Nitrogen

- Nitrogen dynamics depend on plant chemistry (C:N ratio, pH, decomposability), carbon dynamics, microbial community
- Short-cuts for plants: N-fixing organisms, mycorrhizae, direct uptake of organic N (?)
- Human nitrogen loading: fertilizer runoff (*overflows* from previously almost-closed cycles)

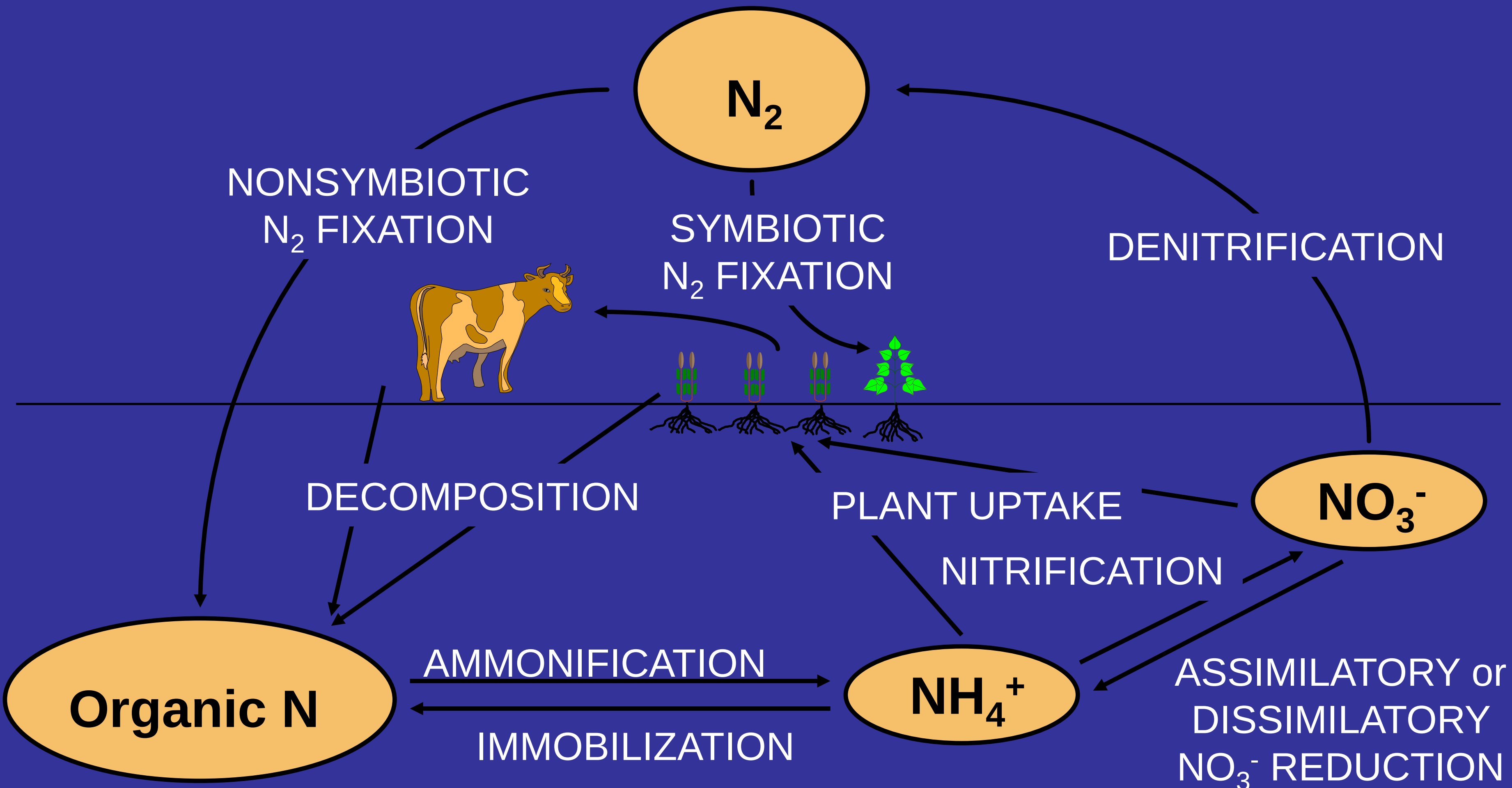
What’s so important about Nitrogen cycling?

essential nutrient (fertilizers, growing legumes as crops) –
changes in native species composition of ecosystem

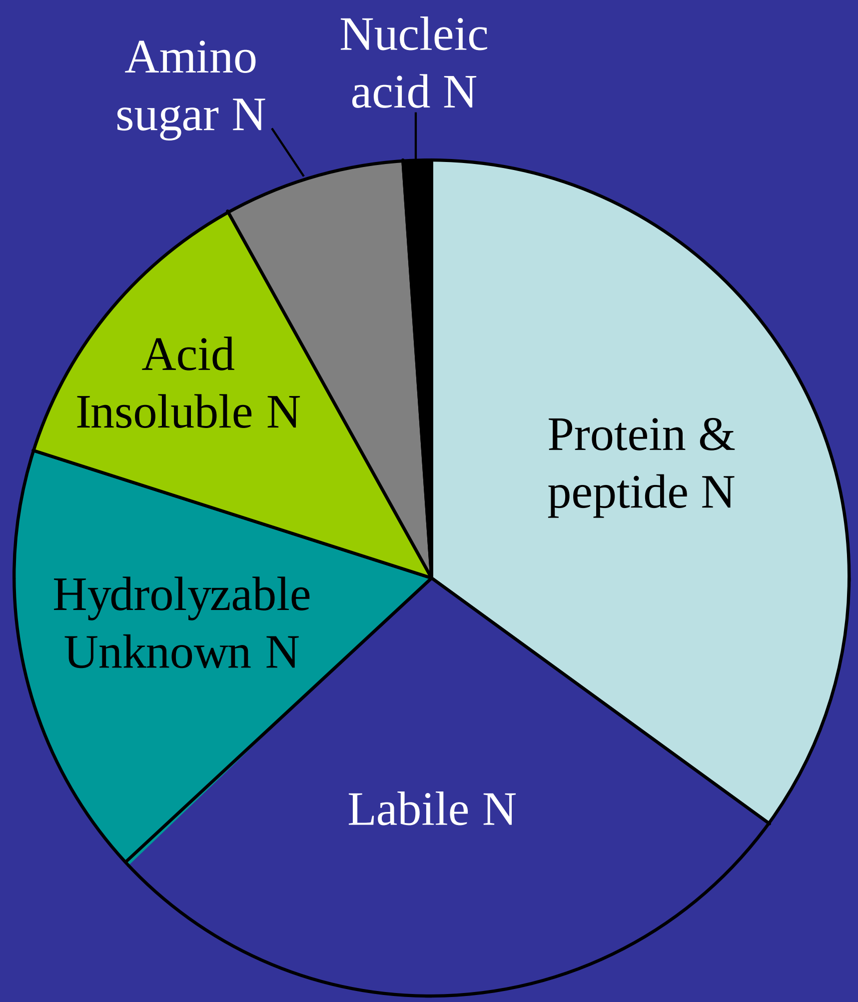
atmospheric pollutant (burning fuels)

groundwater pollutant

Nitrogen Cycle



Forms of Organic N



Major Inorganic N Compounds

Compound	Formula	Oxidation state	Form in soil
Ammonium	NH_4^+	-3	Fixed in clay lattice, dissolved, as gaseous ammonia (NH_3)
Hydroxylamine	NH_2OH	-1	Not detected
Dinitrogen	N_2	0	Gas
Nitrous oxide	N_2O	+1	Gas, dissolved
Nitric oxide	NO	+2	Gas, dissolved
Nitrite	NO_2^-	+3	Dissolved
Nitrate	NO_3^-	+5	Dissolved

Nitrogen Fixation

The nodules on the roots of this bean plant contain bacteria called *Rhizobium* that help convert nitrogen in the soil to a form the plant can utilize.

Dinitrogen Fixation

The alder, whose fat shadow nourisheth–
Each plant set neere him long flourisheth.

–William Browne (1613), *Brittania's Pastorals*, Book I, Song 2

Treatment	Yield (g)	
	Oats	Peas
No N added		
Non-inoculated	0.6	0.8
Inoculated with legume soil	0.7	16.4
Inoculated with sterile soil	—	0.9
112 mg NO_3^-–N per pot added		
Non-inoculated	12.0	12.9
Inoculated with legume soil	11.6	15.3

Hellriegel and Wilfarth (1888)

Types of Biological Nitrogen Fixation

Free-living (asymbiotic)

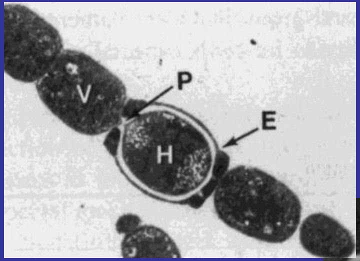
- Cyanobacteria
- *Azotobacter*

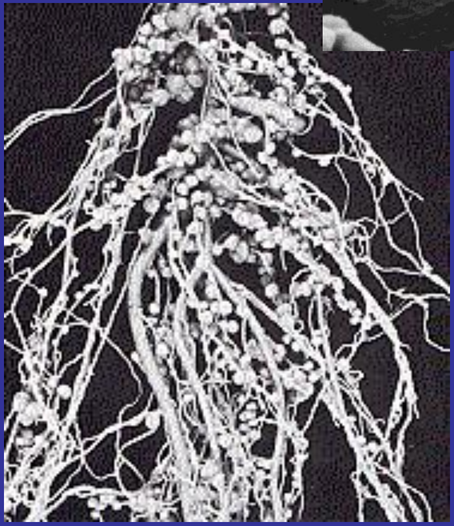
Associative

- Rhizosphere–*Azospirillum*
- Lichens–cyanobacteria
- Leaf nodules

Symbiotic

- Legume-rhizobia
- Actinorhizal-*Frankia*





Free-living N_2 Fixation

Energy

- 20-120 g C used to fix 1 g N

Combined Nitrogen

- *nif* genes tightly regulated
- Inhibited at low NH_4^+ and NO_3^- (1 µg g^{-1} soil, 300 µM)

Oxygen

- Avoidance (anaerobes)
- Microaerophilly
- Respiratory protection
- Specialized cells (heterocysts, vesicles)
- Spatial/temporal separation
- Conformational protection

Associative N_2 Fixation

- Phyllosphere or rhizosphere (tropical grasses)
- *Azosprillum, Acetobacter*
- 1 to 10% of rhizosphere population
- Some establish within root
- Same energy and oxygen limitations as free-living
- *Acetobacter diazotrophicus* lives in internal tissue of sugar cane, grows in 30% sucrose, can reach populations of 10^6 to 10^7 cells g^{-1} tissue, and fix 100 to 150 kg N ha^{-1} y^{-1}

Estimated Average Rates of Biological N_2 Fixation

Organism or system	N_2 fixed ($kg\ ha^{-1}\ y^{-1}$)
Free-living microorganisms	
Cyanobacteria	25
Azotobacter	0.3
Clostridium pasteurianum	0.1-0.5
Grass-Bacteria associative symbioses	
Azospirillum	5-25
Cyanobacterial associations	
Gunnera	10-20
Azolla	300
Lichens	40-80
Leguminous plant symbioses with rhizobia	
Grain legumes (*Glycine, Vigna, Lespedeza, Phaseolus*)	50-100
Pasture legumes (*Trifolium, Medicago, Lupinus*)	100-600
Actinorhizal plant symbioses with *Frankia*	
Alnus	40-300
Hippophaë	1-150
Ceanothus	1-50
Coriaria	50-150
Casuarina	50

- Nitrogen Fixation

- Almost all N is in the atmosphere
- 90-190 Tg N fixed by terrestrial systems
- 40-200 Tg N fixed by aquatic systems
- 3-10 Tg N fixed by lightning
- 32-53 Tg N fixed by crops

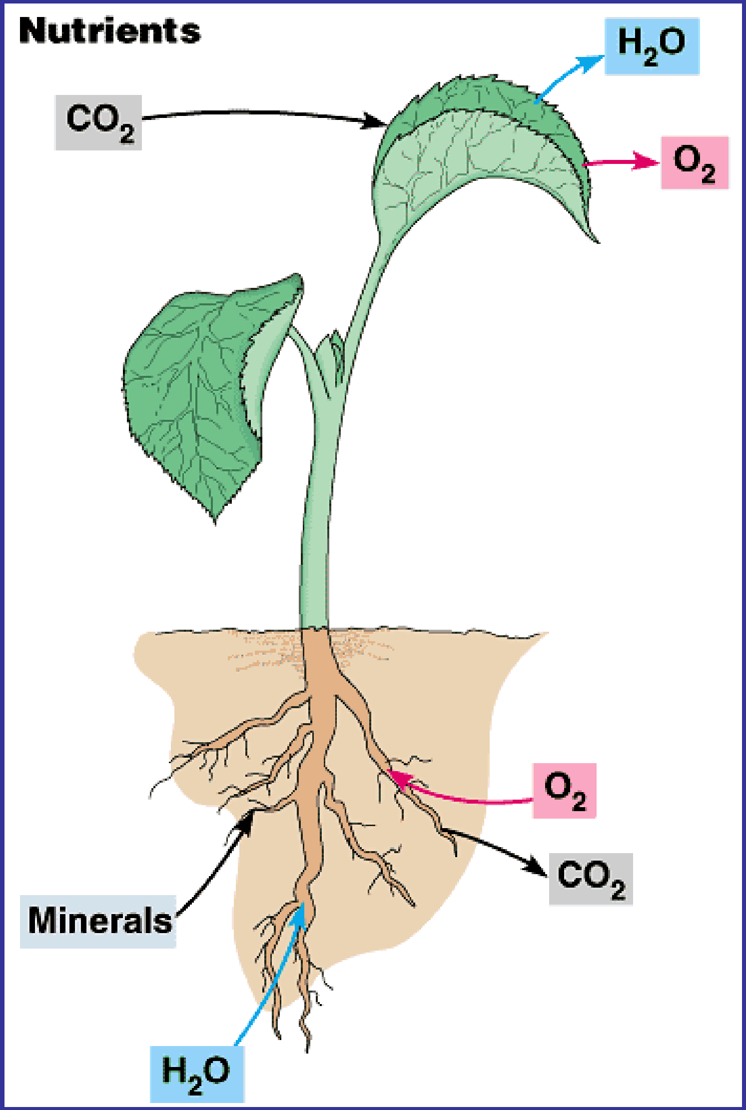
Some biogeochemical cycling key points:

- *cycling occurs at local to global scales*
- *biogeochemical cycles have 2 basic parts: pools and fluxes*
- *elements are recycled among the biosphere, atmosphere, lithosphere and hydrosphere*
- *cycles of each element differ (chemistry, rates, pools, fluxes, interactions)*
- *cycling is important because it can affect many other aspects of the environment and the quality of our lives*

Nitrogen Metabolism

Plant Nutrition

- Plant metabolism is based on sunlight and inorganic elements present in water, air, and soil.
- C, H, and O and energy are used to generate organic molecules via photosynthesis.
- Other chemical elements, such as *mineral nutrients*, are also absorbed from soil.



Plant Nutrients

- Plants absorb many elements, some of which they do not need.
- An element is considered an *essential nutrient* if it meets three criteria:
  - *It is necessary for complete, normal plant development through a full life cycle.*
  - *It itself is necessary; no substitute can be effective.*
  - *It must be acting within the plant, not outside it.*
- Many roles in plant metabolism.

Types of Essential Nutrients

- Nine essential nutrients, called *macronutrients*, are needed in very large amounts
- Eight other essential nutrients, called *micronutrients*, are needed only in small amounts.

Essential Nutrients to Most Plants

Macronutrient	% Dry Weight	Component/Function
Carbon (C)	45.0	Organic compounds
Oxygen (O)	45.0	Organic compounds
Hydrogen (H)	6.0	Organic compounds
Nitrogen (N)	1.0-4.0	Amino acids; nucleic acids, chlorophyll
Potassium (K)	1.0	Amino acids; regulates stomata opening/closing
Calcium (Ca)	0.5	Enzyme cofactor; influences cell permeability
Phosphorus (P)	0.2	ATP; proteins; nucleic acids; phospholipids
Magnesium (Mg)	0.2	Chlorophyll; enzyme activator
Sulfur (S)	0.1	CoA; amino acids

Essential Nutrients to Most Plants

Micronutrient	Component/Function
Iron (Fe)	Cytochromes; chlorophyll synthesis
Chlorine (Cl)	Osmosis; water-splitting in photosynthesis
Copper (Cu)	Plastocyanin; enzyme activator
Manganese (Mn)	Enzyme activator; component of chlorophyll
Zinc (Zn)	Enzyme activator
Molybdenum (Mo)	Nitrogen fixation
Boron (B)	Cofactor in chlorophyll synthesis
Nickel (Ni)	Cofactor for enzyme functioning in nitrogen metabolism

Nitrogen: An Essential Macronutrient

- N is not present in rock, but is abundant in the atmosphere as a gas, N_2.
- The process of converting N_2 to chemically active forms of N is *nitrogen metabolism*.
- Nitrogen metabolism consists of 3 stages:
  - *Nitrogen Fixation* (N_2 -> NO_3^-)
  - *Nitrogen Reduction* (NO_3^- -> NO_2^- -> NH_3 -> NH_4^+)
  - *Nitrogen Assimilation* (transfer of NH_2 groups)
- Runoff, leaching, denitrification, and harvested crops reduce soil nitrogen.

Nitrogen Cycling Processes

Nitrogen Fixation – bacteria convert nitrogen gas (N_2) to ammonia (NH_3).

Decomposition – dead nitrogen fixers release N-containing compounds.

Ammonification – bacteria and fungi decompose dead plants and animals and release excess NH_3 and ammonium ions (NH_4^+).

Nitrification – type of chemosynthesis where NH_3 or NH_4^+ is converted to nitrite (NO_2^{-}); other bacteria convert NO_2^- to nitrate (NO_3^{-}).

Denitrification – bacteria convert NO_2^- and NO_3^- to N_2.

Means of Nitrogen Fixation

1) Human manufacturing of synthetic fertilizers

2) Lightning

3) Nitrogen-fixing bacteria and cyanobacteria

Nitrogen Fixing Bacteria and Cyanobacteria

- Some are free-living in soil (E.g., *Nostoc*, *Azotobacter*); others live symbiotically with plants (E.g., *Frankia*, *Rhizobium*).
- These organisms have *nitrogenase*, an enzyme that uses N_2 as a substrate.
- $N_2 + 8e^- + 8H^+ + 16ATP \rightarrow 2NH_3 + H_2 + 16ADP + 16P_i$
- NH_3 is immediately converted to NH^{4+}.
- Bacterial enzymes sensitive to O_2.
- *Leghemoglobin* binds to O_2 and protects enzymes.
- Symbiotic fixation rate depends on plant stage.

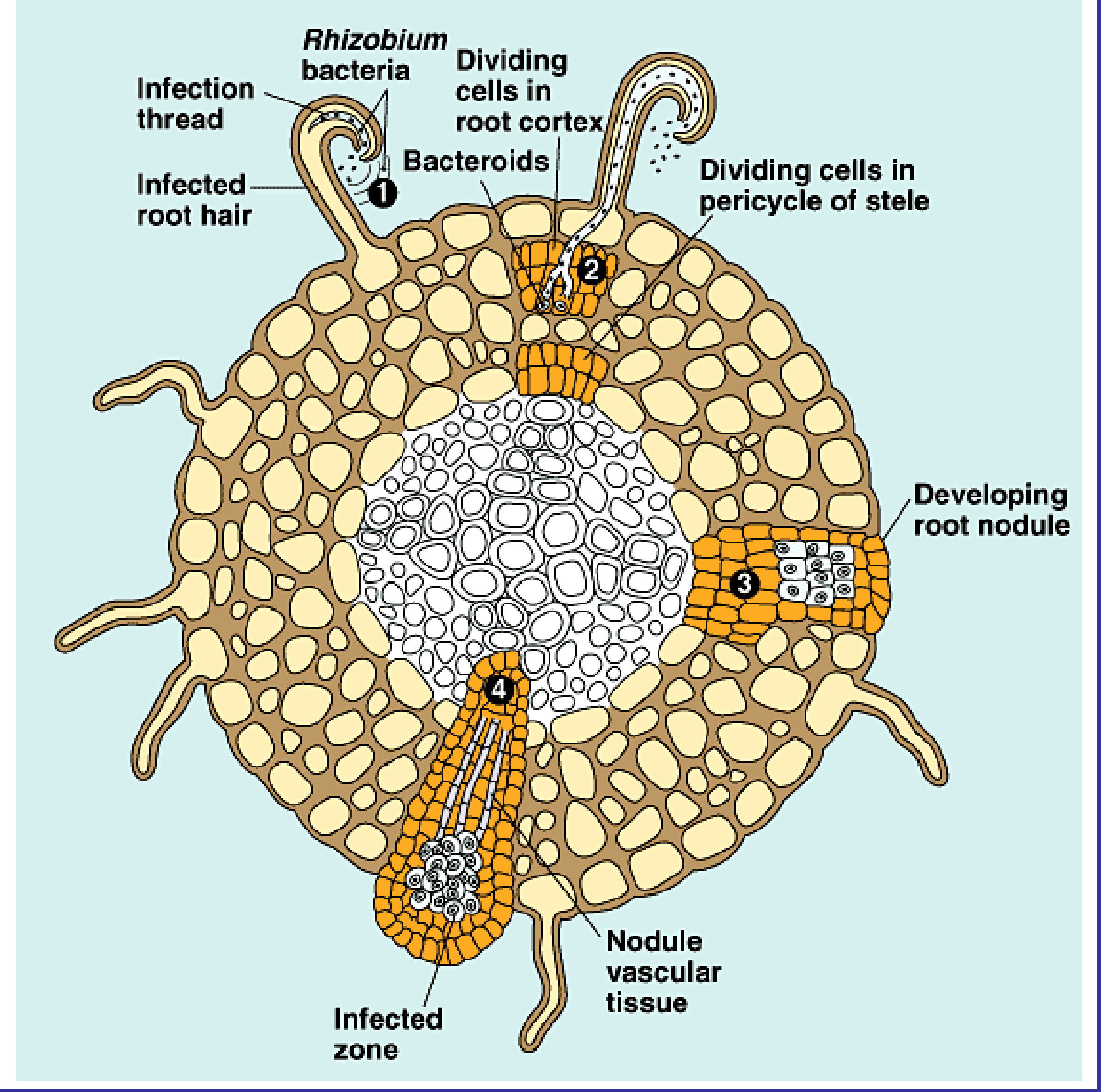
Natural Sources of Organic N

Source	% N
Dried blood	12
Peruvian guano	12
Dried fish meal	10
Peanut meal	7
Cottonseed meal	7
Sludge from sewer treatment plant	6
Poultry manure	5
Bone meal	4
Cattle manure	2

Symbiotic Nitrogen Fixation

- Nitrogen-fixing bacteria fix N (E.g., *Rhizobium*)
- Plants fix sugars (E.g.,legumes).
- Plants form swellings that house N-fixing bacteria, called *root nodules*.
- Mutualistic association.
- Excess NH_3 is released into soil.
- Crop rotation maintains soil fertility.

Development of a Root Nodule

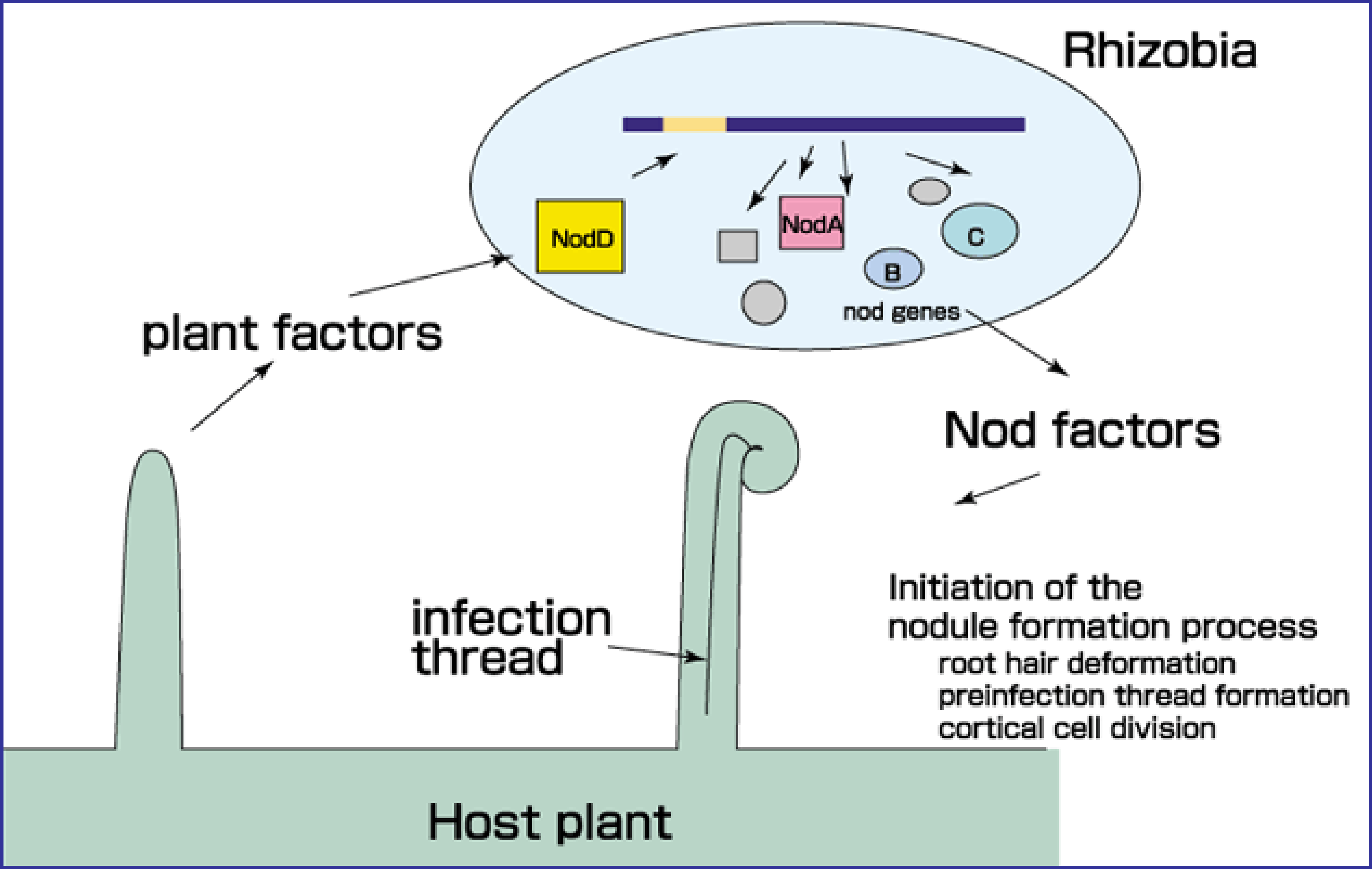


- Bacteria enter the root through an *infection thread*.
- Bacteria are then released into cell and assume form called *bacteroids*, contained within vesicles.

Symbiotic Nitrogen Fixation

The *Rhizobium*-legume association

Bacterial associations with certain plant families, primarily **legume** species, make the largest single contribution to biological nitrogen fixation in the biosphere



Rhizobia
NodD
NodA
B
C
nod genes
plant factors
Nod factors
infection
thread
Initiation of the
nodule formation process
root hair deformation
preinfection thread formation
cortical cell division
Host plant

When this association is not present or functional, we apply nitrogen-containing **fertilizers** to replace reduced nitrogen removed from the soil during repeated cycles of crop production.

This practice consumes fossil fuels, both in fertilizer production and application.

Biological nitrogen fixation is the reduction of atmospheric nitrogen gas (N_2) to ammonium ions (NH_4^+) by the oxygen-sensitive enzyme, **nitrogenase**. Reducing power is provided by NAPH/ferredoxin, via an Fe/Mo centre.

Plant genomes lack any genes encoding this enzyme, which occurs only in prokaryotes (bacteria).

Even within the bacteria, only certain free-living bacteria (*Klebsiella*, *Azospirillum*, *Azotobacter*), blue-green bacteria (*Anabaena*) and a few symbiotic Rhizobial species are known nitrogen-fixers.

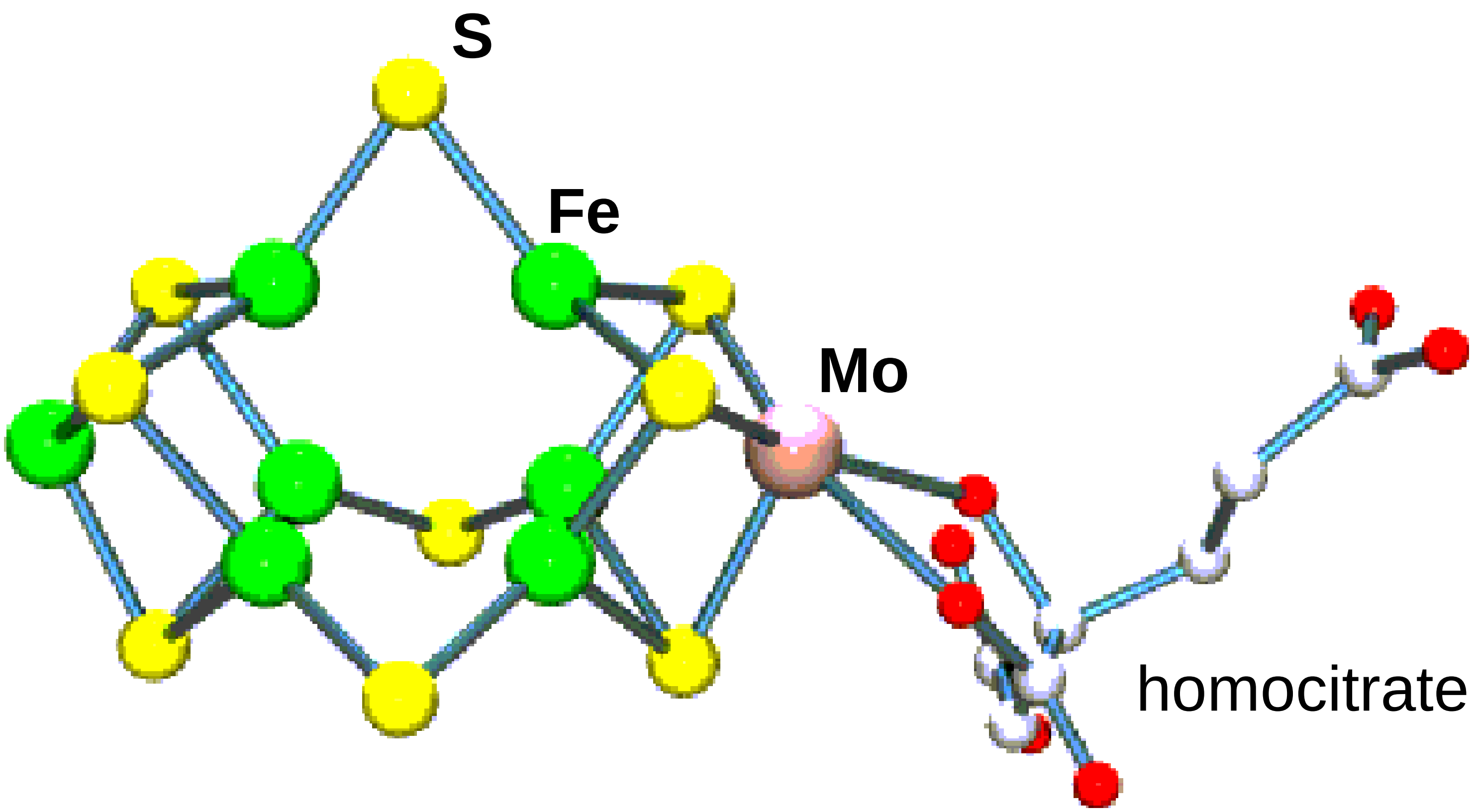
Another nitrogen-fixing association exists between an Actinomycete (*Frankia* spp.) and alder (*Alnus* spp.)

The enzyme **nitrogenase** catalyses the conversion of atmospheric, gaseous dinitrogen (N_2) and dihydrogen (H_2) to ammonia (NH_3), as shown in the chemical equation below:

$$N_2 + 3\,H_2 \Rightarrow 2\,NH_3$$

The above reaction seems simple enough and the atmosphere is 78% N_2, so <u>why is this enzyme so important</u>?

The incredibly strong (triple) bond in N_2 makes this reaction very difficult to carry out efficiently.
In fact, nitrogenase consumes ~16 moles of ATP for every molecule of N_2 it reduces to NH_3, which makes it one of the most energy-expensive processes known in Nature.



Fe - S - Mo electron transfer cofactor in nitrogenase

Biological NH_3 creation (nitrogen fixation) accounts for an estimated 170 x 10^9 kg of ammonia every year. Human industrial production amounts to some 80 x 10^9 kg of ammonia yearly.

The industrial process (Haber-Bosh process) uses an Fe catalyst to dissociate molecules of N_2 to atomic nitrogen on the catalyst surface, followed by reaction with H_2 to form ammonia. This reaction typically runs at ~450º C and 500 atmospheres pressure.

These extreme reaction conditions consume a huge amount of energy each year, considering the scale at which NH_3 is produced industrially.

The Dream.....

If a way could be found to mimic nitrogenase catalysis (a reaction conducted at 0.78 atmospheres N_2 pressure and ambient temperatures), huge amounts of energy (and money) could be saved in industrial ammonia production.

If a way could be found to
transfer the capacity to form N-fixing symbioses
from a typical legume host
to an important non-host crop species such as corn or wheat,
far less fertilizer
would be needed to be produced and applied
in order to sustain crop yields

Because of its current and potential economic importance,
the interaction between Rhizobia and leguminous plants has been intensively studied.

Our understanding of the process by which these two symbionts establish a functional association is still not complete, but it has provided a paradigm for many aspects of cell-to-cell communication between microbes and plants (e.g. during pathogen attack), and even between cells within plants (e.g. developmental signals; fertilization by pollen).

Symbiotic Rhizobia are classified in two groups:

Fast-growing ***Rhizobium*** spp. whose nodulation functions (nif, fix) are encoded on their symbiotic megaplasmids (pSym)

Slow-growing ***Bradyrhizobium*** spp. whose N-fixation and nodulation functions are encoded on their chromosome.

There are also two types of nodule that can be formed:

determinate

and

indeterminate

This outcome is controlled by the plant host

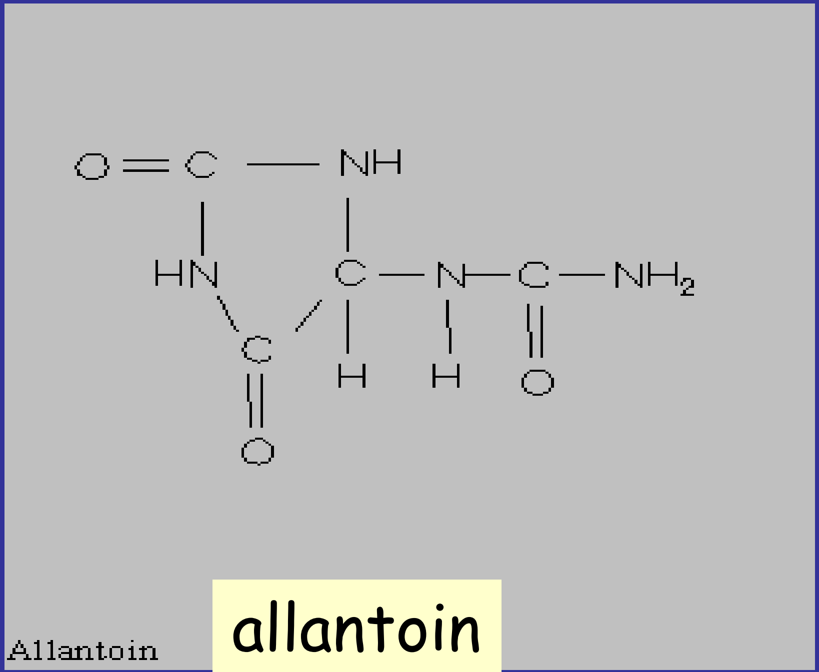
Determinate nodules

Formed on tropical legumes by *Rhizobium* and *Bradyrhizobium*

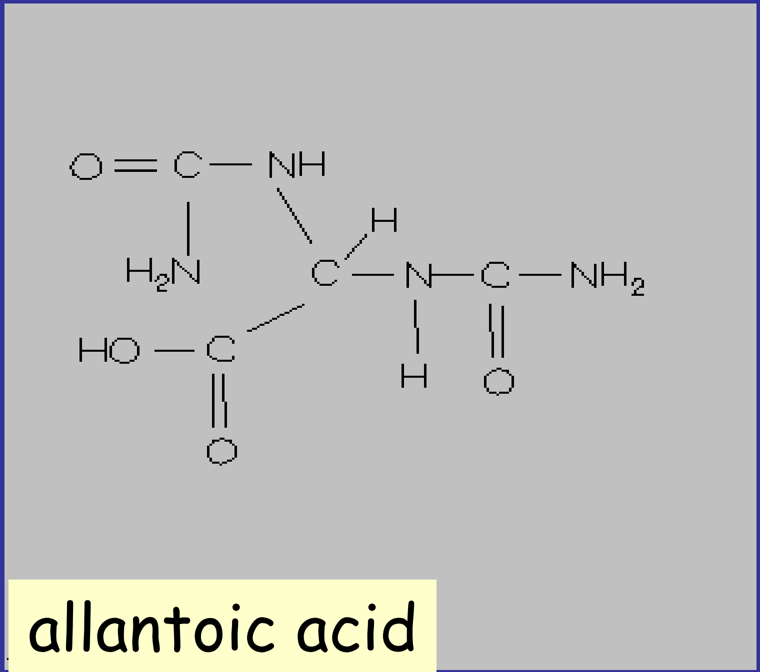
Meristematic activity not persistent - present only
during early stage of nodule formation;
after that, cells simply expand rather than divide, to
form **globose nodules.**

Nodules arise just below epidermis; largely internal
vascular system

Uninfected cells dispersed throughout nodule; equipped to assimilate NH_4^+ as **ureides** (allantoin and allantoic acid)



allantoin

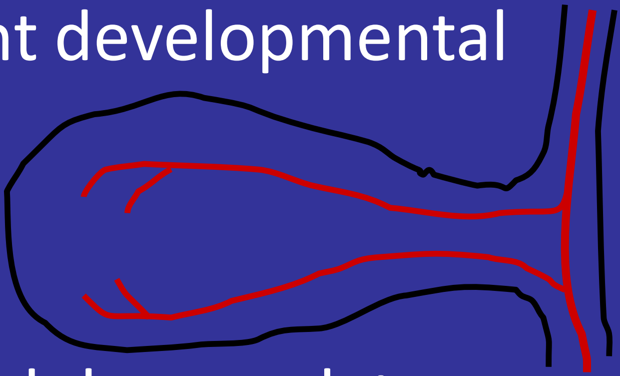


allantoic acid

Indeterminate nodules

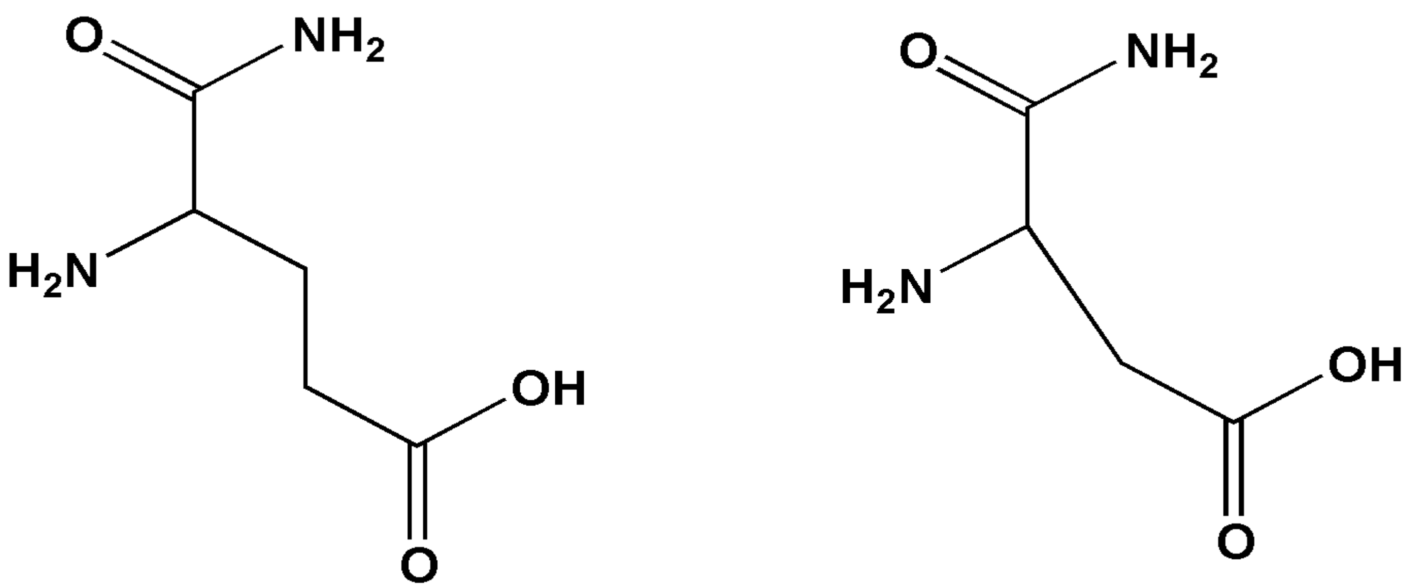
Formed on temperate legumes
(pea, clover, alfalfa);
typically by *Rhizobium* spp.

Cylindrical nodules with a **persistent meristem**;
nodule growth creates zones of different developmental stages



Nodule arises near endodermis, and nodule vasculature clearly connected with root vascular system

Uninfected cells of indeterminate nodules assimilate NH_4^+ as **amides** (asparagine, glutamine)

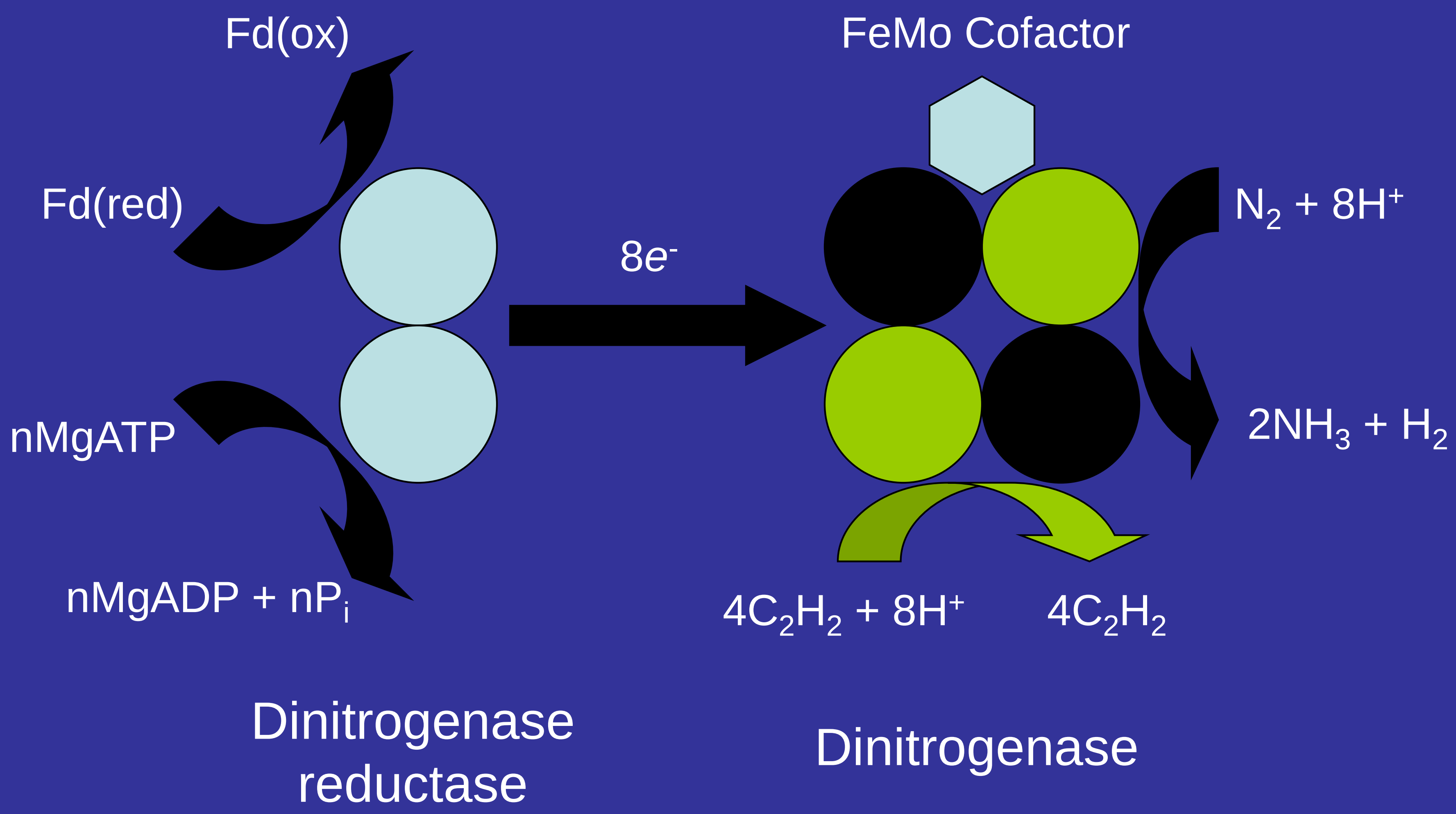


GLUTAMINE ASPARAGINE

Genetics of Nitrogenase

Gene	Properties and function
nifH	Dinitrogenase reductase
nifDK	Dinitrogenase
nifA	Regulatory, activator of most *nif* and *fix* genes
nifB	FeMo cofactor biosynthesis
nifEN	FeMo cofactor biosynthesis
nifS	Unknown
fixABCX	Electron transfer
fixK	Regulatory
fixLJ	Regulatory, two-component sensor/effector
fixNOQP	Electron transfer
fixGHIS	Transmembrane complex

Nitrogenase



$N_2 + 8H^+ + 8e^- + 16\ MgATP \rightarrow 2NH_3 + H_2 + 16MgADP$

Taxonomy of Rhizobia

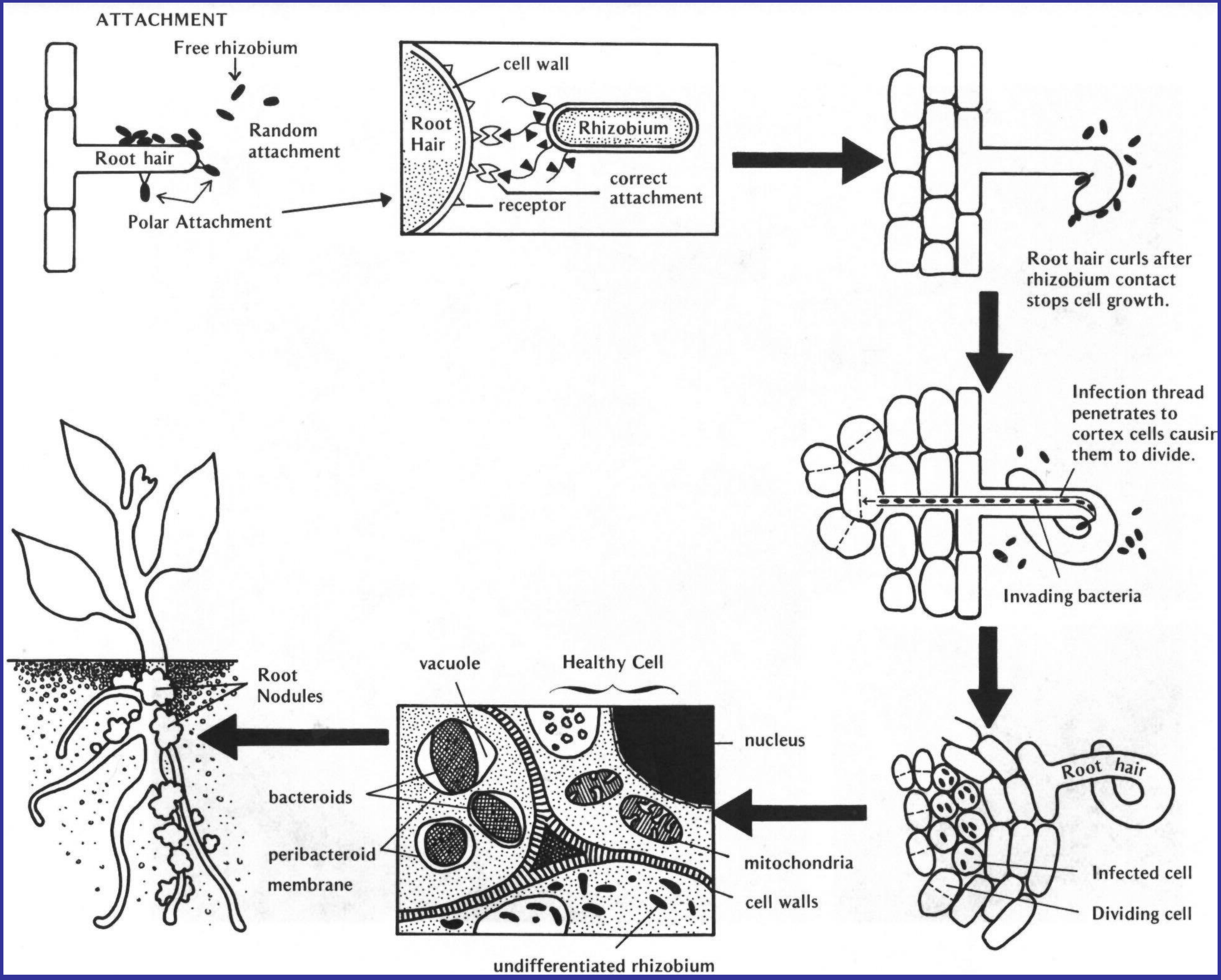
Genus	Species	Host plant
Rhizobium	*leguminosarum bv. trifolii*	*Trifolium* (clovers)
	“ *bv. viciae*	*Pisum* (peas), *Vicia* (field
	“ *bv. phaseoli*	beans), *Lens* (lentils),
	tropici	*Lathyrus*
	etli	*Phaseolus* (bean)
		Phaseolus (bean), *Leucaena*
		Phaseolus (bean)
Sinorhizobium	*meliloti*	*Melilotus* (sweetclover),
	fredii	*Medicago* (alfalfa), *Trigonella*
	saheli	*Glycine* (soybean)
	teranga	*Sesbania*
		Sesbania, Acacia
Bradyrhizobium	*japonicum*	*Glycine* (soybean)
	elkanii	*Glycine* (soybean)
	liaoningense	*Glycine* (soybean)
Azorhizobium	*caulinodans*	*Sesbania* (stem nodule)
‘Meso rhizobium’	*loti*	*Lotus* (trefoil)
	huakuii	*Astragalus* (milkvetch)
	ciceri	*Cicer* (chickpea)
	tianshanense	
	mediterraneum	*Cicer* (chickpea)
[Rhizobium]	*galegae*	*Galega* (goat’s rue), *Leucaena*
Photorhizobium	spp.	*Aeschynomene* (stem nodule)

Nitrogen Fixation

- Energy intensive process :
- $N_2 + 8H^+ + 8e^- + 16\ ATP = 2NH_3 + H_2 + 16ADP + 16\ Pi$
- Performed only by selected bacteria and actinomycetes
- Performed in nitrogen fixing crops

(ex: soybeans)

Nodulation in Legumes



Sources

- Lightning
- Inorganic fertilizers
- Nitrogen Fixation
- Animal Residues
- Crop residues
- Organic fertilizers

Forms of Nitrogen

- Urea → $CO(NH_2)_2$
- Ammonia → NH_3 (*gaseous*)
- Ammonium → NH_4
- Nitrate → NO_3
- Nitrite → NO_2
- Atmospheric Dinitrogen →N_2
- Organic N

Global Nitrogen Reservoirs

Nitrogen Reservoir	Metric tons nitrogen	Actively cycled
Atmosphere	$3.9*10^{15}$	No
Ocean → soluble salts Biomass	$6.9*10^{11}$ $5.2*10^{8}$	Yes Yes
Land → organic matter → Biota	$1.1*10^{11}$ $2.5*10^{10}$	Slow Yes

Roles of Nitrogen

- Plants and bacteria use nitrogen in the form of NH_4^+ or NO_3^-
- It serves as an electron acceptor in anaerobic environment
- Nitrogen is often the most limiting nutrient in soil and water.

Nitrogen is a key element for

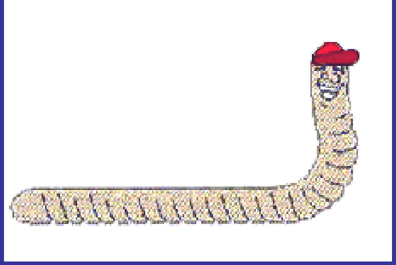
- amino acids
- nucleic acids (purine, pyrimidine)
- cell wall components of bacteria (NAM).

Nitrogen Cycles

- Ammonification/mineralization
- Immobilization
- Nitrogen Fixation
- Nitrification
- Denitrification

Mineralization or Ammonification

- Decomposers: earthworms, termites, slugs, snails, bacteria, and fungi
- Uses extracellular enzymes → initiate degradation of plant polymers
- Microorganisms uses:
- Proteases, lysozymes, nucleases to degrade nitrogen containing molecules



- Plants die or bacterial cells lyse → release of organic nitrogen
- Organic nitrogen is converted to inorganic nitrogen (NH_3)

- When pH<7.5, converted rapidly to NH_4

- Example:

$$\text{Urea} \longrightarrow NH_3 + 2\ CO_2$$

Immobilization

- The opposite of mineralization
- Happens when nitrogen is limiting in the environment
- Nitrogen limitation is governed by C/N ratio
- C/N typical for soil microbial biomass is 20
- C/N < 20 →Mineralization
- C/N > 20 →Immobilization

Microorganisms fixing

- *Azobacter*
- *Beijerinckia*
- *Azospirillum*
- *Clostridium*
- *Cyanobacteria*

- Require the enzyme nitrogenase
- Inhibited by oxygen
- Inhibited by ammonia (end product)

Rates of Nitrogen Fixation

N_2 fixing system	Nitrogen Fixation (kg N/hect/year)
Rhizobium-legume	200-300
Cyanobacteria- moss	30-40
Rhizosphere associations	2-25
Free- living	1-2

Bacterial Fixation

- Occurs mostly in salt marshes
- Is absent from low pH peat of northern bogs
- Cyanobacteria found in waterlogged soils

Denitrification

- Removes a limiting nutrient from the environment
- $4NO_3^- + C_6H_{12}O_6 \rightarrow 2N_2 + 6\ H_20$
- Inhibited by O_2
- Not inhibited by ammonia
- Microbial reaction
- Nitrate is the terminal electron acceptor