Plant Physiology

Nitrogen metabolism

Princy

Nitrogen

- On a dry-weight basis, nitrogen is the fourth most abundant nutrient element in plants.

- It is an essential constituent of proteins, nucleic acids, hormones, chlorophyll, and a variety of other important primary and secondary plant constituents.

- Most plants obtain the bulk of their nitrogen from the soil in the form of either nitrate (NO_3^-) or ammonium (NH_4^+).

- But the supply of nitrogen in the soil pool is limited and plants must compete with a variety of soil microorganisms for what nitrogen is available.

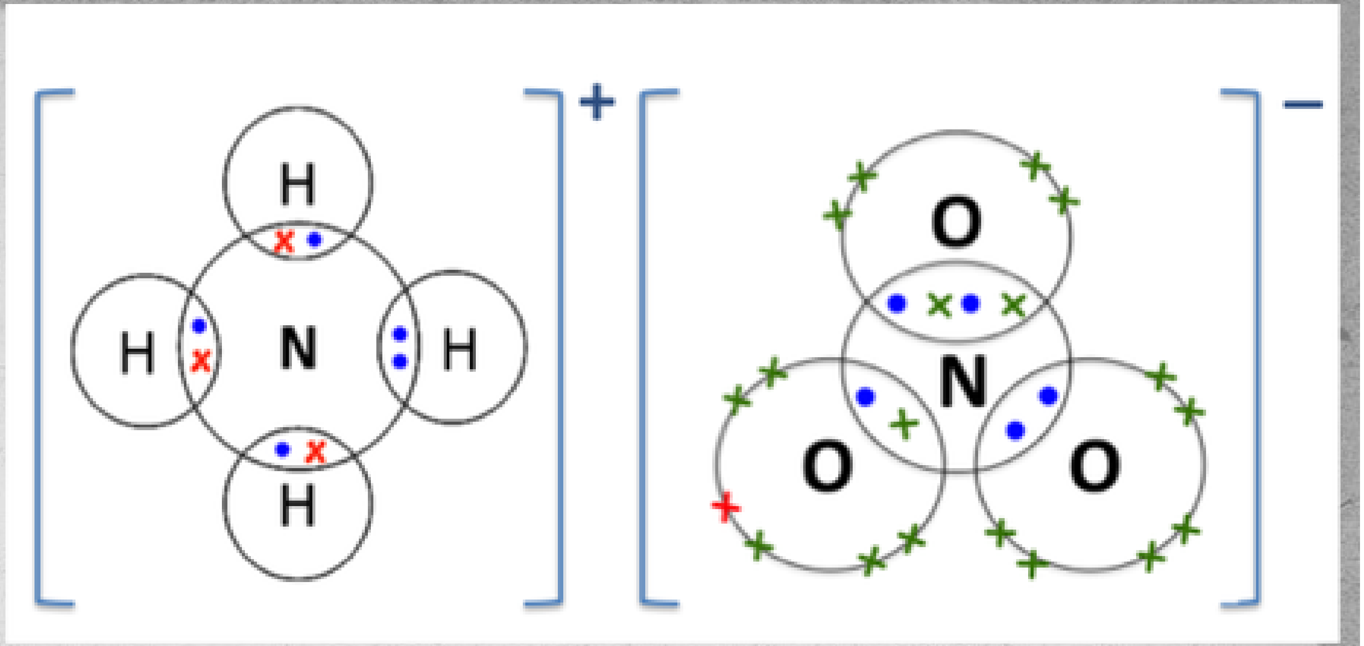
- As a result, nitrogen is often a limiting nutrient for plants.

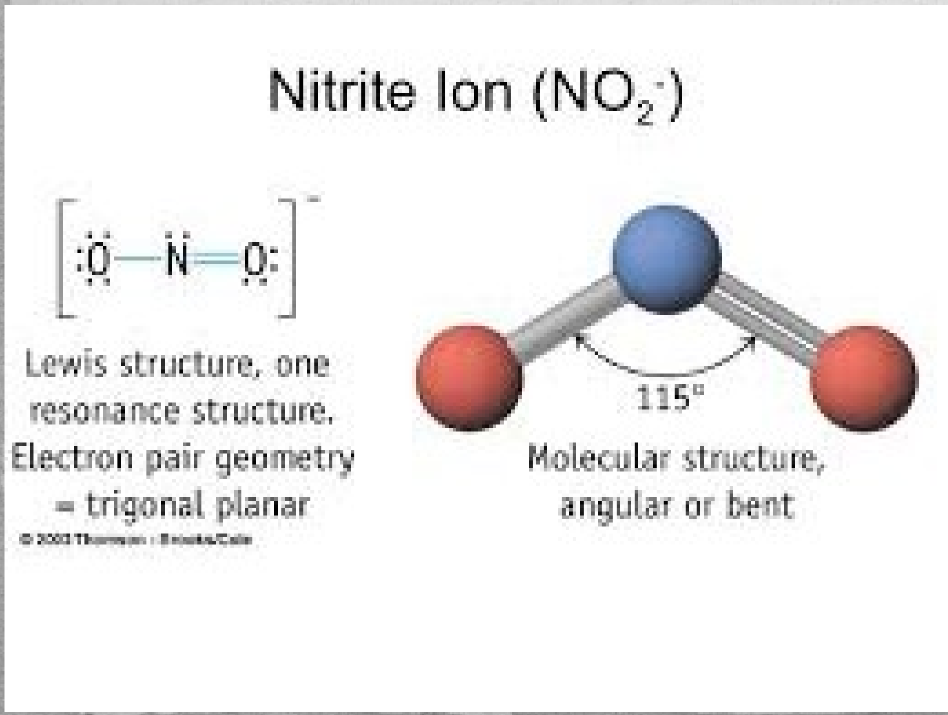
Nitrogen passes through several forms in a biogeochemical cycle

- The bulk of the atmosphere, 78% by volume, consists of molecular nitrogen (N_2 , or **dinitrogen)**, an odorless, colorless gas.

- In spite of its abundance, however, **higher plants are unable to convert dinitrogen into a biologically useful form.**

- Acquisition of nitrogen from the atmosphere requires the breaking of an exceptionally stable triple covalent bond between two nitrogen atoms (N=N) to produce ammonia (NH3) or nitrate (NO_3^-). These reactions, known as nitrogen fixation, can be accomplished by both industrial and natural processes.





- NO_3^- (nitrate)
- NO_2^- (nitrite)
- NH_4^+ (ammonium)

Nitrogen fixation

- The conversion of atmospheric dinitrogen to a combined or *fixed form or the process of reducing dinitrogen to* ammonia or nitrate (usable forrms) is known as **nitrogen fixation or dinitrogen fixation.**

- Biological nitrogen fixation is exclusively **prokaryotic** (by bacteria and cyanobacteria).

- Eukaryotic organisms are unable to fix dinitrogen because they do not have the appropriate biochemical machinery.

- Under elevated temperature (about 200°C) and high pressure (about **200** atmospheres) and in the presence of a metal catalyst (usually iron), N_2 combines with hydrogen to form ammonia.

- The extreme conditions are required to overcome the high activation energy of the reaction.

- This nitrogen fixation reaction, called the *Haber-Bosch process*, is a starting point for the manufacture of many industrial and agricultural products - fertilizers.

- Nitrogen is usually absorbed from the soil in highly oxidized forms and must be reduced by energy-dependent processes before they are incorporated into proteins and other cellular constituents.

- The two nitrogen atoms in dinitrogen are joined by an exceptionally stable bond (N≡N) and plants do not have the enzyme that will reduce this triple covalent bond. **Only certain prokaryote species are able to carry out** this important reaction.

- This situation presents plants with a unique problem with respect to the uptake and assimilation of nitrogen; plants must depend on prokaryote organisms to convert atmospheric dinitrogen into usable form (nitrogen fixation).

Natural processes of nitrogen fixation

- *Lightning*. Lightning is responsible for about 8% of the nitrogen fixed.

- Lightning converts water vapor and oxygen into highly reactive hydroxyl free radicals, free hydrogen atoms, and free oxygen atoms that attack molecular nitrogen (N_2) to form nitric acid (HNO_3). This nitric acid subsequently falls to Earth with rain.

- *Photochemical reactions.* Approximately 2% of the nitrogen fixed derives from photochemical reactions between gaseous nitric oxide (NO) and ozone (O_3) that produce nitric acid (HNO_3).

- *Biological nitrogen fixation*. The remaining 90% results from biological nitrogen fixation, in which **bacteria or blue-green algae** (cyanobacteria) fix N2 into ammonia (NH3). This ammonia dissolves in water to form ammonium (NH_4^+):

$$NH_3 + H_2O \rightarrow NH_4^+ + OH^-$$

- **From an agricultural standpoint**, biological nitrogen fix-ation is critical, because industrial production of nitrogen fertilizers seldom meets agricultural demand.

- Once fixed into ammonia or nitrate, nitrogen enters a biogeochemical cycle and passes through several organic or inorganic forms before it eventually returns to molecular nitrogen.

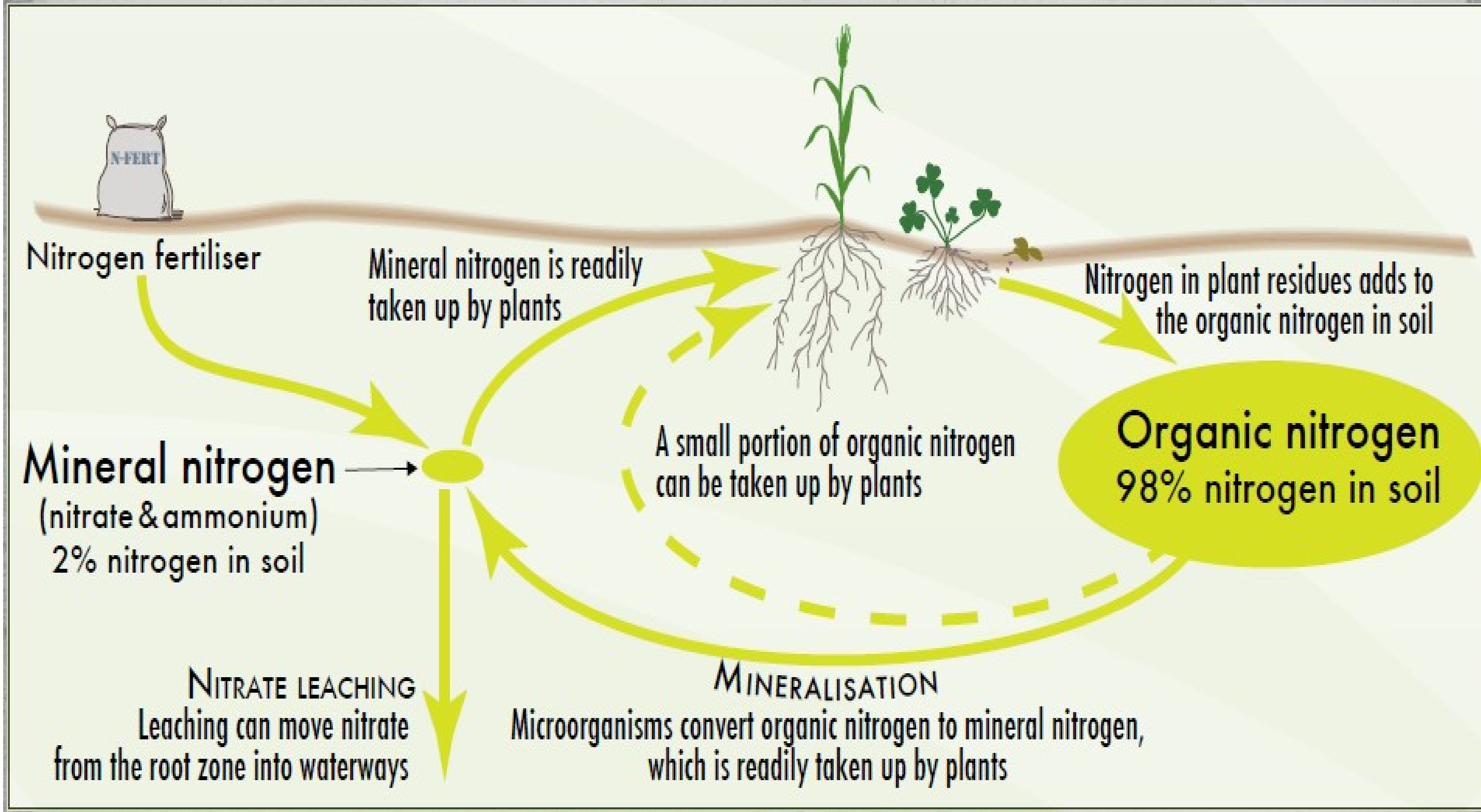
- The ammonium (NH_4^+) and nitrate (NO_3^-) ions in the soil solution that are generated through fixation or released through decomposition of soil organic matter become the object of intense competition among plants and microorganisms.

- To remain competitive, **plants have developed mechanisms for scavenging these ions rapidly from the soil solution**.

- Under the elevated soil concentrations that occur after fertilization, the absorption of ammonium and nitrate by the roots may exceed the capacity of a plant to assimilate these ions, leading to their accumulation within the plant's tissues.

TABLE 12.1
The major processes of the biogeochemical nitrogen cycle

Process	Definition
Industrial fixation	Industrial conversion of molecular nitrogen to ammonia
Atmospheric fixation	Lightning and photochemical conversion of molecular nitrogen to nitrate
Biological fixation	Prokaryotic conversion of molecular nitrogen to ammonia
Plant acquisition	Plant absorption and assimilation of ammonium or nitrate
Immobilization	Microbial absorption and assimilation of ammonium or nitrate
Ammonification	Bacterial and fungal catabolism of soil organic matter to ammonium
Anammox	Anaerobic ammonium oxidation: bacterial conversion of ammonium and nitrate to molecular nitrogen
Nitrification	Bacterial (*Nitrosomonas* sp.) oxidation of ammonium to nitrite and subsequent bacterial (*Nitrobacter* sp.) oxidation of nitrite to nitrate
Mineralization	Bacterial and fungal catabolism of soil organic matter to mineral nitrogen through ammonification or nitrification
Volatilization	Physical loss of gaseous ammonia to the atmosphere
Ammonium fixation	Physical embedding of ammonium into soil particles
Denitrification	Bacterial conversion of nitrate to nitrous oxide and molecular nitrogen
Nitrate leaching	Physical flow of nitrate dissolved in groundwater out of the topsoil and eventually into the oceans


N-FERT
Nitrogen fertiliser
Mineral nitrogen is readily taken up by plants
Nitrogen in plant residues adds to the organic nitrogen in soil
Mineral nitrogen
(nitrate & ammonium)
2% nitrogen in soil
A small portion of organic nitrogen can be taken up by plants
Organic nitrogen
98% nitrogen in soil
NITRATE LEACHING
Leaching can move nitrate from the root zone into waterways
MINERALISATION
Microorganisms convert organic nitrogen to mineral nitrogen, which is readily taken up by plants

Nitrogen cycle

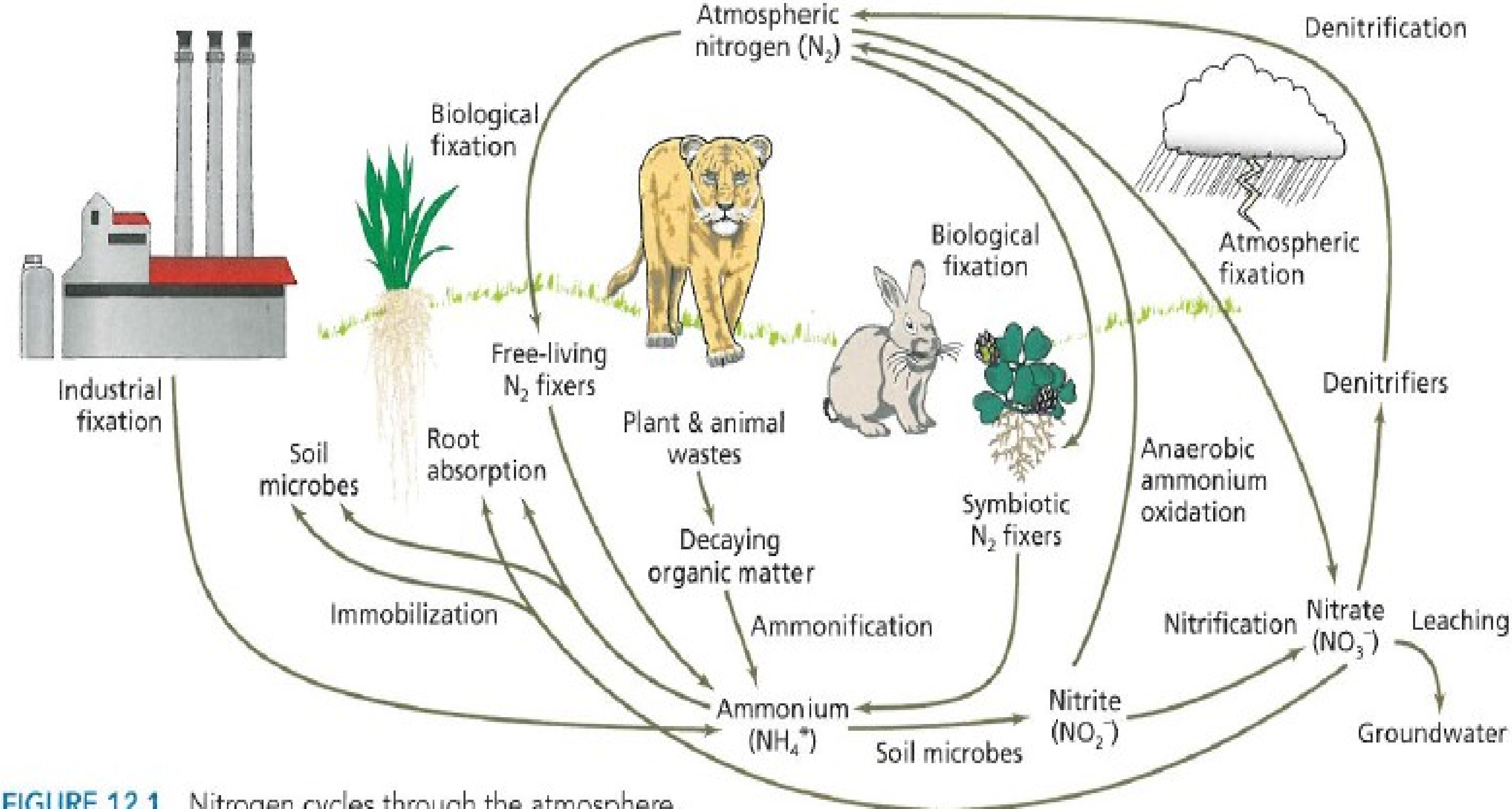


FIGURE 12.1 Nitrogen cycles through the atmosphere, changing from a gas to reduced ions before it is incorporated into organic compounds in living organisms. Some of the steps involved in the nitrogen cycle are shown.

Plants serve as the major conduit through which **nutrients pass from inert geophysical domains into dynamic biological ones.**

Nutrient assimilation

- For many mineral nutrients, they absorbed from the soil by the roots and incorporated into the organic compounds that are essential for growth and development occurs.

- This incorporation of mineral nutrients into organic substances such as pigments, enzyme cofactors, lipids, nucleic acids, and amino acids is termed **nutrient assimilation**.

- Assimilation of some nutrients—particularly nitrogen and sulfur involves a complex series of biochemical reactions that are among the most energy-consuming reactions in living organisms.

- In nitrate (NO_3^-) assimilation, the nitrogen in NO_3^- is converted to a higher-energy form in nitrite (NO_2^-), then to a yet-higher-energy form in ammonium (NH_4^+), and finally into the amide nitrogen of the amino acid glutamine.

$$NO_3^- \longrightarrow NO_2^- \longrightarrow NH_4^+$$

- Plants such as **legumes** form symbiotic relationships with nitrogen-fixing bacteria to convert molecular nitrogen (N_2) into ammonia (NH_3).

- Ammonia (NH_3) is the first stable product of natural fixation (N reducton); at physiological pH, however, ammonia is protonated to form the ammonium ion (NH_4^+).

Unassimilated ammonium or nitrate may be dangerous

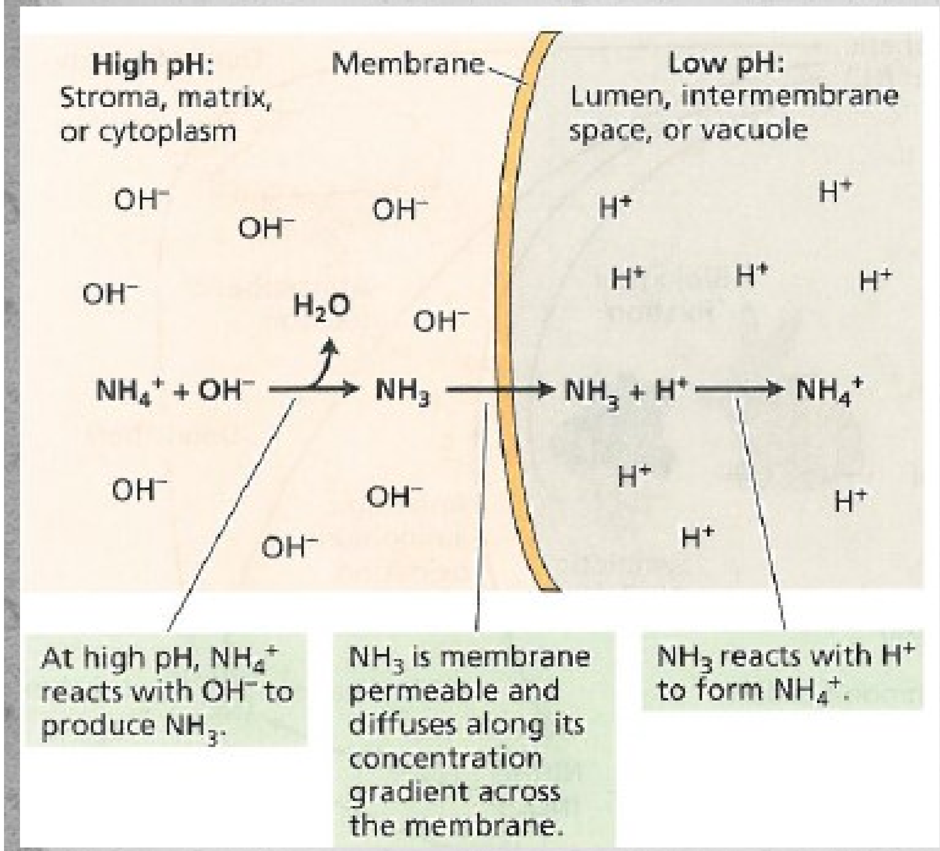
- Ammonium, if it accumulates to high levels in living tissues, is toxic to both plants and animals.

- Ammonium dissipates transmembrane proton gradients that are required for both photosynthetic and respiratory electron transport and for sequestering metabolites in the vacuole.

- Because high levels of ammonium are dangerous, animals have developed a strong aversion to its smell.

- Plants assimilate ammonium near the site of absorption or generation and rapidly store any excess in their vacuoles, thus avoiding toxic effects on membranes and the cytosol.

NH_4^+ toxicity can dissipate pH gradients.



- The left side represents the stroma, matrix, or cytoplasm,where the pH is high;
- the right side represents the lumen, intermembrane space, or vacuole, where the pH is low; and the membrane represents the thylakoid, inner mitochondrial, or tonoplast membrane for a chloroplast, mitochondrion, or root cell, respectively.
- The net result of the reaction shown is that both the OH^- concentration on the left side and the H^+ concentration on the right side have been diminished; that is, the pH gradient has been dissipated

- **In contrast to ammonium, plants can store high levels of nitrate**, or they can translocate it from tissue to tissue without deleterious effect.

- Yet if livestock or humans consume plant material that is high in nitrate, they may suffer methemoglobinemia, a disease in which the liver reduces nitrate to nitrite, which *combines with hemoglobin and renders hemoglobin unable to bind oxygen*.

- Plants assimilate nitrate into organic compounds via the enzymatic reduction of nitrate first into nitrite,
- next into ammonium, and
- then into amino acids.

Nitrate Assimilation

- Plant roots actively absorb nitrate from the soil solution via several low- and high-affinity nitrate-proton cotransporters.

- Plants eventually assimilate most of this nitrate into organic nitrogen compounds.

- The first step of this process is the **reduction** of nitrate to nitrite in the cytosol, a reaction that involves the transfer of two electrons.

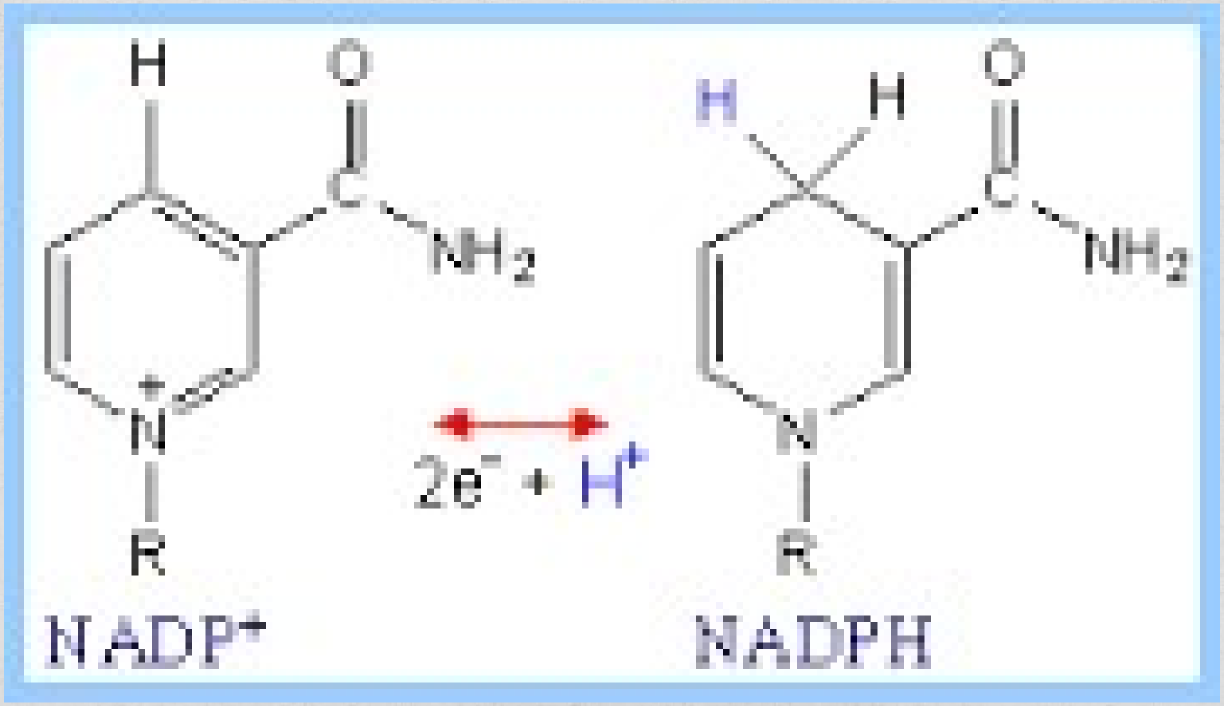
- The enzyme **nitrate reductase** catalyzes this reaction:

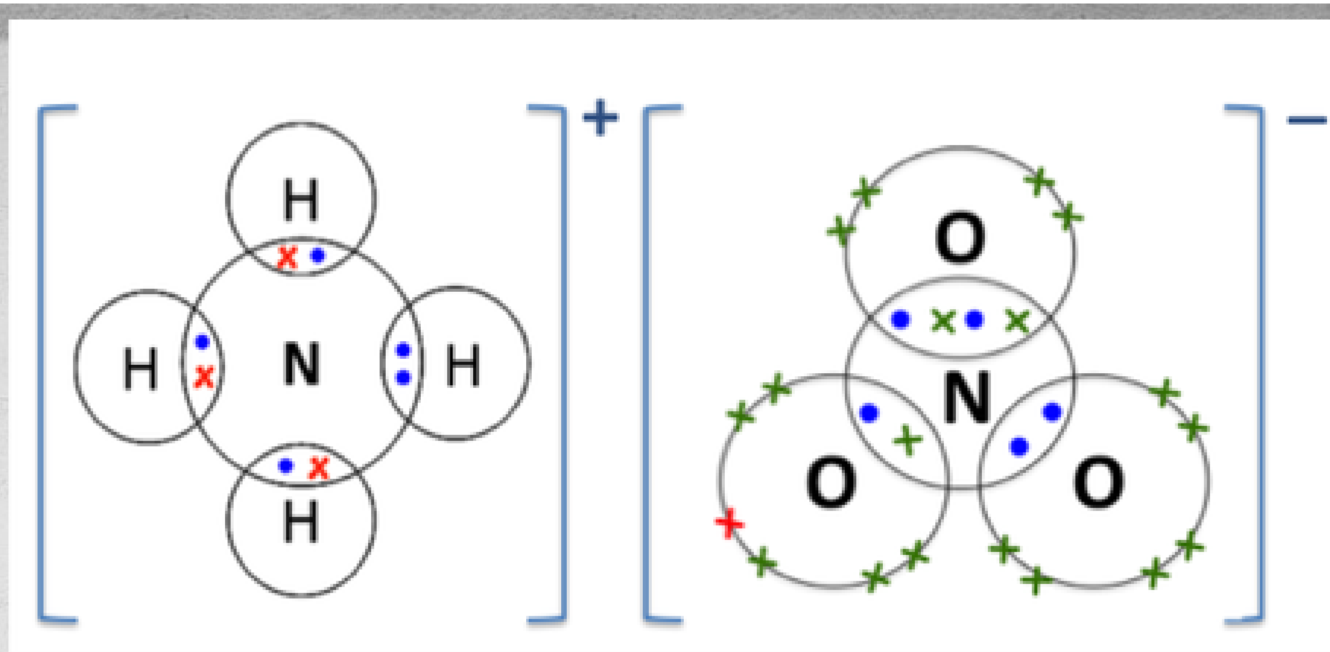
$$NO_3^- + NAD(P)H + H^+ \rightarrow$$
$$NO_2^- + NAD(P)^+ + H_2O$$

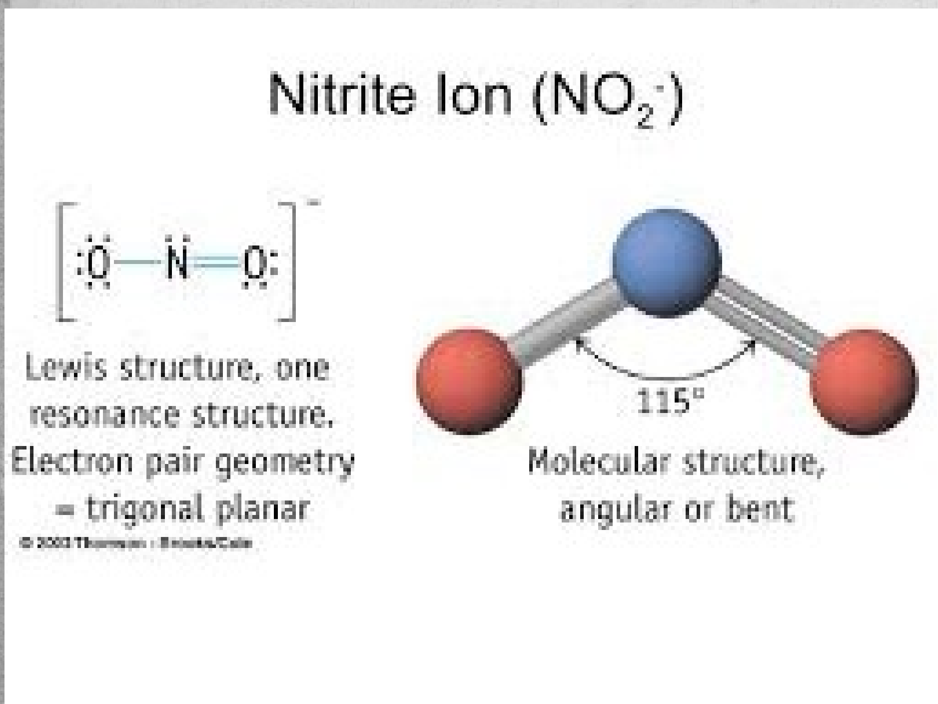
$$NO_3^- \xrightarrow[\text{Nitrate reductase}]{NADH \rightarrow NAD} NO_2^-$$

Cytosol of root or shoot cells

- Where NAD(P)H indicates NADH or NADPH. The most common form of nitrate reductase uses only NADH as an electron donor; another form of the enzyme that is found predominantly in non-green tissues such as roots can use either NADH or NADPH.







- NO_3^- (nitrate)
- NO_2^- (nitrite)
- NH_4^+ (ammonium)

- The nitrate reductases of higher plants are composed of two identical subunits,

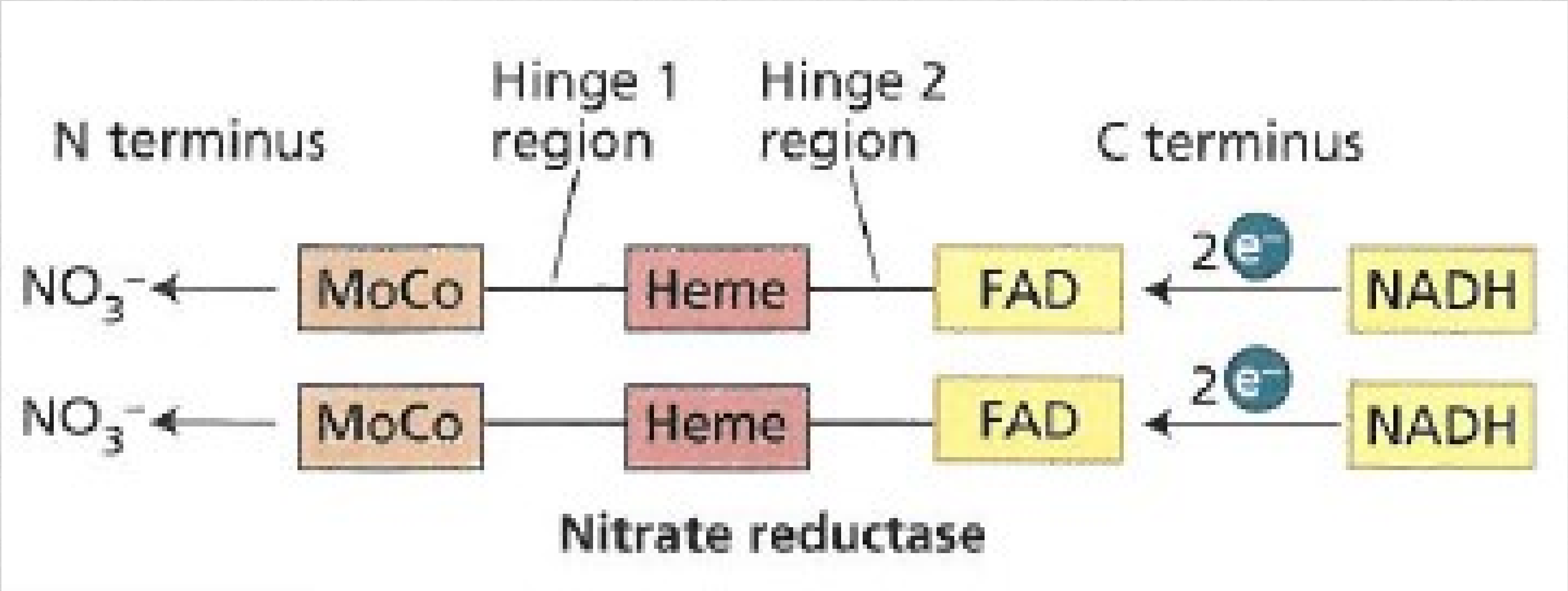
- **Each containing three prosthetic groups:**

  1. Flavin Adenine Dinucleotide (FAD),
  2. Heme, and
  3. A molybdenum atom complexed to an organic molecule called a *pterin*.

- Nitrate reductase is the main molybdenum-containing protein in vegetative tissues; **one symptom of molybdenum deficiency is the accumulation of nitrate** that results from diminished nitrate reductase activity.

- There is multiple-domain model for nitrate reductase;

  The FAD-binding domain accepts two electrons from NADH or NADPH. The electrons then pass through the heme domain to the molybdenum complex, where they are transferred to nitrate.



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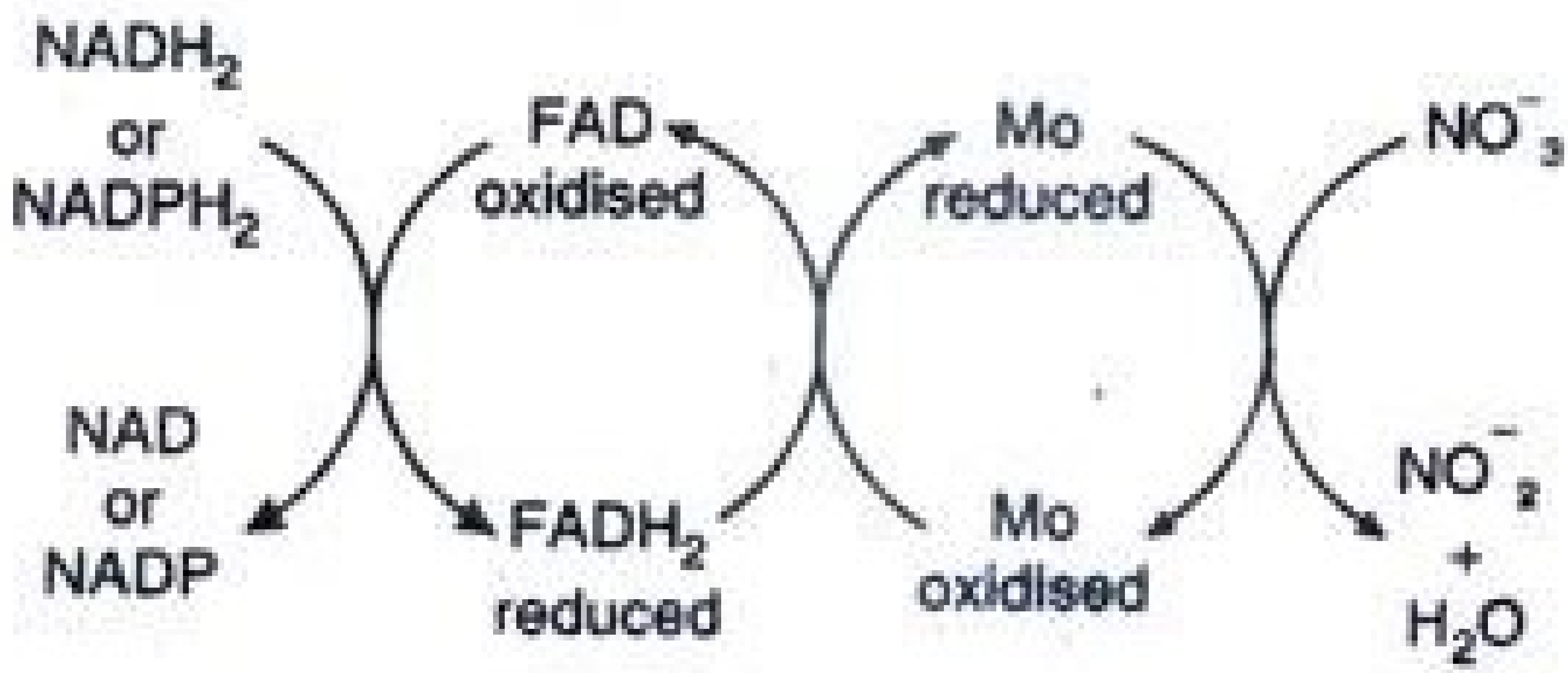


Fig. 9.1 Reduction of nitrate to nitrite

Factors regulate nitrate reductase

- **Nitrate**, light, and carbohydrates influence nitrate reductase at the transcription and translation levels
- **Induction of nitrate** linearly increases nitrate reductase activity.
- **Light, carbohydrate levels**, and other environmental factors stimulate a protein phosphatase that dephosphorylates a key serine residue in the hinge 1 region of nitrate reductase and thereby activates the enzyme.
- **Darkness and Mg_2^+** stimulate a protein kinase that phosphorylates the same serine residues, which then interact with a inhibitor protein, and thereby inactivate nitrate reductase.

Nitrite reductase converts nitrite to ammonium

- Nitrite (NO_2^-) is a highly reactive, potentially toxic ion.

- Plant cells immediately transport the nitrite generated by nitrate reduction from the cytosol into **chloroplasts** in leaves and **plastids** in roots.

- In these organelles, the enzyme **nitrite reductase reduces nitrite to ammonium**, a reaction that involves the transfer of six electrons.

$$NO_2^- + 6\,Fd_{red} + 8\,H^+ \rightarrow NH_4^+ + 6\,Fd_{ox} + 2\,H_2O$$

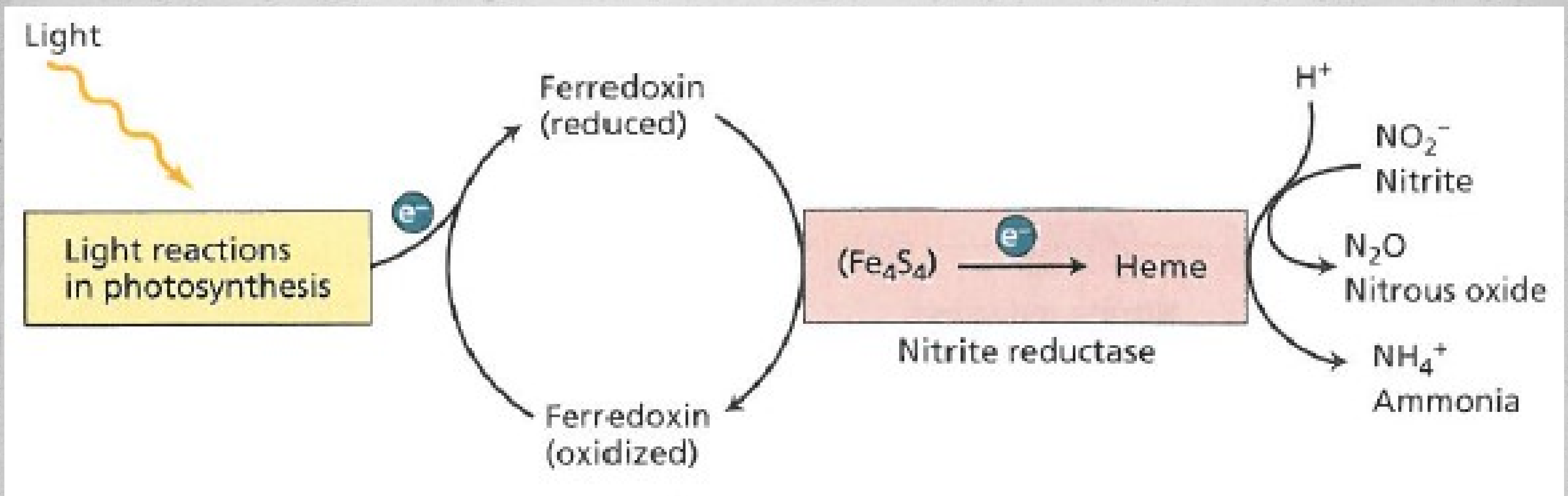
- Where Fd is ferredoxin, and the subscripts *red* and *ox* stand for *reduced* and *oxidized,* respectively.

- Reduced ferredoxin is derived from photosynthetic electron transport in the chloroplasts and from NADPH generated by the oxidative pentose phosphate pathway in non-green tissues.

- Enzyme consist of a single polypeptide containing two prosthetic groups:

    1. An iron-sulfur cluster (Fe_4S_4) and
    2. A specialized heme .

- A small percentage (**0.02-0.2%**) of the nitrite reduced is released as nitrous oxide (N_2O), a greenhouse gas.



- Model for coupling of photosynthetic electron flow, via ferredoxin, to the reduction of nitrite by nitrite reductase. The enzyme contains two prosthetic groups, Fe4S4 and heme, which participate in the reduction of nitrite to ammonium.

Both roots and shoots assimilate nitrate

- In many plants, when the roots receive small amounts of nitrate, nitrate is reduced primarily in the roots.

- As the supply of nitrate increases, a greater proportion of the absorbed nitrate is translocated to the shoot and assimilated there.

- Generally, species native to ***temperate regions*** rely more heavily on nitrate assimilation ***by the roots*** than do species of tropical or sub-tropical origins.

Ammonium Assimilation

- Plant cells avoid ammonium toxicity by rapidly converting the ammonium generated from nitrate assimilation or photorespiration, into amino acids.

- The primary pathway for this conversion involves the sequential actions of **glutamine synthetase** and **glutamate synthase.**

- **Converting ammonium to amino *acids requires two enzymes***
- Glutamine synthetase (GS) - combines ammonium with glutamate to form glutamine.

$$\text{Glutamate} + NH_4^+ + \text{ATP} \rightarrow \text{glutamine} + \text{ADP} + P_i$$

- Plants contain **two classes of GS**, one in the cytosol and the other in root plastids or shoot chloroplasts.

- The GS in root plastids generates amide nitrogen for local consumption; the GS in shoot chloroplasts reassimilates photorespiratory $\mathbf{NH_4^+}$ **.**

- Elevated plastid levels of glutamine stimulate the activity of **glutamate synthase .**

- This enzyme transfers the amide group of glutamine to **2-**oxoglutarate, yielding two molecules of glutamate.

- Plants contain **two types of glutamate synthase** : One accepts electrons from NADH; the other accepts electrons from ferredoxin(Fd).

$$\text{Glutamine} + \text{2-oxoglutarate} + \text{NADH} + H^+ \rightarrow 2\ \text{glutamate} + \text{NAD}^+$$

In **plastids** of non-photosynthetic tissues

$$\text{Glutamine} + \text{2-oxoglutarate} + \text{Fd}_{red} \rightarrow 2\ \text{glutamate} + \text{Fd}_{ox}$$

In **chloroplast** of photosynthetic tissues

Transamination reactions transfer nitrogen

- Once assimilated into glutamine and glutamate, nitrogen is incorporated into other amino acids via transamination reactions.

- The enzymes that catalyze these reactions are - **Aminotransferase .**

E.g. Aspartate aminotransferase which catalyzes the following reaction

Glutamate + oxaloacetate —> aspartate + **2**-oxoglutarate

- Here the amino group of glutamate is transferred to the carboxyl group of aspartate (an amino acid).

Asparagine and glutamine link carbon and nitrogen metabolism

- Asparagine, was the first amide to be identified.

- It serves not only as a component of proteins, but as a key compound for nitrogen transport and storage because of its stability and high nitrogen-to-carbon ratio (2 N to 4 C for asparagine in comparison to 2 N to 5 C for glutamine or 1 N to 5 C for glutamate).

- The major pathway for asparagine synthesis involves the transfer of the amide nitrogen from glutamine to asparagine.

$$\text{Glutamine} + \text{aspartate} + \text{ATP} \rightarrow \text{asparagine} + \text{glutamate} + \text{AMP} + \text{PP}_i$$

- Asparagine synthetase (AS), the enzyme that catalyzes this reaction, is found in the cytosol of leaves and roots and in nitrogen-fixing nodules .

- High levels of light and carbohydrate—conditions that stimulate plastid GS and Fd- glutamate synthase—inhibit the expression of genes coding for AS and the activity of the enzyme.

- The opposing regulation of these competing pathways helps balance the metabolism of carbon and nitrogen in plants.

- Conditions of ample energy (i.e., high levels of light and carbohydrates) stimulate GS and glutamate synthase, and inhibit AS; thus they favor nitrogen assimilation into glutamine and glutamate, compounds that are rich in carbon and participate in the synthesis of new plant materials.

- In contrast, energy-limited conditions inhibit GS and glutamate synthase, stimulate AS, and thus favor nitrogen assimilation into asparagine, a compound that is rich in nitrogen and sufficiently stable for long-distance transport or long-term storage.

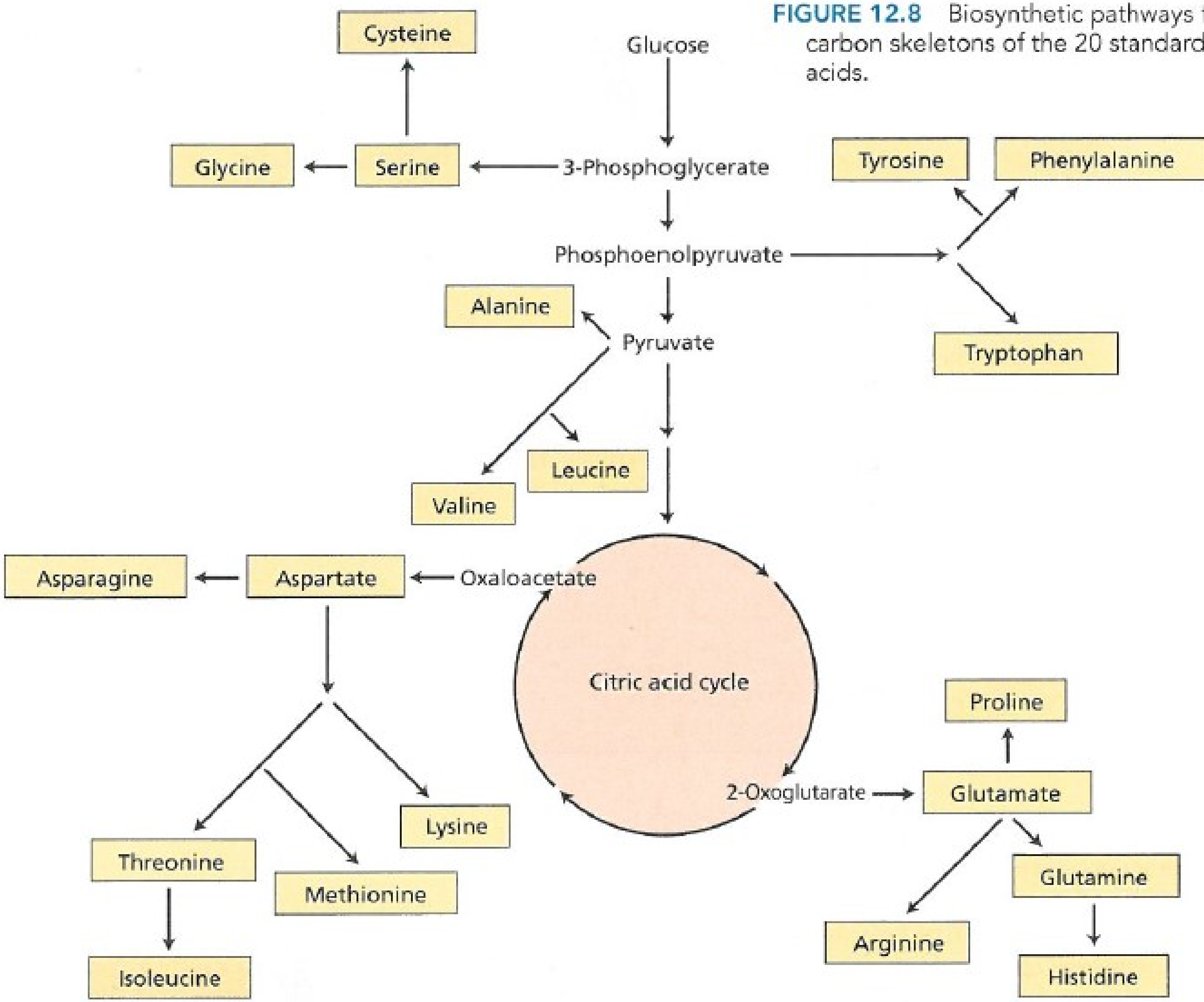


FIGURE 12.8 Biosynthetic pathways for the carbon skeletons of the 20 standard amino acids.

Amino Acid Biosynthesis

- Humans and most animals cannot synthesize certain amino acids—histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine, and, in the case of young humans, arginine (human adults can synthesize arginine)—and thus must obtain these so-called essential amino acids from their diet.

- In contrast, plants synthesize all the **20** or so amino acids that are common in proteins.

- The nitrogen-containing amino group, derives from trans-amination reactions with glutamine or glutamate.

- The carbon skeleton for amino acids derives from 3 phosphoglycerate, phosphoenolpyruvate, or pyruvate generated during glycolysis

or

- from 2-oxoglutarate or oxaloacetate generated in the citric acid cycle, Parts of these pathways required for synthesis of the essential amino are appropriate targets for herbicides (such as "Roundup"), because they are missing from animals, so substances that block these pathways are lethal to plants but in low concentrations do not injure animals.

Biological Nitrogen Fixation

- Biological nitrogen fixation accounts for most of the conversion of atmospheric N_2 into ammonium, and thus serves as the key entry point of molecular nitrogen into the biogeochemical cycle of nitrogen.

- ***Free-living and symbiotic bacteria fix nitrogen***
- Some bacteria can convert atmospheric nitrogen into ammonium.

- Most of these nitrogen-fixing prokaryotes live in the soil, generally independent of other organisms.

- A few form symbiotic associations with higher plants in which the prokaryote directly provides the host plant with fixed nitrogen in exchange for other nutrients and carbohydrates

The most common type of symbiosis

	Plant symbiont (as host)	Nitrogen fixing prokaryote
1.	Members of the plant family **Fabaceae** (Leguminosae)	**Soil bacteria** *Azorhizobium* *Bradyrhizobium* *Photorhizobium* *Rhizobium* and *Sinorhizobium* *Collectively called* Rhizobia
2.	**Actinorhizal plants** woody plant species -Alder trees	**Soil bacteria** *Frankia* (actinobacteria*)*
3.	**Herb** – *Gunnera*	**Cyanobacteria** – *Nostoc*
4.	**Water fern** – *Azolla*	**Cyanobacteria –** *Anabaena*
5.	**Grasses –** *Paspalum notatum* *Miscanthus* Sugarcane Other cereals	**Soil bacteria –** *Azotobacter paspali* *Azospirillum* sp. *Acetobacter diazotrophicus* *Azospirillum brasilense, Klebsiella*

TABLE 12.2
Examples of organisms that can carry out nitrogen fixation

Host plant	N-fixing symbionts
SYMBIOTIC NITROGEN FIXATION	
Leguminous: legumes, *Parasponia*	*Azorhizobium, Bradyrhizobium, Photorhizobium, Rhizobium, Sinorhizobium*
Actinorhizal: alder (tree), *Ceanothus* (shrub), *Casuarina* (tree), *Datisca* (shrub)	*Frankia*
Gunnera	*Nostoc*
Azolla (water fern)	*Anabaena*
Sugarcane	*Acetobacter*
Miscanthus	*Azospirillum*
FREE-LIVING NITROGEN FIXATION	
Type	**N-fixing genera**
Cyanobacteria (blue-green algae)	*Anabaena, Calothrix, Nostoc*
Other bacteria	
Aerobic	*Azospirillum, Azotobacter, Beijerinckia, Derxia*
Facultative	*Bacillus, Klebsiella*
Anaerobic	
Nonphotosynthetic	*Clostridium, Methanococcus* (archaebacterium)
Photosynthetic	*Chromatium, Rhodospirillum*

Actinorhizal plants are a group of angiosperms characterized by their ability to form a symbiosis with the nitrogen fixing actinobacteria Frankia. This association leads to the formation of nitrogen-fixing root nodules.

Gunnera

FIGURE 12.9 Root nodules on a common bean (*Phaseolus vulgaris*). The nodules are a result of infection by *Rhizobium* sp. (Photo by David McIntyre.)

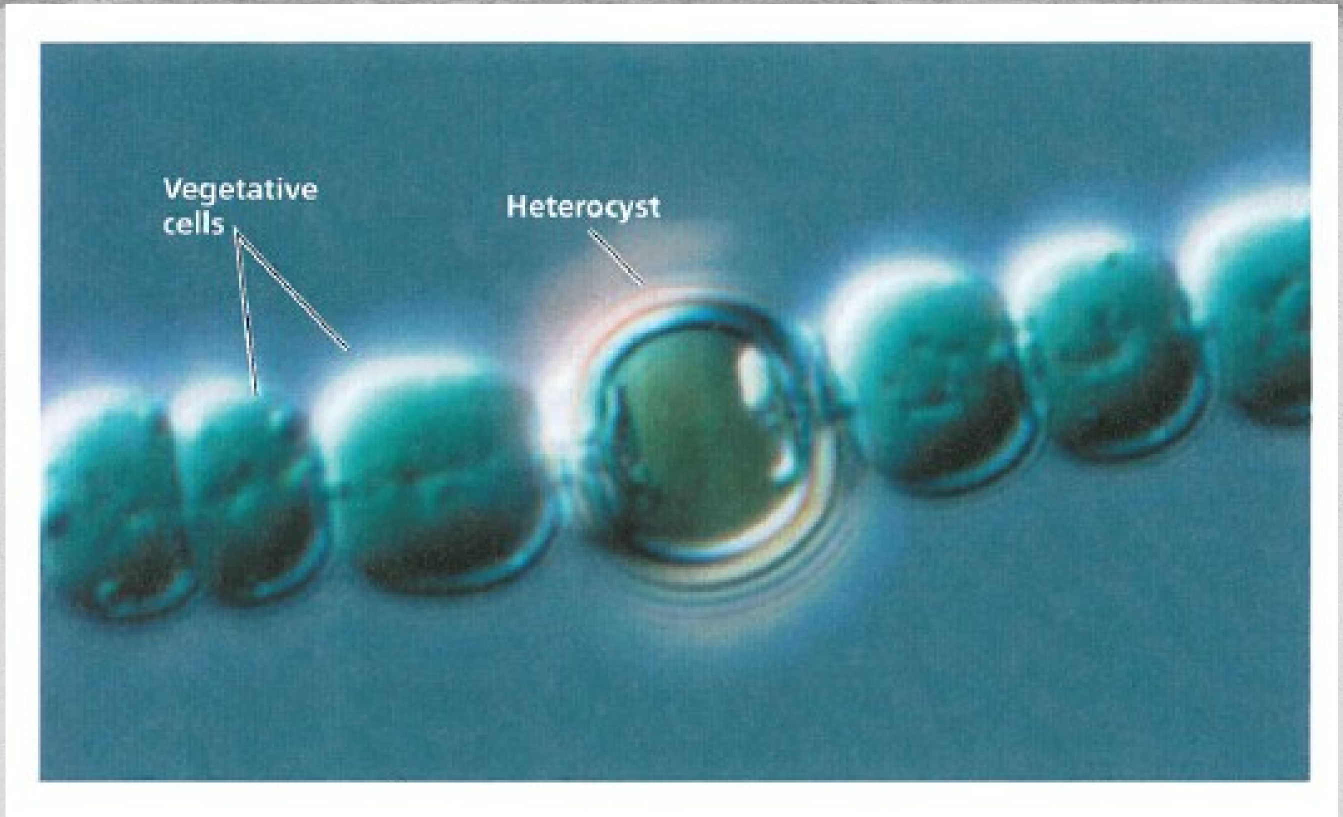
Nitrogen fixation requires anaerobic conditions

- Because nitrogen fixation involves the expenditure of large amounts of energy, the nitrogenase enzymes that catalyze these reactions have sites that facilitate the high-energy exchange of electrons.

- Oxygen, being a strong electron acceptor, can damage these sites and irreversibly inactivate nitrogenase, so **nitrogen must be fixed under anaerobic conditions.**

- Each of the nitrogen-fixing organisms either functions under natural anaerobic conditions or creates an internal, local anaerobic environment in the presence of oxygen.

- In cyanobacteria, anaerobic conditions - *heterocysts.*
- Heterocysts are thick-walled cells that differentiate when filamentous cyanobacteria are deprived of NH_4^+.
- These cells lack photosystem II, the oxygen-producing photosystem of chloroplasts, so they do not generate oxygen.
- Heterocysts appear to represent an adaptation for nitrogen fixation, in that they are widespread among aerobic cyanobacteria that fix nitrogen.
- Cyanobacteria can fix nitrogen under anaerobic conditions such as those that occur in flooded fields and they die as the fields dry, releasing the fixed nitrogen to the soil.



- A heterocyst in a filament of the nitrogen-fixing cyanobacterium *Anabaena.* The thick-walled heterocysts, interspersed among vegetative ceils, have an anaerobic inner environment that allows cyanobacteria to fix nitrogen in aerobic conditions.

TABLE 12.3
Associations between host plants and rhizobia

Plant host	Rhizobial symbiont
Parasponia (a nonlegume, formerly called *Trema*)	*Bradyrhizobium* spp.
Soybean (*Glycine max*)	*Bradyrhizobium japonicum* (slow-growing type); *Sinorhizobium fredii* (fast-growing type)
Alfalfa (*Medicago sativa*)	*Sinorhizobium meliloti*
Sesbania (aquatic)	*Azorhizobium* (forms both root and stem nodules; the stems have adventitious roots)
Bean (*Phaseolus*)	*Rhizobium leguminosarum* bv. *phaseoli*; *Rhizobium tropicii*; *Rhizobium etli*
Clover (*Trifolium*)	*Rhizobium leguminosarum* bv. *trifolii*
Pea (*Pisum sativum*)	*Rhizobium leguminosarum* bv. *viciae*
Aeschenomene (aquatic)	*Photorhizobium* (photosynthetically active rhizobia that form stem nodules, probably associated with adventitious roots)

- **Free-living bacteria that are capable of fixing nitrogen are aerobic, facultative, or anaerobic**
- *Aerobic* nitrogen-fixing bacteria such as *Azotobacter* are thought to maintain a low oxygen concentration (microaerobic conditions) by evolve O_2 photosynthetically during the day and fix nitrogen during the night when respiration lowers oxygen levels.
- *Facultative* organisms, which are able to grow under both aerobic and anaerobic conditions, generally fix nitrogen only under anaerobic conditions.
- For *anaerobic* nitrogen-fixing bacteria, oxygen does not pose a problem, because it is absent in their habitat.
  Photosynthetic (e.g., *Rhodospirillum)*
  Nonphotosynthetic (e.g., *Clostridium)*

Symbiotic nitrogen fixation occurs in specialized structures

- Symbiotic nitrogen-fixing prokaryotes dwell within **nodules**, the special organs of the plant host that enclose the nitrogen-fixing bacteria.

- In the case of *Gunnera*, these organs are existing stem glands that develop independently of the symbiont.

- In the case of legumes and actinorhizal plants, the nitrogen-fixing bacteria induce the plant to form root nodules.

- Grasses can also develop symbiotic relationships with nitrogen-fixing organisms, but in these associations root nodules are not produced.

- Instead, the nitrogen-fixing bacteria seem to colonize plant tissues or anchor to the root surfaces, mainly around the elongation zone and the root hairs.

- Legumes and actinorhizal plants **regulate gas permeability in their nodules**, maintaining a level of oxygen within the nodule that can support respiration but is sufficiently low to avoid inactivation of the nitrogenase.

- Gas permeability increases in the light and decreases under drought or upon exposure to nitrate.

- Nodules contain an oxygen-binding heme protein called **leghemoglobin**.

- Leghemoglobin is present in the cytoplasm of infected nodule cells at high concentrations and gives the nodules a **pink color.**

- The host plant produces the globin portion of leghemoglobin in response to infection by the bacteria; the bacterial symbiont produces the heme portion.

- Leghemoglobin has a high affinity for oxygen about ten times higher than that of human hemoglobin.

- Although leghemoglobin was once thought to provide a buffer for nodule oxygen, more recent studies indicate that it stores only enough oxygen to support nodule respiration for a few seconds.

- Its function is to help transport oxygen to the respiring symbiotic bacterial cells, analogous to the way hemoglobin transports oxygen to respiring tissues in animals.

Establishing symbiosis requires an exchange of signals

- **The symbiosis between legumes and rhizobia is not obligatory**.
- Legume seedlings germinate without any association with rhizobia, and may remain unassociated throughout their life cycle. Rhizobia also occur as free-living organisms in the soil.
- Under nitrogen-limited conditions, however, the symbionts seek each other out through an elaborate exchange of signals.
- This signaling, the subsequent infection process, and the development of nitrogen-fixing nodules involve specific genes in both the host and the symbionts.

- Plant genes specific to nodules are called nodulin *(Nod)* genes;

- Rhizobial genes that participate in nodule formation are called nodulation *(nod)* genes .

- The *nod* genes are classified as

  common *nod* genes or **host-specific *nod* genes**

- The common *nod* genes— ***nod A***, ***nod B***, and ***nod C***—are found in all rhizobial strains;

- the host-specific *nod* genes—such as ***nod P***, ***nod Q***, and ***nod H***, or ***nod F***, ***nod E***, and ***nod L***—differ among rhizobial species and determine the host range (the plants that can be infected).

- Only one of the *nod* genes, the regulatory *nod D,* is constitutively expressed, its protein product (NodD) regulates the transcription of the other *nod* genes.

- The first stage in the formation of the symbiotic relationship between the nitrogen-fixing bacteria and their host is migration of the bacteria toward the roots of the host plant.

- This migration is a chemotactic response mediated by chemical attractants, especially (**iso)flavonoids** and **betaines**, secreted by the roots.

- These **attractants activate the rhizobial Nod D protein**, which then induces transcription of the other *nod* genes.

Nod factors produced by bacteria act as signals for symbiosis

- The *nod* genes, which NodD activates, code for nodulation proteins, most of which are involved in the biosynthesis of nod factors.

- Nod factors are lipochitin oligosaccharide signal molecules.

- Three of the *nod* genes *(nod A, nod B,* and *nod C)* encode enzymes (Nod A, Nod B, and Nod C, respectively) that are required for synthesizing this basic structure.

- Host-specific *nod* genes that vary among rhizobial species are involved in the modification of the fatty acyl chain or the addition of groups important in determining host specificity.

- A particular legume host responds to a specific Nod factor.

- The legume receptors for Nod factors appear to involve sugar-binding LysM domains (for lysine motif) in the root hairs.

- Nod factors activate these domains, inducing calcium oscillations in root epidermal cells. Recognition of the calcium oscillations requires a caldum/calmodulin-dependent protein kinase (CaMK).

- Once the plant epidermal cell recognizes ongoing calcium oscillations. Nod factor-specific transcriptional regulators, directly associate with the promoters of Nod factor-inducible genes.

- The overall process links Nod factor perception at the plasma membrane to gene expression changes in the nucleus and is called the symbiotic pathway because it has many similarities with the process through which arbuscular mycorrhizae initially interact with their hosts.

Nodule formation involves phytohormones

- Two processes—infection and nodule organogenesis— occur simultaneously during root nodule formation.

- During the infection process, rhizobia attached to the root hairs release Nod factors that induce a pronounced curling of the root hair cells.

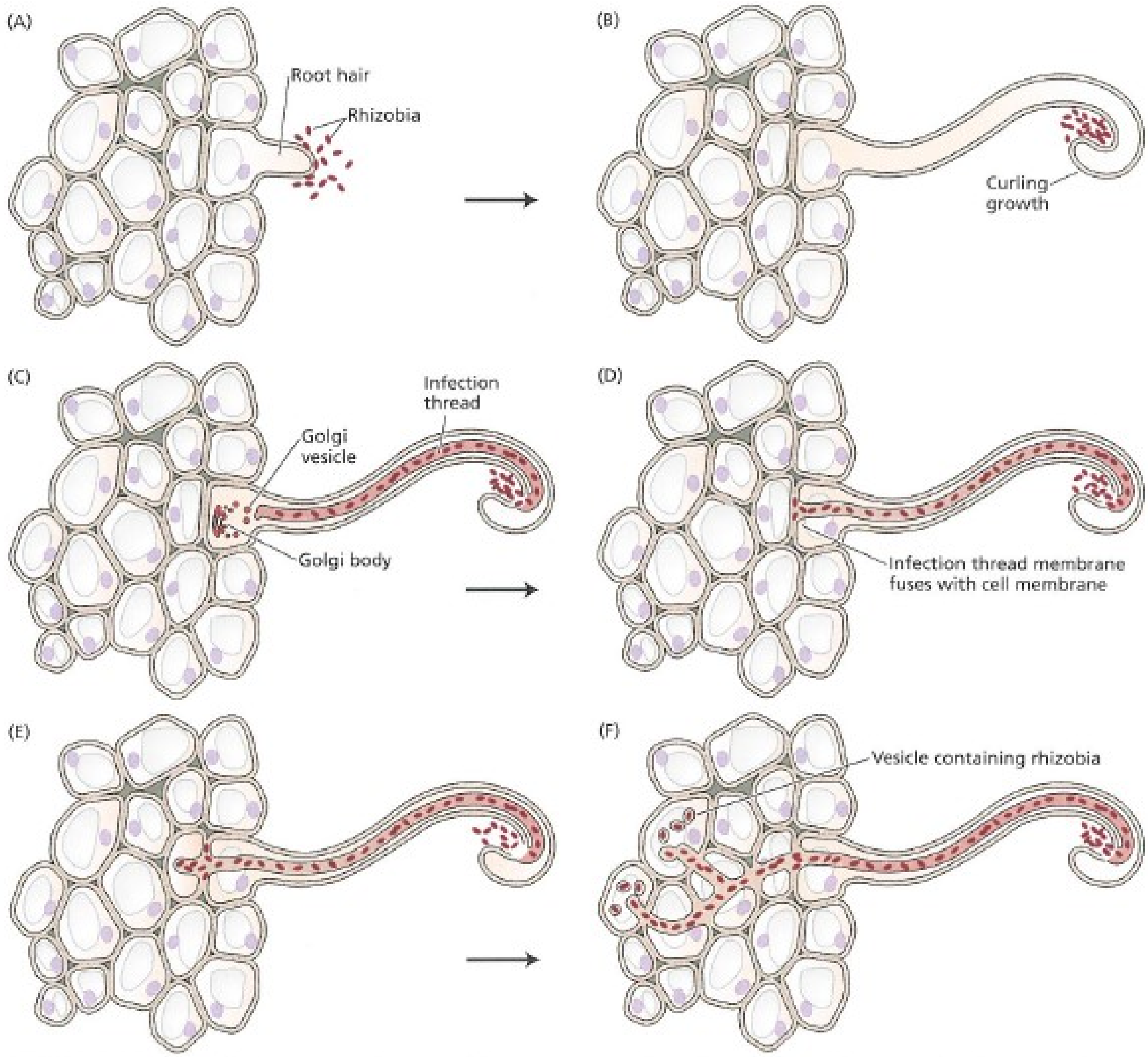
- The rhizobia become enclosed in the small compartment formed by the curling.

- The cell wall of the root hair degrades in these regions, also in response to Nod factors, allowing the **bacterial cells direct access to the outer surface of the plant plasma membrane**.

- The next step is **formation of the infection thread**, an internal tubular extension of the plasma membrane that is produced by the fusion of Golgi-derived membrane vesicles at the site of infection.

- The thread grows at its tip by the fusion of secretory vesicles to the end of the tube.

- Deeper into the root cortex, near the xylem, cortical cells dedifferentiate and start dividing, forming a **distinct area within the cortex,** called a *nodule primordium*, from which the nodule will develop.



(A)
Root hair
Rhizobia
(B)
Curling growth
(C)
Infection thread
Golgi vesicle
Golgi body
(D)
Infection thread membrane fuses with cell membrane
(E)
(F)
Vesicle containing rhizobia

- The nodule primordia form opposite the protoxylem poles of the root vascular bundle.

- Different signaling compounds, acting either positively or negatively, control the position of nodule primordia.

- The nucleoside **uridine** diffuses from the stele into the cortex in the protoxylem zones of the root and stimulates cell division.

- Ethylene is synthesized in the region of the pericycle, diffuses into the cortex, and blocks cell division opposite the phloem poles of the root.

- The infection thread filled with proliferating rhizobia elongates through the root hair and cortical cell layers, in the direction of the nodule primordium.

- When the infection thread reaches specialized cells within the nodule, its tip fuses with the plasma membrane of the host cell, releasing bacterial cells that are packaged in a membrane derived from the host cell plasma membrane.

- Branching of the infection thread inside the nodule enables the bacteria to infect many cells.

- At first the bacteria continue to divide, and the surrounding membrane increases in surface area to accommodate this growth by fusing with smaller vesicles.

- Soon thereafter, upon an undetermined signal from the plant, the bacteria stop dividing and begin to enlarge and to differentiate into nitrogen-fixing endosymbiotic organelles called **bacteroids.**

- The membrane surrounding the bacteroids is called the *peribacteroid membrane.*

- The nodule as a whole develops such features as a vascular system (which facilitates the exchange of fixed nitrogen produced by the bacteroids for nutrients contributed by the plant) and a layer of cells to exclude O_2 from the root nodule interior.

The nitrogenase enzyme complex fixes N₂

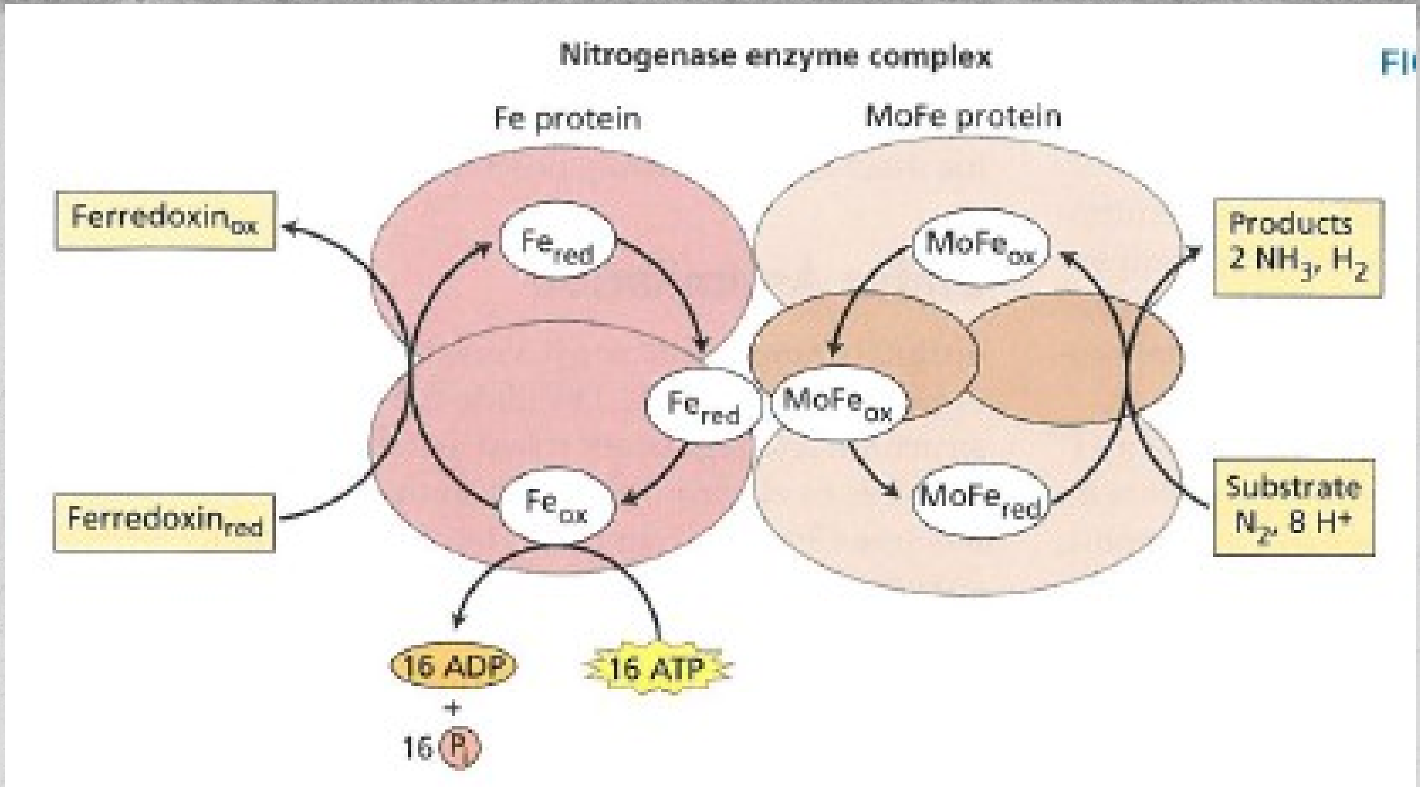
- Biological nitrogen fixation, like industrial nitrogen fixation, produces ammonia from molecular nitrogen.

- The overall reaction is

$$N_2 + 8\,e^- + 8\,H^+ + 16\,ATP \rightarrow 2\,NH_3 + H_2 + 16\,ADP + 16\,P_i$$

- Here in the reduction of N_2 to **2NH3,** a six-electron transfer, is coupled to the reduction of two protons to evolve H2.

- The nitrogenase enzyme complex catalyzes this reaction.



- The reaction catalyzed by nitrogenase.
- Ferredoxin reduces the Fe protein. Binding and hydrolysis of ATP to the Fe protein is thought to cause a conformational change of the Fe protein that facilitates the redox reactions.
- The Fe protein reduces the MoFe protein, and the **MoFe protein reduces the N2.**

- The nitrogenase enzyme complex can be separated into two components—the Fe protein and the MoFe protein—neither of which has catalytic activity by itself.

- 1. The Fe protein is the smaller of the two components and has two identical subunits of 30 to 72 kDa each, depending on the organism.

- Each subunit contains an iron-sulfur cluster (4 Fe and 4 S_2^-) that participates in the redox reactions involved in the conversion of **N2** to **NH3.**

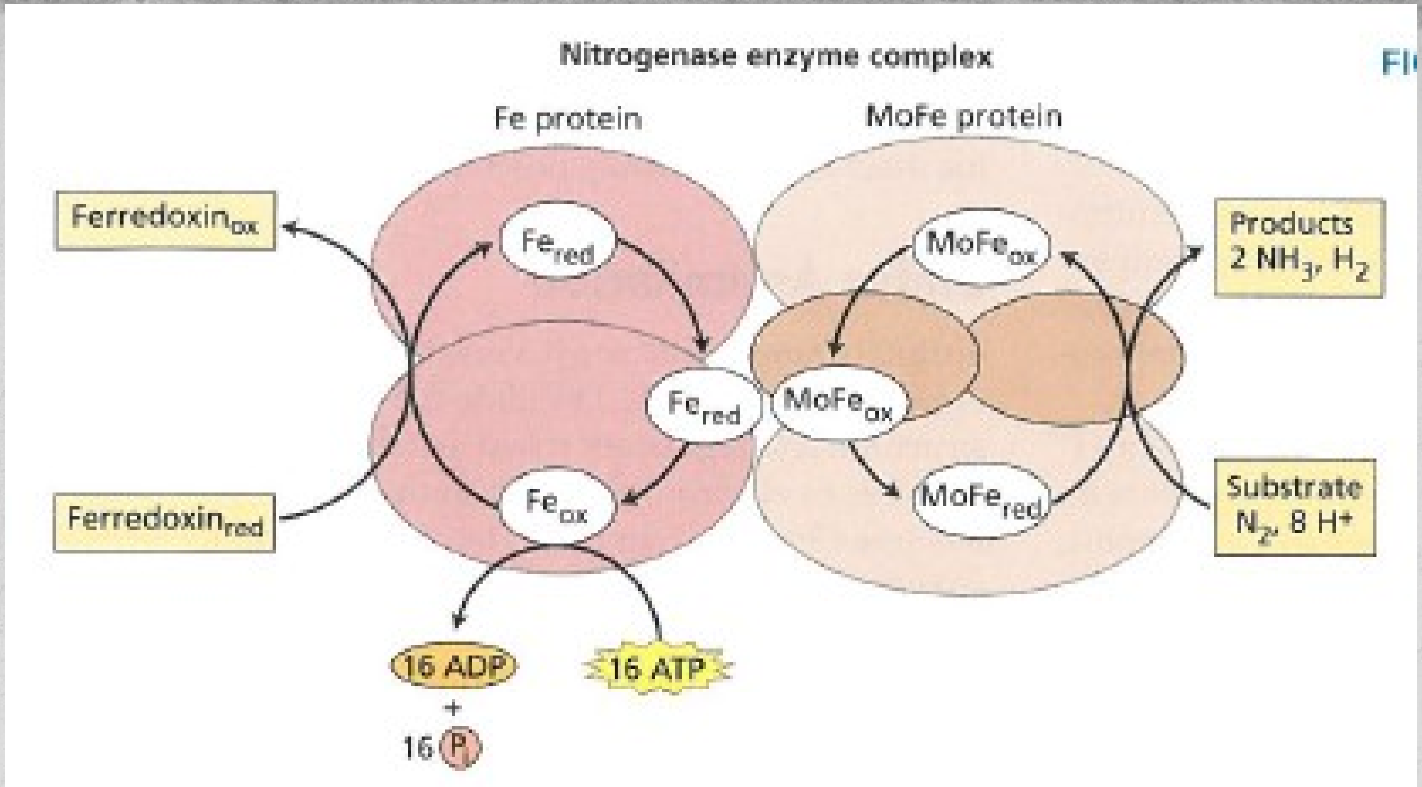
- The Fe protein is irreversibly inactivated by O2.

- 2. The MoFe protein has four subunits, with a total molecular mass of 180 to 235 kDa, depending on the species.

- Each subunit has two Mo-Fe-S clusters. The MoFe protein is also inactivated by oxygen.

- In the overall nitrogen reduction reaction, **ferredoxin serves as an electron donor to the Fe protein**, which in turn hydrolyzes ATP and reduces the Mo- Fe protein.

- The MoFe protein can then reduce numerous substrates, although under natural conditions it reacts only with N_2 and H^+.



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- The energetics of nitrogen fixation is complex.
- The production of NH_3 from N_2 and H_2 is an exergonic reaction.
- However, industrial production of NH_3 from N_2 and H_2 is *endergonic*, requiring a very large energy input because of the activation energy needed to break the triple bond in N_2.
- For the same reason, the enzymatic reduction of N_2 by nitrogenase also requires a large investment of energy although the exact changes in free energy are not yet known.

- Under natural conditions, substantial amounts of H^+ are reduced to gas, and this process can compete with N_2 reduction for electrons from nitrogenase.

- In rhizobia, 30 to 60% of the energy supplied to nitrogenase may be lost as H_2, diminishing the efficiency of nitrogen fixation.

- Some rhizobia, however, contain hydrogenase, an enzyme that can split the H_2 formed and generate electrons for N_2 reduction, thus improving the efficiency of nitrogen fixation.

Amides and ureides are the transported forms of nitrogen

- In most plant species, amino acids are the predominant chemical forms in which nitrogen is transported.
- Synthesis of amino acids mainly occurs in roots or mature leaves (sources) that export N to supply sinks such as root tips, flowers, fruits, and seeds.
- Xylem and phloem connect source and sink organs and serve as routes for long-distance transport of the organic nitrogen.

- The final step in nitrogen fixation is the export of the fixed nitrogen from the nodule to other regions of the host plant.
- The symbiotic nitrogen-fixing prokaryotes release ammonia that, to avoid toxicity, must be rapidly converted into organic forms in the root nodules before being transported to the shoot via the xylem.
- Export of the organic nitrogen products from nodules is primarily through the xylem.
- Consequently, the form in which the nitrogen is exported has been identified primarily by analysis of xylem sap.

- The studies have shown that although glutamine is the principal organic product of nitrogen fixation, it rarely accounts for a significant fraction of the nitrogen exported, at least in legumes.

- In some groups of legumes, largely those of temperate origins, such as pea and clover, the amino acid **asparagine is the predominant form translocated.**

- Legumes of tropical origins, for example, soybean and cowpea, appear to export predominantly derivatives of urea, known as **ureides**

- In nodulated tropical or subtropical legumes, ureides represent the major form for long-distance transport of N.
- Nitrogen-fixing legumes can be classified as two based on the composition of the xylem sap.
  - Amide exporters
  - Ureide exporters

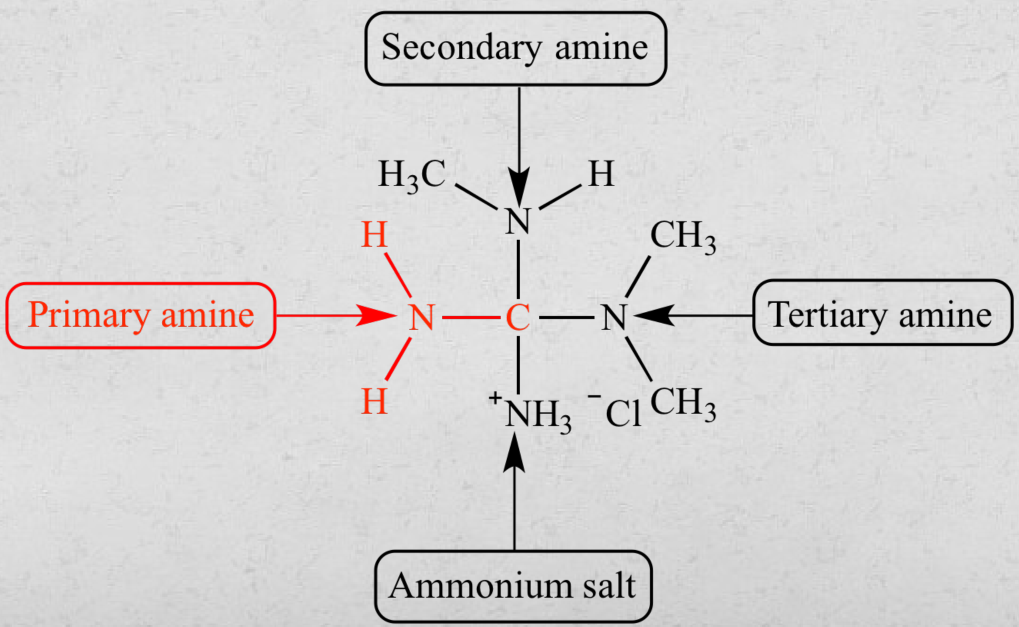
- **Amides** are usually regarded as derivatives of carboxylic acids in which the hydroxyl group has been replaced by an amine or ammonia.

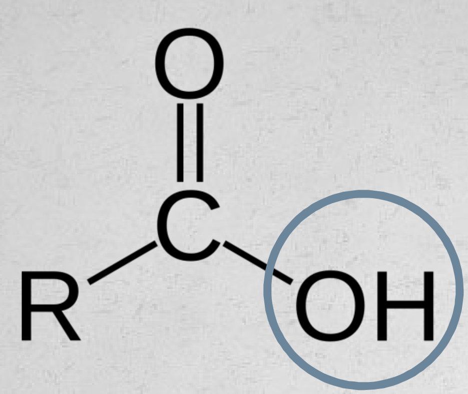
- **Ureide** any of a group of compounds which are acyl derivatives of urea. $CO(NH_2)_2$.

- Acyl - denoting a radical of general formula —C(O)R, where R is an alkyl group, derived from a carboxylic acid.

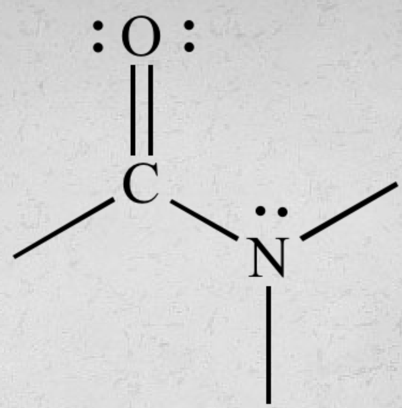
Extra reading……….

- In organic chemistry, amines are compounds and functional groups that contain a basic nitrogen atom with a lone pair. Amines are formally derivatives of ammonia, wherein one or more hydrogen atoms have been replaced by a substituent such as an alkyl or aryl group

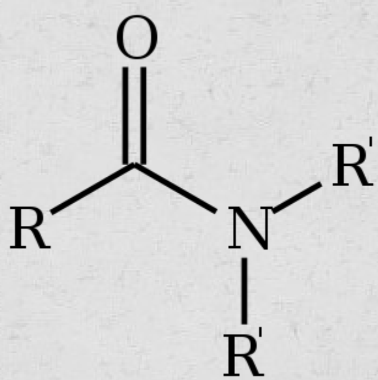




carboxylic acids

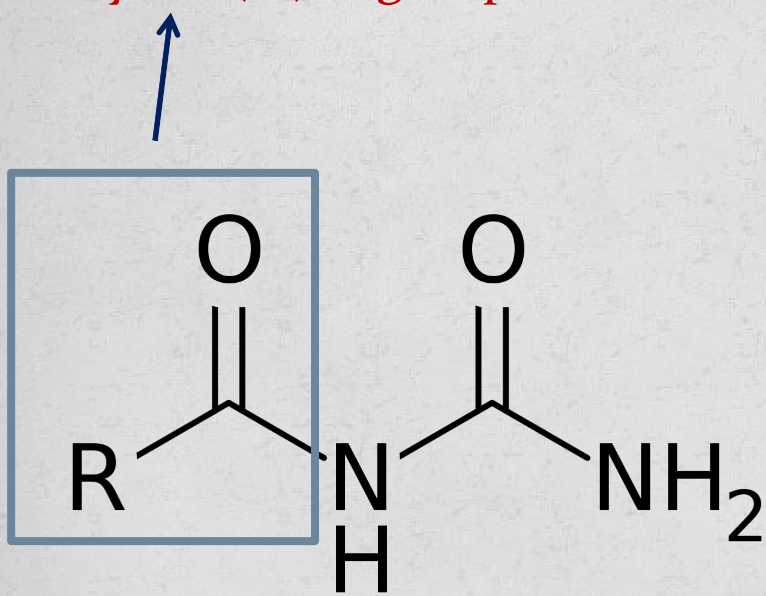


General amide structure

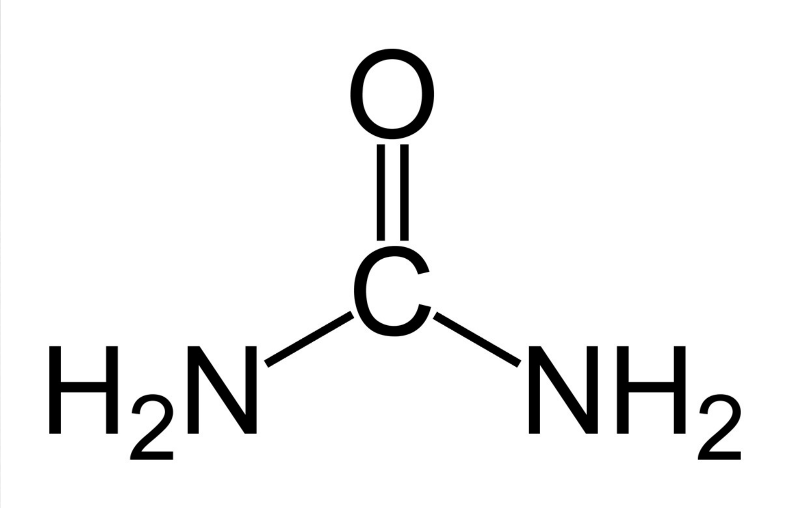


organic
amide

Acyl - **C(O)R** group



Ureide



Urea

Amides (principally the amino acids **asparagine** or **glutamine**) are exported by temperate-region legumes, such as

- pea *(Pisum)*
- clover *{Trifolium)*
- broad bean *(Vida)*, and
- lentil (*Lens)*

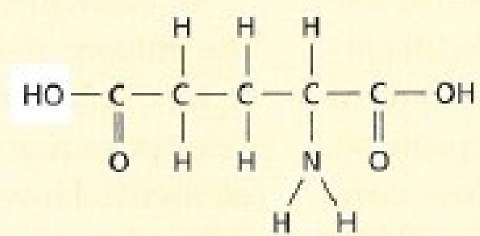
Ureides are exported by legumes of tropical origin, such as

- soybean *(Glydne)*
- common bean *(Phaseolus)*
- peanut *(Arachis)*, and
- southern pea *(Vigna)*

- Nitrogen is found in the phloem largely in amino acids – especially glutamate and aspartate – and their respective amides – **glutamine** and **asparagine.**

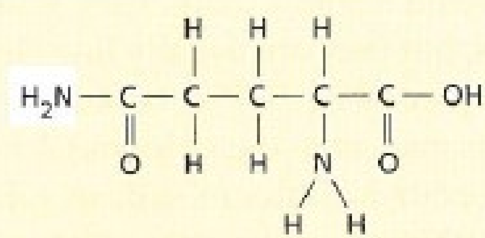
- As the older leaves yellow and die, the nitrogen is mobilized, largely in the form of soluble amines and amides, and exported from the older leaves to the younger, more rapidly developing leaves.

Glutamic acid, an amino acid, and glutamine, its amide, are important nitrogenous compounds in the phloem, in addition to aspartate and asparagine.



Glutamic acid

Amino acid



Glutamine

Amide

- A common form of mobile nitrogen in some legumes is a family of urea-based compounds known as **ureides.**

- The three major ureides are ***Allantoin, allantoic acid*** *or* ***citrulline.***

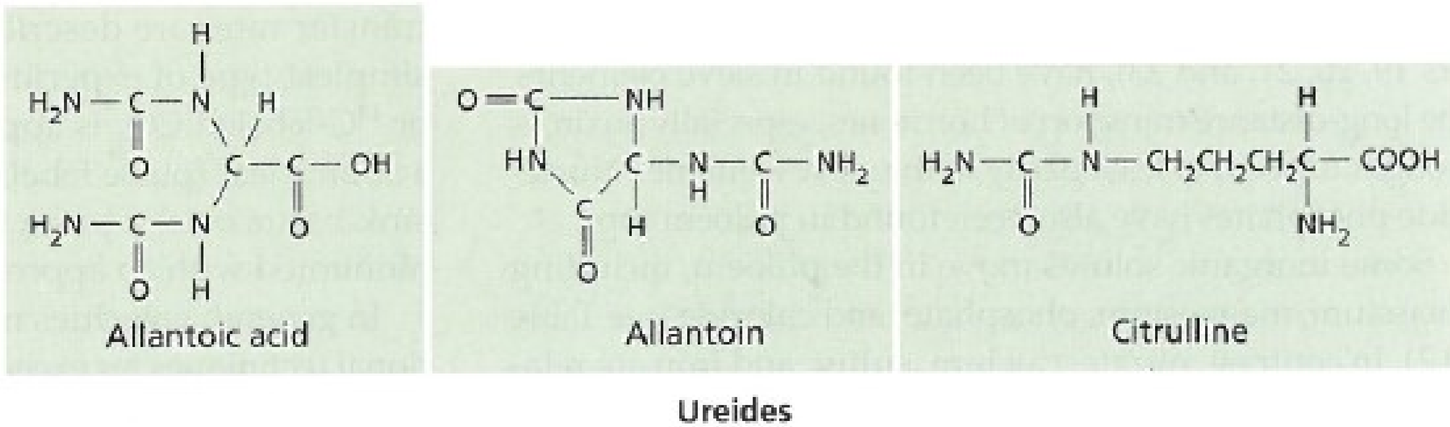
- Allantoin is synthesized in peroxisomes from uric acid.

- Allantoic acid is synthesized from allantoin in the endoplasmic reticulum.

- The site of citrulline synthesis from the amino acid ornithine has not yet been determined.

- All three compounds are ultimately released into the xylem and transported to the shoot, where they are **rapidly catabolized to ammonium.** This ammonium enters the assimilation pathway.

Species with nitrogen-fixing nodules also utilize ureides as transport forms of nitrogen.



- Ureides are formed in root nodules during nitrogen fixation and transported via the xylem throughout the host plant.

- Ureides are also formed in senescing leaves and transported out to the developing seeds for storage.

- The breakdown of ureides produces urea, which accumulates to toxic levels in nitrogen deficient plants.

- While root to shoot transport of amino acids and ureides occurs in the xylem, transport of organic N from source leaves to sinks occurs in the phloem.

- Although, along the long-distance transport pathway, transfer of amino acids or ureides from the xylem to the phloem (i.e. transport phloem) can occur for direct N delivery to fast growing sinks

Thank you