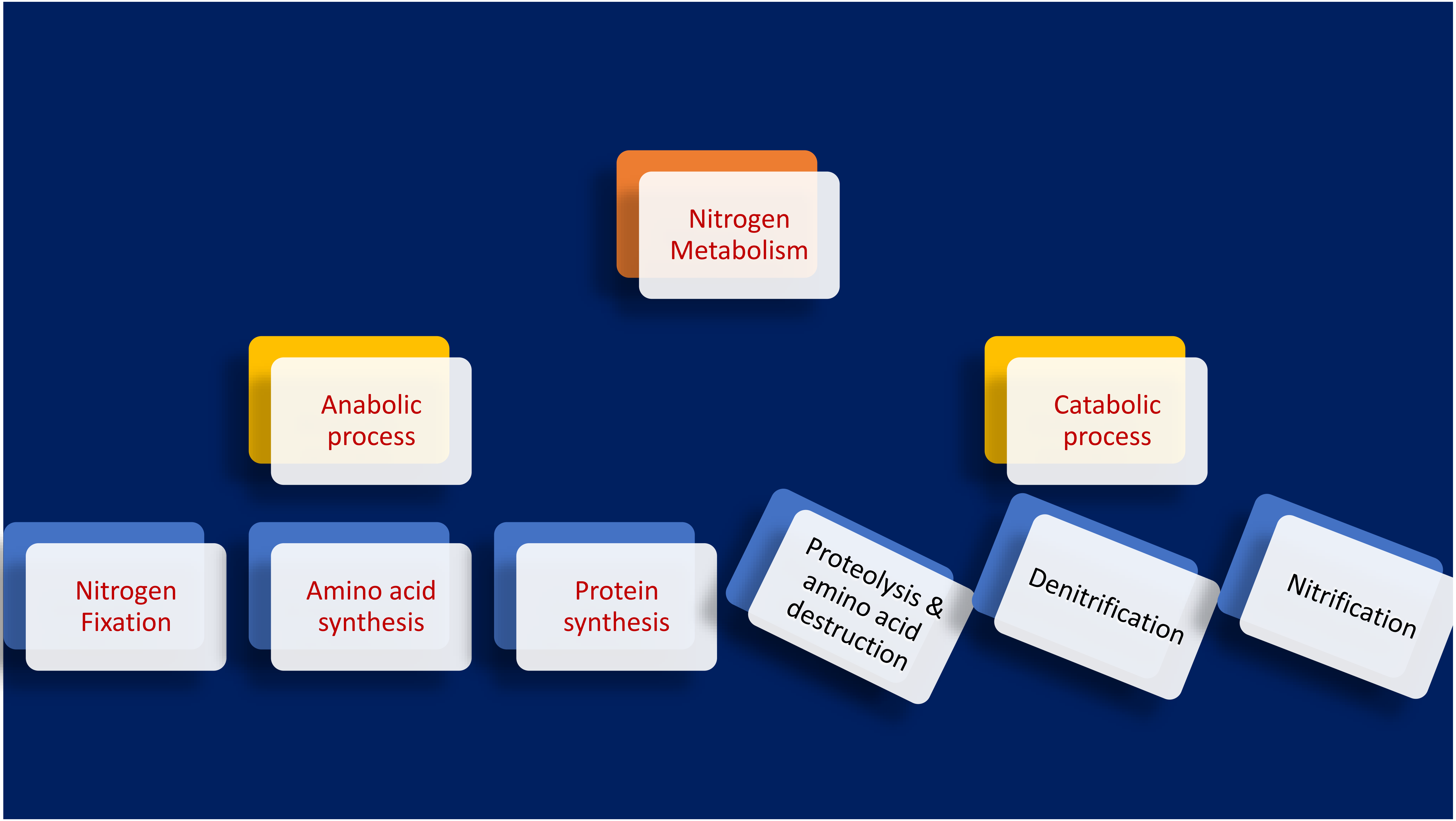
Plant physiology

Nitrogen metabolism

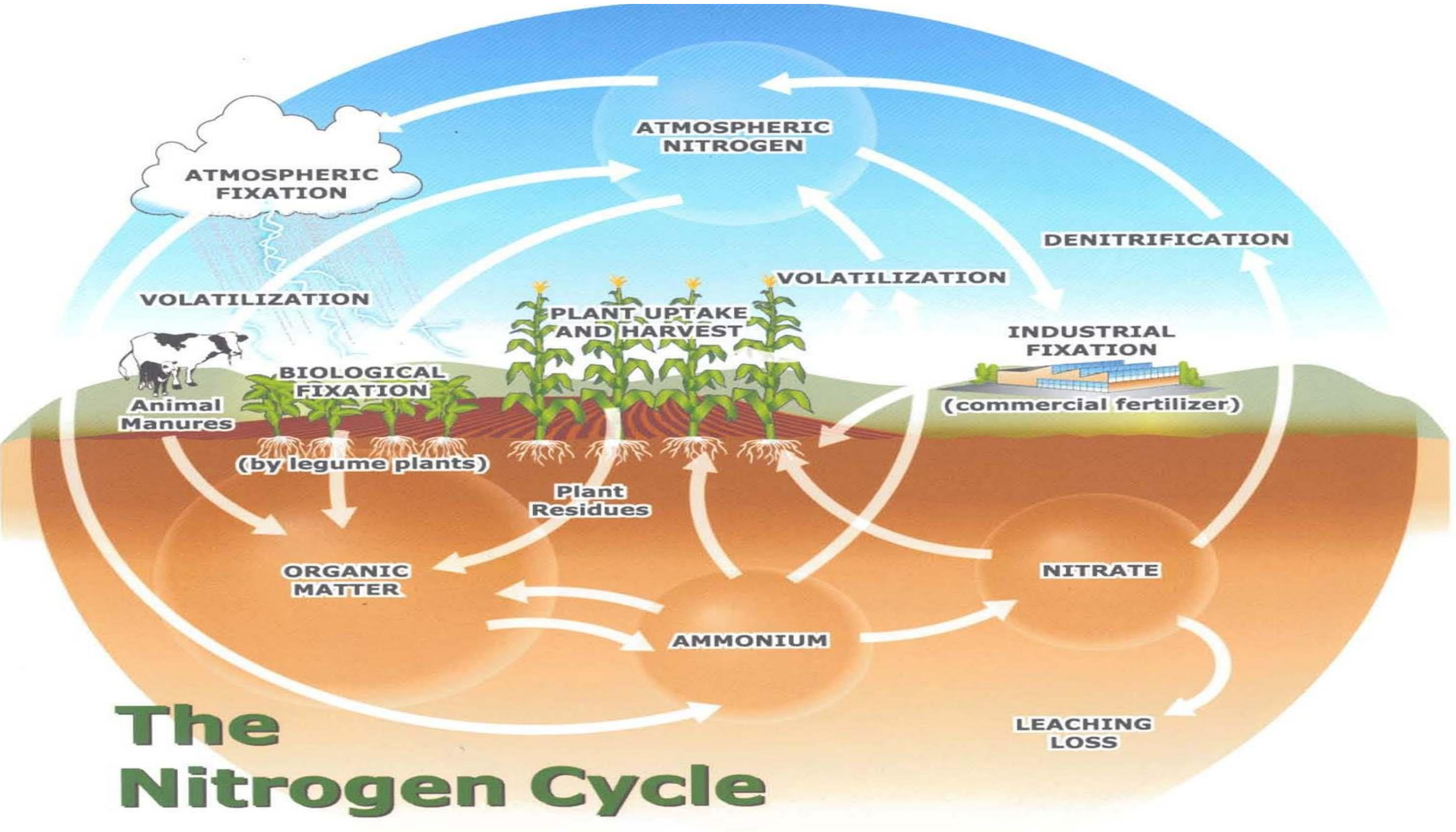
Introduction :

Like carbon,hydrogen and oxygen,nitrogen is also one of the most prevalent essential macroelements which regulates plant growth.

As well as making up approximately 78% of the Earth’s atmosphere, **nitrogen** is a vital component of amino acids and nucleic acids which make up proteins and DNA. **Nitrogen metabolism** is therefore essential to the function and structure of many molecules and for the survival of all living organisms.


Nitrogen Metabolism
Anabolic process
Catabolic process
Nitrogen Fixation
Amino acid synthesis
Protein synthesis
Proteolysis & amino acid destruction
Denitrification
Nitrification

Nitrogen is available in several forms for plant system, like nitrate,ammonia,organic nitrogen and molecular nitrogen in the environment.
The continuous interconversion of these forms to maintain the constancy of the amount of nitrogen in atmosphere, by physical, chemical and biological processes the constitute the nitrogen cycle.



The Nitrogen Cycle

Amonification :

Conversion of organic nitrogen to NH_4^+ by soil microbes is called **ammonification**.

Amonifying saprophytic bacteria are Bacillus mycoides,bacillus vulgaris and Bacillus ramosus.

Certain fungi and actinomycetes can release ammonia from the natural organic compounds.

Enzymes involved are:

GS: Gln Synthetase (Cytosolic & Plastic)

GOGAT: Glu 2-oxoglutarate aminotransferase (Ferredoxin & NADH-dependent)

GDH: Glu Dehydrogenase:

Minor Role in ammonium assimilation.

Important in amino acid catabolism.

Nitrification : The conversion of ammonium to nitrate is performed primarily by soil-living bacteria and other nitrifying bacteria.

In the primary stage of nitrification, the oxidation of ammonium (NH_4^+) is performed by bacteria such as the *Nitrosomonas* species, which converts ammonia to nitrites (NO−2). Other bacterial species such as *Nitrobacter*, are responsible for the oxidation of the nitrites (NO−2) into nitrates (NO−3). It is important for the ammonia (NH3) to be converted to nitrates or nitrites because ammonia gas is toxic to plants.

Nitrate assimilation:

Plants can absorb nitrate or ammonium from the soil via their root hairs. If nitrate is absorbed, it is first reduced to nitrite ions to the ammonia before incorporation into amino acids, nucleic acids, and chlorophyll.

For the frist time, nitrate is reduced to nitrite by enzyme nitrate reductase, respiratory energy.

NO_3^- +NADH(NADPH) + H^+ → NO_2^- + NAD^+($NADP^+$) + H_2O

+5 nitrate reductase +3(oxidation of N2)

The process is repeated in further reduction of nitrate through the intermediates, hyponitrite reductase and hydroxylamine reductase, to form NH_3.

$$NO_3^- \xrightarrow[\text{nitrate reductase}]{2e^-} NO_2^- \xrightarrow[\text{nitrite reductase}]{2e^-} N_2O_2^{-2} \xrightarrow[\text{hyponitrite reductase}]{2e^-} NH_2OH^- \xrightarrow[\text{hydroxylamine reductase}]{2e^-} NH_3$$

Each step involves the addition of two electrons by reduced NAD^+ ($NADP^+$) .

Thish reduction process of NO_3^- to NH_3 and its incorporation into the cellular proteins by aerobic microorganism and higher plants,is reffered to as nitrate assimilation.

Nitrite reductase(NiR) transfers electrons from ferredoxin to nitrite as follows:

NO_2+6Fd_{red}+8H^++6e-→NH_4^++6Fd_{ox} +2H_2O

The source of electrons is reduced ferrodoxin(Fd_{red}),produced in chloroplasts by photosynthetic noncyclic electron transfer.

In non photosynthetic tissues nitrite reduction also utilizes Fdred in plastids where NADPHproduced from oxidative pentose phosphate pathway reduces ferredoxin by an enzyme Fd-NADP+reductase.

In plants that have a symbiotic relationship with rhizobia, some nitrogen is assimilated in the form of ammonium ions directly from the nodules. It is now known that there is a more complex cycling of amino acids between *Rhizobia* bacteroids and plants. The plant provides amino acids to the bacteroids so ammonia assimilation is not required and the bacteroids pass amino acids (with the newly fixed nitrogen) back to the plant, thus forming an interdependent relationship.[6] While many animals, fungi, and other heterotrophic organisms obtain nitrogen by ingestion of amino acids, nucleotides, and other small organic molecules, other heterotrophs (including many bacteria) are able to utilize inorganic compounds, such as ammonium as sole N sources. Utilization of various N sources is carefully regulated in all organisms.

Dentrification:

The conversion of nitrate and nitrite into ammonia, nitrogen gas nitrous oxide() is called denitrification.

The process ending in the release of gaseous nitrogen into the atmosphere,complete the nitrogen cycle.

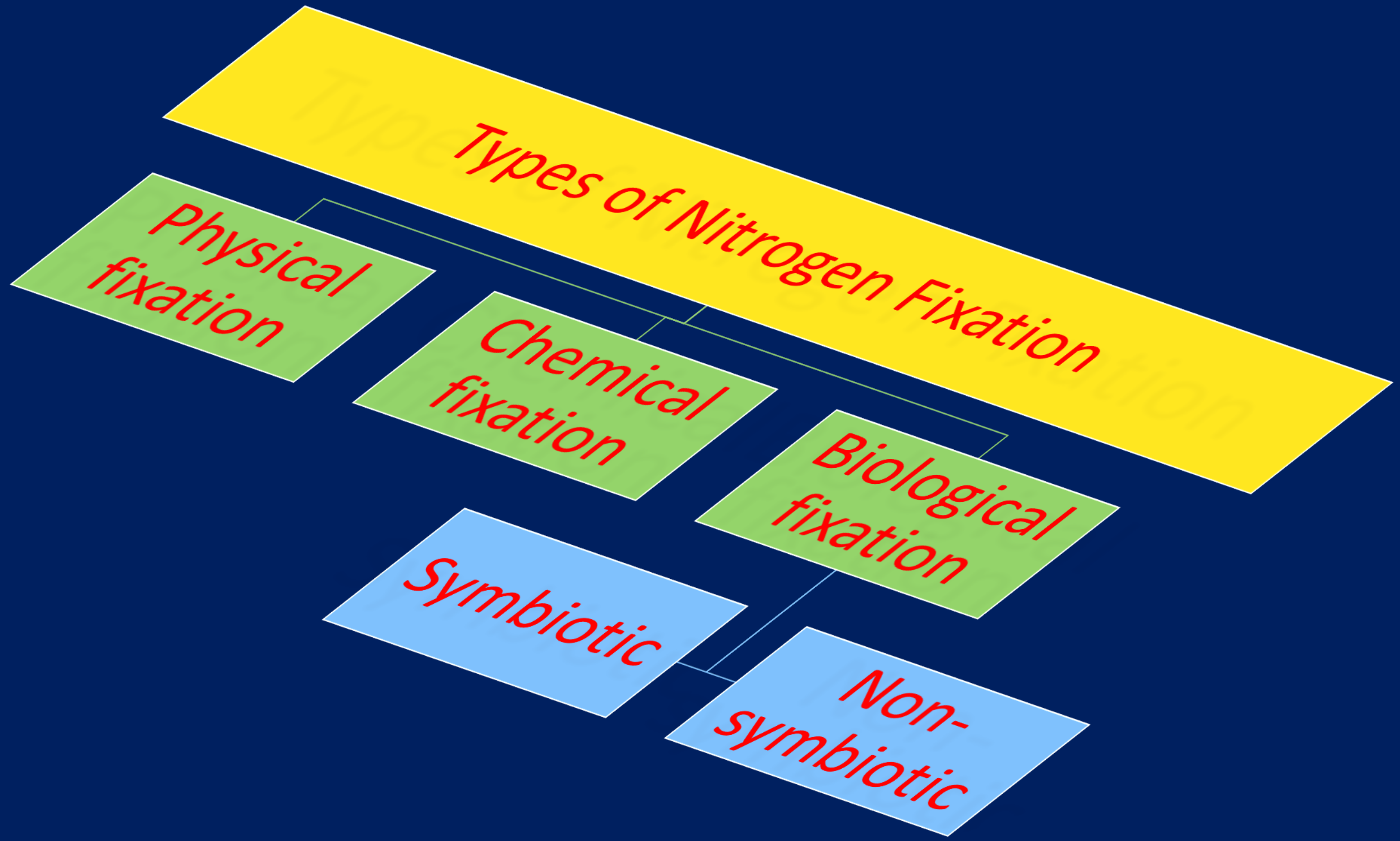
In this process through a series of reactions nitrates are reduced to ammonia and free nitrogen by anaerobic bacteria like *Pseudomonas denitrificans,Bacillus subtilis, Thiobacillus denitrificans, Micrococcus, Azotobactor, Clostridium* etc.

These bacteria use NO_3^- as an electron acceptor during respiration instead of O_2.

Denitrification:

Denitrification is the reduction of nitrates back into nitrogen gas (N_2), completing the nitrogen cycle. This process is performed by bacterial species such as *Pseudomonas* and *Clostridium* in anaerobic conditions.[5] They use the nitrate as an electron acceptor in the place of oxygen during respiration. These facultatively anaerobic bacteria can also live in aerobic conditions. Denitrification happens in anaerobic conditions e.g. waterlogged soils. The denitrifying bacteria use nitrates in the soil to carry out respiration and consequently produce nitrogen gas, which is inert and unavailable to plants.

The **nitrogen cycle** is the biogeochemical cycle by which nitrogen is converted into multiple chemical forms as it circulates among the atmosphere, terrestrial, and marine ecosystems. The conversion of nitrogen can be carried out through both biological and physical processes. Important processes in the nitrogen cycle include fixation, ammonification, nitrification, and denitrification.


Types of Nitrogen Fixation
Physical fixation
Chemical fixation
Biological fixation
Symbiotic
Non-symbiotic

BIOLOGICAL NITROGEN FIXATION:
Fixation of atmospheric nitrogen into nitrogenus salts with the help of microorganisms
Two types
Symbiotic
Non-symbiotic
Non-symbiotic:
Fixation carried out by free living microorganisms
Aerobic,anaerobic and blue green algae
Bacteria:special types
Free living aerobic: azotobactor,Beijerenkia
Free living anaerobic: Clostridium
Free living photosynthetic: Chlorobium, Rhodopseudomonas
Free living chemosynthetic: Desulfovibro, Thiobacillus
Free living fungi: yeasts and pillularia
Blue green algae
Unicellular: Gloeothece, synechococcus
Filamentous(non heterocystous): oscillatoria
Filamentous(heterocystous):Tolypothrix,Nostoc, Anabaena

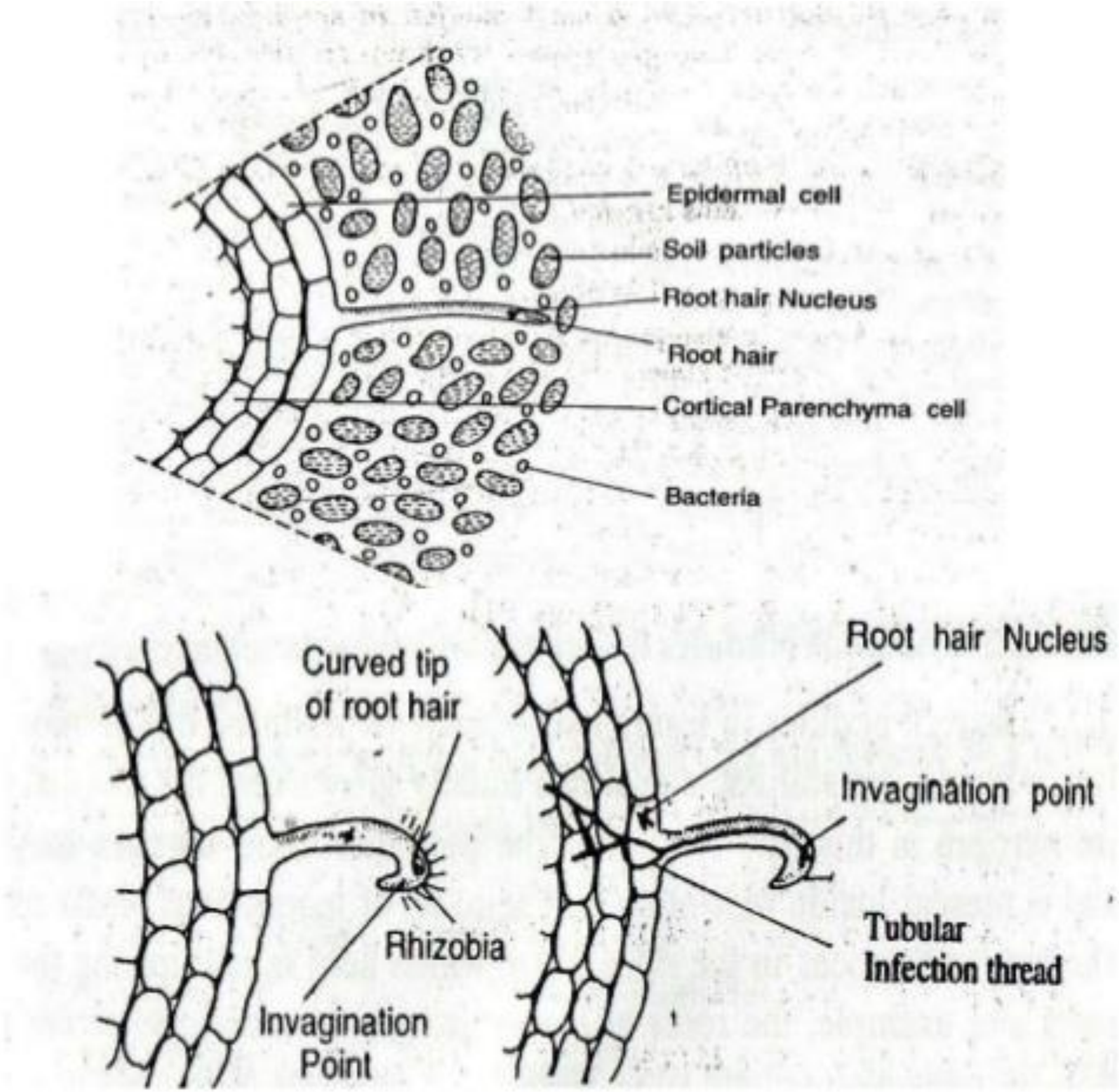
Symbiotic:
Fixation of free n2 by microorganisms in soil living symbiotically inside the plants.
Symbiosis coined by DE BARY
Three categories:
Nodule formation in leguminous plants
Nodule formation in non leguminous plants
Non nodulation

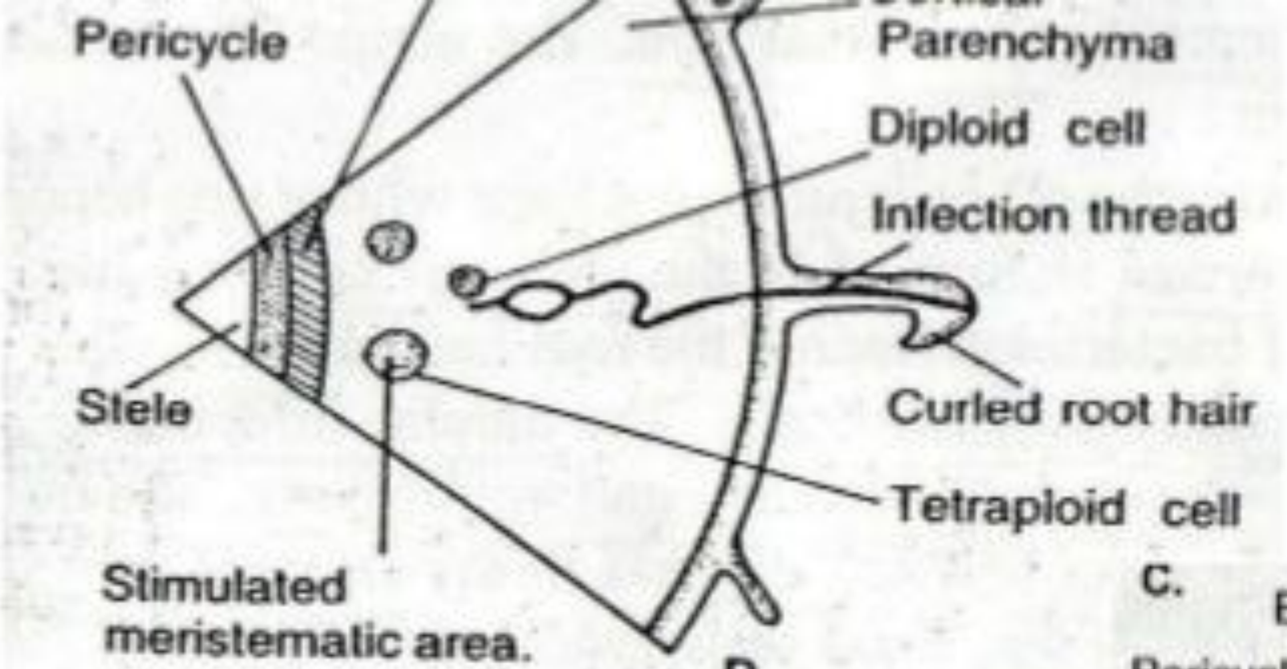
Nodule formation in leguminous plants:
13000 spp. Of legumes (Cicer arientium, pisum, Cajanus, Arachis) produce root nodules with gram negative Rhizobium spp.
They fix nitrogen only inside the root nodules.
Association provides- food and shelter to bacteria,bacteria supply fixed nitrogen to plant
Nodules may buried in soil even after harvesting – continue nitrogen fixation

Nodule formation in non leguminous plants:
Some other plants also produces root nodules
Causuarina equisetifolia-Frankia
Alnus- Frankia
Myrica gale – Frankia
Parasponia- Rhizobium
Leaf nodules are also noted:
Dioscorea, Psychotria
Gymnosperms root- Podocarpus
leaves- pavetta zinumermanniana, Chomelia

Non-nodulation:
Lichens-cyanobacteria
Anthoceros-Nostoc
Azolla-Anabaena azollae
Cycas-Nostoc and Anabaena
Gunnera macrophylla- Nostoc
Digitaria, maize and sorghum- Spirillum notatum
Paspalum notatum- Azotobactor paspali

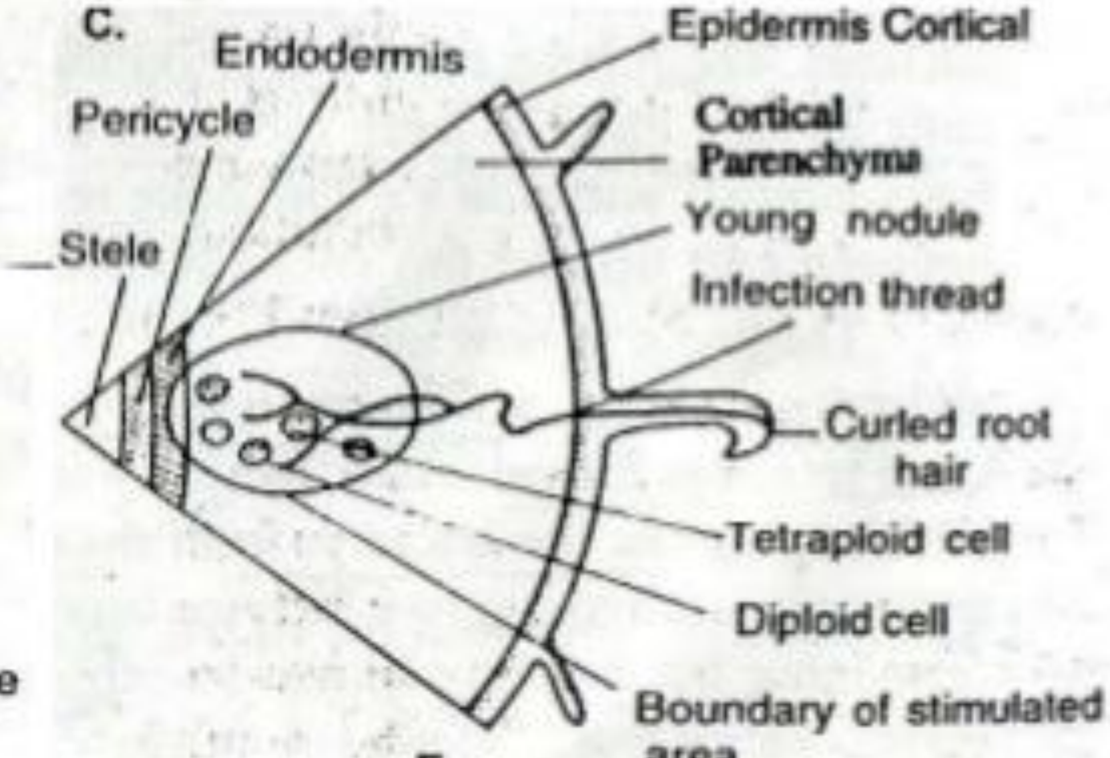
Symbiotic nitrogen fixation:
Formation of rot nodules in legumes plants:
Root nodules fromed due to infection of Rhizobium
Occur between root hairs and young root hair
Free living bacteria growing near root of legumes unable to fix nitrogen in free condition
Roots of the legumes secrete some growth factors helps in fast multiplication of bacteria
e.g. pisum sativum secretes homo serine also carbohydrate containing protein lectins over their surface
And bacteria release nod factors(lipochitin oligosaccharide signal,product of three nod genes- nod A, nod B, nod C,host specific)
This helps in recognization and attachment of rhizobial cells
Rhizobial cells have carbohydrate receptor on their surface
Lectins interact with the carbohydrate receptor of rhibial cells
Bacteria enter the roots through soft infected hairs
Tips are deformed and curved
Tubular infection thread is formed(by membrane vesicles) in the root hair cell and bacteria enters into it


Epidermal cell
Soil particles
Root hair Nucleus
Root hair
Cortical Parenchyma cell
Bacteria
Curved tip
of root hair
Rhizobia
Invagination
Point
Root hair Nucleus
Invagination point
Tubular
Infection thread

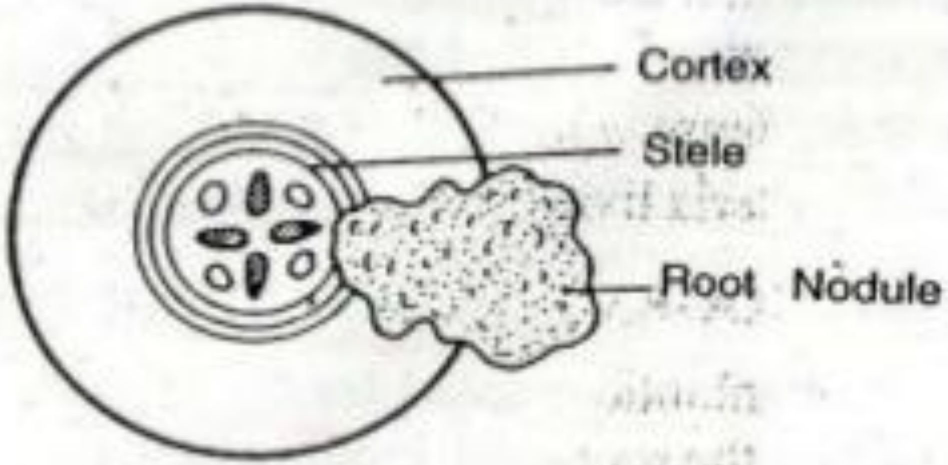

Pericycle
Parenchyma
Diploid cell
Infection thread
Stele
Curled root hair
Tetraploid cell
Stimulated meristematic area.

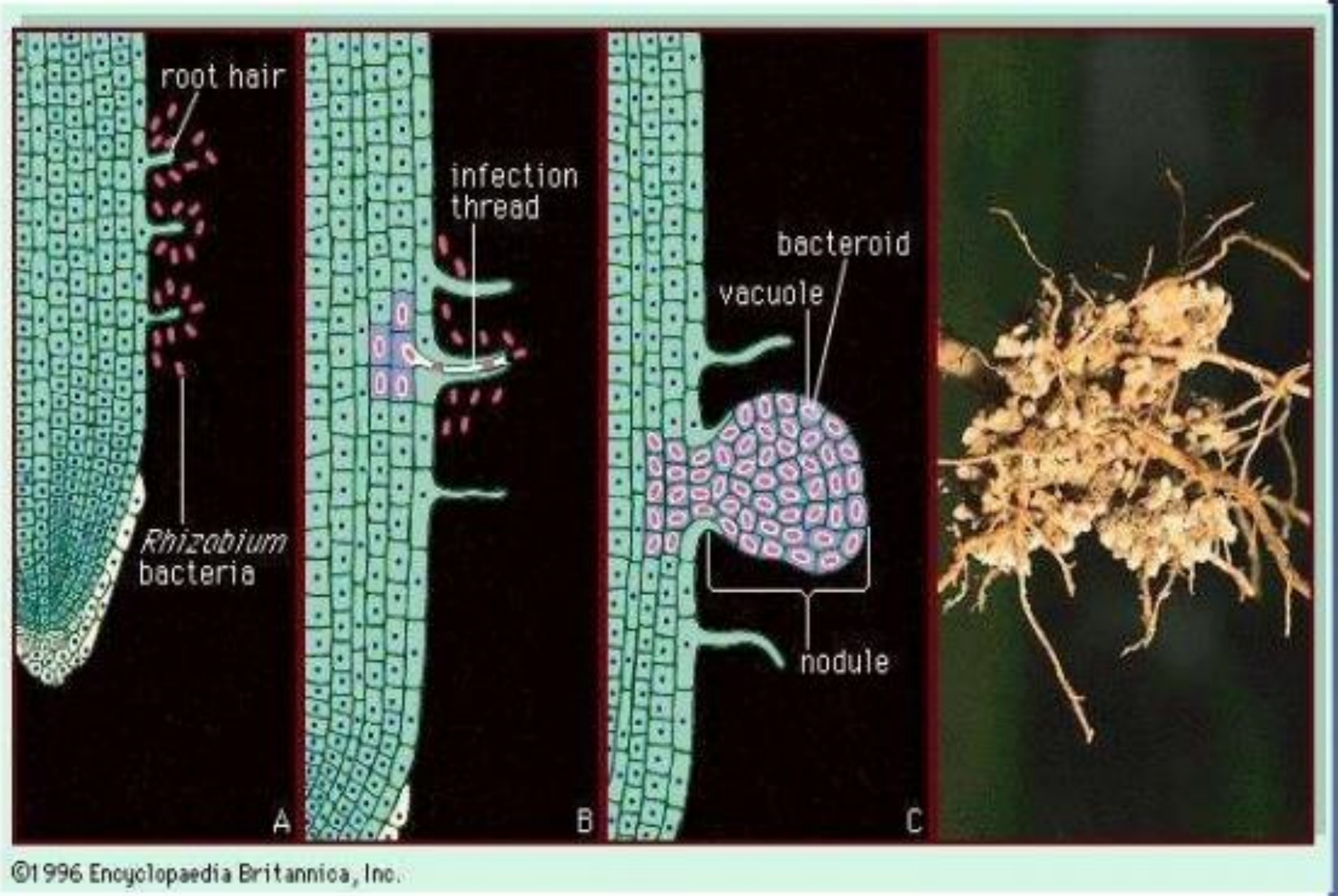
D.

C.


Epidermis Cortical
Endodermis
Pericycle
Cortical Parenchyma
Young nodule
Stele
Infection thread
Curled root hair
Tetraploid cell
Diploid cell
Boundary of stimulated area

E.


Cortex
Stele
Root Nodule


root hair
infection
thread
bacteroid
vacuole
Rhizobium
bacteria
nodule
A
B
C
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After entry, new cell wall is formed
Tubular infection contains mucoploysaccharides where bacteria embedded and start multiplication
It grows much and reaches the inner layers of cortex,endodermis,pericycle.
Adjoining areas cells dedifferentiate and start proliferation forming nodule primordium in cortex.
It induces the cortical cells to multiply which result in the formation of nodule on the surface
When the infection thread reaches the primordial cells,the tip of the infected thread fuses with the cell membrane and bacteria is released in host cells.
The bacterial cells then packaged in membrane
Branching of the thread enables the bacteria to infect many cells.
The bacterial cells multiplies and colonize in the multiplying host cells
After host cells are completely filed bacterial cells becomes dormant-bacteriods
The membrane surrounding the bacteroids is called symbiosome membrane
Float in leghaemoglobin-reddish pigment in cytoplasm of host cells
efficient o2 scavenger
maintains steady state of o2
stimulates ATP production
The mature nodules remain connected with the root via vascular tissuesand
A layer of cells surrounding th ebacteriods exclude o2 from the nodule interior.
Nitrogenous compounds synthesized is translocated through vascular tissuses
The cortical cells of the nodule occur in higher ploidy level.

These bacteria have the nitrogenase enzyme that combines gaseous nitrogen with hydrogen to produce ammonia,
The nitrogenase enzyme made up of two proteins subunits
non heme iron protein(Fe-protein or dinitrogen reductase)
Iron molybdenum protein(Mo-Fe-protein or dinitrogenase)
The reduction of N2 to NH3 requires 6 protons and 6 electrons
12 mols of ATP requires
One pair of electron requires 4 ATP
Hydrogen produced is catalyzed into protons and electrons by hydrogenase.

Pathway :

Glucose-6- phosphate acts as a electron donor

Glucose-6- phosphate is converted to phosphogluconic acid

Glucose -6-phosphate + $NADP^+$ + $H_2O \rightarrow$ 6-phosphogluconic acid + NADPH + H^+

NADPH donates electrons to ferrodoxin. protons released and ferrodixin is reduced

Reduced ferrodoxin acts as electron carrier,

Donate electron to Fe-Protein to reduce it

Electron released from ferrodoxin thus oxidized

Reduced Fe-protein combines with ATP in the presence of Mg+2

Second sub unit is activated and reduced

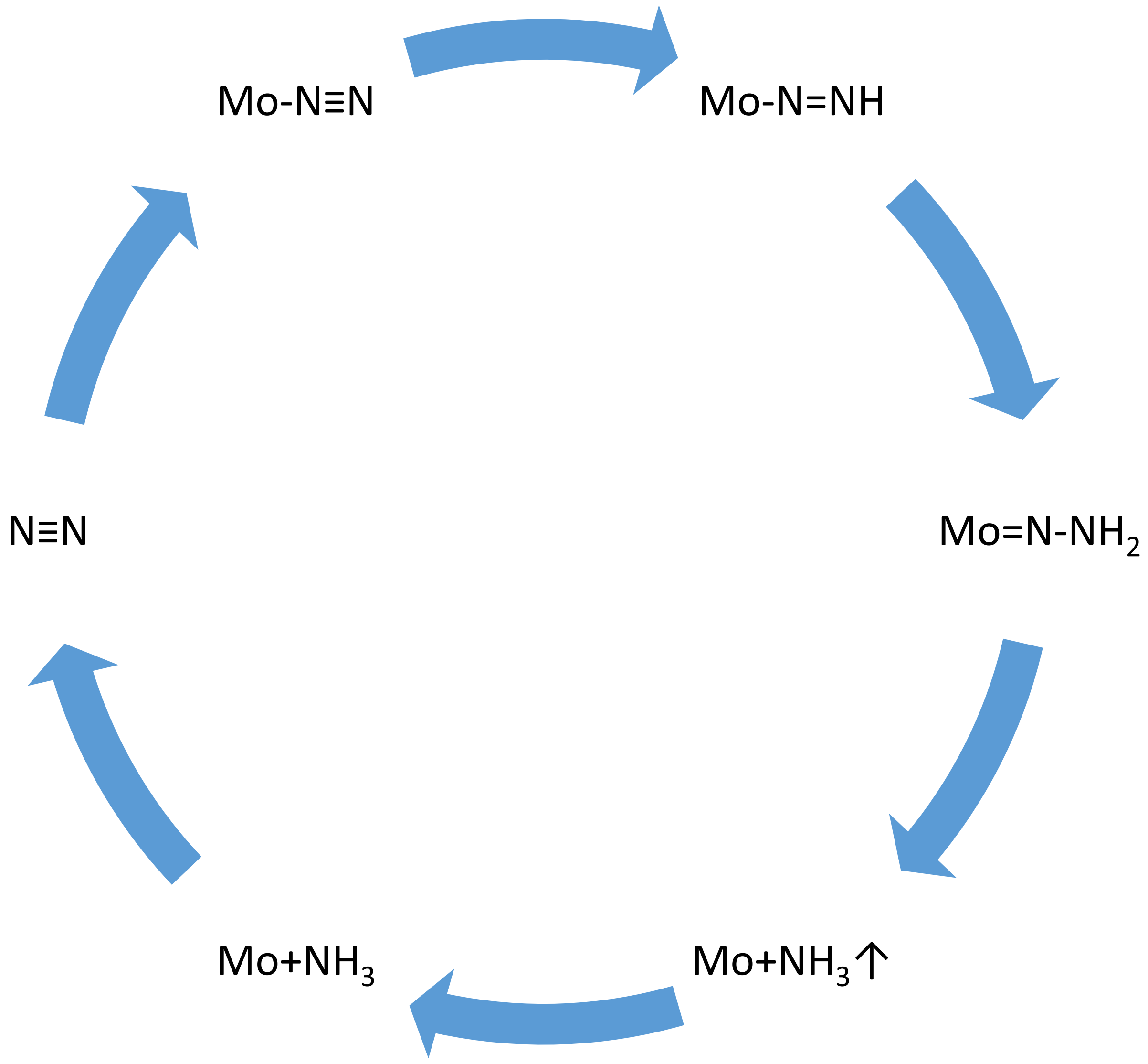
It donates electrons to N2 to NH3

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

Hydrogen produced is catalised into protons and electrons by hydrogenase

$H_2 \rightarrow 2H^+ + 2e^-$

Enzyme set free after complete reduction of N2 to NH3


Mo-N≡N
Mo-N=NH
Mo=N-NH2
Mo+NH3↑
Mo+NH3
N≡N

Process:

Nitrogen is present in the environment in a wide variety of chemical forms including organic nitrogen, ammonium (NH+
4), nitrite (NO−
2), nitrate (NO−
3), nitrous oxide (N_2O), nitric oxide (NO) or inorganic nitrogen gas (N_2). Organic nitrogen may be in the form of a living organism, humus or in the intermediate products of organic matter decomposition. The processes of the nitrogen cycle transform nitrogen from one form to another. Many of those processes are carried out by microbes, either in their effort to harvest energy or to accumulate nitrogen in a form needed for their growth. For example, the nitrogenous wastes in animal urine are broken down by nitrifying bacteria in the soil to be used as new. The diagram besides shows how these processes fit together to form the nitrogen cycle.

Nitrogen fixation:

Conversion of nitrogen into nitrates and nitrites through atmospheric, industrial and biological processes is called as nitrogen fixation. Atmospheric nitrogen must be processed, or "fixed", in a usable form to be taken up by plants. Between $5x10^{12}$ and $10x10^{12}$ g per year are fixed by lightning strikes, but most fixation is done by free-living or symbiotic bacteria known as diazotrophs. These bacteria have the nitrogenase enzyme that combines gaseous nitrogen with hydrogen to produce ammonia, which is converted by the bacteria into other organic compounds. Most biological nitrogen fixation occurs by the activity of Mo-nitrogenase, found in a wide variety of bacteria and some Archaea. Mo-nitrogenase is a complex two-component enzyme that has multiple metal-containing prosthetic groups.[3] An example of free-living bacteria is *Azotobacter*. Symbiotic nitrogen-fixing bacteria such as *Rhizobium* usually live in the root nodules of legumes (such as peas, alfalfa, and locust trees). Here they form a mutualistic relationship with the plant, producing ammonia in exchange for carbohydrates. Because of this relationship, legumes will often increase the nitrogen content of nitrogen-poor soils. A few non-legumes can also form such symbioses. Today, about 30% of the total fixed nitrogen is produced industrially using the Haber-Bosch process,[4] which uses high temperatures and pressures to convert nitrogen gas and a hydrogen source (natural gas or petroleum) into ammonia.[5]

Assimilation :

Plants can absorb nitrate or ammonium from the soil via their root hairs. If nitrate is absorbed, it is first reduced to nitrite ions and then ammonium ions for incorporation into amino acids, nucleic acids, and chlorophyll.

In plants that have a symbiotic relationship with rhizobia, some nitrogen is assimilated in the form of ammonium ions directly from the nodules.

It is now known that there is a more complex cycling of amino acids between *Rhizobia* bacteroids and plants. The plant provides amino acids to the bacteroids so ammonia assimilation is not required and the bacteroids pass amino acids (with the newly fixed nitrogen) back to the plant, thus forming an interdependent relationship.[6] While many animals, fungi, and other heterotrophic organisms obtain nitrogen by ingestion of amino acids, nucleotides, and other small organic molecules, other heterotrophs (including many bacteria) are able to utilize inorganic compounds, such as ammonium as sole N sources. Utilization of various N sources is carefully regulated in all organisms.

Amonification :

When a plant or animal dies or an animal expels waste, the initial form of nitrogen is organic. Bacteria or fungi convert the organic nitrogen within the remains back into ammonium (NH_4^+), a process called ammonification or mineralization. Enzymes involved are:

GS: Gln Synthetase (Cytosolic & Plastic)

GOGAT: Glu 2-oxoglutarate aminotransferase (Ferredoxin & NADH-dependent)

GDH: Glu Dehydrogenase:

Minor Role in ammonium assimilation.

Important in amino acid catabolism.

NITRIFICATION:

The conversion of ammonium to nitrate is performed primarily by soil-living bacteria and other nitrifying bacteria. In the primary stage of nitrification, the oxidation of ammonium (NH_4^+) is performed by bacteria such as the *Nitrosomonas* species, which converts ammonia to nitrites (NO_2^-). Other bacterial species such as *Nitrobacter*, are responsible for the oxidation of the nitrites (NO_2^-) into nitrates (NO_3^-).[5] It is important for the ammonia (NH_3) to be converted to nitrates or nitrites because ammonia gas is toxic to plants.

Due to their very high solubility and because soils are highly unable to retain anions, 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.[9][10] Where groundwater recharges stream flow, nitrate-enriched groundwater can contribute to eutrophication, a process that leads to high algal population and growth, especially blue-green algal populations. While not directly toxic to fish life, like ammonia, nitrate can have indirect effects on fish if it contributes to this eutrophication. Nitrogen has contributed to severe eutrophication problems in some water bodies. Since 2006, the application of nitrogen fertilizer has been increasingly controlled in Britain and the United States. This is occurring along the same lines as control of phosphorus fertilizer, restriction of which is normally considered essential to the recovery of eutrophied waterbodies.

Denitrification :

Denitrification is the reduction of nitrates back into nitrogen gas (N_2), completing the nitrogen cycle. This process is performed by bacterial species such as *Pseudomonas* and *Clostridium* in anaerobic conditions.[5] They use the nitrate as an electron acceptor in the place of oxygen during respiration. These facultatively anaerobic bacteria can also live in aerobic conditions. Denitrification happens in anaerobic conditions e.g. waterlogged soils. The denitrifying bacteria use nitrates in the soil to carry out respiration and consequently produce nitrogen gas, which is inert and unavailable to plants.

References:

https://en.m.Wikipedia.org/wiki/Nitrogen_cycle