudzu, clovers, soybeans, alfalfa, lupines, peanuts, and rooibos.

- They contain symbiotic bacteria called rhizobia within nodules in their root systems, producing nitrogen compounds that help the plant to grow and compete with other plants.
- When the plant dies, the fixed nitrogen is released, making it available to other plants; this helps to fertilize the soil.
- The great majority of legumes have this association, but a few genera (e.g., *Styphnolobium*) do not.
- In many traditional and organic farming practices, fields are rotated through various types of crops, which usually include one consisting mainly or entirely of clover or buckwheat (non-legume family Polygonaceae), often referred to as "green manure".

Centurion UNIVERSITY
Shaping Lives...
Empowering Communities...

The efficiency of nitrogen fixation in soil is dependent on many factors, including the legume as well as air and soil conditions. For example, nitrogen fixation by red clover can range from 50 - 200 lb/acre depending on these variables.

Inga alley farming relies on the leguminous genus *Inga*, a small tropical, tough-leaved, nitrogen-fixing tree.

Non-leguminous

A sectioned alder tree root nodule.

Centurion University
Shaping Lives...
Empowering Communities...

Although by far the majority of plants able to form nitrogen-fixing root nodules are in the legume family Fabaceae, there are a few exceptions:

Parasponia, a tropical genus in the Cannabaceae also able to interact with rhizobia and form nitrogen-fixing nodules

Actinorhizal plants such as alder and bayberry can also form nitrogen-fixing nodules, thanks to a symbiotic association with *Frankia* bacteria. These plants belong to 25 genera distributed among 8 plant families.