Biological Nitrogen Fixation: Supplying Nitrogen needs of a Sustainable Agriculture

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Ecological Agriculture

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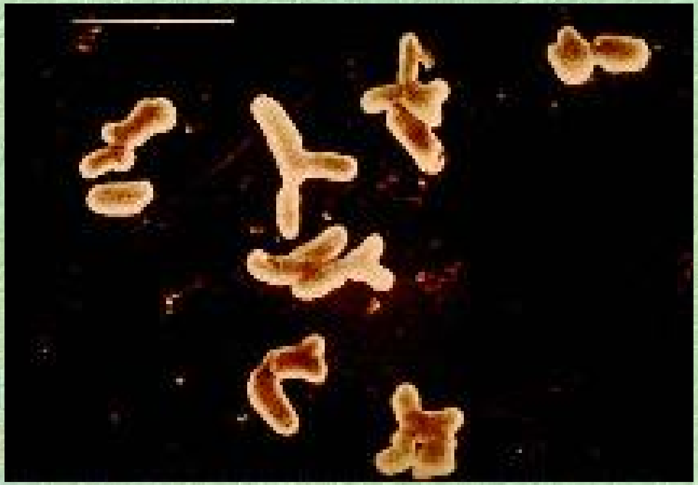
Outline



Nodules
Barak, UW

I. What is BNF?
II. Types of BNF
III. How it works- nitrogenase enzyme
IV. How to measure
V. Ecology and how to increase
VI. Where are the levers to decrease N amplification in ecosystem?
VII. Study questions

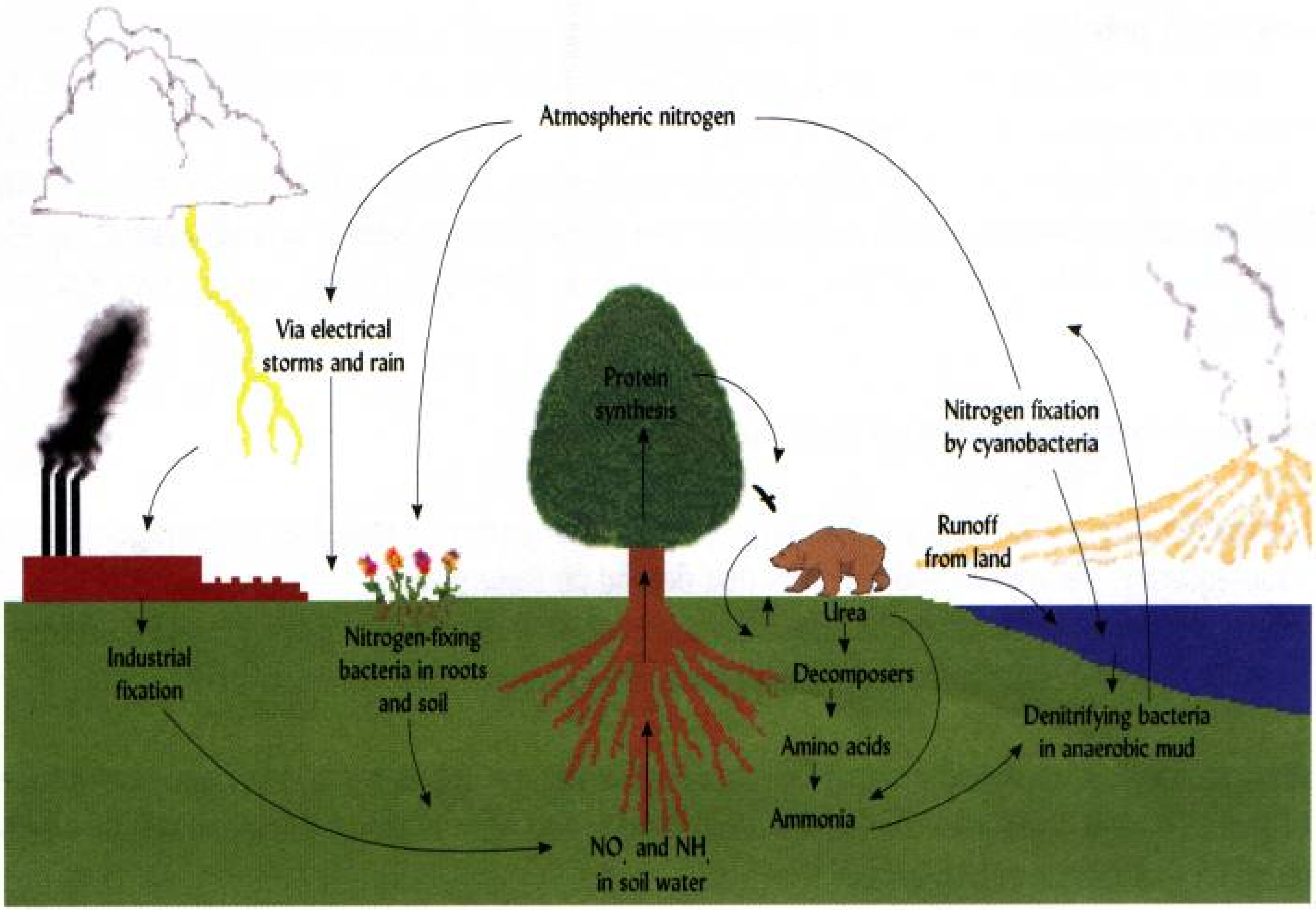


Figure 1-Simplified diagram of the nitrogen cycle. Adapted from *Environmental Science*, Third Edition by Jonathon Turk and Amos Turk, ©1984 by Saunders College Publishing, reproduced by permission of the publisher.

Ecological Society of America. 1997. Human Alteration of the Global Nitrogen Cycle: Causes and Consequences. *Issues in Ecology*, No. 1.

Human-caused NF =
Natural terrestrial BNF (Table 13.3)

BNF from crops	35×10^6	Mg N fixed/yr
Fertilizer industry	77	
Fossil fuel burning	20 (Not in Table 13.3)	
Total human-caused	**142**	

BNF terrestrial ecosystems 139

Severe soil N change (inorganic fertilizer) affects plant diversity

Figure 8-Native grasslands in Minnesota often contain 20 to 30 or more plant species per square meter, as does this plot. This plot is a "control" plot that received no nitrogen, and that retained its original plant diversity.

Ecological Society of America. 1997. Human Alteration of the Global Nitrogen Cycle: Causes and Consequences. *Issues in Ecology*, No. 1.

Figure 9-Nitrogen addition to this plot, located near that shown in Figure 8, led to the loss of almost all native prairie species and to dominance by the weedy European quackgrass. In 1982 this plot looked much like the one shown in Figure 8.

Ecological Society of America. 1997. Human Alteration of the Global Nitrogen Cycle: Causes and Consequences. Issues in Ecology, No. 1.

I. What is BNF?

- **Second most important biochemical reaction of ecosystem after photosynthesis to life on earth**
- Involves the reduction of N from molecular gaseous state N_2 (triple bond) to NH_3 in which form it can be incorporated into an organic (C containing) molecular structure

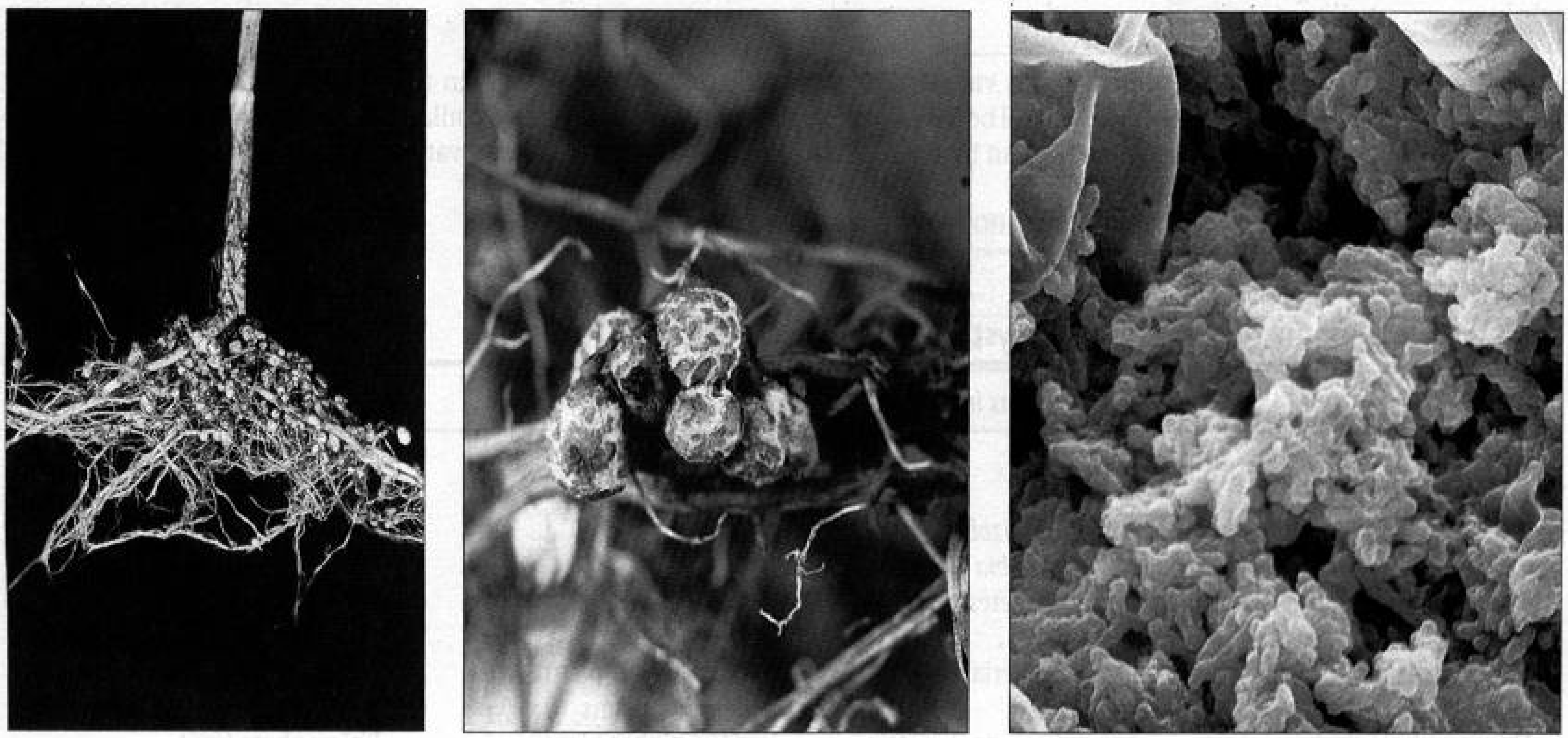
BNF

- Regardless of organism, it uses nitrogenase enzyme for to fix N_2 to ammonia NH_3
- NH_3 + org. acids → amino acids → proteins

- Irony that in an atmosphere of N, all useful N needs to pass through the eye of the needle-- BNF!

II. Types of N fixation (Table 13.4, simplified)

- Symbiotic, obligatory bacteria with legumes (herbacous and trees) (*Rhizobium* and *Bradyrhizobium*)
- Symbiotic, obligatory actinomycetes with non-legumes, e.g. woody shrubs and trees (Alders and *Frankia*)
- Symbiotic, associative without nodules: cyanobacteria on surface plant leaf (*Nostoc* or *Anabaena*)
- Symbiotic, associative cyanobacteria with fungus-lichen
- Symbiotic, associative N-fixation (*Azospirillium*, *Azotobacter*) in rhizosphere of grasses
- Non-symbiotic free-living- *Azotobacter*, *Beijerinkia* in soil, water



(a) (b) (c)

FIGURE 13.14 Photos illustrating soybean nodules. In (*a*) the nodules are seen on the roots of the soybean plant, and a closeup (*b*) shows a few of the nodules associated with the roots. A scanning electron micrograph (*c*) shows a single plant cell within the nodule stuffed with the bacterium *Bradyrhizobium japonicum*. (Courtesy of W. J. Brill, University of Wisconsin)

Brady, N.C. and R.R. Weil. 1999. *The Nature and Properties of Soils*. 12th ed. Prentice Hall, New Jersey.

Root Knot Nematode *galls* can be mistaken for *nodules*

Root knot nematode of bean

Cardona et al. 1982

Nodules vs. Gall?

Capon 1990

Soybeans inoculated with *Bradyrhizobium japonicum*

Yellow, stunted rows are uninoculated, indicating a severe N shortage
Where soybeans other members of cross-inoc group not been grown

Photo: Barak

Crop	Organism	kg fixed N/ha
Alfalfa	*Rhizobium*	150-250
Bean	"	30-50 (3-91)
Soybean	*Brady-rhizobium*	50-150 (26-188)
Peanut	"	68-206
Chickpea	"	60-84
Alders	*Frankia*	50-150
Azolla	*Anabaena*	150-300
Bahia grass	*Azotobacter*	5-30

Modified from B&W Table 13.6

Alder with N-fixer *Frankia* (actino-mycete) in PNW

Increase in growth of Douglas Fir when intercropped with Alder

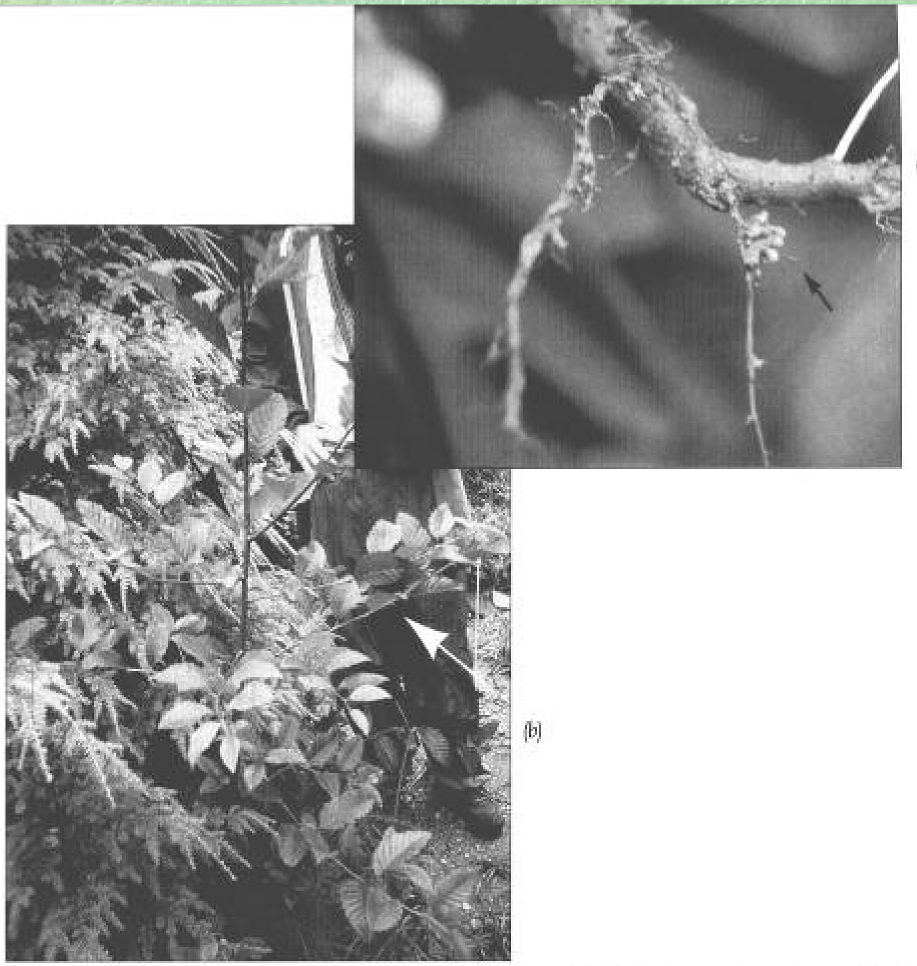


FIGURE 13.19 Soil actinomycetes of the genus *Frankia* can nodulate the roots of certain woody plant species and form a nitrogen-fixing symbiosis that rivals the legume–rhizobia partnership in efficiency. The actinomycete-filled root nodule (*a*) is the site of nitrogen fixation. The red alder tree (*b*) is among the first pioneer tree species to revegetate disturbed or badly eroded sites in high-rainfall areas of the Pacific Northwest in North America. This young alder is thriving despite the nitrogen-poor, eroded condition of the soil, because it is not dependent on soil nitrogen for its needs. (Photos courtesy of R. Weil)

Brady, N.C. and R.R. Weil. 1999. *The Nature and Properties of Soils*. 12th ed. Prentice Hall, New Jersey.

Actinomycete nodulated Non-legumes (we will see Alder in lab)

TABLE 13.9 Number and Distribution of Major Actinomycete-Nodulated Nonlegume Angiosperms

In comparison, there are about 13,000 legume species.

Genus	*Family*	*Species*[a] *nodulated*	*Geographic distribution*
Alnus	Betulaceae	33/35	Cool regions of the northern hemisphere
Ceanothus	Rhamnaceae	31/35	North America
Myrica	Myricaceae	26/35	Many tropical, subtropical, and temperate regions
Casuarina	Casuarinaceae	24/25	Tropics and subtropics
Elaeagnus	Elaeagnaceae	16/45	Asia, Europe, North America
Coriaria	Coriariaceae	13/15	Mediterranean to Japan, New Zealand, Chile to Mexico

[a] Number of species nodulated/total number of species in genus.
Selected from Torrey (1978).

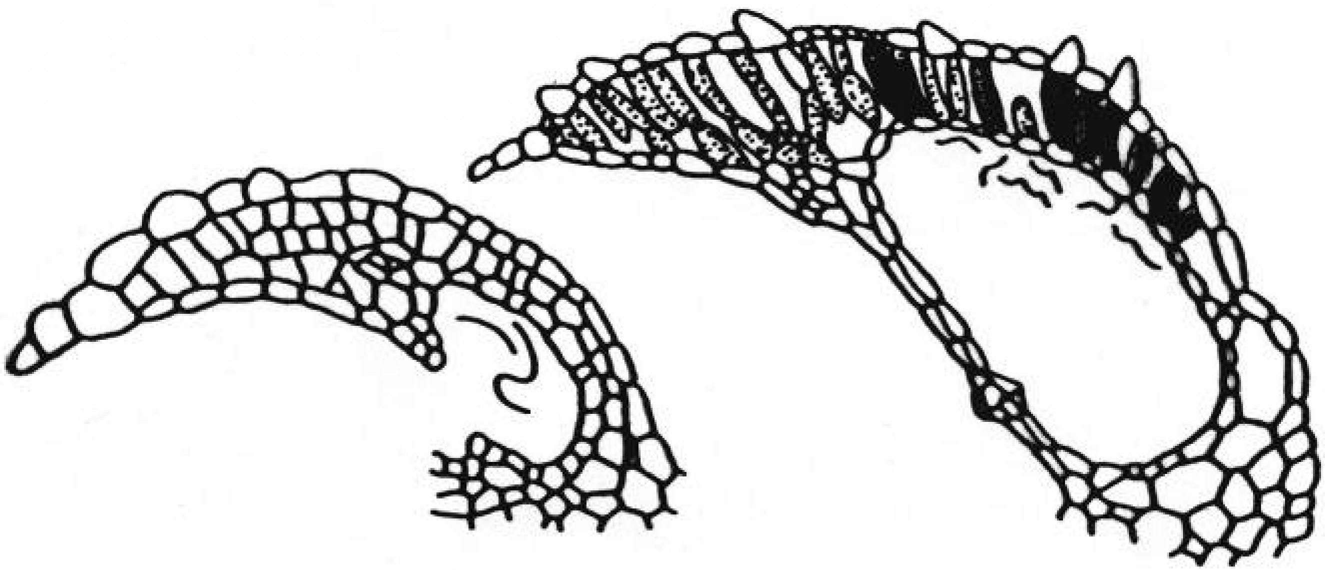
Brady, N.C. and R.R. Weil. 1999. *The Nature and Properties of Soils*. 12th ed. Prentice Hall, New Jersey.

N is limiting in rice agroeosystem

Azolla /*Anabaena* (150-300 kg N/ha)

Azolla spp. (floating fern) N-fixing cyanobacteria symbiont *Anabaena*

FIGURE 5.12
Drawing illustrating the symbiotic relationship between the cyanobacterium *Anabaena* and the aquatic fern *Azolla*. (From Smith, 1938; reprinted by permission; copyright McGraw-Hill.)



N addition to a subsequent crop **with legume as a crop and harvested for seed is a *N saver*** but doesn't contribute much

To have net increase in soil N:

- Amount of N fixed > % total N removed in seed + amount lost from the system by leaching and volatilization

Example: Legume crop biomass contained 100 kg/ha total N with 80% fixed. If 50% is lost in grain, then approximately 40 kg fixed N is theoretically available for next crop. With N losses through volatilization and nitrate leaching, the N available to next crop would be much less.

If your next crop is high yielding it is harder to fulfill next crop's needs!

Table 12.3. Typical N contents, yields and N offtake for several important tropical staple crops (based on recent FAO statistics for Africa and Asia)

Crop	Grain/tuber N content (kg N t^{-1})	Grain/tuber DW yields in the tropics (t ha^{-1})	Calculated N offtake (kg N ha^{-1})[a]
Sorghum	19	1–3	19–57
Maize	16	1–3	16–48
Cassava	6	6–10	36–60
Potatoes	10	5–15	50–150

[a] Assuming crop residues are not removed.
DW, dry weight.

K.E. Giller et al.

↑ This offtake will need to be covered in sustainable system.

Breeding N harvest index (% removed in crop) for the system vs the yield

Table 12.2. N_2-fixation by grain legumes grown as monocrops in the tropics (modified from Giller and Wilson, 1991)

Legume food crop	N fixed		N harvest index (%)
	%	kg ha^{-1}	
Groundnut (*Arachis hypogaea*)	47–92	68–206	35–64
Pigeonpea (*Cajanus cajan*)	65–88	68–88	25–62
Chickpea (*Cicer arietinum*)	60–80	60–84	70
Soyabean (*Glycine max*)	70–87	26–188	60–90
Common bean (*Phaseolus vulgaris*)	15–72	3–91	50–70
Black gram (*Vigna mungo*)	95–98	119–140	52
Mung bean (*V. radiata*)	89–90	58–107	49
Cowpea (*V. unguiculata*)	32–76	47–201	31–70

K.E. Giller et al.

Green manure vs. cover crop term

- ***Green manures* (**temp. zone): Crops planted for nutrient addition to cropping system, not for harvested portion. Usually plowed into the soil at flowering. Usually legumes.
- ***Cover crops***: Planted to cover soil to prevent erosion, crusting and nutrient loss.
- In tropics often not turned in, so gm=cc

N addition to subsequent crop if legume is grown as **green manure,** i.e. turned in just before reproductive

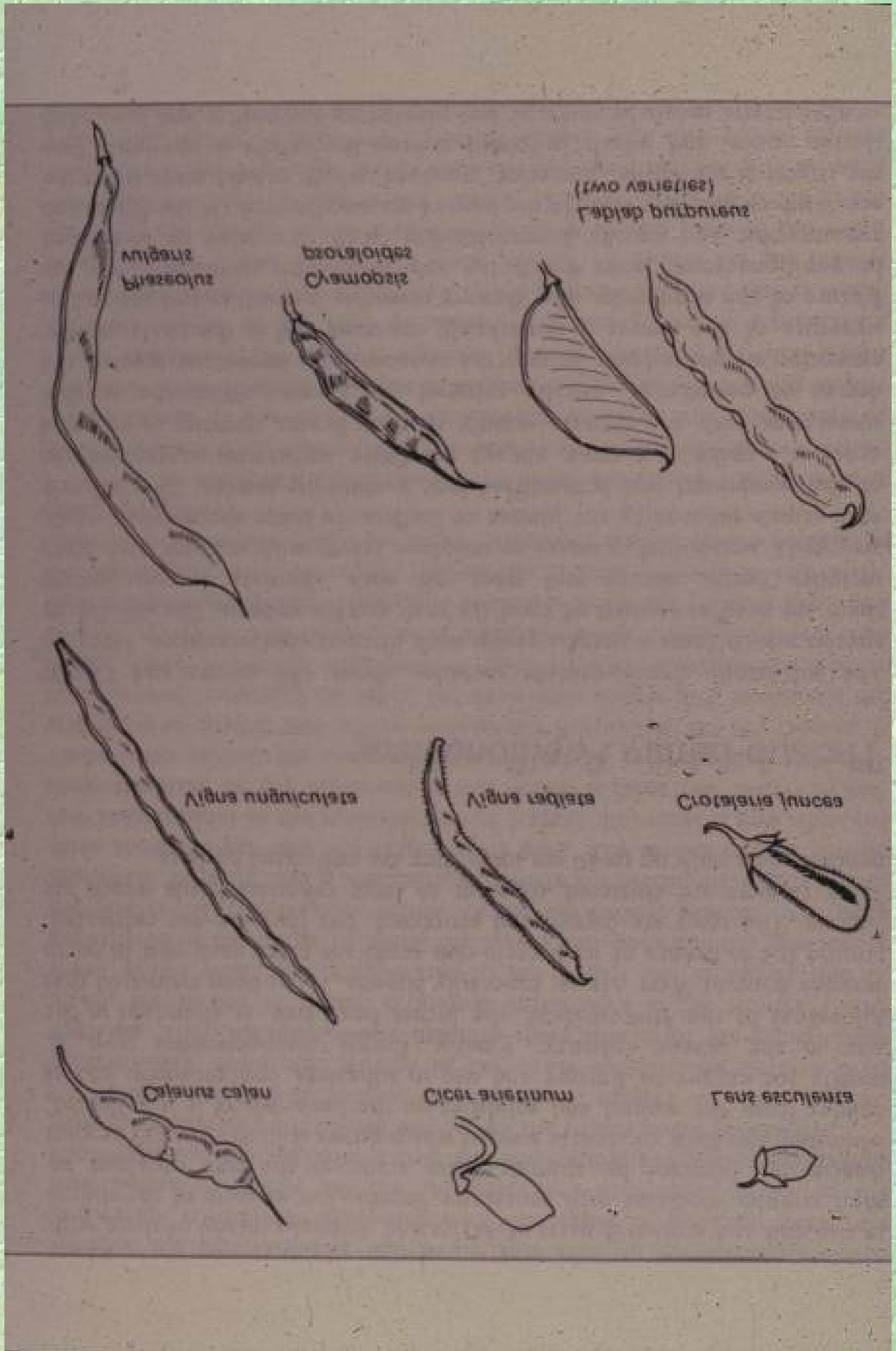
- No export of N as crop so all fixed N can potentially contribute
- Fixation in temperate zone
  - Fava (Bell) beans (*Vicia)* 50-150 kgN /ha
  - Vetch *(Vicia vilosa)* 50-150 kgN /ha
  - Lupine (*Lupinus*) 50-150 kgN /ha
  - Clover (*Trifolium pratense)* 100-150 kgN /ha

Territorial Seed Chart

When to Sow	Cover Crop	Height	Description	Sow per 1000 sq ft
Summer	Alfalfa, Nitro	12-24"	• Fixes up to 200 lbs N per acre • Attracts beneficial insects	$1\frac{1}{2}$ lbs
	Buckwheat	12-24"	• Phosphorus accumulator • Weed smother crop	2 lbs
	Clover, Yellow Blossom Sweet	24-120"	• Fixes up to 125 lbs N per acre • Loosens hard packed soil • Tap roots bring minerals to surface	$\frac{3}{4}$ lb
	Medic Mix	6-16"	• Best choice for dryland cover crop • Smothers weeds	1 lb
	Oats	24-36"	• Quick germinating • Provides erosion control • Must be mowed	1-2 lbs
	Soybeans	18-24"	• Fixes up to 130 lbs N per acre • Likes summer heat • Edible • Tolerates wide range of soil conditions with adequate water	2-3 lbs
	Sudan Grass, Piper	36-120"	• Controls harmful nematodes & symphylans • Chokes weeds	1-2 lbs
Fall	Austrian Field Peas	18-30"	• Fixes 70-120 lbs N per acre • Tolerates poorly drained soils • Adaptable to wide range of conditions • Hardy to 15° F	2-4 lbs
	Clover, Berseem	18-30"	• Fixes up to 300 lbs N per acre • Excellent hay • Hardy to 18°F	1 lb
	Clover, Crimson	12-18"	• Fixes up to 125 lbs N per acre • Shade tolerant • Does not perform well in water-logged soils	1-2 lbs
	Clover, Mammoth Red	18-36"	• Fixes up to 200 lbs N per acre • Breaks up clay soils • Tolerant of acid soils • Vigorous in cold weather	$\frac{1}{2}$-1 lb
	Clover, New Zealand White	6-8"	• Fixes up to 170 lbs N per acre • Drought tolerant • Adaptable to wide range of soil contitions	1 lb
	Clover, Subterranean	6-10"	• Fixes up to 80 lbs N per acre • Good in orchards • Performs well in poor soil • Burrowing seed heads self-seed	1 lb
	Fava Beans	24-36"	• Fixes up to 150 lbs N per acre • Brittle stalks till easily • Good forage crop • Hardy to 15°F	5-10 lbs
	Tyfon	8-12"	• Strong tap root breaks up clay soils • Easily hand tilled	1 lb
	Vetch, Common	18-24"	• Fixes up to 175 lbs N per Acre • Valuable forage crop • Attracts beneficial insects • Hardy to 0°F	2-3 lbs
	Vetch, Hairy	18-24"	• Fixes up to 180 lbs N per acre • Tolerates acid soils • Shade tolerant • Good choke crop • Hardy to 10°F	1-2 lbs
	Winter Wheat	24-40"	• Quick germinating • Adaptable to wide range of soils	1-2 lbs

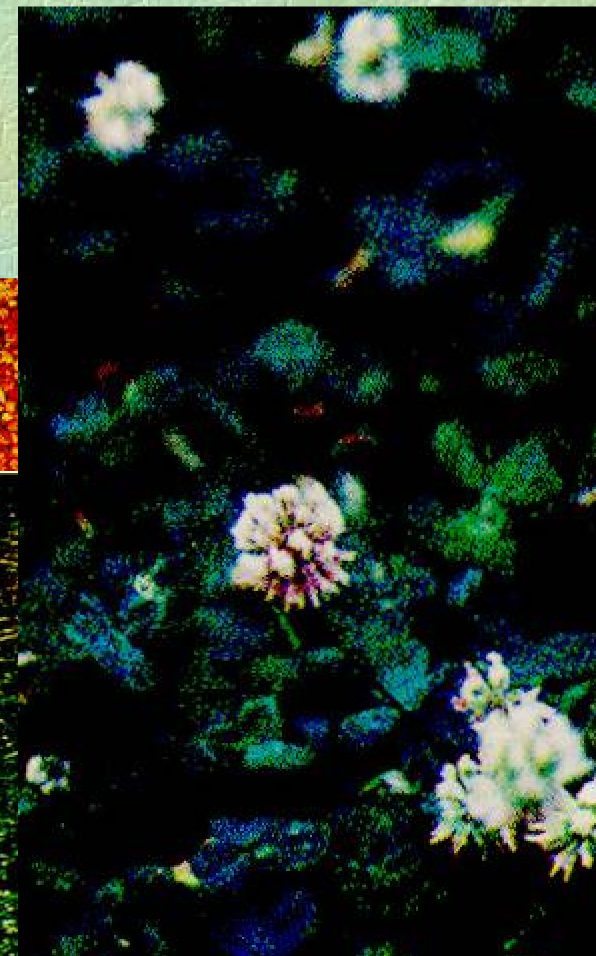
Fabaceae or Leguminosae

- Fruit is a pod
- Third largest family
- Most, but not all members, can fix N



White clover, *Trifolium repens* (Fabaceae)

Origin: Mediterranean
Common in New World since 1746



Subterranean clover
Trifolium subterranean

Origin: Mediterranean
Used with corn in MW and CA

http://www.agric.nsw.gov.au/reader/dpi268-sub-clover-hr.jpg

Vetch, *Vicia sativa*
Fabaceae

Origin: Mediterranean to Asia

Medics, *Medicago sp.* (Fabaceae)

Origin: *M. truncatula* Barrel, native to Mediterranean

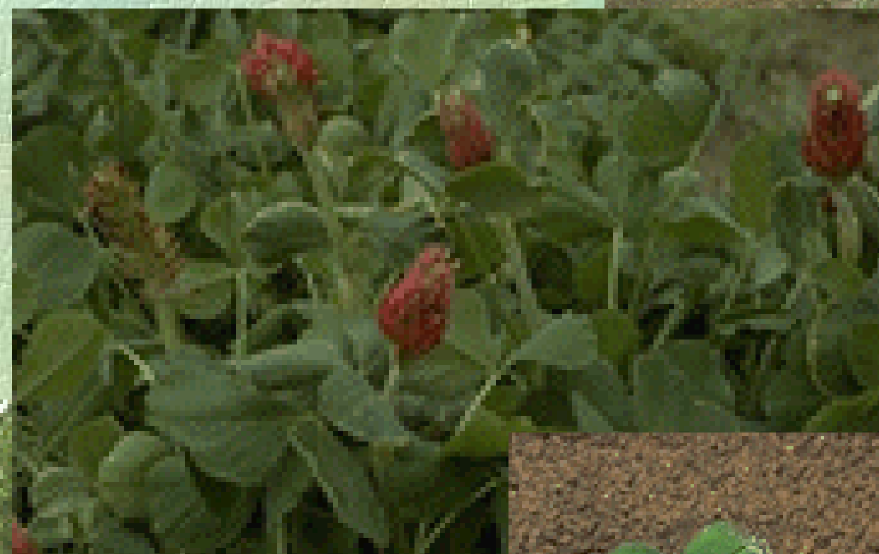
Summer annual

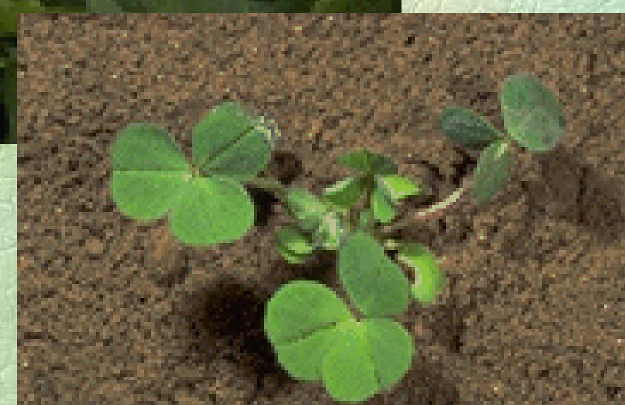
Good smother crop for weeds

Good dryland cover crop

Crimson clover, *Trifolium incarnatum* (Fabaceae)

Native to ?
Fall cover crop
Easy to till in
Shade tolerant





Fava bean, *Vicia faba* (Fabaceae)

Origin: Native to Mediterranean

Fall cover crop in W Wa

After 3 years can reduce infestation of symphylans (Solomon)

Extrafloral nectaries attract beneficial insects

Control of Symphylans

- Fava bean 3 years and turn in, leave dry in summer (Solomon)
- Use plant var with vigorous root systems

Can fix much N avail. to next, e.g in tropics

- Kudzu, *Pueraria* 100-140 kg/ha
- Velvetbean, *Mucuna* 150 kg N/ha per crop
- Andean lupine, *Lupinus* 400 kg/ha
- Tree, *Inga* 200 kg/ha

Velvetbean, *Mucuna pruriens*
“*terciopelo, frijol de abono*”

R. Bunch,
Honduras

Velvet bean, *Mucuna pruriens* “*terciopelo, frijol de abono*”

Use of velvet bean in US 1920s

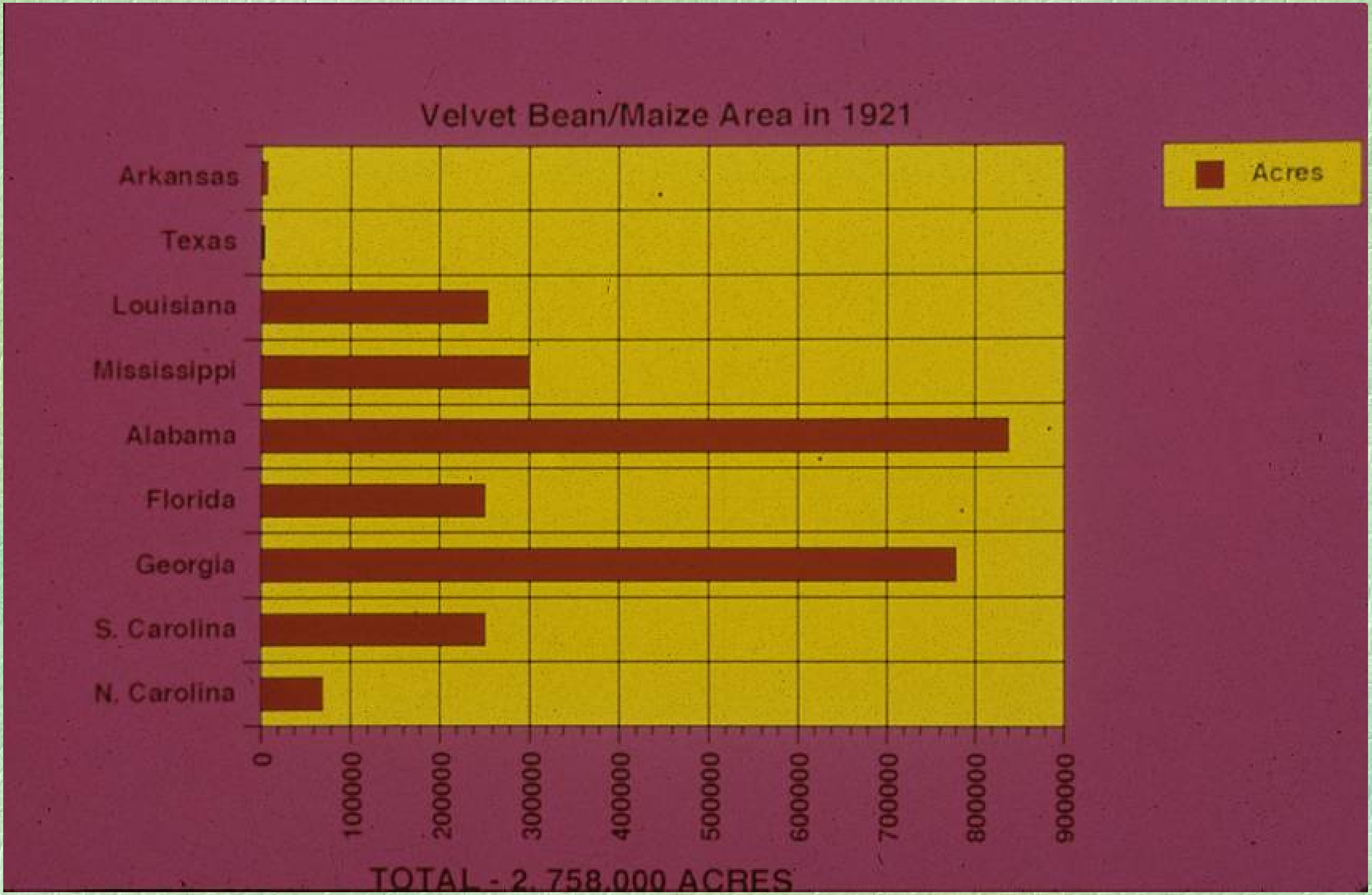
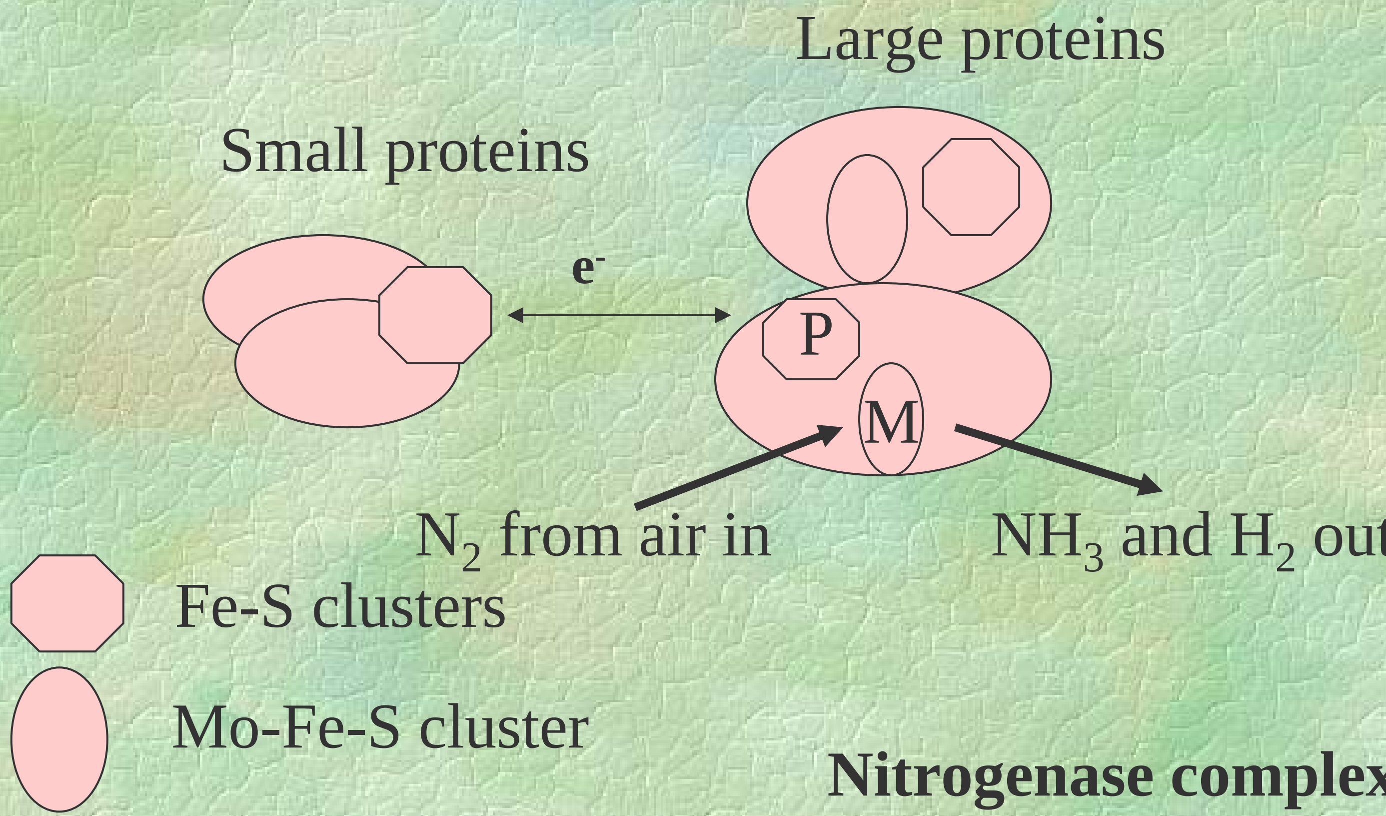


Photo: Thurston, Cornell

Jack bean, *Canavalia ensiformis* "*Canavalia*"

Origin: Central America
Long pods
Takes dry weather
Cuba: potatoes (50% N), coffee

III. How does nitrogenase work?



Nitrogenase complex

Brady Fig 13.16

Requires much energy-estimated 10% of C-fixed translocated to nods!

Nitrogenase destroyed by free O_2

- In root nodules the nitrogenase is protected from the oxygen by ***leghemaglobin***
- Virtually same molecule that gives blood its color and performs similar function
- Pink substance when cut open a nodule
- Demonstrates natures conservative tendency

Inside the nodule the $[O_2]$ is low

FIGURE 13-10.
Scanning electron micrograph of cells from the root nodule of a soybean plant, showing the mass of *Rhizobium* bacteria occupying these cells. Magnification about 5000×. (Photograph by P. Sihanonth and R. Todd, University of Georgia.)

Cox, G. and M. Atkins. 1964. *Agricultural Ecology. An Analysis of World Food Production Systems*. W.H. Freeman and Co., San Francisco.

IV. How BNF measured?

- For many years with acetylene reduction

  $N{\equiv}N \longrightarrow NH_3$

  nitrogen gas ammonia

  $HC \equiv CH \quad H_2C{=}CH_2$

  acetylene $\longrightarrow$ ethylene
- good relative measure, not absolute
- Now apply stable ^{15}N isotope and look at dilution due to N-fixation
- Comparison with non-nodulating isolines, meaning same variety but doesn't nodulate

In polyculture: Effect on soil N level through mineralization of root tissue

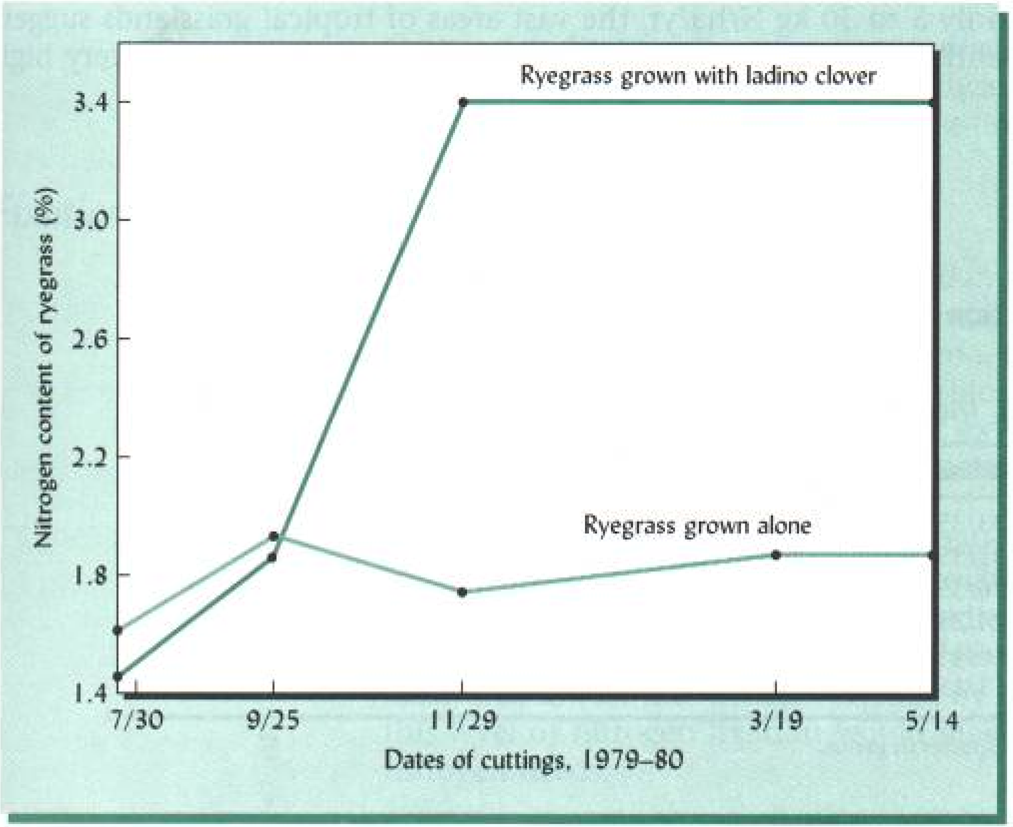


Figure 13.21 in 13th Edn

FIGURE 13.18 Nitrogen content of five field cuttings of ryegrass grown alone or with ladino clover. For the first two harvests, nitrogen fixed by the clover was not available to the ryegrass and the nitrogen content of the ryegrass forage was low. In subsequent harvests, the fixed nitrogen apparently was available and was taken up by the ryegrass. This was probably due to the mineralization of dead ladino clover root tissue. [From Broadbent, et al. (1982)]

Brady, N.C. and R.R. Weil. 1999. *The Nature and Properties of Soils*. 12th ed. Prentice Hall, New Jersey.

Intercropping dynamics

Is there N transfer?

Wiley

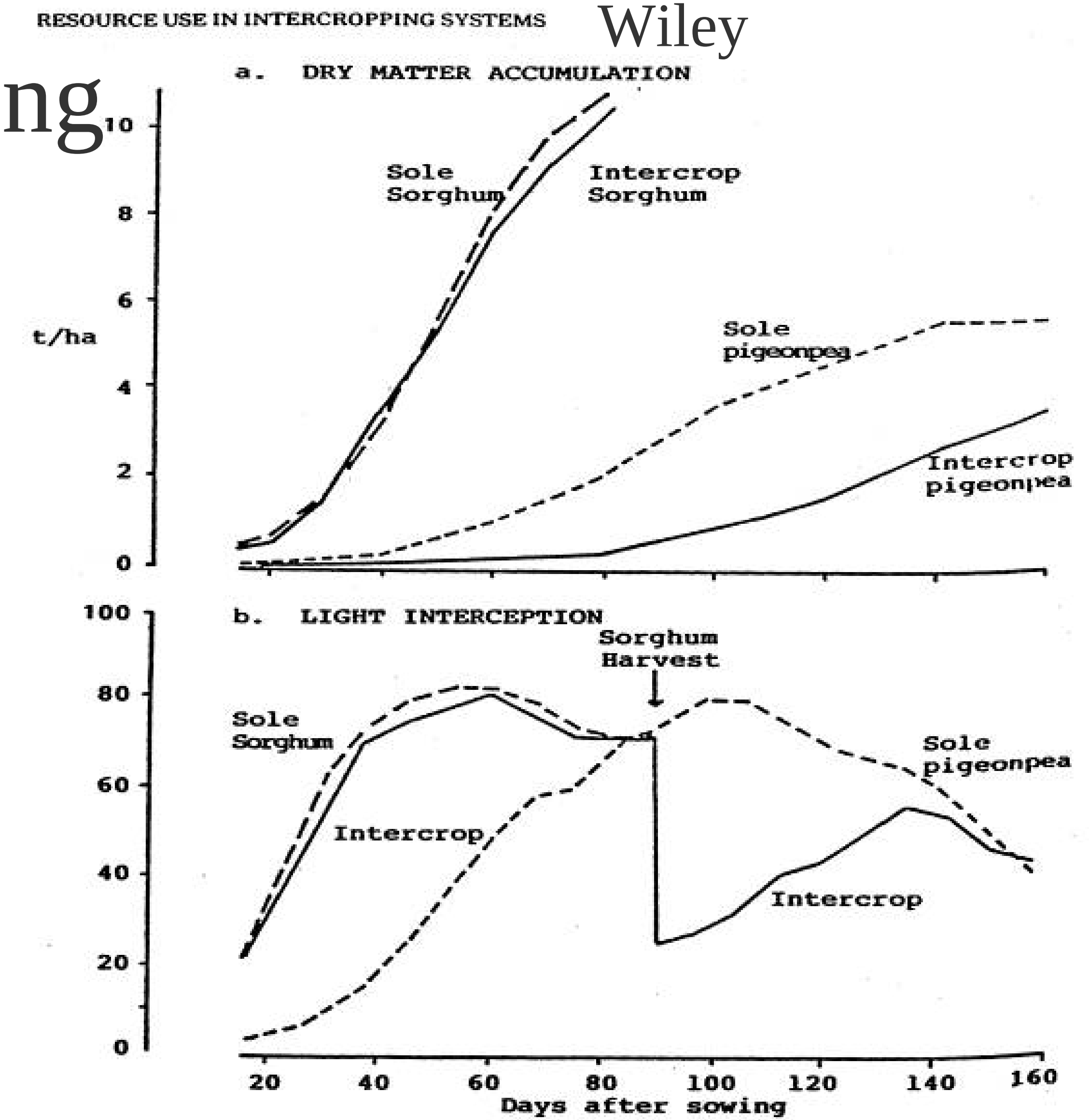


Figure 1 Dry matter accumulation and light interception in sorghum and pigeonpea sown as sole crops and as a 2-row sorghum : 1-row pigeonpea intercrop (means of 3 years – after Willey et al, 1983).

Pine soil N increases with locust (Fig 13.20 in 13th Edn.)

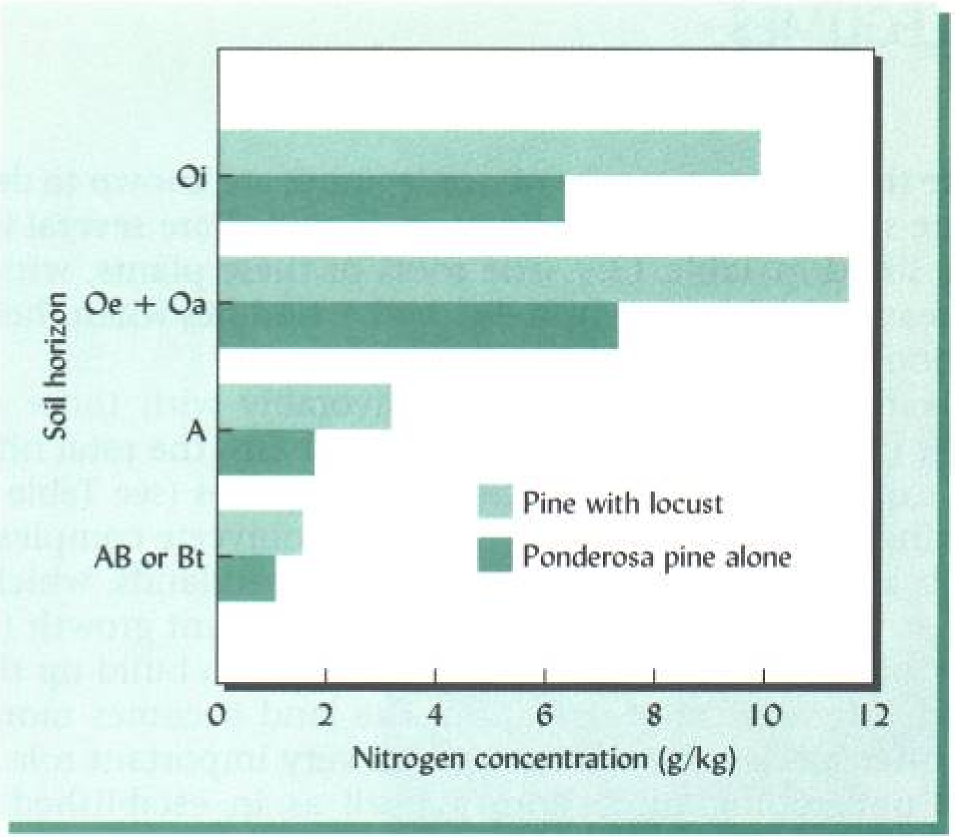


FIGURE 13.17 Nitrogen contents of forest soil horizons showing the effects of New Mexican locust trees (*Robinia neomexicana*) growing in association with ponderosa pine (*Pinus ponderosa*) in a region of Arizona receiving about 670 mm rainfall per year. The data are means from 20 stands of pondersosa pine, half of them with the nitrogen-fixing legume trees (locust) in the understory. The soils are Eutrustalfs and Argiustolls with loam and clay loam textures. [Data from Klemmedson (1994)]

Brady, N.C. and R.R. Weil. 1999. *The Nature and Properties of Soils*. 12th ed. Prentice Hall, New Jersey.

Ecology of N fixation: complex

Affected by nitrate in environment, pH, OM, Fe, Mo, S

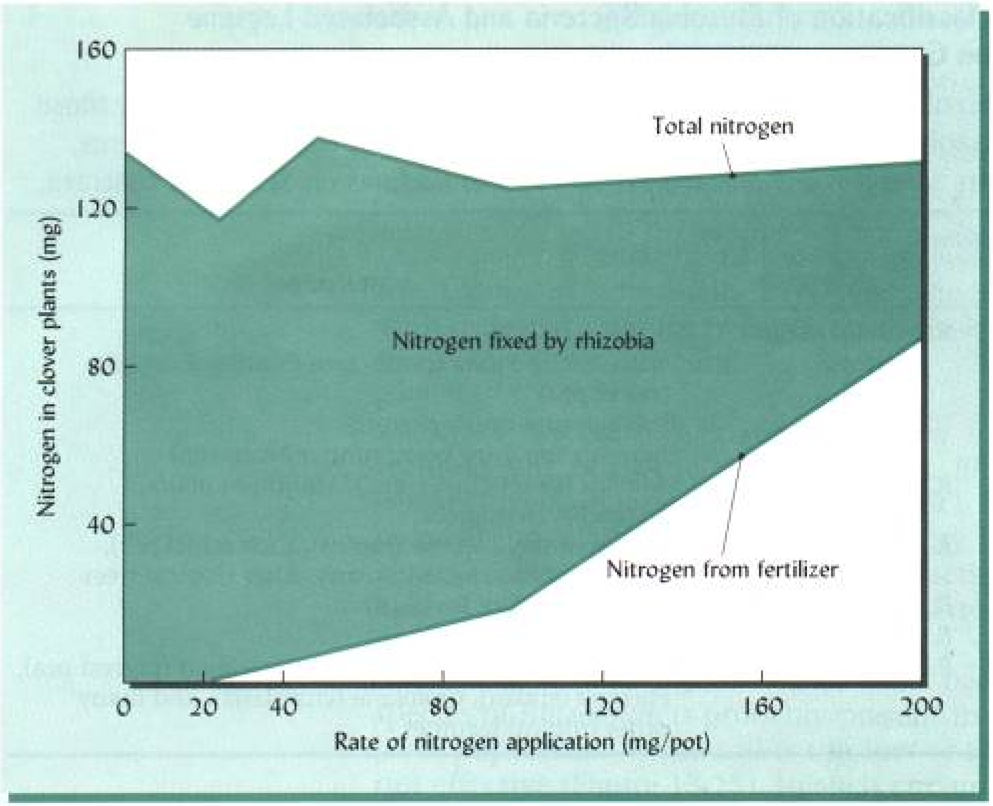


Figure 13.19

FIGURE 13.16 Influence of added inorganic nitrogen on the total nitrogen in clover plants, the proportion supplied by the fertilizer, and that fixed by the rhizobium organisms associated with the clover roots. Increasing the rate of nitrogen application decreased the amount of nitrogen fixed by the organisms in this greenhouse experiment. [From Walker, et al. (1956)]

Brady, N.C. and R.R. Weil. 1999. *The Nature and Properties of Soils*. 12th ed. Prentice Hall, New Jersey.

N fixation greater where no applied N (left)

Alfalfa with 175 lb/ac N applied on right, none on left
Photo: Barak, UW

How to increase BNF?

- Include legumes in farming systems, including trees
  - *Robinia* and alder in temperate zone
  - *Inga*, *Calliandra*, *Leucaena*, *Glyricidia* in tropics
- Use fertilizers judiciously-- P and K can double N fixation, Mo, Fe critical, N reduces
- Select well-adapted combinations of legumes and rhizobia
  - Cuba supplying 80% of N needs of legumes with *Rhizobium* strains (Gersper et al. 1993)
- Breed for high N-fixation
- Use of associative N-fixing organisms
- Develop ways to introduce N-fixing symbiosis to non-symbionts (like corn fixing its own N)?

Cover crops can be combined with cash crop

Corn-soybean-wheat/red clover

Corn-oat/alfalfa-alfalfa

Cover crops can be combined easily with perennials: Kudzu in oil palm

Needs to be cut back in 1st years
Shaded out after 5 years

VI. Can organic agriculture provide enough biologically fixed N?

- In tropics, BNF can supply needs-- but at moderate outputs (Giller et al. 1994)

- Cuba providing 40-50% of non-legumes with *Azotobacter*
  - also shortens crop production (Gersper et al. 1993)

Principal uses of biofertilizers in Cuba (Funes-Monzote, In Press).

Rhizobium	Beans, peanuts, and cowpeas	75-80% of the N fertilizer
Bradyrhizobium	Soybeans and forage legumes	80% of the N fertilizer
Azotobacter	Vegetables, cassava, sweet potato, maize, rice	15-50% of the N fertilizer
Azospirillum	Rice	25% of the N fertilizer

Source: Martínez Viera and Hernández, 1995; Treto et al., 2002.

Organic agriculture provide N (cont.)?

- But 3-4 g vegetable protein/1 g animal protein and Latin America increasing consumption of meat
- Change to vegetarianism unlikely, therefore BNF cannot supply world desires for meat (Smil. 1997)

What's the lever in regaining control of N?

Studies of the N cost of Food Production in Norway have concluded (Bleken and Bakken 1997):

- N in food consumed is 10% applied N
- Plant production leaky
- N cost (N in ÷ N out) = wheat 3, dairy 14, meat 24
- Recycling at high trophic level more (human diet) more effective than low (composting) ∴ Her conclusion was that as foodstuffs are currently produced, ***Human diet is the lever!***
- However, does it matter how the animals are raised?
- If animals are range fed vs. feedlot fed do the input/output ratios of N change?

Study Questions

1. It has been estimated that the Haber-Bosch industrial fixation of N supply 2/5 of the world's food supply. Summarize the changes that may needed in order to maintain the world's population when fossil fuels are depleted.
2. Green manures are one key to cropping systems sustainability. What are the three destinations/path of N fixed by a green manure?
3. What different factors do you need to know in order to determine if your legume is contributing N to the next crop? ie is it a net importer of N or exporter to your cropping system?

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