Fate of Nitrogen & Biological Nitrogen Fixation

Tiny Microscopic Organism's Precious Gift to the Living World for the Synthesis of Organic Food (Organic Matter)

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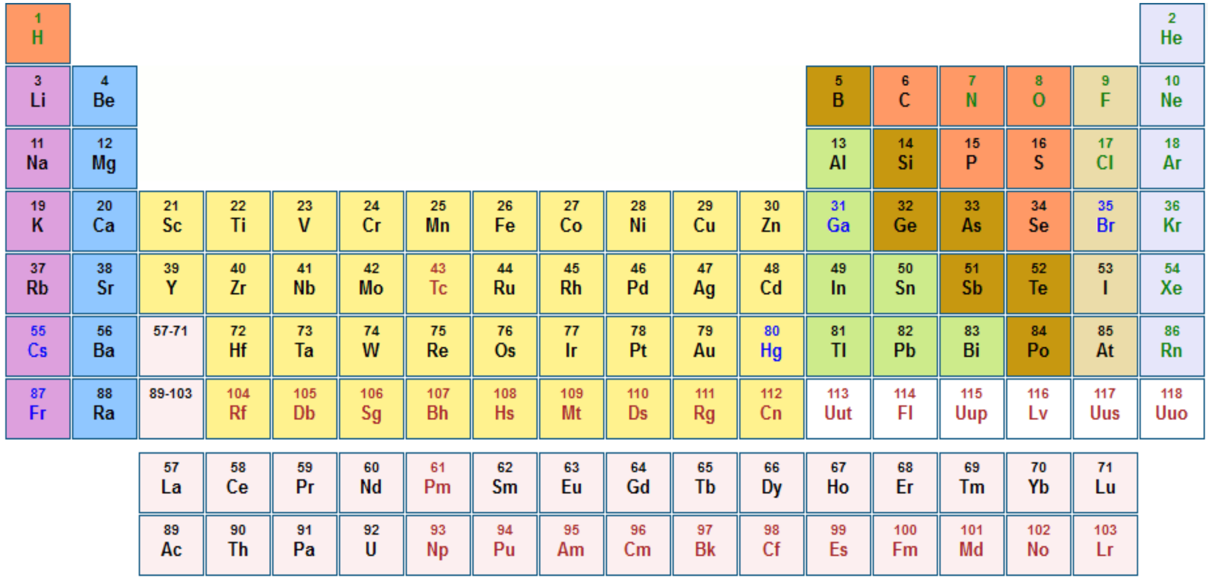
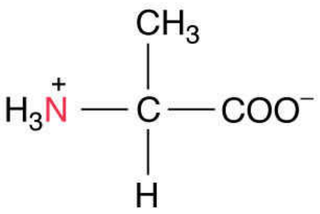
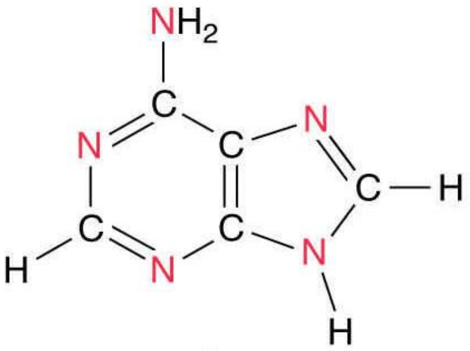
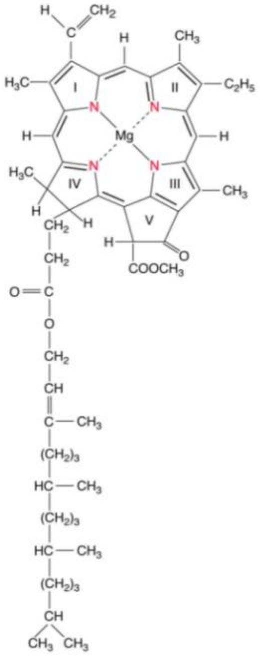
Nitrogen: an essential element

- The most abundant element in the earth's atmosphere.
- Nitrogen is the fourth most common element in a plant (after C, H & O).
- Living beings contain a large amount of nitrogen incorporated in proteins, nucleic acids, and many other biomolecules like, amino acids (proteins), nucleic acids (DNA, RNA), chlorophyll, and countless small molecules (PGRs, Chlorophyll, Cytochromes, Flavanoids, Alkaloids, Coumarins, Lignin, etc.
- Nitrogen is one of the three major macronutrients found in most fertilizers
- **78% of atmosphere is N_2 most of this is NOT available to living organisms.**
- **Paradox: limiting** in environment for growth but plenty available in atmosphere as N_2
    - Biologically unavailable!
    - Need prokaryotes to help with this…
    - Often the limiting nutrient for plant growth









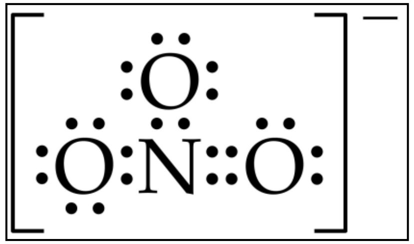
Deficiency Symptoms - N

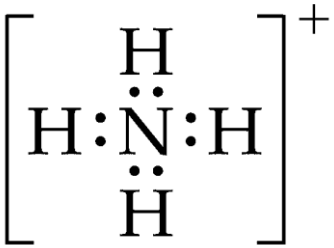
- General chlorosis.
- Chlorosis progresses from light green to yellow.
- Entire plant becomes yellow under prolonged stress.
- Growth is immediately restricted and drop older leaves.

http://plantsci.sdstate.edu/woodardh/soilfert/Nutrient_Deficiency_Pages/soy_def/SOY-N1.JPG

- During autotrophic growth the nitrogen demand for the formation of cellular matter is met by inorganic nitrogen in two alternative ways:
  1. Fixation of molecular nitrogen from air; or
  2. Assimilation of the nitrate or ammonia contained in water or soil.

- Bioavailable forms are nitrate (**NO_3^-**) and ammonia (**NH_4^+**)



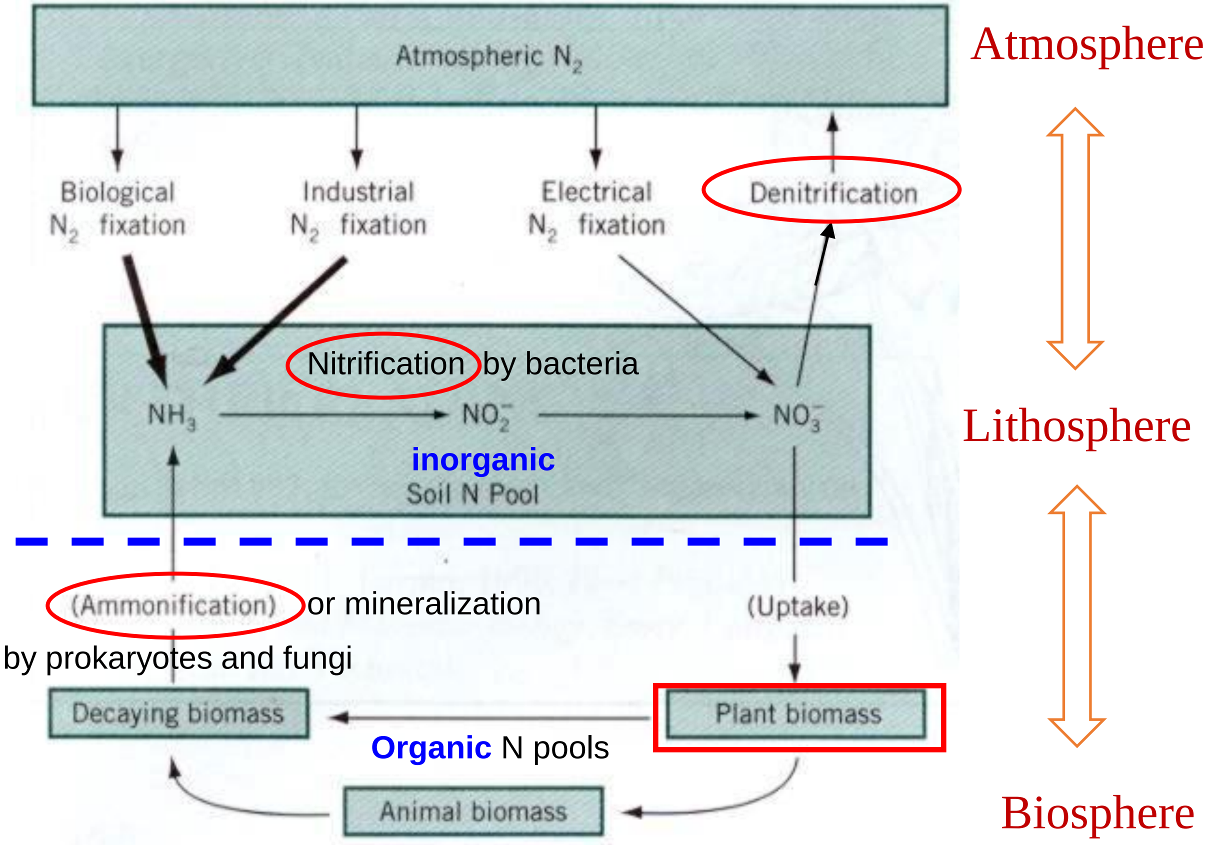


- Plants absorb nitrogen from the soil in the form of nitrate (NO3−) and ammonia (NH3).

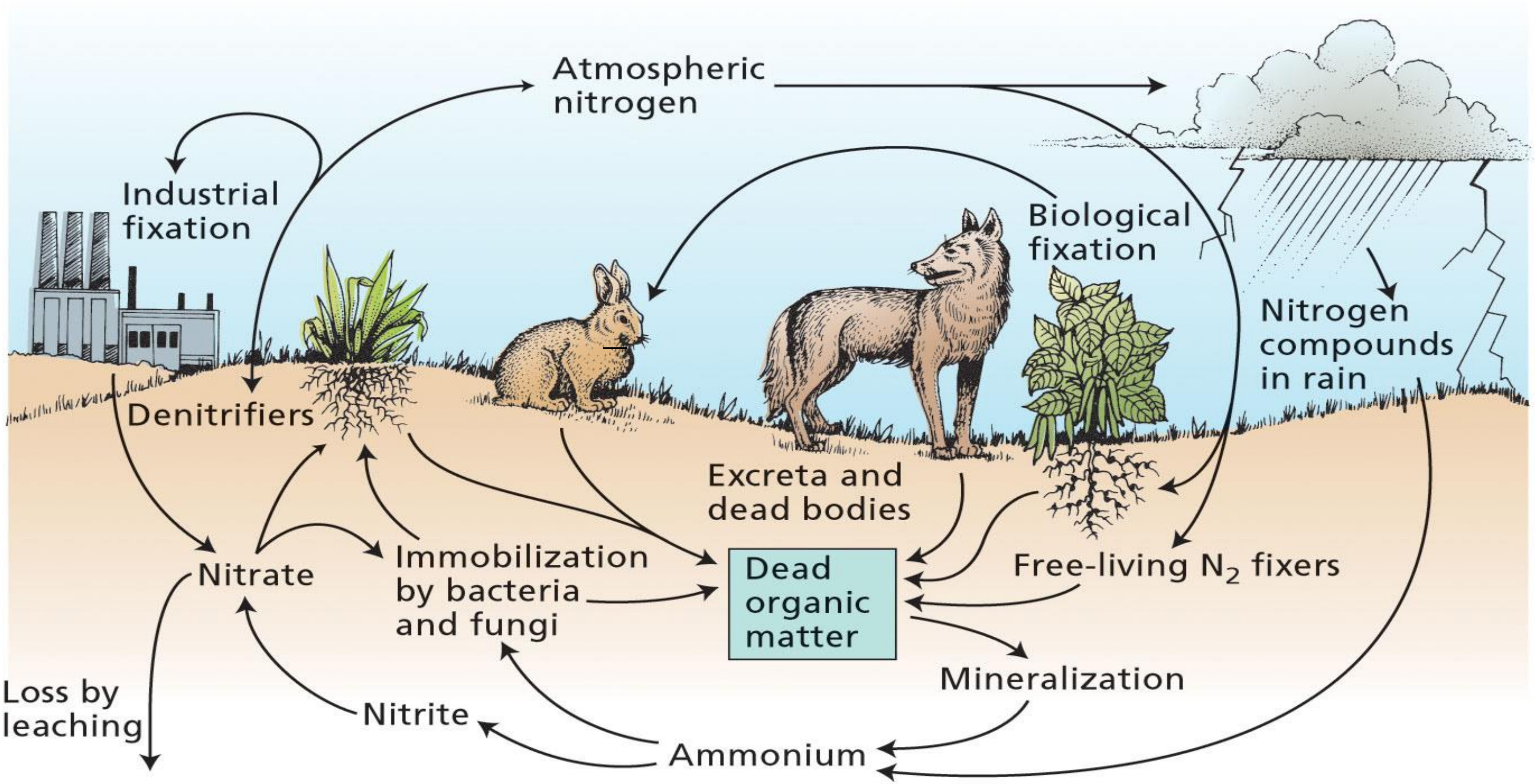
- In aerobic soils where nitrification can occur, nitrate is usually the predominant form of available nitrogen that is absorbed.

The Nitrogen cycle circulates N in the biosphere:

- **3 major N pools:**
  - Atmosphere,
  - Soil (Lithosphere),
  - Biomass (Biosphere)
- Plants convert inorganic soil N to organic N (amino acids, NAs, etc.)
- Organic N moves up the chain to animals (they eat plants!)
- Returns to soil in animal waste and decomposition after death



Nitrogen in the environment



PLANT PHYSIOLOGY , Third Edition, Figure 12.1

Essential N cycle processes include ammonification, nitrification and denitrification

- **Ammonification** by prokaryotes and fungi returns N to soil:
    - organic N → ammonia (NH_4^+)
- Make ammonia biologically available by its sequential oxidization to nitrite and nitrate by soil bacteria during **nitrification**
- Plants must compete for nitrate with soil bacteria that reduce NO_3^- to N_2: **denitrification**
    - 93 to 130 Mt / year back to atmosphere

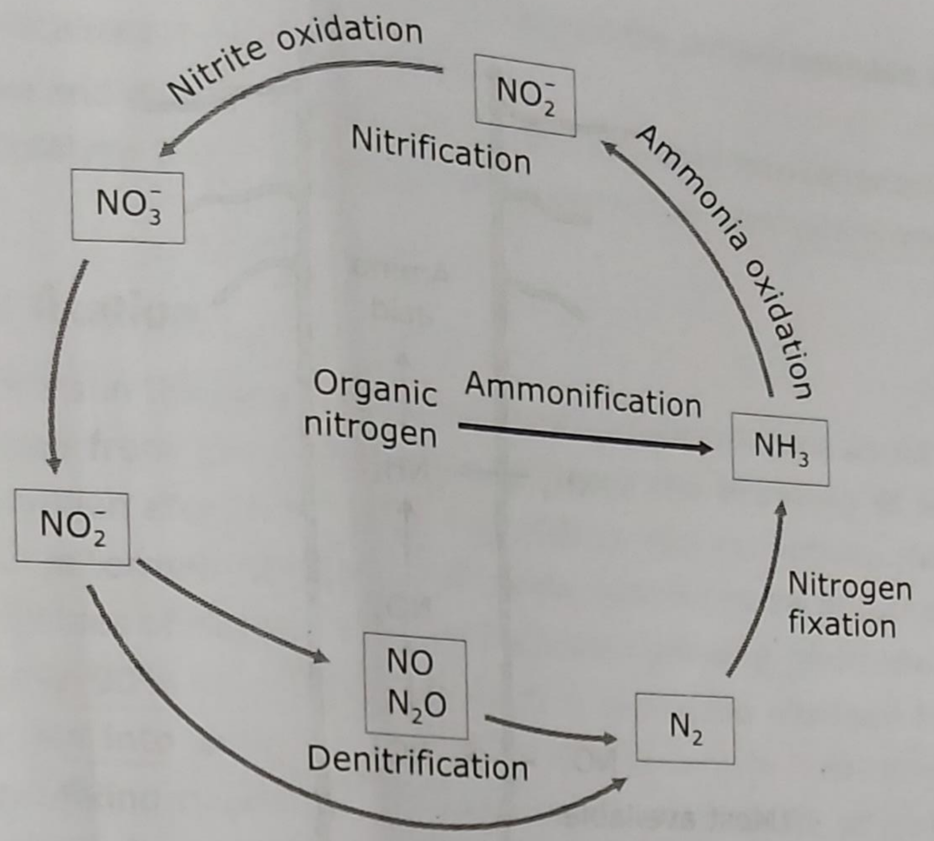


Figure 3.10 The nitrogen cycle is the process by which nitrogen is converted between its various chemical forms. This transformation can be carried out by both biological and non-biological processes. Important processes in the nitrogen cycle include fixation, mineralization (ammonification), nitrification and denitrification.

TABLE 12.1
The major processes of the biogeochemical nitrogen cycle

Process	Definition	Rate (10^{12} g yr^{-1})[a]
Industrial fixation	Industrial conversion of molecular nitrogen to ammonia	80
Atmospheric fixation	Lightning and photochemical conversion of molecular nitrogen to nitrate	19
Biological fixation	Prokaryotic conversion of molecular nitrogen to ammonia	170
Plant acquisition	Plant absorption and assimilation of ammonium or nitrate	1200
Immobilization	Microbial absorption and assimilation of ammonium or nitrate	N/C
Ammonification	Bacterial and fungal catabolism of soil organic matter to ammonium	N/C
Nitrification	Bacterial (*Nitrosomonas* sp.) oxidation of ammonium to nitrite and subsequent bacterial (*Nitrobacter* sp.) oxidation of nitrite to nitrate	N/C
Mineralization	Bacterial and fungal catabolism of soil organic matter to mineral nitrogen through ammonification or nitrification	N/C
Volatilization	Physical loss of gaseous ammonia to the atmosphere	100
Ammonium fixation	Physical embedding of ammonium into soil particles	10
Denitrification	Bacterial conversion of nitrate to nitrous oxide and molecular nitrogen	210
Nitrate leaching	Physical flow of nitrate dissolved in groundwater out of the topsoil and eventually into the oceans	36

Note: Terrestrial organisms, the soil, and the oceans contain about 5.2×10^{15} g, 95×10^{15} g, and 6.5×10^{15} g, respectively, of organic nitrogen that is active in the cycle. Assuming that the amount of atmospheric N_2 remains constant (inputs = outputs), the *mean residence time* (the average time that a nitrogen molecule remains in organic forms) is about 370 years [(pool size)/(fixation input) = $(5.2 \times 10^{15}\text{ g} + 95 \times 10^{15}\text{ g})/(80 \times 10^{12}\text{ g yr}^{-1} + 19 \times 10^{12}\text{ g yr}^{-1} + 170 \times 10^{12}\text{ g yr}^{-1})$] (Schlesinger 1997).

[a]N/C, not calculated.

N fixation reduces N_2 to NH_4^+

- Soil N pool loses N to atmosphere but regains N through action of fixing bacteria
- $N_2 \rightarrow NH_4^+$ does not happen spontaneously: highly endergonic = **very energetically costly**

Where does the biologically available soil N come from?

- 10% of N fixed into **N oxides**: lightning, UV, air pollution
- 30% via industrial N fixation: make N using fossil fuels via **Haver-Bosch** process at high T and pressure
- **60% via biological N fixation** by microorganisms: poorly understood but extremely valuable process

Nitrogen in the environment

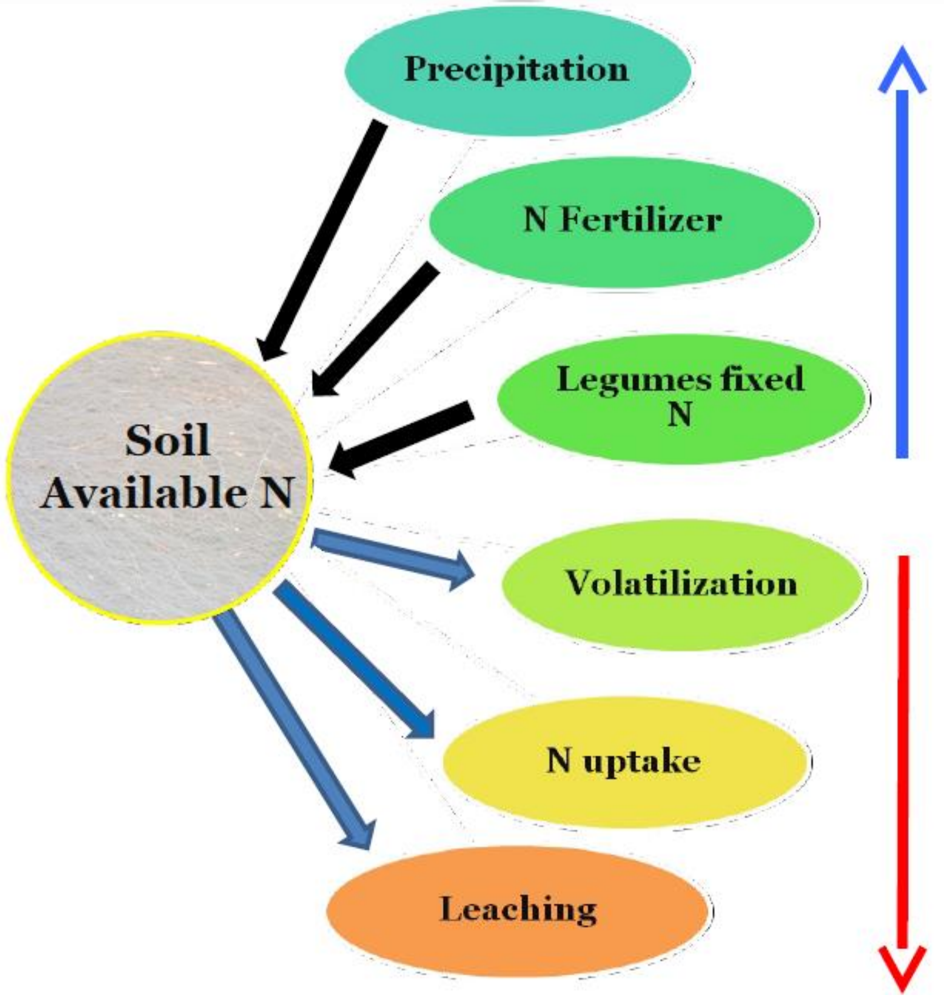
- This occurs naturally by:-***Lightning***:
  - **8%:** splits H_2O: the free O and H attack N_2 – forms HNO_3 (nitric acid) which fall to ground with rain
- ***Photochemical reactions***:
  - **2%:** photochemical reactions between NO gas and O_3 to give HNO_3
- ***Nitrogen fixation***:
  - **90%:** biological – bacteria fix N_2 to ammonium (NH_4^+)

Table 16.2 Rates of natural and anthropogenic nitrogen fixation

Source	Amount of N fixed[a]
Lightning	<10 Tg/year
Biological N-fixation in terrestrial systems[b]	90–140 Tg/year
Biological N-fixation in marine systems	30–300 Tg/year[c]
N fertilizer synthesis	80 Tg/year
Fossil fuel combustion	>20 Tg/year

[a]The standard unit of measure is the teragram (Tg), 10^{12} g, equal to 10^6 metric tons.
[b]This estimate includes both natural ecosystems and agricultural nitrogen fixation.
[c]Estimates differ because of variable data.

Key events responsible for soil available N

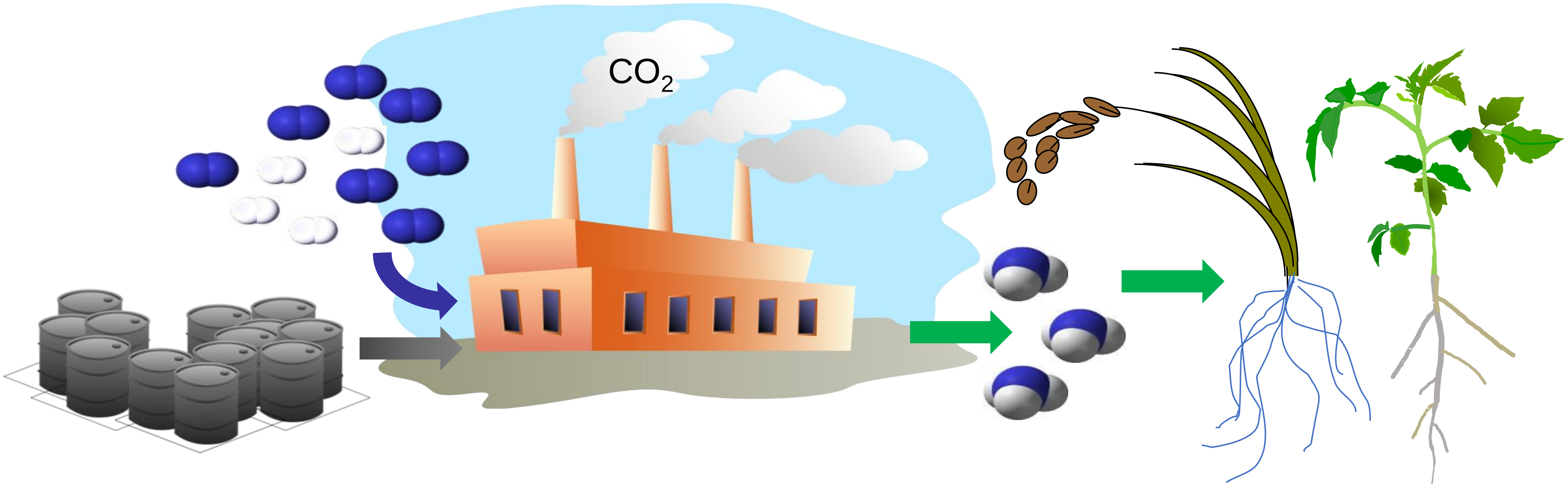


- In natural terrestrial ecosystems, 80% - 90% of the nitrogen available to plants is estimated to originate from biological nitrogen fixation.
- Of that total, approx. 80% is generated in symbiotic associations.
- If the plant resources, required to establish nodules, fix nitrogen and transport the resulting ammonia throughout the plant are taken in to account; obtaining nitrogen through symbiosis consumes 12- 17 g carbohydrates per gram of N fixed.
- Unlike the Haber- Bosch process the biological nitrogen fixation catalyzed by the enzyme nitrogenase occurs at ambient temperature and atmospheric pressure.

Fixing nitrogen to fertilize plants accounts for ~2% of global energy use

$$N_2 + 3H_2 \xrightarrow[150\text{- }400\text{ atm}]{400\text{- }650\ ^{o}C} 2NH_3 \quad (\text{delta } H_f^0 = -46.1\text{ KJ mol}^{-1}$$

$$7CH_4 + 10\ H_2O + 8\ N_2 + 2O_2 \longrightarrow 16NH_3 + 7CO_2$$



Biological Nitrogen Fixation

Types of Diazotrophs involved in Biological Nitrogen Fixation

- Free-living (asymbiotic)
    - Cyanobacteria
    - Azotobacter
- Associative
    - Rhizosphere– Azospirillum
    - Lichens–cyanobacteria
    - Leaf nodules
- Symbiotic
    - Legume- Rhizobia
    - Actinorhizal- Frankia
    - Plants- Cynobacteria

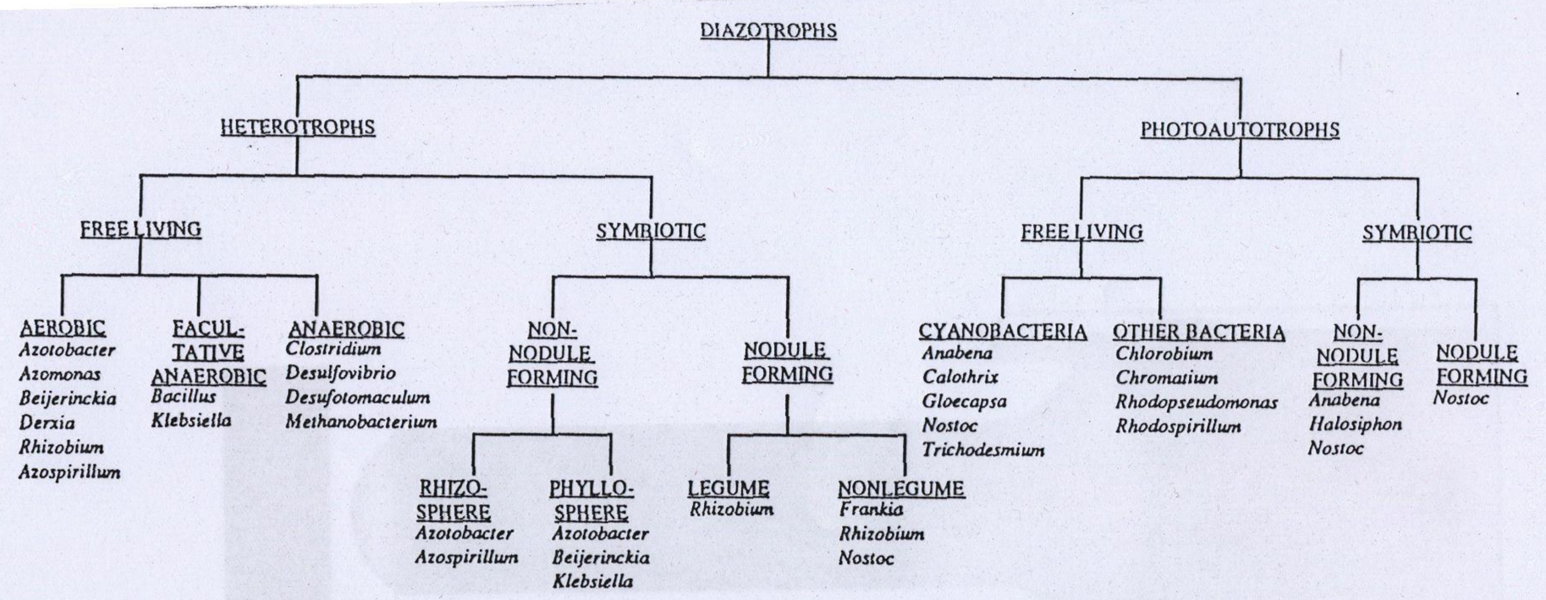
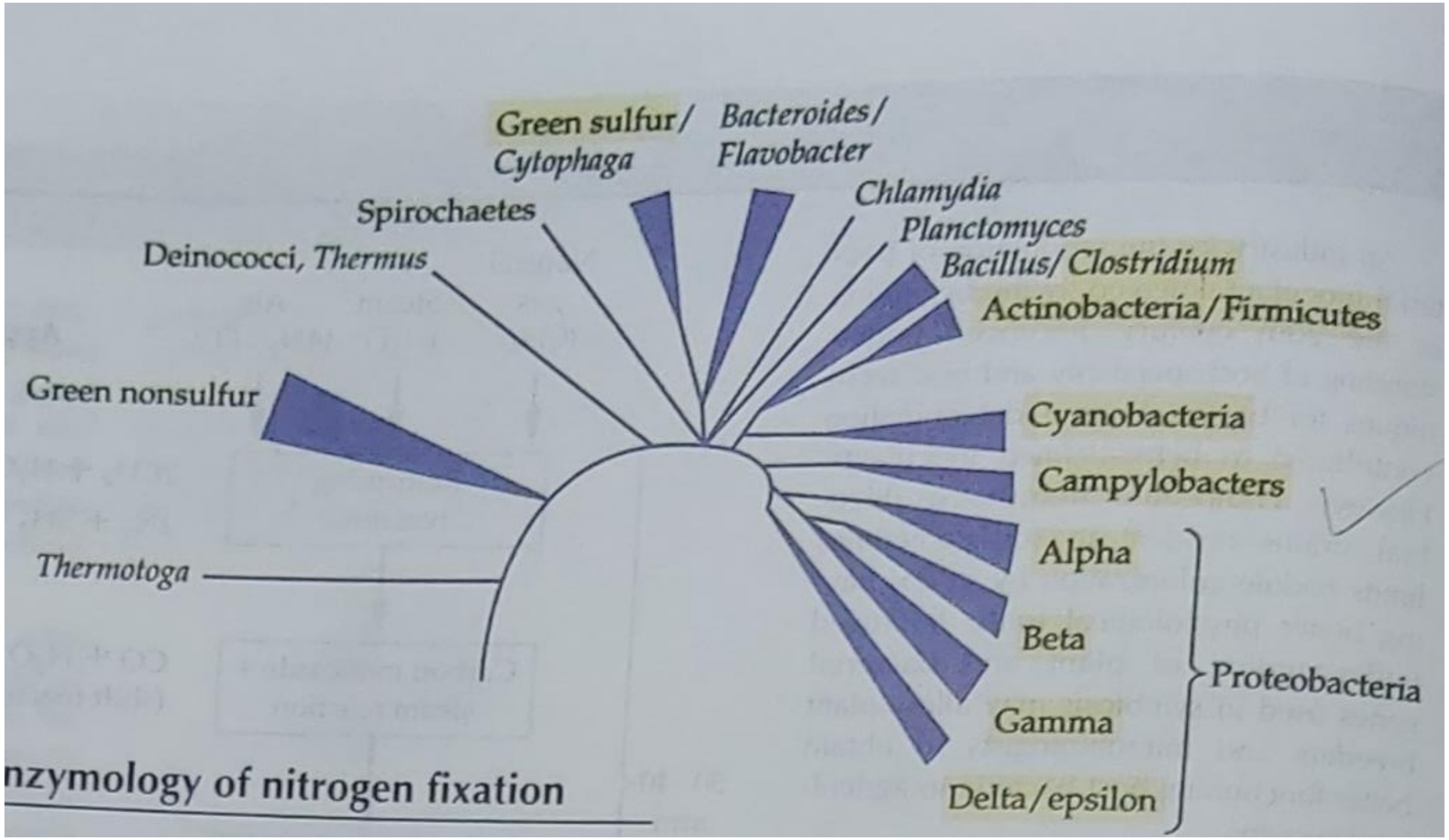


Figure 2 Diagrammatic representation of the primary nitrogen-fixing genera.

- Enzymatic nitrogen fixation is limited to prokaryotes only.
- This trait is associated with members of many eubacterial phylogenies as well as some methanogenic archaea.



Phylogenetic Distribution of Nitrogen Fixing Eubacteria. A simplified taxonomy of these bacteria shows that although many groups contain nitrogen fixing species (highlighted) in yellow), nitrogen fixation is not carried out by every representative of these groups.

- A few nitrogen fixing bacteria from diverse taxa, i.e. cyanobacteria, actinomycetes, alphaproteobacteria, etc. are able to establish symbiotic association with plants.

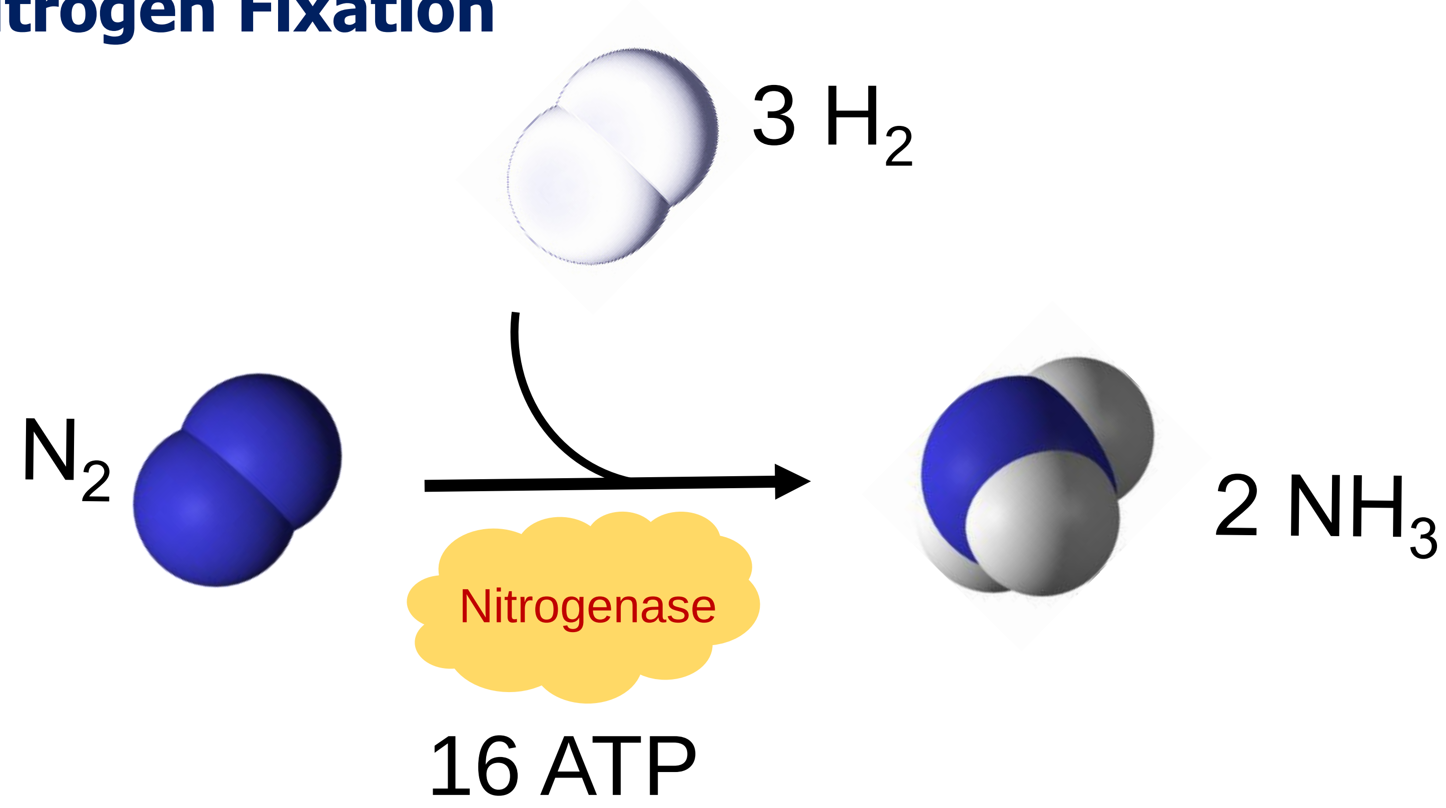
Table 3.3 Examples of nitrogen fixing bacterial and archaeal genus and their lifestyle

Genus	*Phylogenetic affiliation*	*Lifestyle*
Nostoc, Anabaena	Bacteria	Free-living, aerobic, photolithotrophic
Pseudomonas, Azotobacter	Bacteria	Free-living, aerobic, chemotroph
Thiobacillus	Bacteria	Free-living, aerobic, chemotroph
Methanococcus	Archaea	Free-living, anaerobic, chemotroph
Chromatium, Chlorobium	Bacteria	Free-living, anaerobic, phototroph
Desulfovibrio, Clostridium	Bacteria	Free-living, anaerobic, chemotroph
Rhizobium, Frankia	Bacteria	Symbiotic, aerobic, chemotroph

- In such symbiosis nitrogen fixed by bacteria is exchanged for Carbon fixed by the plant.

- Nitrogen fixation by symbiotic bacteria can be highly productive because interaction with the plant allows fixation to occur under optimized physiological conditions, overcoming constraints that often limit nitrogen fixation by non-symbiotic bacteria.

Biological Nitrogen Fixation



1. Rare, extremely energy consuming conversion because of stability of triply bonded N_2.
2. Many prokaryotes can fix nitrogen using an enzyme called nitrogenase. This process uses a great deal of cellular energy, ATP
3. Produces fixed N in the form of NH_4^+ which can be either directly accumulated and assimilated into N containing biomolecules or converted to Nitrate via bioactivity of certain microbes (nitrifying bacteria) in a process known as nitrification

Biological Nitrogen Fixation

$$N_2 + 8\ \text{flavodoxin}^- + 8H^+ + 16\ MgATP^{2-} + 18\ H_2O$$

$$\xrightarrow{\text{Nitrogenase}}$$

$$2NH_4^+ + 2OH^- + 8\ \text{flavodoxin} + 16\ MgADP^- + 16H_2PO_4^- + H_2$$

N-fixation requires energy input:

- Reduction reaction, e^- must be added (sensitive to O_2)

- Requires ~35 kJ of energy per mol of N fixed (theoretically)

- Actual cost: ~15-30g CH per g of NH_3 produced

- Assimilation of NH_3 into organic form takes 3.1-3.6 g CH

- It (nitrogen fixation) is a unique biochemical reaction that consumes energy rich compounds while requiring strong biological reductants.
- Because nitrogenase and some of the proteins that supply it with reductant are sensitive to oxygen, many nitrogen fixing bacteria are anaerobes.
- Neither fermentation nor respiration oxidizes reduced carbon compounds as efficiently as aerobic respiration, so anaerobic bacteria must process large quantities of substrate to generate the ATP required for dinitrogen fixation.
- In contrast, aerobes have the advantage of high ATP production from aerobic metabolism but must contend with the oxygen sensitivity of nitrogenase.

- In some cases, free- living nitrogen- fixing organisms use mechanical or biochemical barriers to keep oxygen away from the biological catalysts of nitrogen fixation.
- In other cases, the nitrogen fixation is segregated specially in specialised structures.
- For example, some filamentous cyanobacteria generate heterocysts, thick walled cells that fix nitrogen but cannot complete all the reactions of oxygenic photosynthesis.
- Heterocysts produce the ATP needed for nitrogen fixation by way of cyclic photophosphorylation, a light dependent process that doesn't create Oxygen gas.
- Some non- filamentous cyanobacteria segregate photosynthesis from nitrogen fixation temporally, performing oxygenic photosynthesis in the light and nitrogen fixation in the dark.

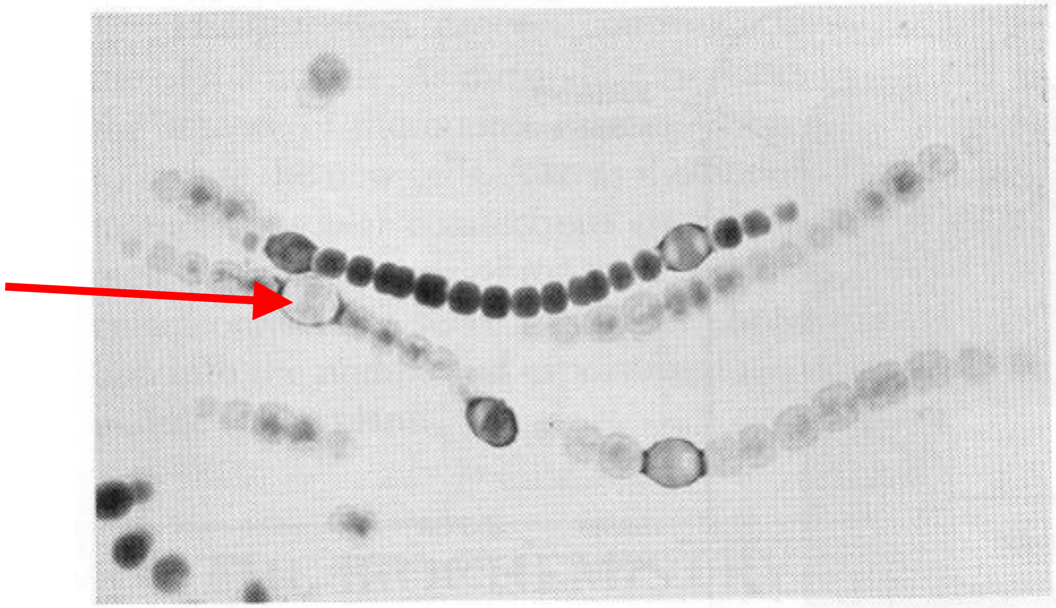
Oxygen inhibits dinitrogenase

- Irreversibly denatures both constituent proteins
- But need cellular respiration to make ATP!

❖ Strategies

- Free living bacteria maintain an anaerobic lifestyle or only fix N2 when under anaerobisis

- Cyanobacteria structurally isolate nitrogen fixing cells (heterocysts): thick walls, high respiratory capacity limits O_2 levels, lack PSII and thus can't evolve O_2
- Nodules restrict O_2 to an O_2-binding protein, leghemoglobin
  - Synthesized by host, present in bacteroid infected host cells
  - Keeps respiration high while sequestering O_2 from dinitrogenase



- **Rhizobia fix nitrogen in symbiotic association with leguminous plants**
- **Rhizobia fix N for the plant and plant provides Rhizobia with carbon substrates**
- **All nitrogen fixing systems appear to be identical**
- **They require nitrogenase, a reductant (reduced ferredoxin), ATP, O-free conditions and regulatory controls (ADP inhibits and NH_4^+ inhibits expression of nif genes).**

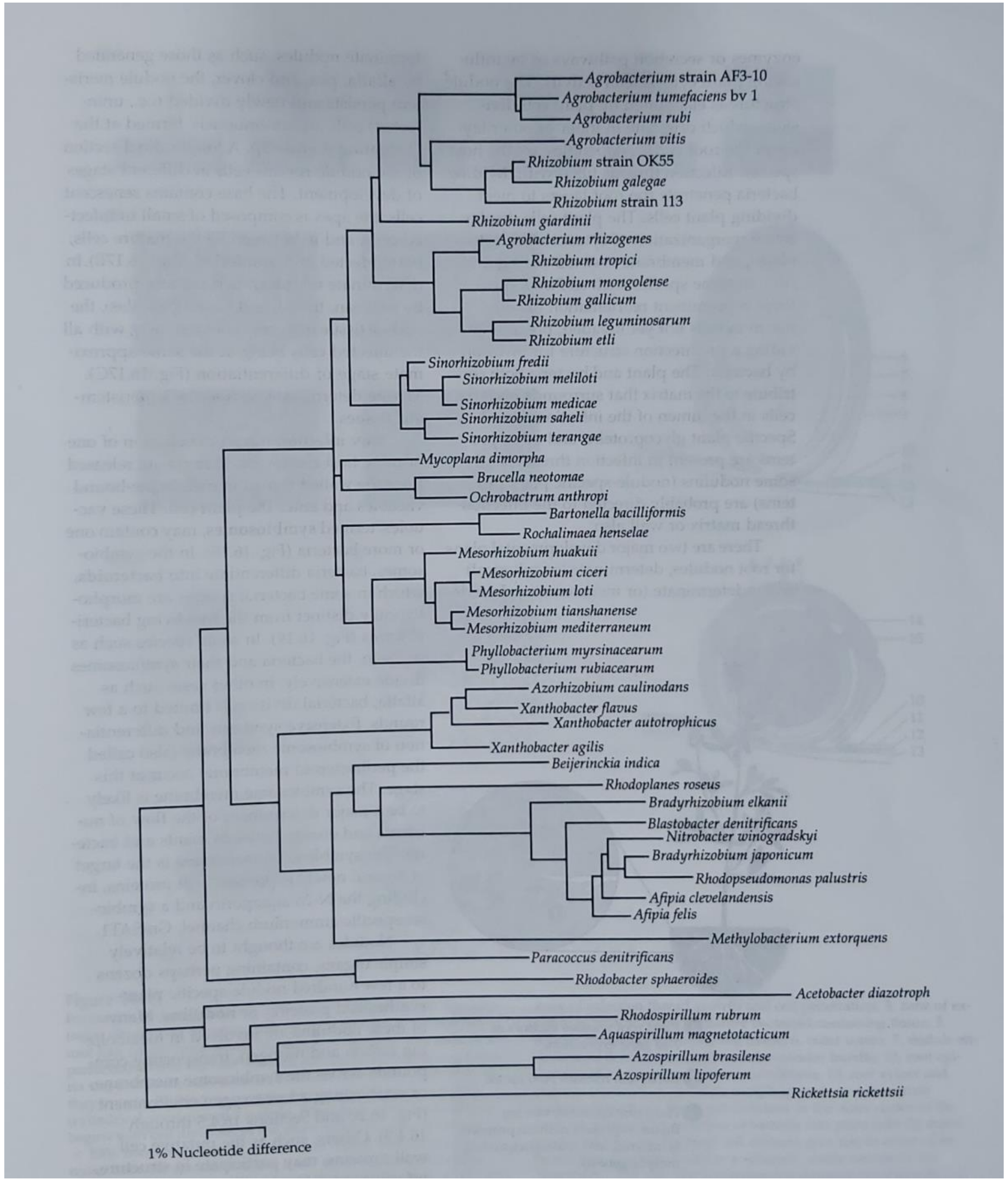
Table 16.4 *Rhizobium* and related bacteria that form symbioses with legumes

Bacterial species	Host plants[a]
Sinorhizobium meliloti[b]	*Medicago* (alfalfa), *Trigonella* (fenugreek), *Melilotus* (sweetclover)
S. fredii[b]	*Glycine* (soybean), *Vigna* (cowpea)
Sinorhizobium sp. NGR234	Broad host range, many genera: *Vigna*, *Leucaena*, *Macroptilium* (siratro), *Parasponia*[c]
Rhizobium leguminosarum biovar *viciae*	*Vicia* (vetch), *Pisum* (pea), *Cicer* (chickpea), *Lathyrus* (sweet pea)
R. leguminosarum biovar *trifolii*	*Trifolium* (clover)
R. leguminosarum biovar *phaseoli*	*Phaseolus* (bean)
R. tropici	*Phaseolus*, *Leucaena*, *Medicago*, *Macroptilium*
R. etli	*Phaseolus*
Mesorhizobium loti	*Lotus* (trefoil), *Anthyllis* (kidney vetch), *Lupinus* (lupine)
Bradyrhizobium japonicum	*Glycine*, *Macroptilium*, *Vigna*
Azorhizobium caulinodans	*Sesbania rostrata*

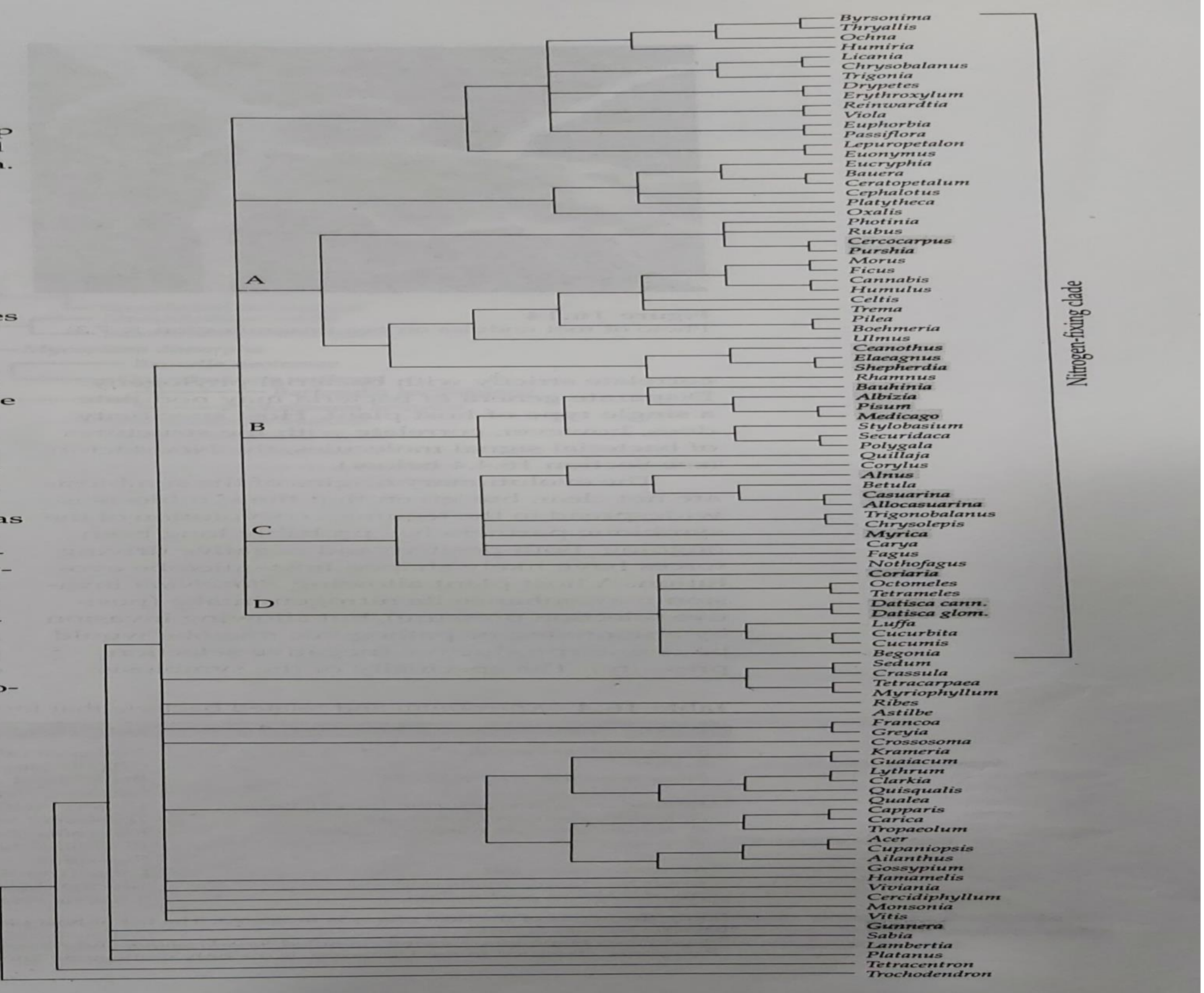
[a]Only the genus of the host plants is indicated. The list of host plants is not comprehensive, particularly for broad-host-range bacteria such as *S. fredii*.

[b]A previous taxonomic scheme classified *Sinorhizobium* and *Mesorhizobium* as *Rhizobium*.

[c]*Parasponia*, classified in the Ulmaceae, is the only nonlegume known to associate with *Rhizobium*.

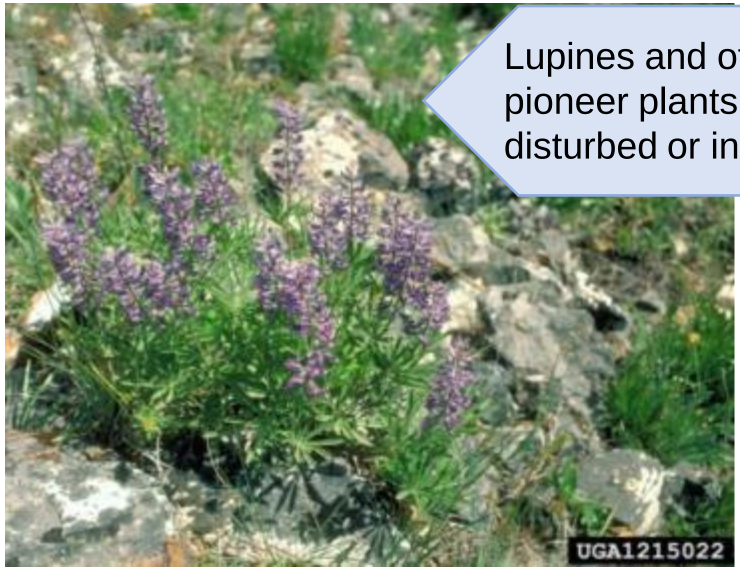


- Phylogenetic tree of the alpha- proteobacteria, showing the dispersed groups of rhizobia as well as free living organisms (eg., *Rhodobacter*) and animal pathogens (eg. Brucella, *Bartonella*, *Rickettsia*).
- Note that *Rhizobium* and *Sinorhizobium* are very closely related to *Agrobacterium* and more distantly related to *Bradyrhizobium*.
- Thus, the symbiotic habit and host range do not correlate easily with relatedness, as estimated from 16S rRNA sequence comparisons.



- ❖ Phylogenetic analysis of 99 rbcl genes (encoding the large subunit of Rubisco) from genera representing the subclass Rosidae and additional angiosperm taxa.
- ❖ Note that a single clade includes all the nodules- forming genera included in the tree.
- ❖ Subclass A, C, and D include actinorhizal symbiotic hosts; the legume family (Fabaceae) falls into subclade B. The subfamily Celtoideae, w/c containd the nitrogen fixing genus *Parasponia*, is represented here by the genera *Celtis* and *Trema*.

Legumes are important ecologically and as food and fodder crops



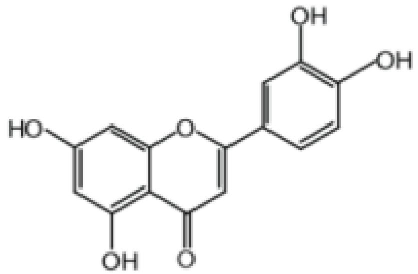
Lupines and other legumes are pioneer plants that can grow in disturbed or infertile soils

Some legumes are too successful and become a pest, such as the invasive legume kudzu (*Pueraria montana*)

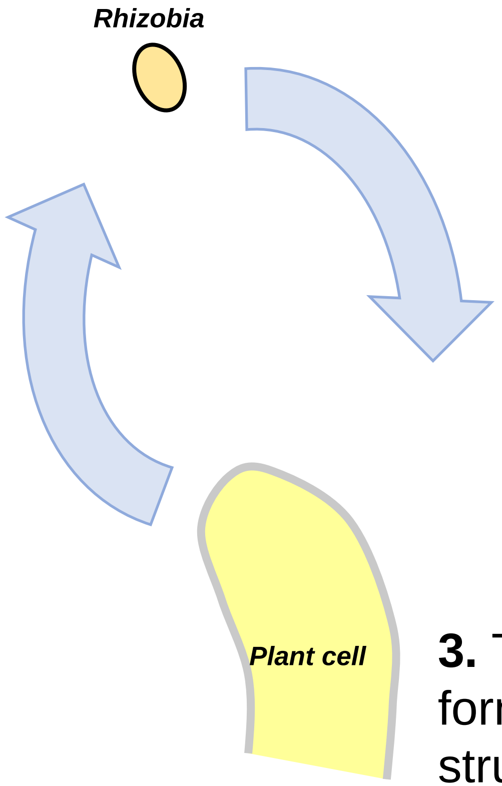
Legumes provide protein to humans and other animals

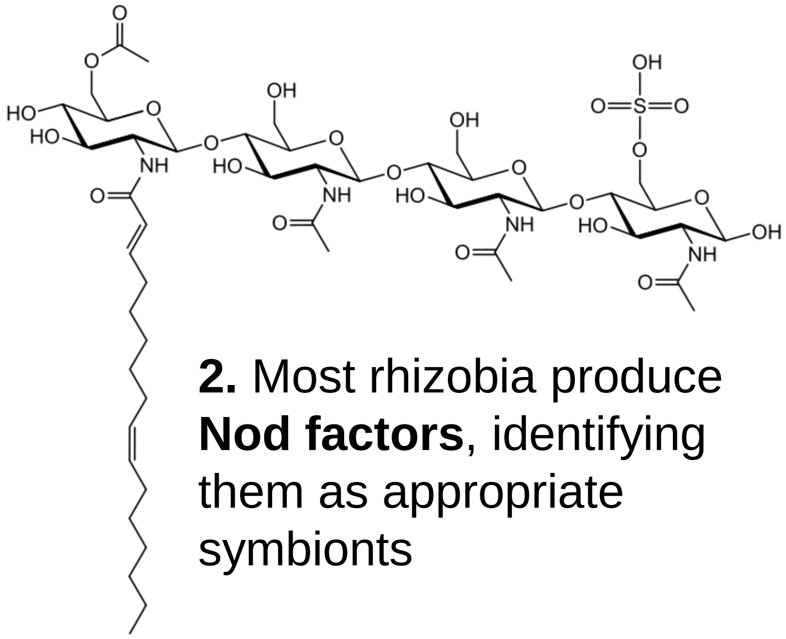
Photo credits: Dave Powell, USDA Forest Service, Bugwood.org; Scott Ehardt; Drypot; IITA; Tofu; MedIndia; HayandForage

Communication: Flavonoids and Nod factors



1. The plant root produces specific **flavonoids** that attract rhizobia

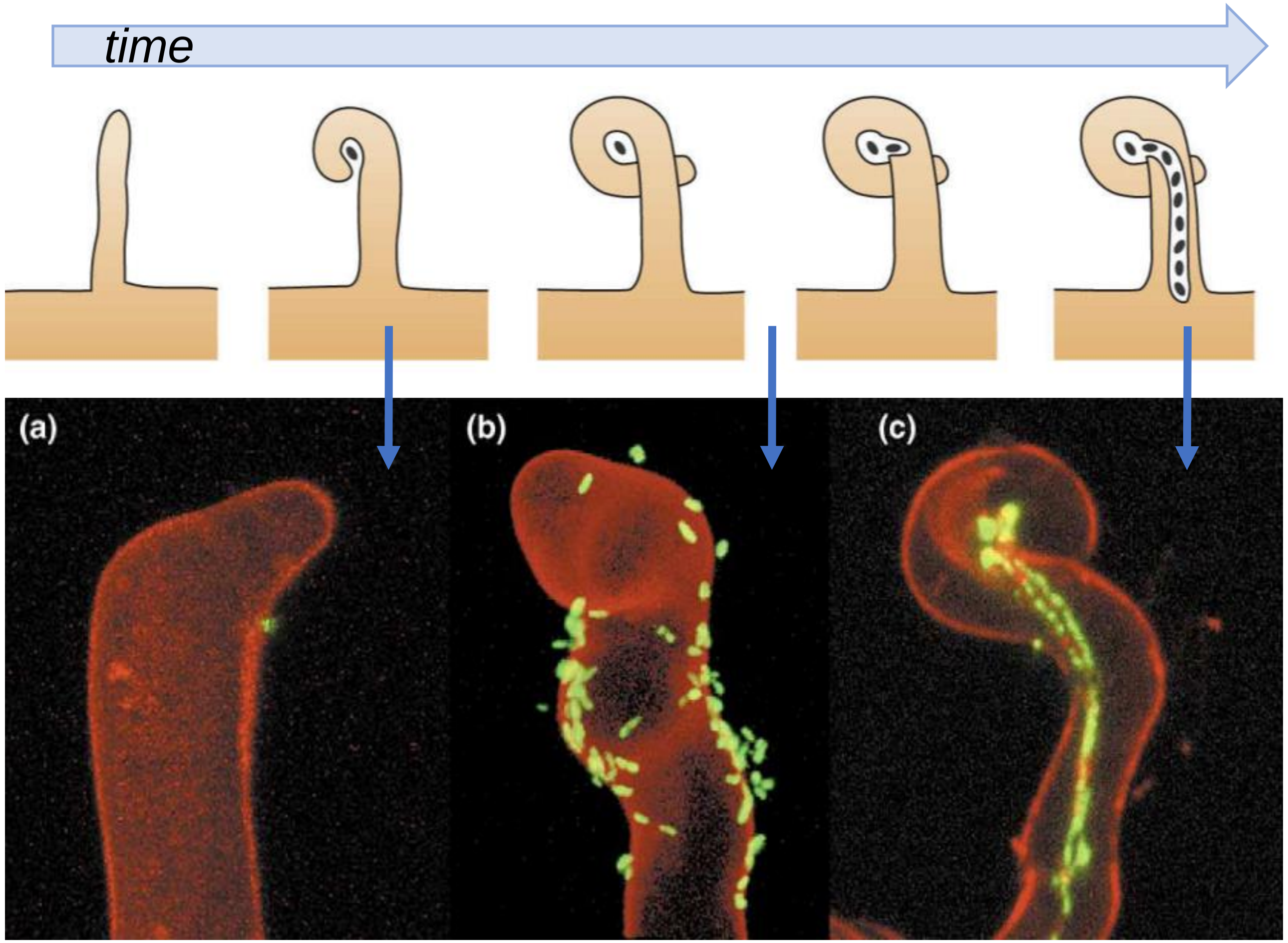




2. Most rhizobia produce **Nod factors**, identifying them as appropriate symbionts

3. The plant prepares to form a symbiotic nodule structure

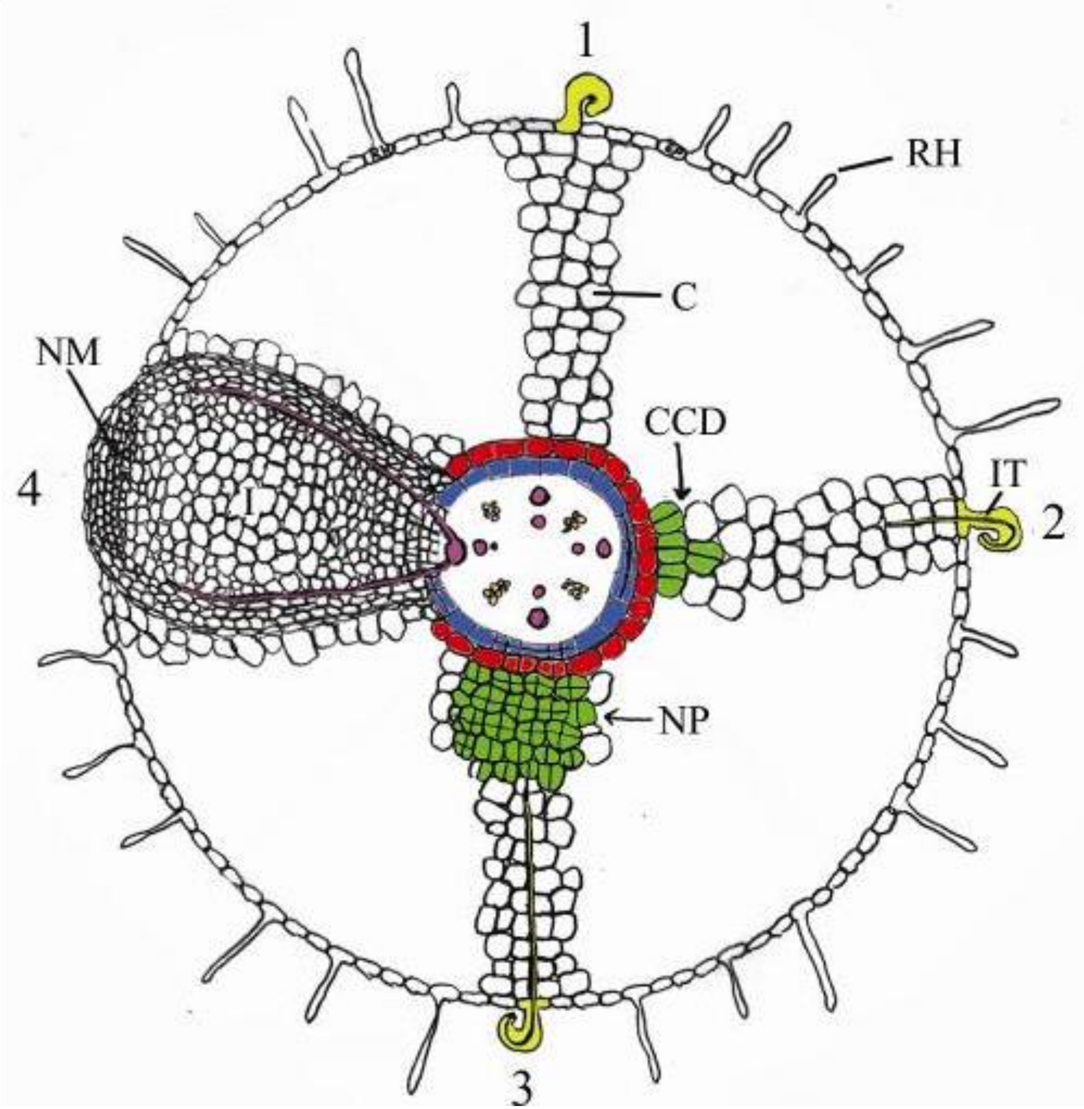
Nod factor perception induces root hair curling



- Nod-factors are concentrated in the cell wall and are almost immobile
- Nod-factors cause redirection of tip growth (shown in *a*)
- Only a few bacteria actually redirect the growth of the root hair successfully and become enveloped in an infection thread

From: Buchanan, B.B., Gruissem, W. and Jones, R.L. (2000) Biochemistry and Molecular Biology of Plants. American Society of Plant Physiologists.

Bacterial entry and nodulation process



1 Root hair curling

2 Infection thread (IT) formation and cortical cell division (CCD)

3 Nodule primordium (NP) formation

4 Nodule development with formation of nodule meristem (in some legumes)

Image by U Mathesius

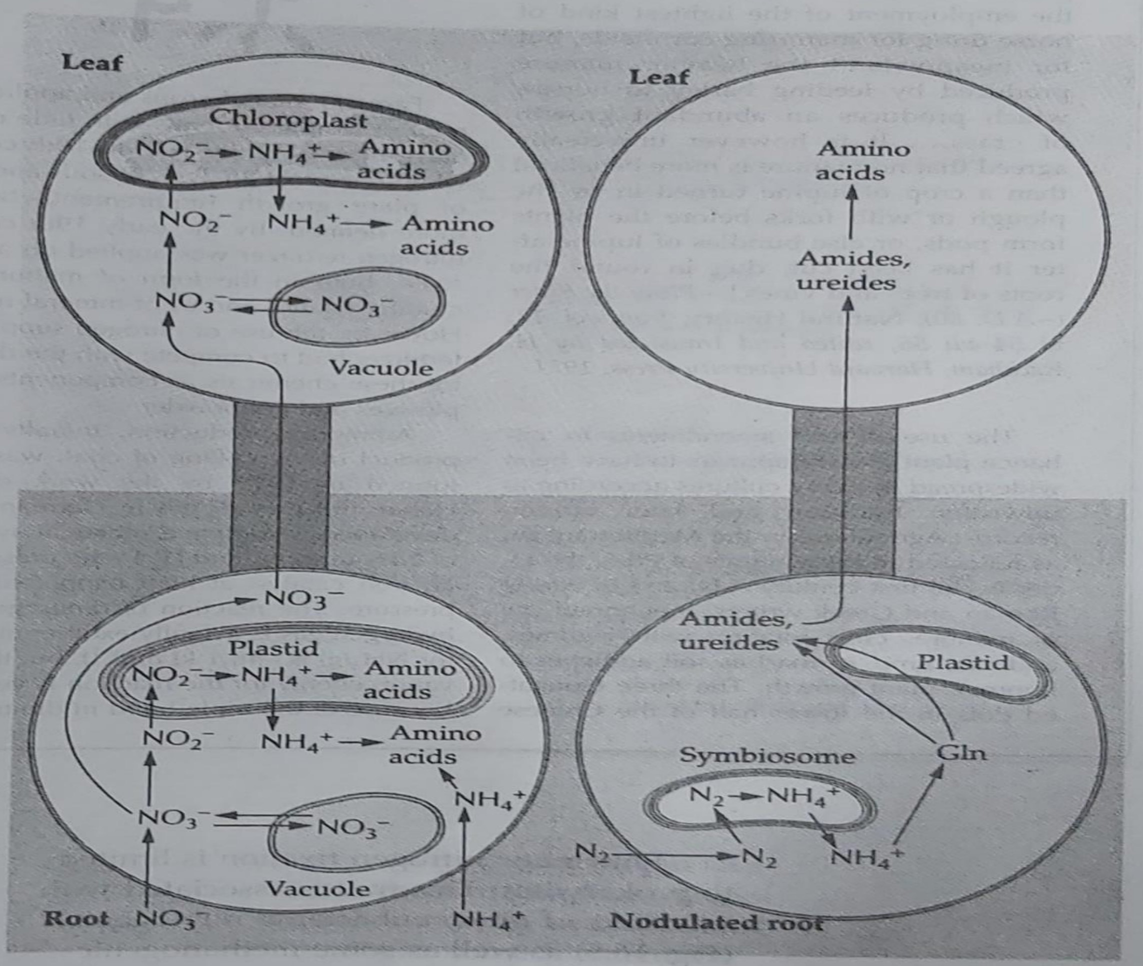
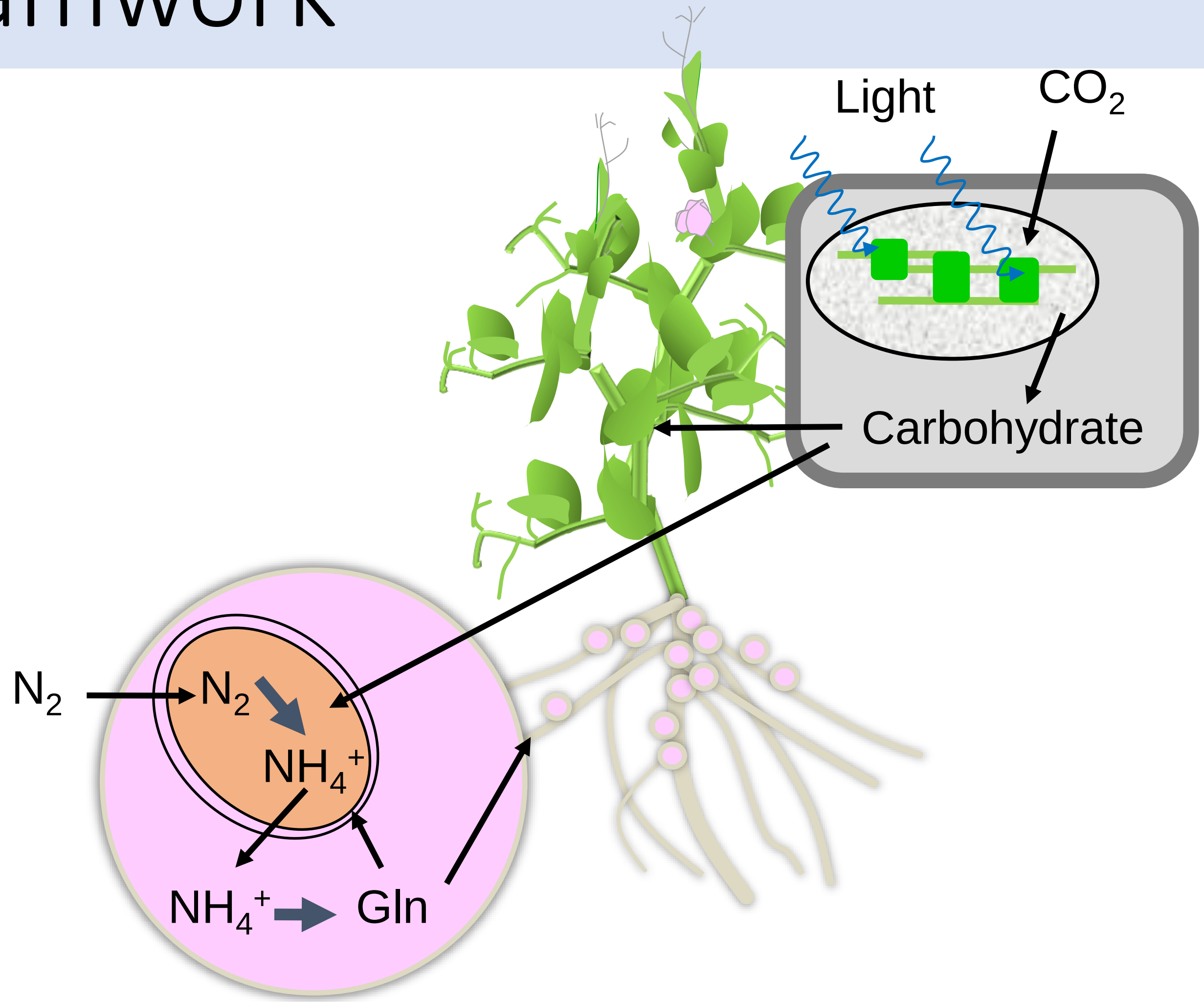


Figure 16.4
Overview of N uptake by a nonnodulated plant (left), and by a nodulated plant with N-fixing symbionts (right). There is considerable variation in the details of nitrogen assimilation in different plants. Plant roots can import nitrate, ammonium, and other nitrogenous compounds from the soil. For use in synthesis of amines and amides, nitrate must be reduced to nitrite and then to ammonium. Nitrate reduction in the cytosol and storage in the vacuoles are processes that can occur in either the roots or the leaves. Nodulated plants are able to take up fixed nitrogen from the soil (not shown) but, through the action of symbiotic bacteria, can generate ammonium also by reducing N_2. The ammonium from nitrogen fixation is assimilated into amino acids and ultimately incorporated into amide amino acids (glutamine or asparagine) or ureides (see Section 16.4.9) for export to the leaves. These conversions are shown in more detail in Figures 16.32 and 16.33.

Symbiotic nitrogen fixation requires teamwork



Plants cannot fix nitrogen on their own, and most rhizobia cannot fix nitrogen on their own.

Symbiotic nitrogen fixation is a true partnership.

The bacteria provide **nitrogenase**.

The host plant provides **leghemoglobin**, **homocitrate**, **carbon sources**, **organic nitrogen**…..

Thank you!