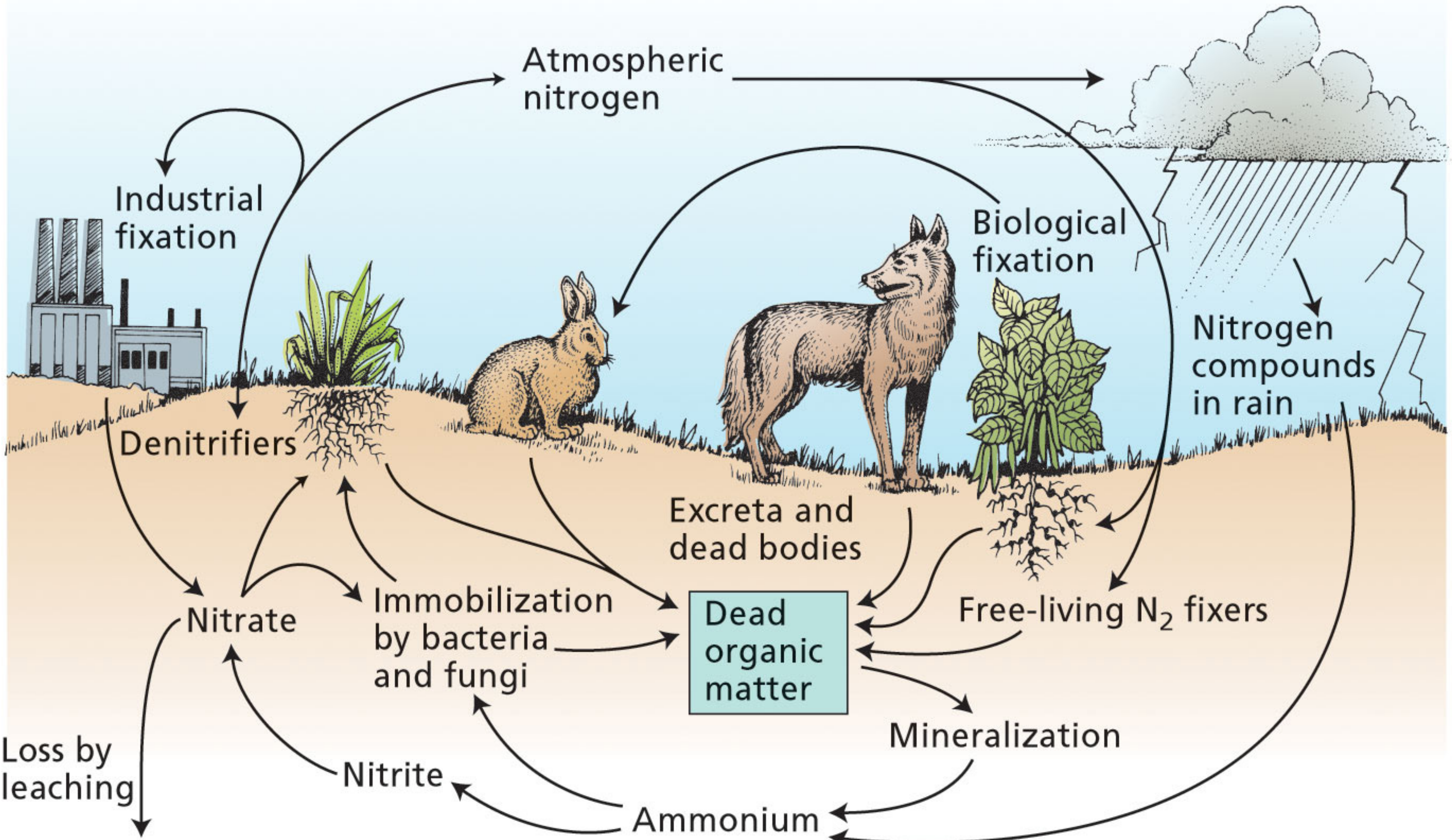
AGRY 515 2012

- Major Strategies for N Fixation
- Inoculation Process
- Biochemistry of N Fixation
- Ecosystem Level Factors

Fig. 1. The Nitrogen Cycle



PLANT PHYSIOLOGY, Third Edition, Figure 12.1 © 2002 Sinauer Associates, Inc.

Table 1. Major N Cycle Processes

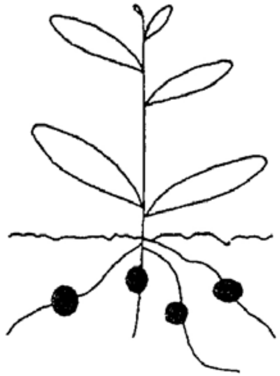
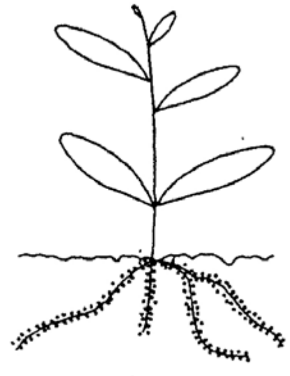
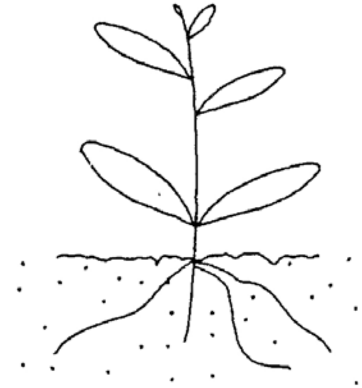
TABLE 12.1
The major processes of the biogeochemical nitrogen cycle

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) = $(5.2 \times 10^{15}$ g $+ 95 \times 10^{15}$ g$)/(80 \times 10^{12}$ g yr$^{-1} + 19 \times 10^{12}$ g yr$^{-1} + 170 \times 10^{12}$ g yr^{-1})] (Schlesinger 1997).

[a] N/C, not calculated.

3 Classes of N fixation systems (Fig. 7.1, Marschner, 1995)

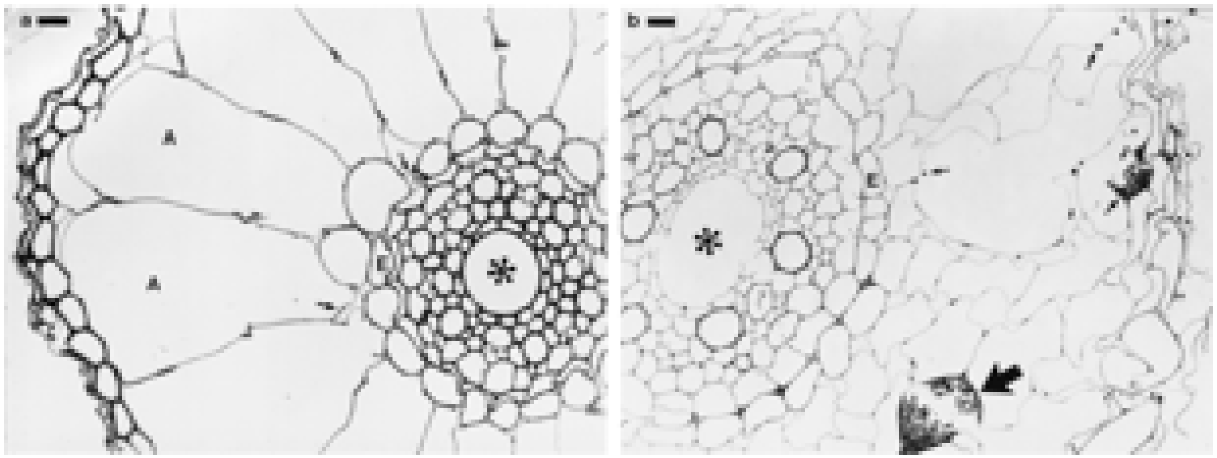
System of N_2 fixation ($N_2 \rightarrow NH_3$)	 Symbiosis	 Association	 Free living	
Micro-organisms involved	e.g. Rhizobium Actinomycetes	e.g. Azospirillium Azobacter	e.g. Azobacter, Klebsiella, Rhodospirillium	
Energy source (organic C)	Sucrose & its metabolites (from host)	Root exudates (from host)	Heterotroph (plant residue)	Autotroph (photo-synthesis)
Estimated N fixed (kg N ha^{-1} yr^{-1})	**50 – 400** (20 – 300 nodulated non-legumes)	**10 - 200**	**1 – 2**	**10 – 80**

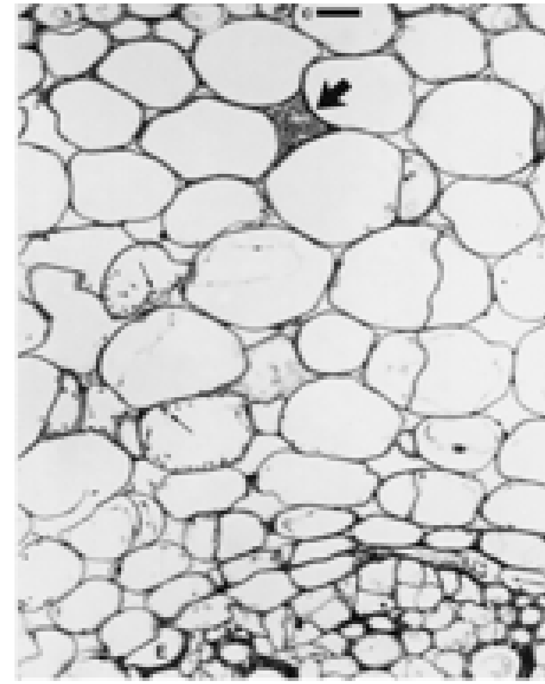
ANF estimates for crop plants (Table 7.9, Marschner, 1995 w/ additions)

Non-legume plant species	**Total N from ANF**
Maize	**Slight to no benefit***
Rice	0 – 35 %
Wheat	0 – 47 %
Sugar cane	Est 1: 2 – 56 % Est 2: 60 – 80 % **Est 3: 0 – 72 %**[†]
Forage grass: *Brachiaria* sp.	Est 1: 30 – 40 % **Est 2: 0 – 26 %**[††]
Forage grass: *Leptochloa* sp.	2 – 41 %
Forage grass: *Pennisetum* sp.	**0 – 42 %**[††]

* Numerous studies; † Boddey et al., 2003; †† Reis et al., 2001

Where are the ANFers?





- Live
  - Mostly (?) outside living tissue (membranes).
  - In rhizosphere or in cell wall between adjacent cells → better access to C than "free livers" but C limited.
- Availability of fixed N may depend on bacteria death.

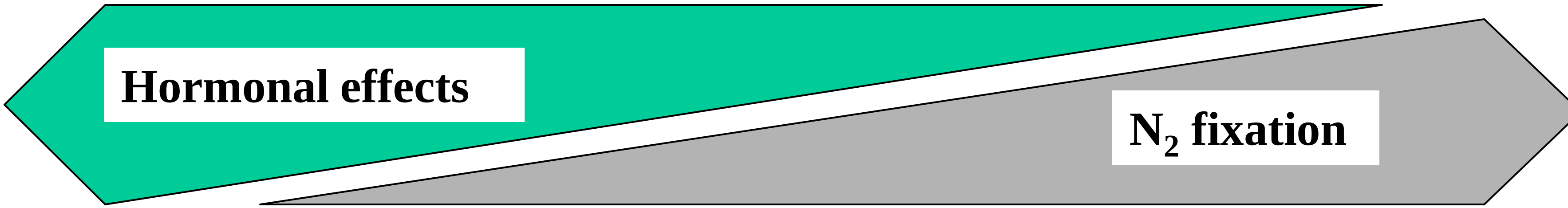
What factors contribute to variation in ANF contributions to plant N status?

- Methodological / experimental protocols
- Genotype / ecotype
- G x E: 5 to 10 kg N ha-1 for cereals (C_3) in temperate environments; > 200 kg ha^{-1} for sugar cane
  - Host / bacteria specificity
  - Exogenous N: high N reduces N contribution
  - Quality of exudates (sugar cane / malate): type of C cmpds in rhizosphere, role of P, Zn, Fe, other nutrients in N-fixation
  - Temperature and moisture: In sugar cane – high soil temps w/ appropriate moisture create microaerophilic conditions in rhizosphere
  - Endophyte "behavior" – tissue invasion may be required (injury or bio carrier present)

Confounding effects of ANF organisms on host plant performance

- Some yield / productivity improvements are independent of soil N or mineral N improvements.
- Rhizosphere "beneficials"
  - Secrete hormones that enhance root / shoot growth, excretion of compounds
  - Excreted compounds dissolve poorly soluble nutrients (P, Zn, Mn)
  - Uptake of poorly soluble / mobile nutrients improves
  - Suppress pathogens

Where do Miscanthus g., switchgrass and native prairies fit? (Marschner, 1995)



Plants	C3 grasses, other non-legumes	C4 grasses, sugarcane
Plant-microbe interaction	Low specificity, rhizosphere associations	High specificity, endophytic properties
Climate	Temperate	Tropical, subtropical
Soils	Low to high nitrogen	Low nitrogen, high moisture

Table 2. Example organisms

TABLE 12.2
Examples of organisms that can carry out nitrogen fixation

Symbiotic nitrogen fixation	
Host plant	**N-fixing symbionts**
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*

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*

Table 3. Example host plants and symbionts (See Table 16.1 of Marschner 2012)

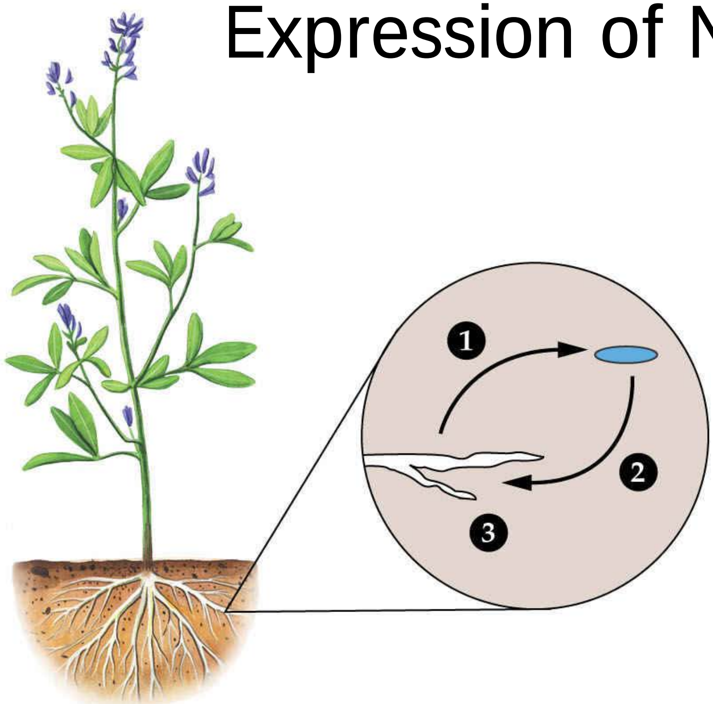
TABLE 12.3
Associations between host plants and rhizobia

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)

Table 4. Cross Inoculation Groups & Rhizobium-Legume Assoc. (See Table 16.1 of Marschner 2012)

Cross Inoc. Groups	**Rhizobium Species**	**Host Genera**	**Legumes Included**
Alfalfa Grp	R. meliloti	Medicago, Melilotus, Trigongella	Alfalfa, Sweet clover, Fenugreek
Clover Grp	R. trifolii	Trifolium	Clovers
Pea Grp	R. leguminosarium	Pisum, Vicia, Lathyrus, Lens	Pea, Vetch, Sweetpea, Lentil
Bean Grp	R. phaseoli	Phaseolus	Beans
Lupine Grp	B. lupini	Lupinus, Ornithopus	Lupines, Serradella
Soybean Grp	B. japonicum	Glycine	Soybean
Cowpea Grp	B. japonicum spp. “cowpea”	Vigna, Lespedeza, Crotalaria, Pueraria, Arachis, Phaseolus	Cowpea, Lespedeza, Kudzu, Peanut, Lima bean

Fig. 2. Overview of Events That Lead to Formation of Legume-Rhizobium Symbiosis, Including Expression of Nodulin Genes in Nodule Apex



1 Plant root releases elicitors of *Nod* gene expression.

2 Bacterium releases Nod factor.

3 Plant root demonstrates ion fluxes, expresses nodulin proteins, is infected, and undergoes nodule morphogenesis.

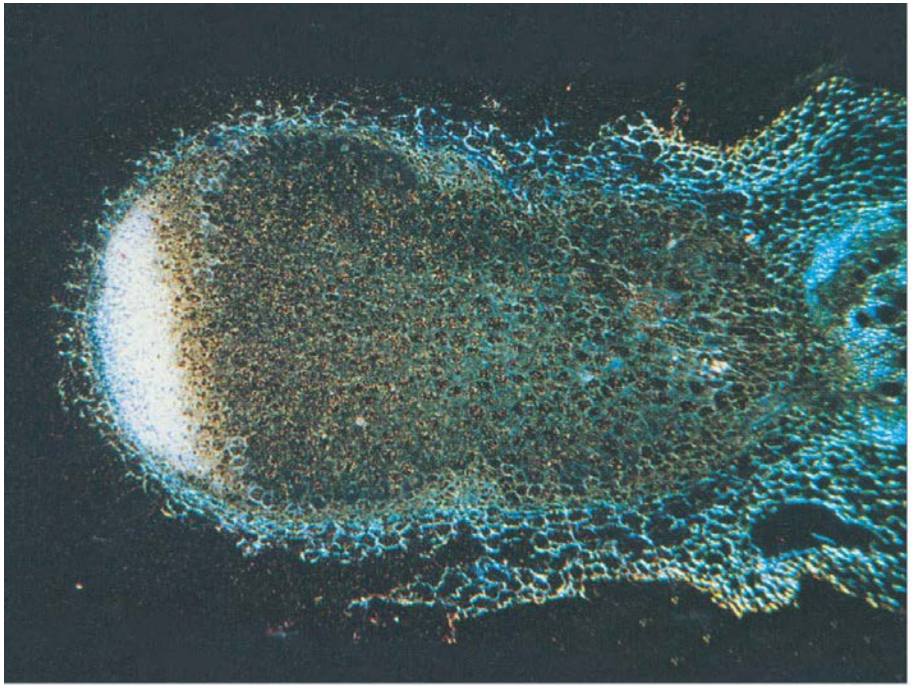


Fig. 3. The Process of Infection and
Early Nodule Formation in Roots of a Leguminous Plant
as it Interacts With Rhizobium. (Similar to Fig. 7.4 in Marschner, 1995)

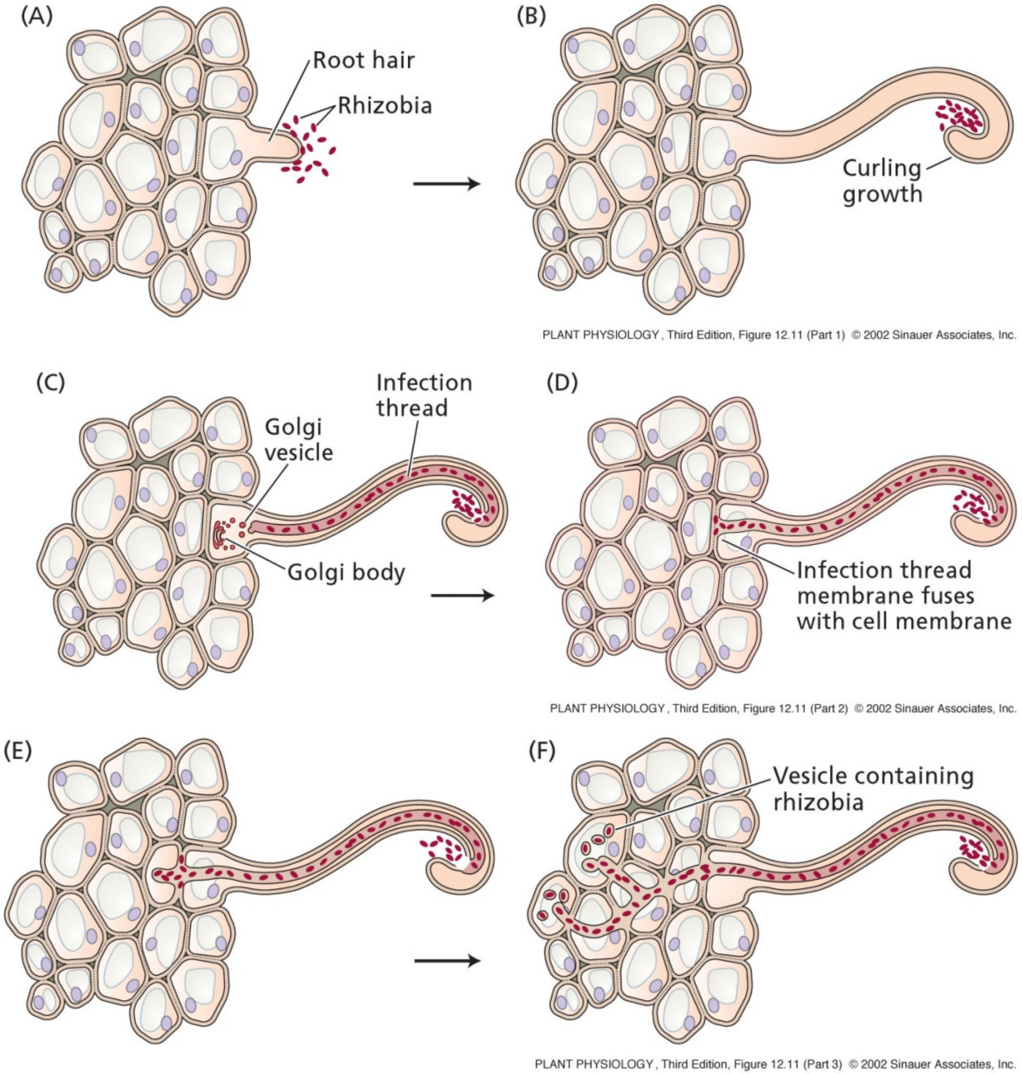


Fig. 3a. Another schematic of the nodulation process

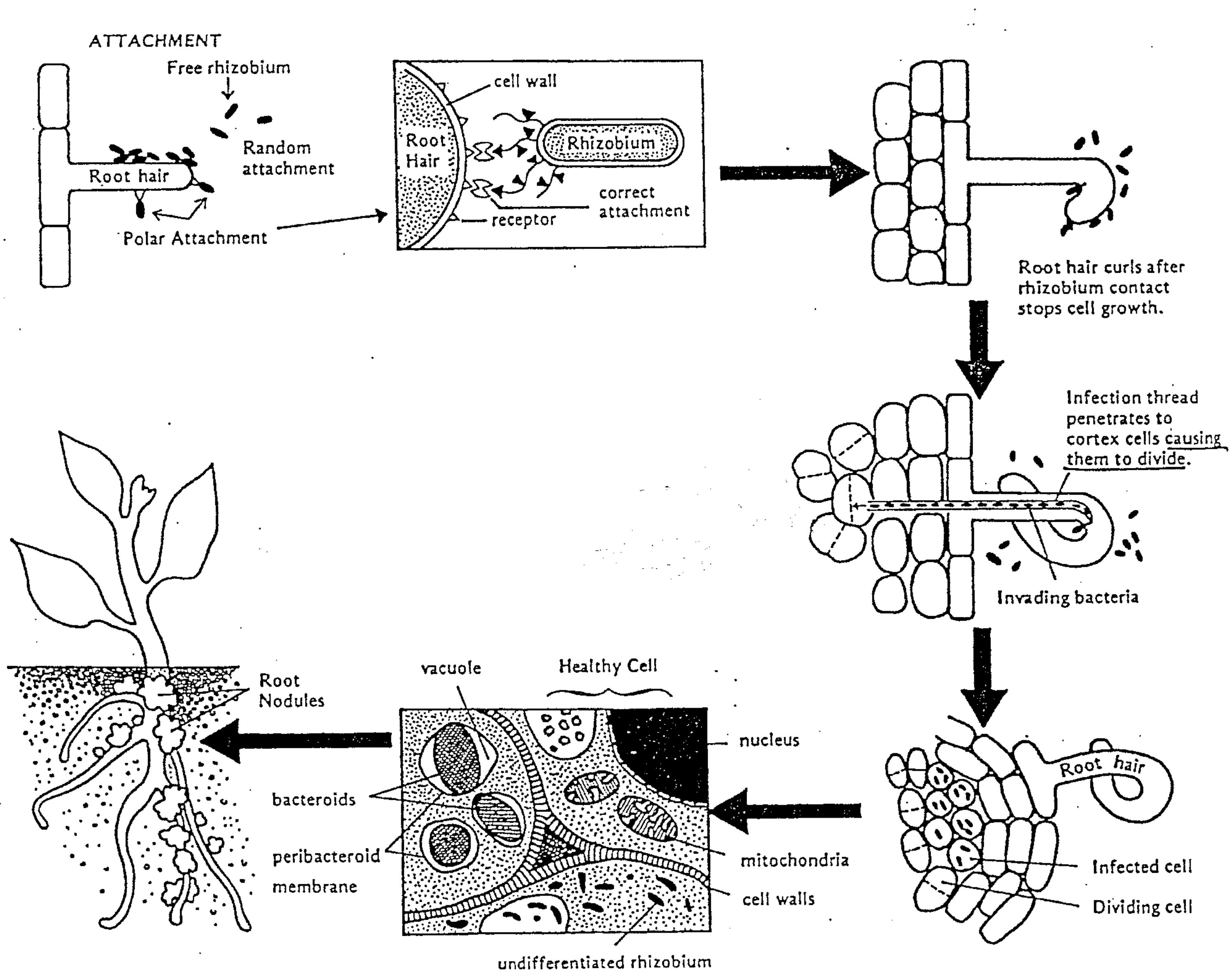


Figure 10.6. Stages in the infection of legume roots by rhizobia. (From Ahmadjian and Paracer, 1986).

Fig. 4. Release of Bacteria From a Walled Infection Thread into a Target Cortical Cell. The Cortical Cell's Plasmalemma Serves to Envelope the Bacteria, Which Begin to Divide Forming Leghemoglobin-fill Bacteriods

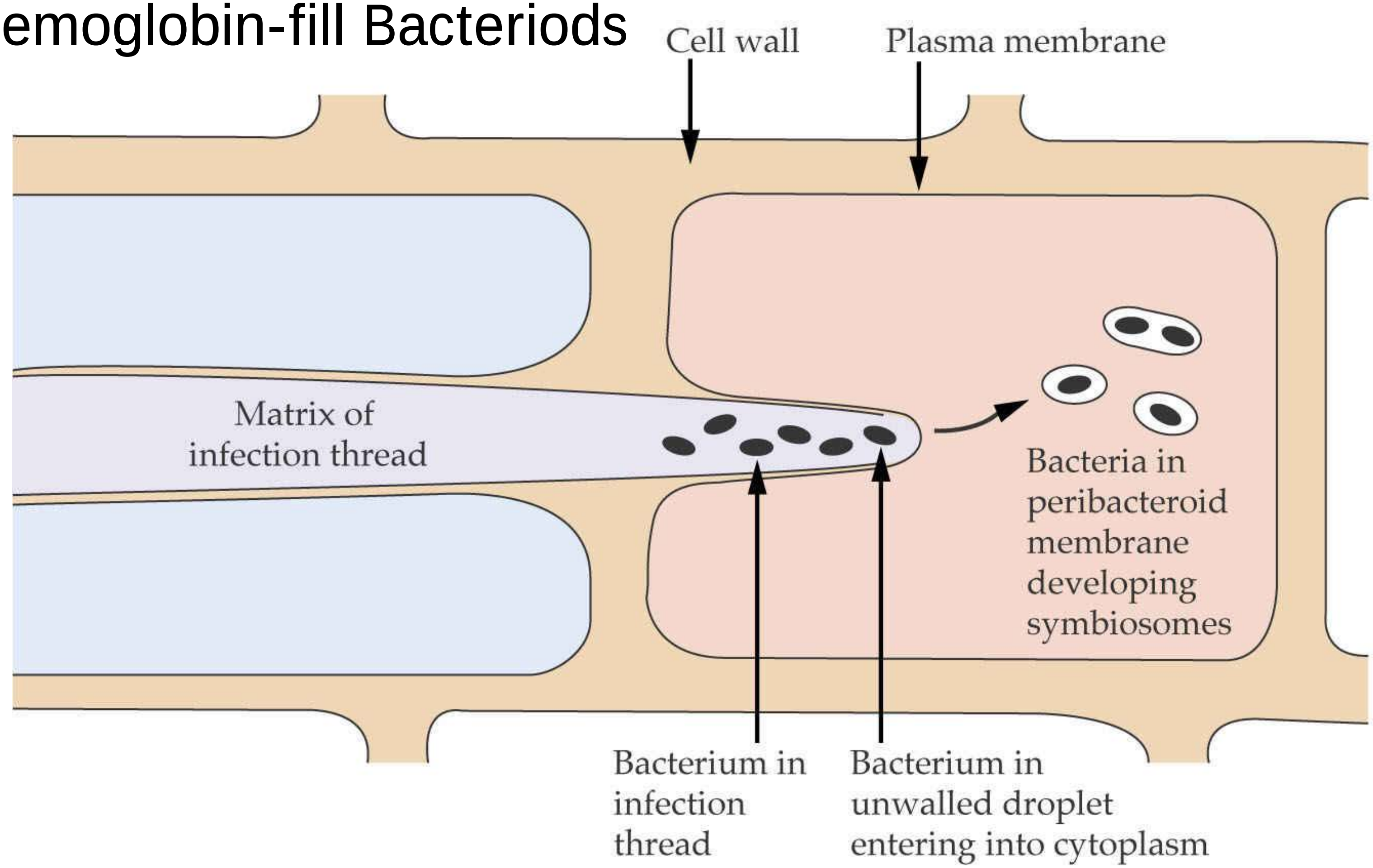
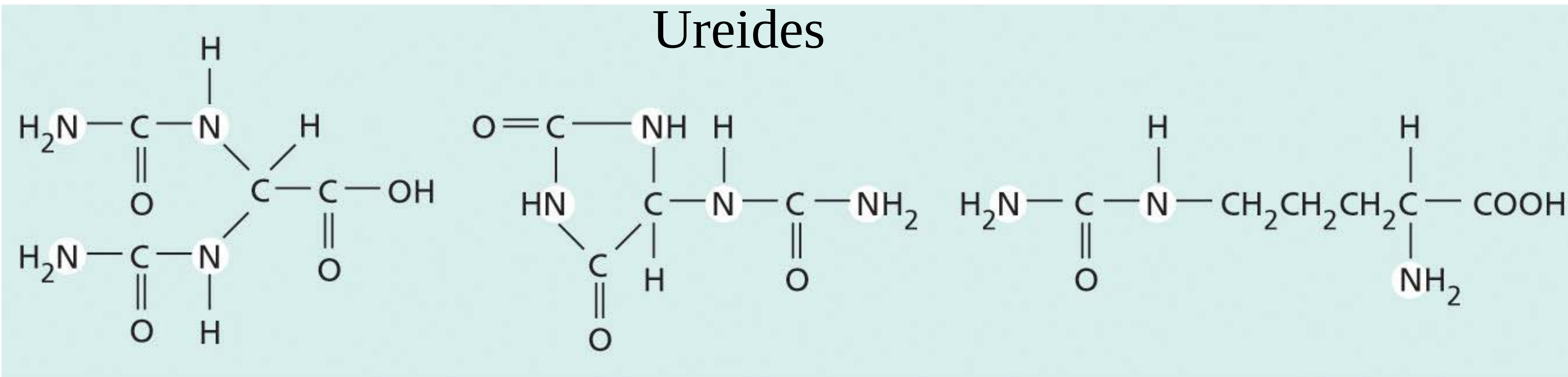


Fig. 5. Nodule morphology of soybean (left) and pea (right)

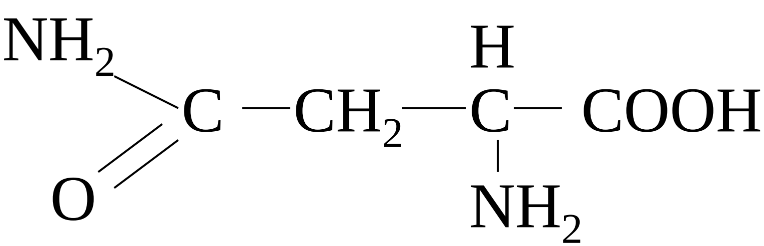
Table 5.Comparisons of Indeterminate and Determinate Nodules

Parameter	**Indeterminate**	**Determinate**
Nodule initiation	Inner cortex	Outer cortex
Cell infection	Infection threads	Infection threads & cell division
Meristem	Persistent (months)	Nonpersistent (days)
Bacteriod size	Larger than bacteria	Variable, although usually not too much larger than bacteria
Peribacteriod membrane unit	1 bacteriod per unit	Several bacteriods per uint
Poly-B-hydroxy-butyrate	None in bacteriods	Large deposits in bacteriods
N2-fixation products transported	Amides usually	Ureides, usually
Infected cells	Vacuolate	Nonvacuolate
Origin	Temperate	Tropical to subtropical
Genera	Medicago, Trifolium, Pisum	Glycine, Phaseolus, Vigna

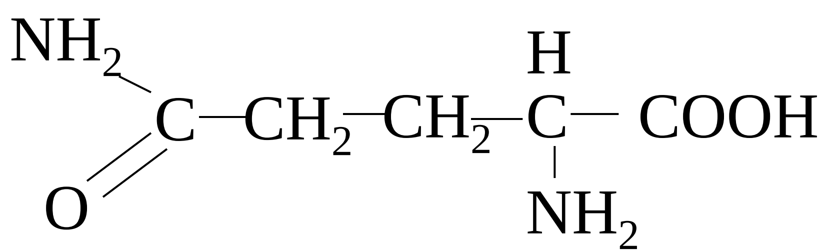
Fig. 6. Chemical structure of three ureides (tend to have 1:1 ratio of C to N) and one amide (2:1 C:N ratio)



Allantoic acid **Allantoin** **Citrulline**



Amides: Asparagine



Amides: Glutamine

Fig. 7. Nitrogen Composition of Xylem Sap From Several Plants. Note the Presence of Ureides in Xylem Sap of Legumes With Determinant Nodules.

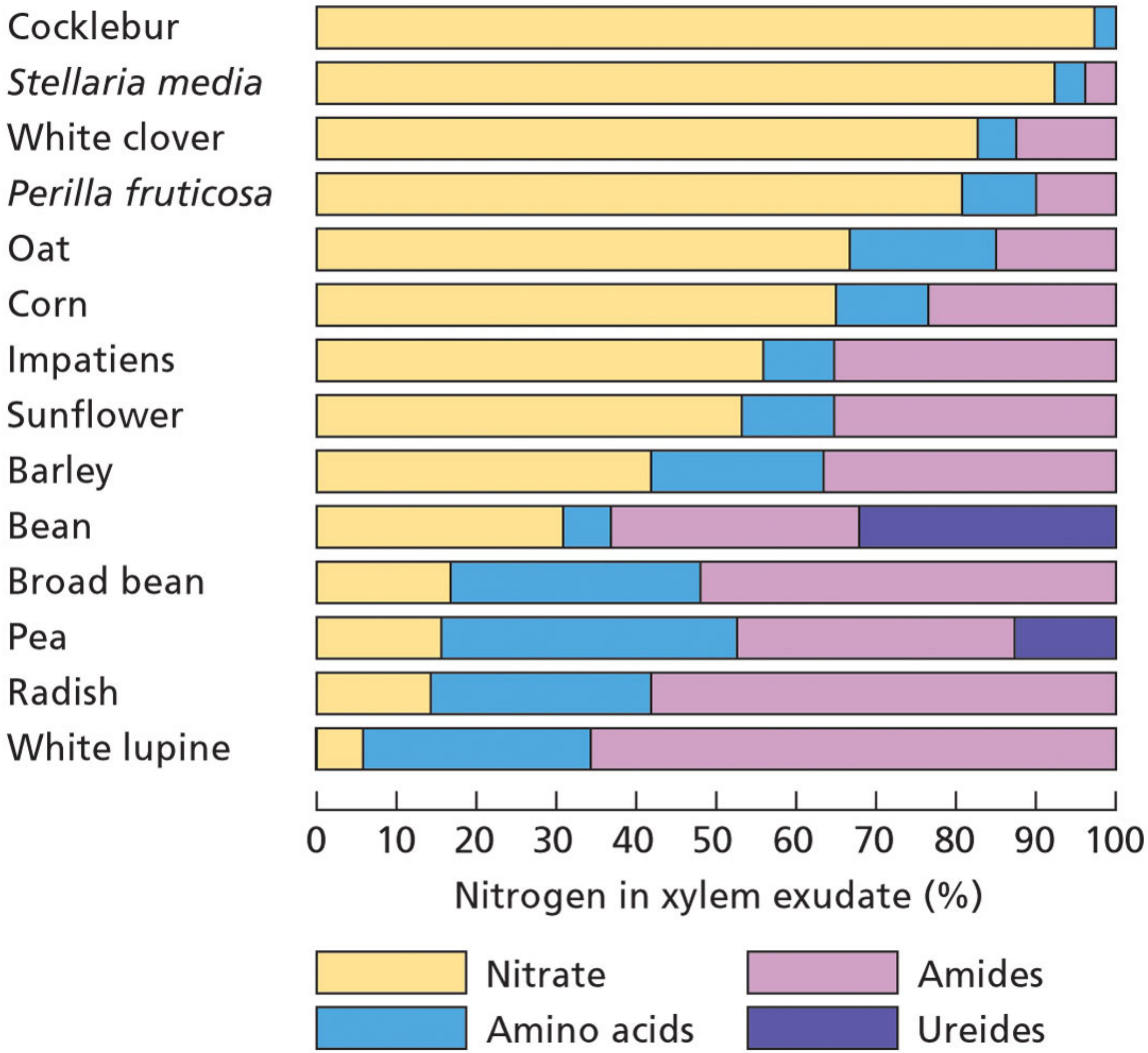


Fig. 8. Nodules possess mechanisms to maintain a relatively O_2 depleted environment (barrier; leghemoglobin), while producing the ATP Needed for N_2 fixation (cytochrome oxidase)

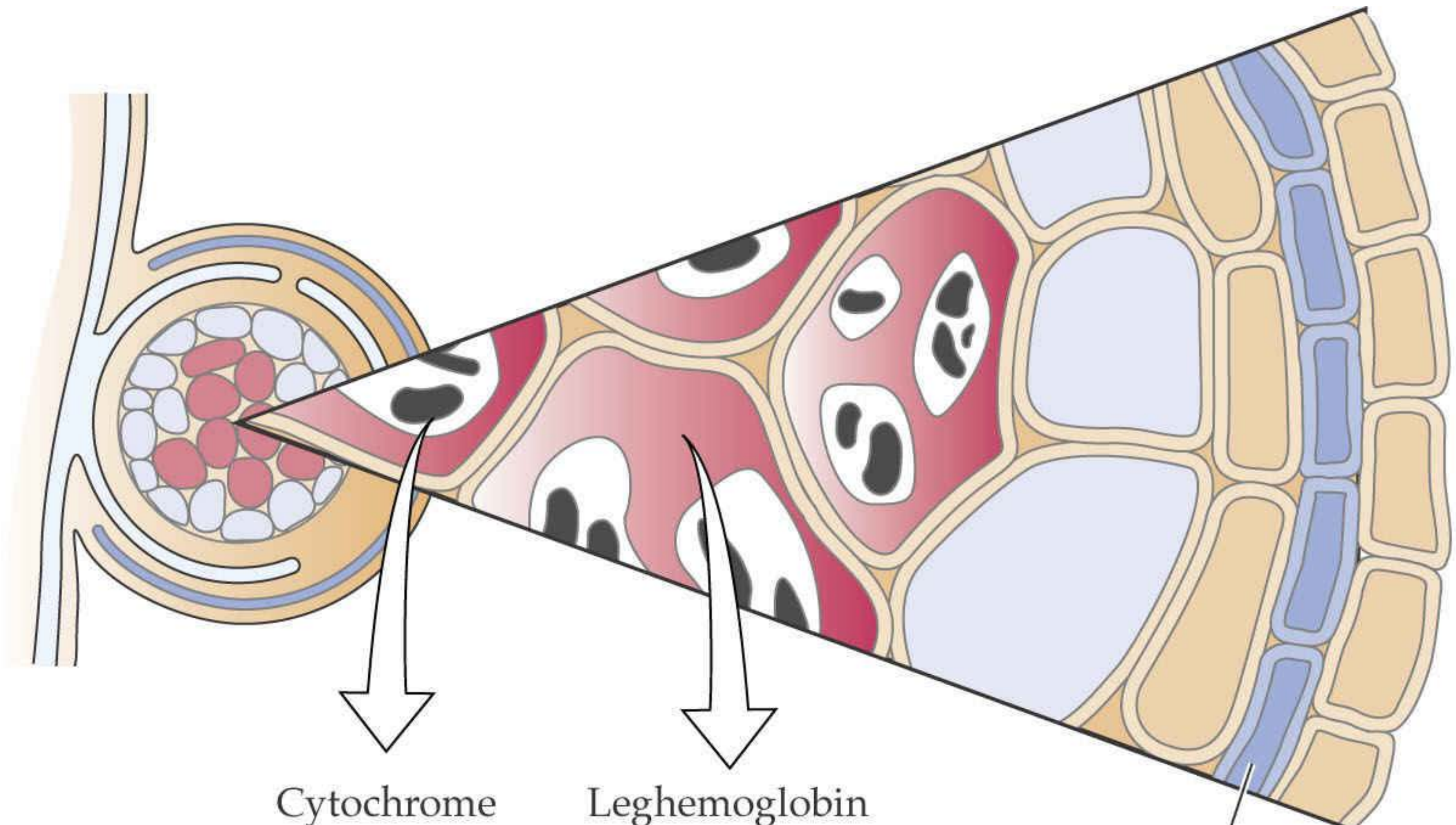


Fig. 9. (Fig. 7.6 from Marschner, 1995)

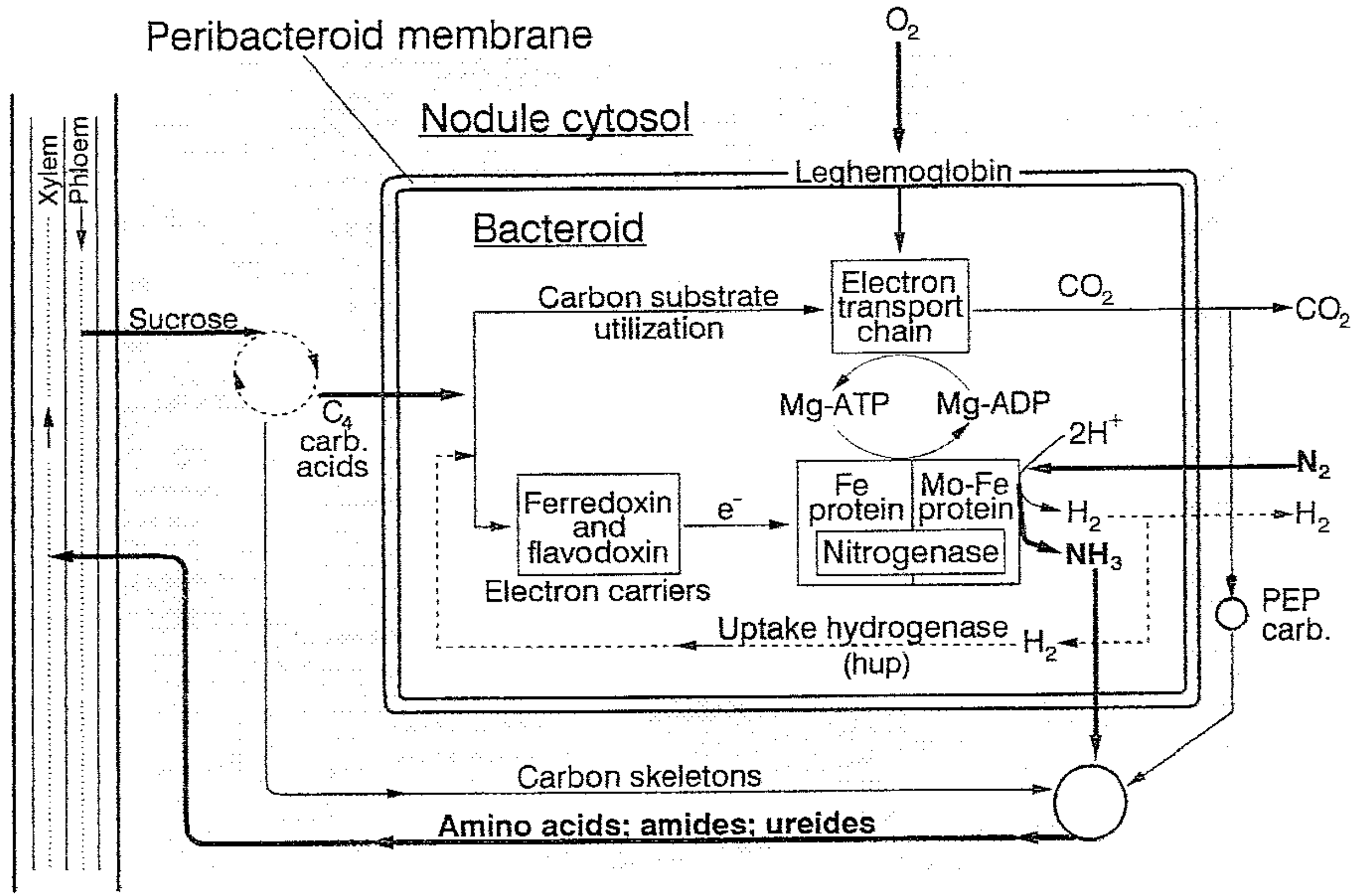


Fig. 7.6 Model of relationships between nitrogenase and related reactions in bacteroids and the cytosol of the host in legume nodules.

Fig. 10. Schematic showing the structural features of nitrogenase and how these function to reduce dinitrogen to ammonia

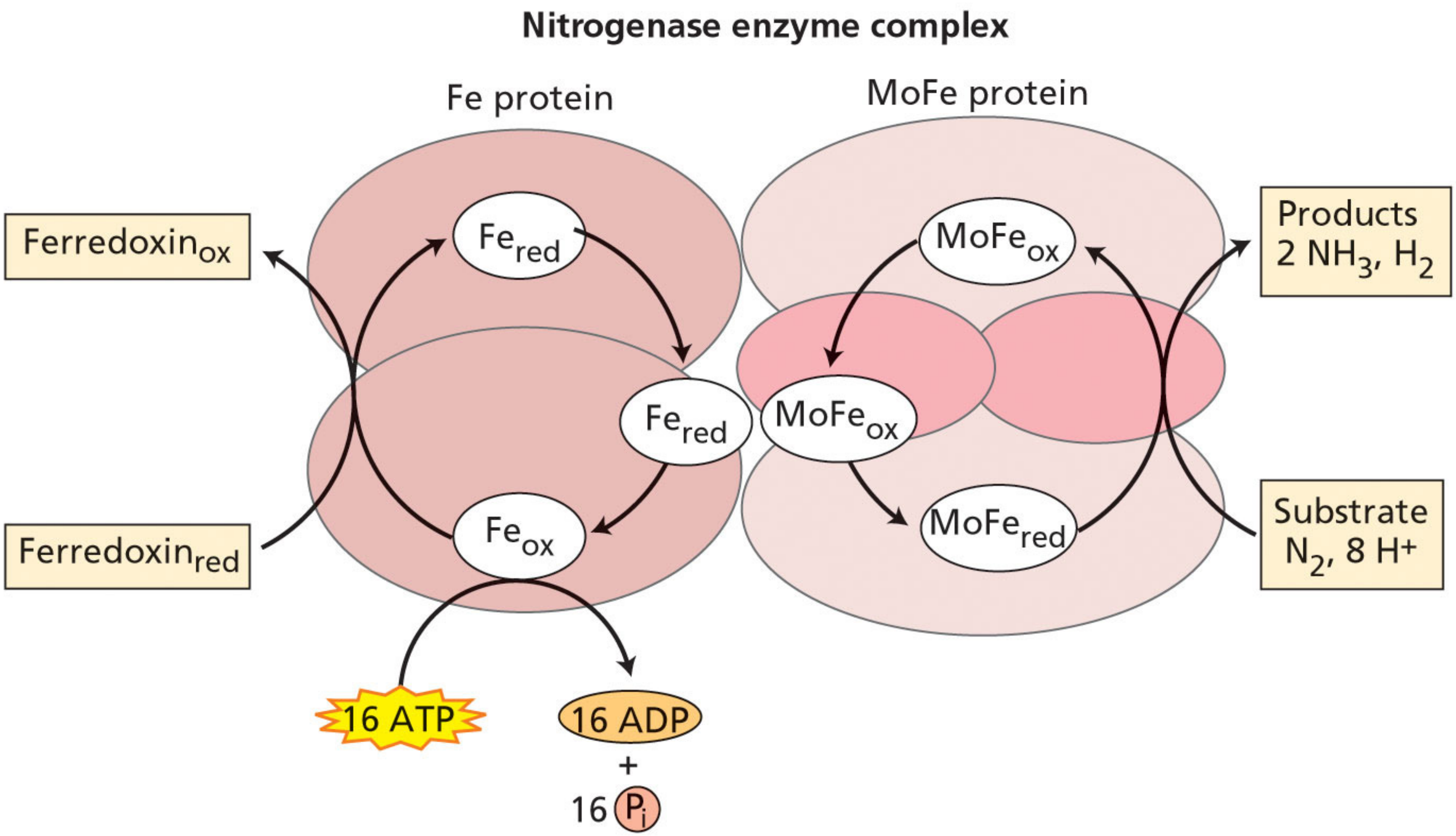


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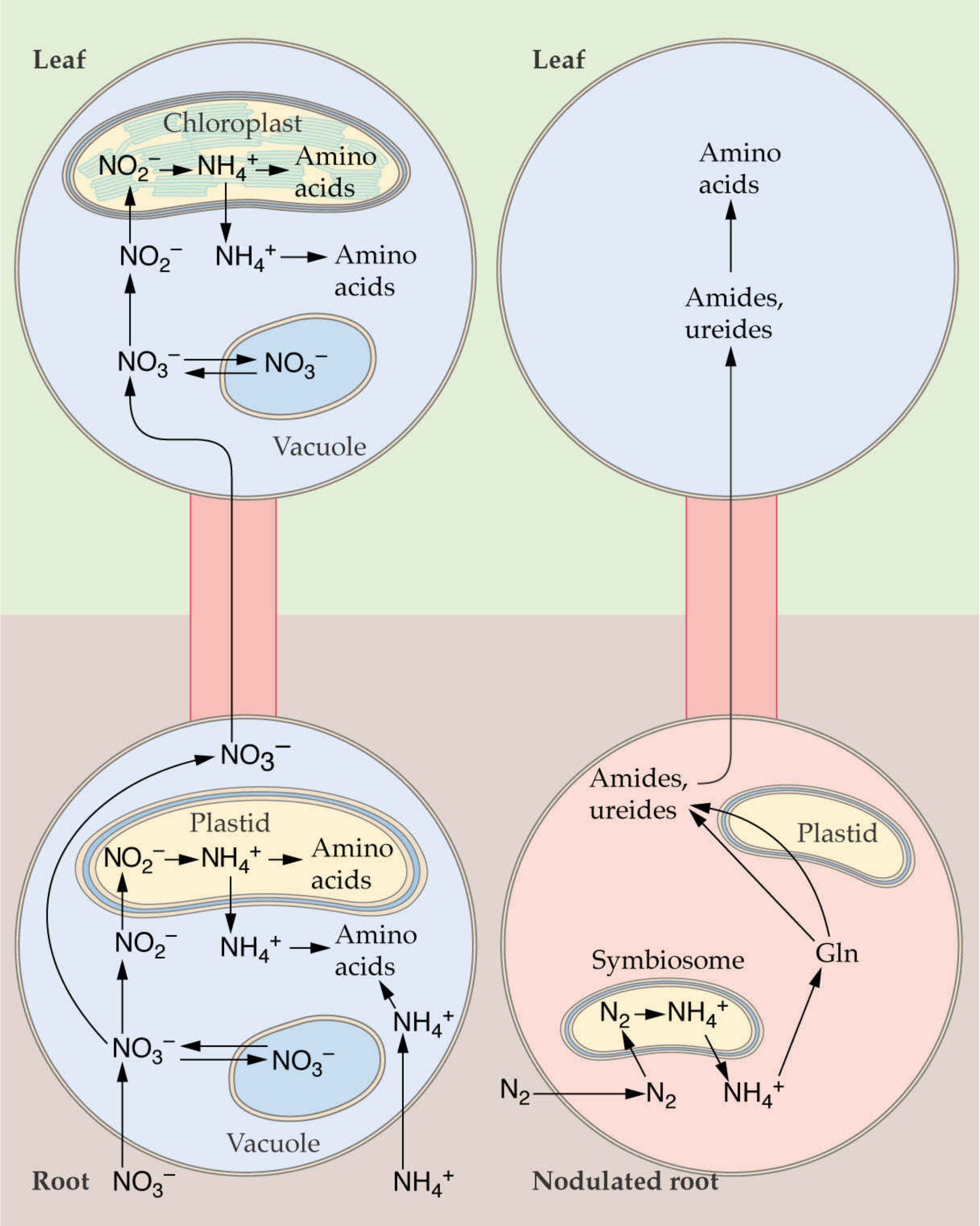
Table. 6. Experiments can use the ability of MoFe protein to reduce many substrates as tools to estimate N-fixation capability.

TABLE 12.4
Reactions catalyzed by nitrogenase

$N_2 \rightarrow NH_3$	Molecular nitrogen fixation
$N_2O \rightarrow N_2 + H_2O$	Nitrous oxide reduction
$N_3^- \rightarrow N_2 + NH_3$	Azide reduction
$C_2H_2 \rightarrow C_2H_4$	Acetylene reduction
$2\ H^+ \rightarrow H_2$	H_2 production
$ATP \rightarrow ADP + P_i$	ATP hydrolytic activity

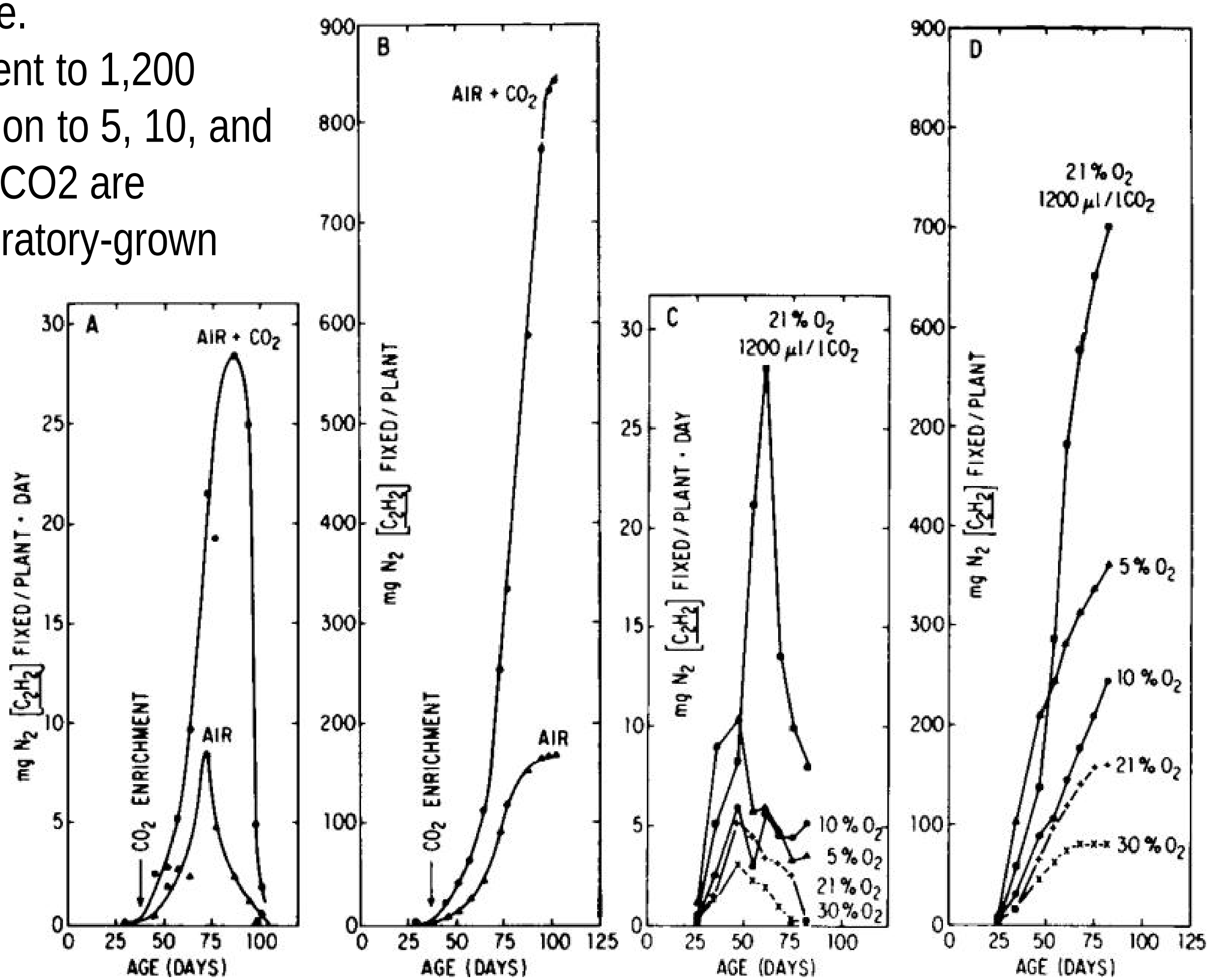
Source: After Burris 1976.

Fig. 11. Comparison of N assimilation in roots and leaves of nitrate- or ammonium-fed plants (left) or nitrogen-fixing legume (right)



Photosynthate as a major limiting factor for N2 fixation based on effect of altered canopy CO2/O2 ratio on N2 fixation.

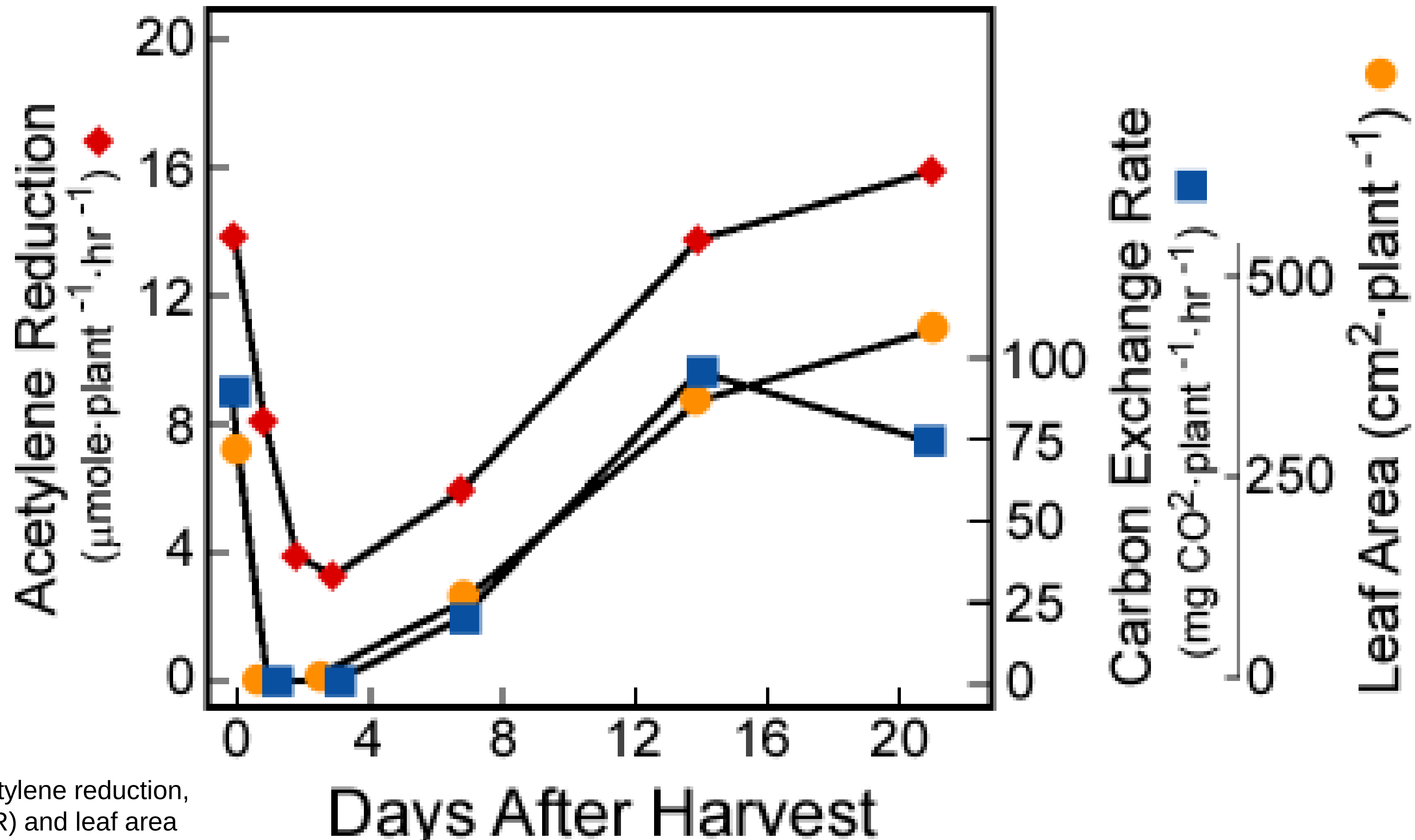
1. In A and B, CO2 enrichment to 800 to 1,200 µliters/liter of field-grown soybeans occurred in open-top enclosures from 40 days of age to senescence.
2. In C and D, CO2 enrichment to 1,200 µliters/liter and O2 alteration to 5, 10, and 30% at 300 µliters/liter of CO2 are compared with air for laboratory-grown soybeans.



10.2134/agronmonogr22.c10
p. 365-422
Agronomy Monograph 22. Nitrogen in Agricultural Soil.. Biological Nitrogen Fixation
U. D. Havelka, M. G. Boyle and R. W. F. Hardy

Response of N_2 Fixation to Assimilate Supply

1. Decreasing assimilate supply (shading, girdling, harvesting shoots) decreases nitrogenase activity and nodule respiration rate.
2. The drop in N_2 fixation is due mostly to reduced activity of nodules. Recovery of N_2 fixation closely follows capacity for assimilate production



Nitrogenase activity (acetylene reduction, AR) photosynthesis (CER) and leaf area (LA) of alfalfa during recovery from harvest. Fishbeck and Phillips, 1982.

Diurnal and Seasonal Variation in Dinitrogen Fixation (Acetylene Reduction) Rates by Field-Grown Soybeans. R. F. Denison and T. R. Sinclair. Agronomy Journal 1985 77: 5: 679-684

1. Altering the assimilate supply via de-topping, shading, de-leafing reduces N2 fixation.
2. These responses persist for several days
3. Large (2.5-fold) plant-to-plant variation in N2 fixation among these individual soybean plants.

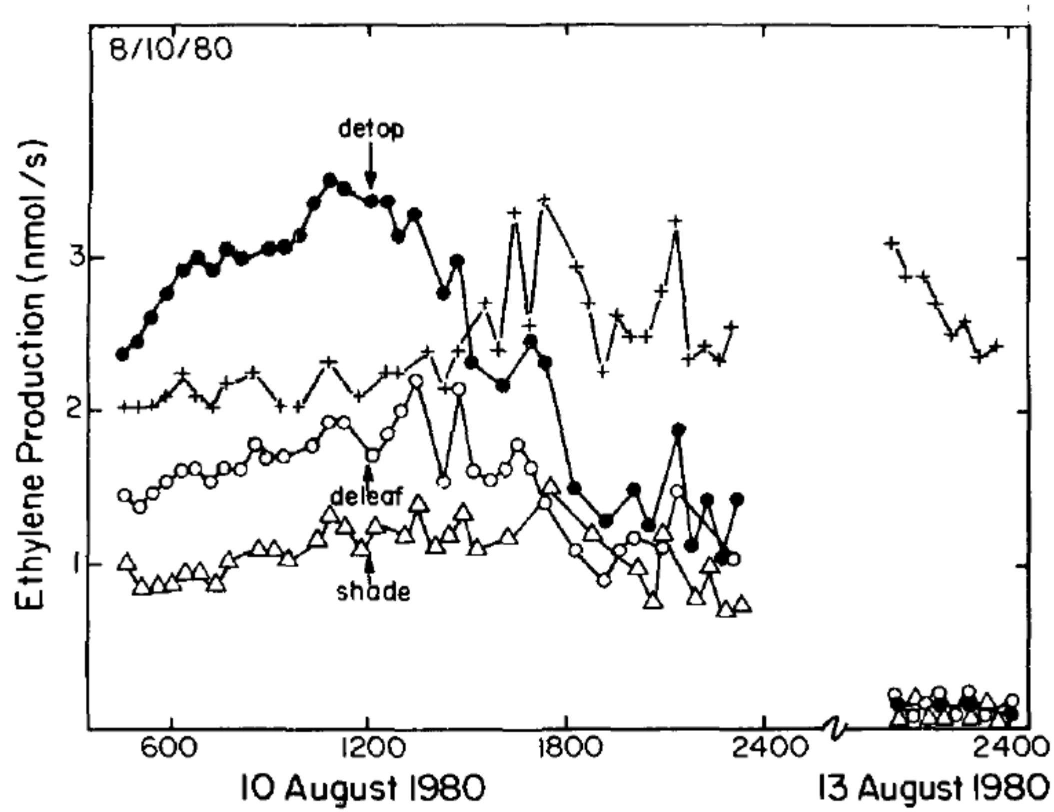
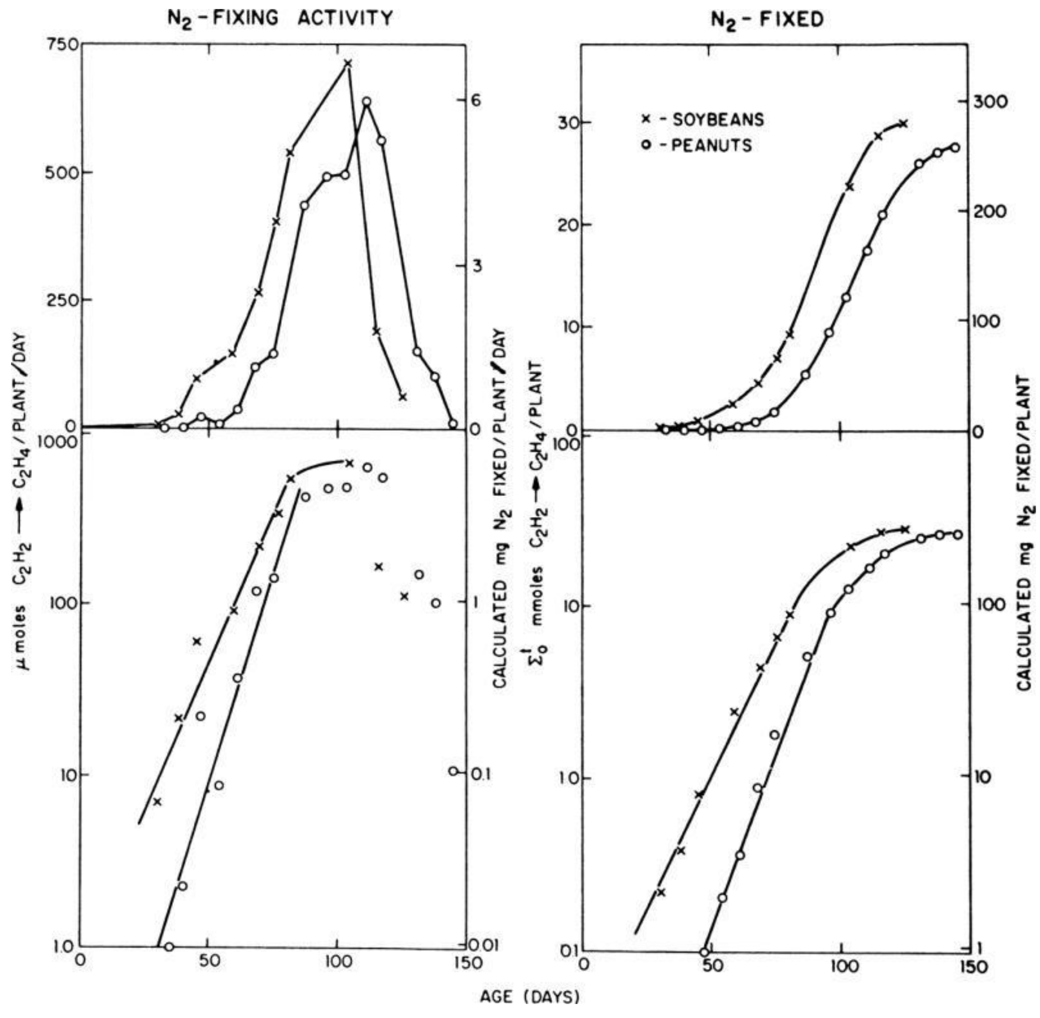


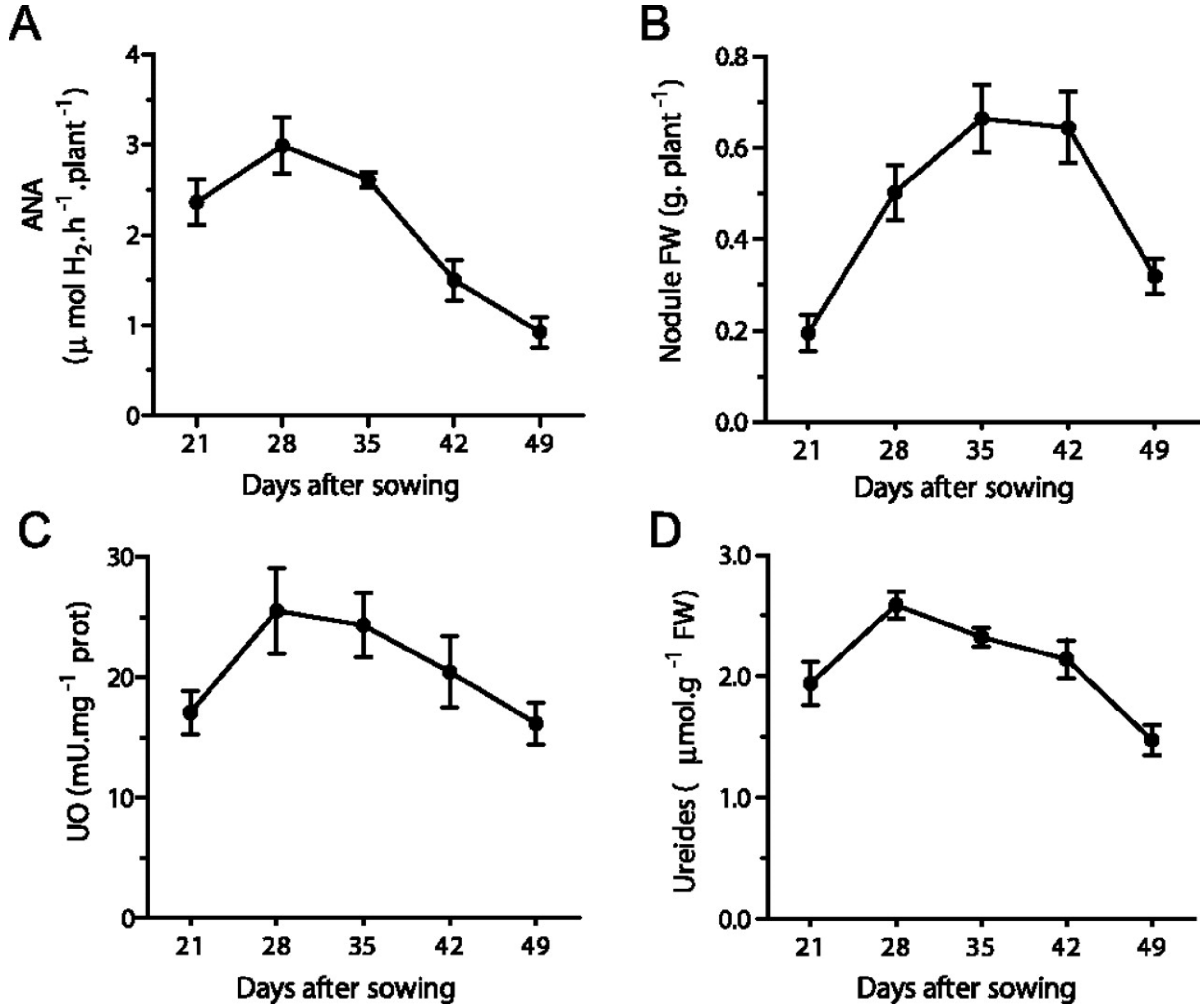
Fig. 4. Effects of shading and organ removal on acetylene reduction rate of Chippewa 64 plants in 1980. One of the three plants for each treatment is shown. At the time indicated by the arrows, the plant indicated by triangles was subjected to > 90% shade, while open and closed circles represent plants that were defoliated and detopped, respectively. Plus signs represent an untreated control plant.

Time courses for N2 fixation by soybeans measured with the C2H2-C2H4 assay and reported as N2 fixed per plant per day and total N2 fixed per plant on regular and semilogarithmic plots (Hardy et al., 1971).

1. N2 fixation of both species is very low for 50 days
2. Maximum N2 fixation rate coincides with highest LAI
3. N2 fixations declines rapidly as leaves senesce after Day 100.



Developmental effects in N2 fixation map with changes in nodule mass, uricase (UO) activity, and ureide contents in bean nodules

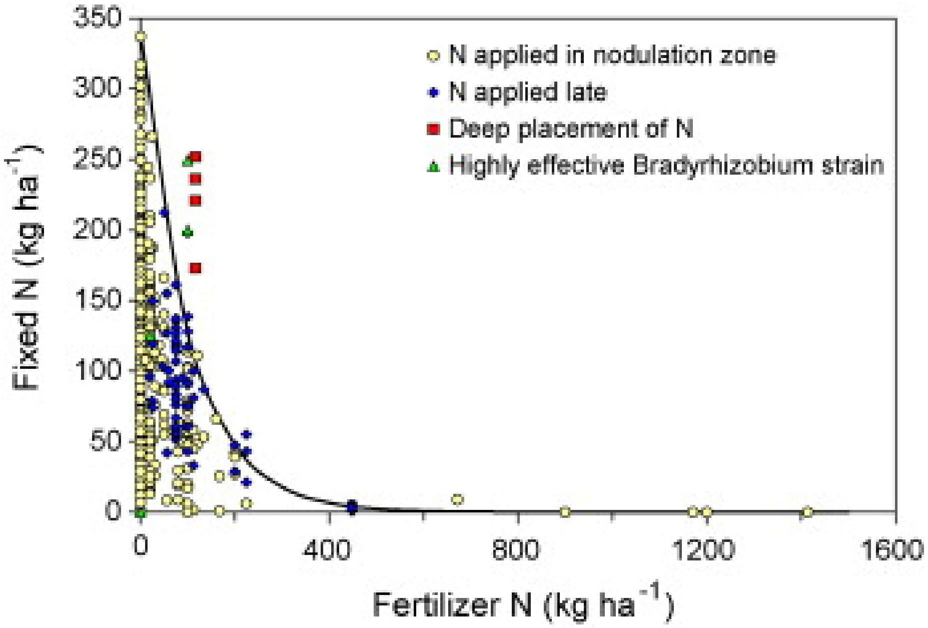


Díaz-Leal J L et al. J. Exp. Bot. 2012;jxb.ers090

JOURNAL OF Experimental Botany

Boundary Analysis of N Fertilizer Impact on N2 Fixation of Soybean

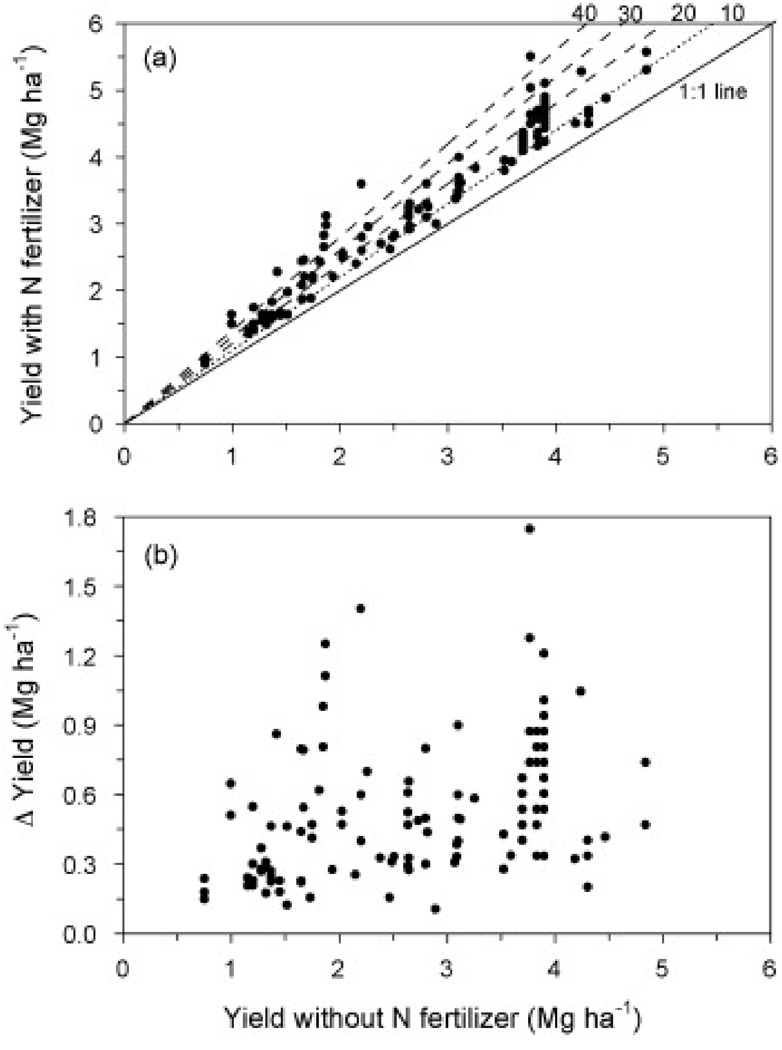
1. Tremendous variation in N2 fixation rate under 0 N conditions
2. Late-season N application similar to conventional timing
3. Deep placement of N outside boundary suggesting that it is less suppressive; however, how much of this N did plants take up?
4. Genetically improved Rhizobium similar to regular strains.



Nitrogen uptake, fixation and response to fertilizer N in soybeans: A review
F. Salvagiotti, K.G. Cassman, J.E. Specht, D.T. Walters, A. Weiss, A. Dobermann. Field Crops Research
Volume 108, Issue 1, 11 July 2008, Pages 1–13

Yield Responses of Soybean in Response to N Fertilization

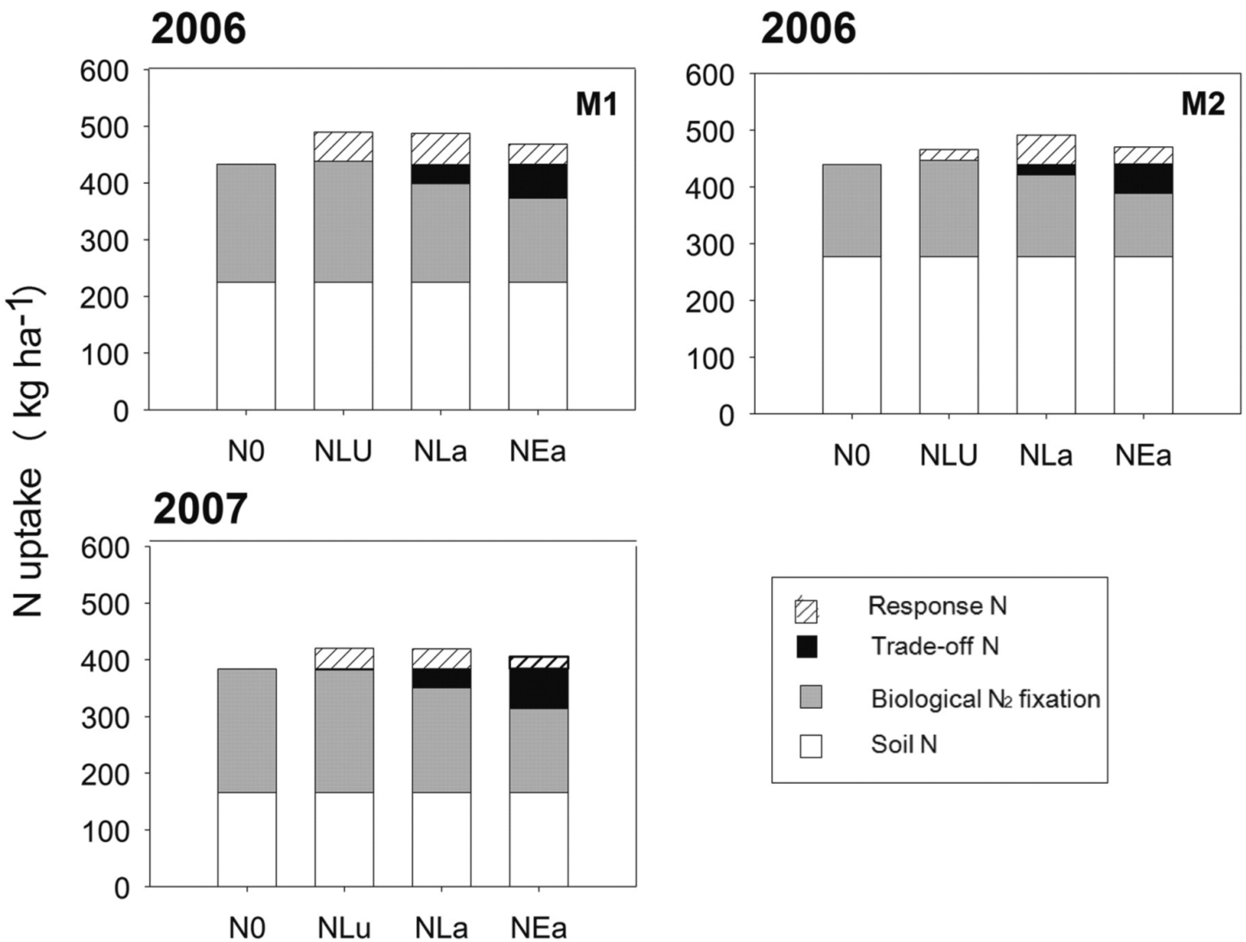
1. N fertilization increased soybean yields, albeit slightly in most cases. All data in “a” are above the 1:1 line
2. Most yield increases were in the 10 to 20% range
3. Yield response to N was largely independent of yield without N (“b”) suggesting that factors other than N were constraining yield under low yield conditions.



Nitrogen uptake, fixation and response to fertilizer N in soybeans: A review. F. Salvagiotti, K.G. Cassman, J.E. Specht, D.T. Walters, A. Weiss, A. Dobermann. Field Crops Research
Volume 108, Issue 1, 11 July 2008, Pages 1–13

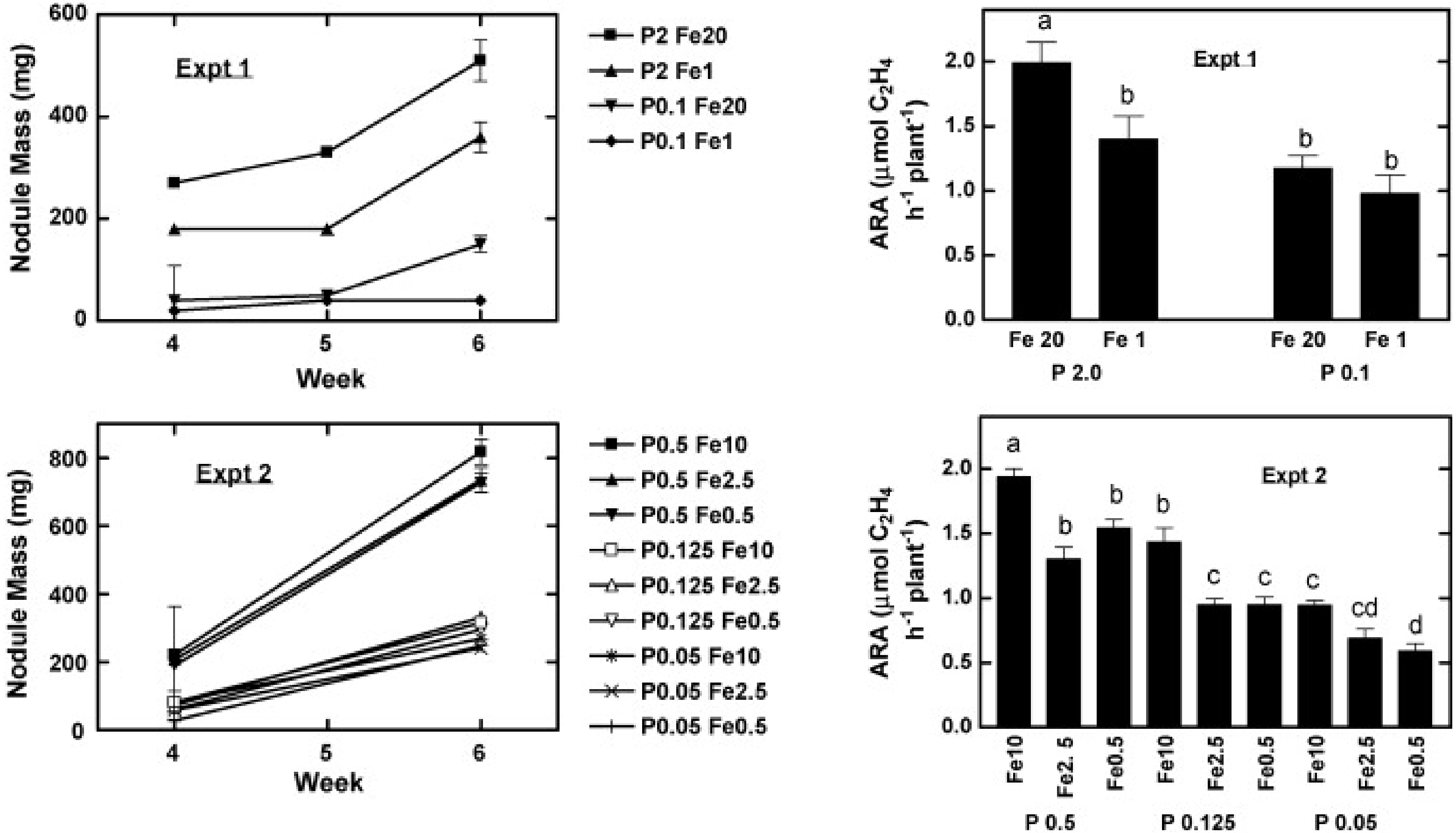
Contribution of Soil N, N2 Fixation, and N fertilizer to Yield of Soybean Under High-yield Conditions

1. Soil N contributes about 50% of plant N uptake in all years.
2. N2 fixation contributes 40 to 50% of plant N.
3. N applied early (NEa) gives reduces N2 fixation the greatest (tradeoff N)
4. Additional N uptake due to N fertilization is small irrespective of mgmt



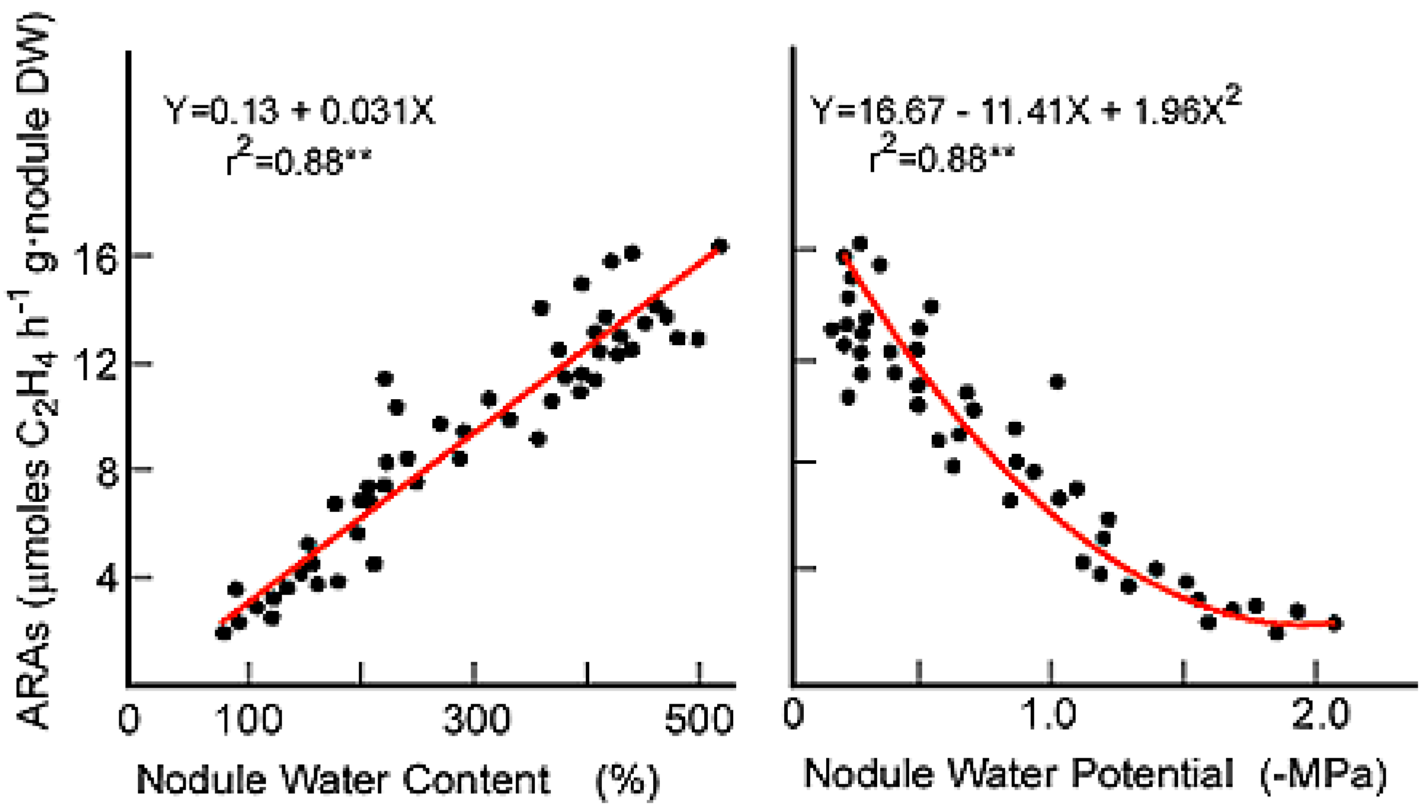
Growth and Nitrogen Fixation in High-Yielding Soybean: Impact of Nitrogen Fertilization. Fernando Salvagiotti, James E. Specht , Kenneth G. Cassman, Daniel T. Walters, Albert Weiss and Achim Dobermann. 2009. Agron. J. Vol. 101 p. 958-970

Adequate Phosphorus and Iron Nutrition is Essential for Nodule Development and High Rates of N2 Fixation.



Interactive influence of phosphorus and iron on nitrogen fixation by soybean. Vladimir Rotaru, Thomas R. Sinclair. Environmental and Experimental Botany Volume 66, Issue 1, April 2009, Pages 94–99

Water Stress Reduces N2 Fixation Rates. This Response is Consistent with Reduced Diffusion Rates of Gasses into Nodules, and Reduced Rates of Photosynthesis and Assimilate Supply.



Djekuon and Planchon, 1991