NITROGEN FIXATION OF HAY AND PASTURE LEGUMES

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SUMMARY

Nitrogen is the major limiting nutrient for grain crops and for forages and grasslands of the United States. Nitrogen also is the most costly of the major nutrients on a land area basis because of the quantities applied and because of its dependence for manufacture upon increasingly scarce and expensive natural gas.

Nitrogen fixed by symbiosis in legume root nodules can be substituted for fertilizer N, and thus effect financial and energy savings, by adoption of management practices that result in a net return of symbiotically fixed N_2 to the soil. Legumes can be grown in rotation with nonlegumes, and the legume residues plowed down to provide N to the succeeding nonlegume. Also, legumes in legume-grass swards may furnish N to the grass by decomposition of plant residues or through animal excreta.

In either of these practices, the amount of fertilizer N that can be displaced by legume N depends upon (a) the quantity of N_2 fixed by the legume, (b) the quantity of legume residues returned to the soil, (c) the proportion of N in the residues derived from symbiotic fixation, and (d) the rate at which the N in the residues becomes available to the associated nonlegume crop.

The benefits from substituting a hay or pasture legume for N fertilizer in a cropping system will depend on how the legume is managed. If the legume is managed for maximum hay production, little net N input to the soil may occur if sparce regrowth is plowed down at the end of the cropping cycle. If a lush stand of herbage is incorporated, the prospect of a net N addition to the cropping system is enhanced. Grazing of legume-grass swards may result in substantial transfer of legume N through animal excreta to the associated grass.

INTRODUCTION

Symbiotic N_2 fixation is a mutually beneficial process involving two partners. The plant partner provides an environment in which the bacterial partner receives nutrition from the plant for growth and reproductive processes. The plant provides sugars generated by photosynthesis and other nutrients to bacteria, which are sequestered inside a specialized root structure called a nodule. The bacteria also use the plant-derived sugars to provide energy to reduce unusable gaseous N_2 in the soil and above-ground atmosphere to nutritionally valuable ammonium ions, which the plant converts to amino acids for protein synthesis. The symbiosis is important to agriculture because it provides up to 100 percent of the total nitrogen (N) needs of plants which carry out this activity, replacing the need for applications of manufactured N fertilizers. With proper management, the partnership of plant and bacteria can meet the nutritional needs of the host plant for N as well as provide a net N input into crop rotations and pastoral cropping systems.

The purpose of this paper is to discuss the N_2 fixation capabilities of hay and pasture legumes potentially useful in cropping systems developed for hill lands, and to illustrate how legume management influences fixation of N_2 and its return to the soil.

THE LEGUME ROOT NODULE

Root nodules are highly organized masses of tissue derived from cortical cells of roots. There are three major patterns of nodule development; (1) elongate, cylindrically shaped, frequently branched nodules with persistent infection threads and a long-lived apical meristem, (2) spherically shaped nodules with short-lived infection threads and a transient apical meristem, and (3) collar type nodules that completely encircle the root. Only the morphology of type (1) or indeterminant nodules will be considered further.

An established alfalfa (*Medicago sativa* L.) nodule has four distinct regions. The white terminal end of the nodule is an apical meristem, a tissue of plant origin composed of rapidly dividing and elongating cortical cells. The thread invasion region located immediately behind the apical meristem contains many cells which have been invaded by infection threads to release nodule forming bacteria. In this region of the nodule, bacteria commence a change from motile rods to a new morphological form called bacteroids which are capable of N_2 fixation. The fully functional bacteroids contain the N_2 fixing enzyme nitrogenase, and fill the cells in the pink to red early symbiotic portion of the nodule in which most N_2 fixation occurs. The red pigment, leghemoglobin, has a role in providing an optimum oxygen concentration for N_2 fixation. Frequently, a senescent zone of the nodule adjacent to its attachment to the root contains bacteroids undergoing breakdown or senescence owing to age or stresses such as drought, grazing or herbage harvest. This region of the nodule is gray to green and highly disorganized. Nutrients from the shoot and root of the plant enter, and products of N_2 fixation exit, the nodule through a net-like system of vascular bundles surrounding its periphery.

MECHANISM OF NITROGEN FIXATION

Industrial reduction of atmospheric N_2 to produce ammonia for fertilizer requires the transfer of hydrogen from natural gas to the N_2 molecule under high temperatures (300 to 600 C) and pressures (20 to 80 M Pa). Plants achieve the reduction of N_2 at ambient temperatures and pressures with enzymes and energy from the oxidative phosphorylation of sugars produced by photosynthesis.

Nitrogenase

Nitrogenase is the enzyme in bacteroids that facilitates the reduction of gaseous N_2 to ammonia. It has two components that cooperate in fixation. The smaller component is an iron protein, while the larger is an iron-molybdenum protein. The gaseous N_2 molecule combines with the molybdenum of the large component while the small enzyme component gathers the electrons necessary for the reduction of N_2 to ammonia. Metabolic energy in the form of adenosine triphosphate (ATP) is used to split the triple bond of the N_2 molecule:

$$N \equiv N + 16\ ATP + 8e^- + 10\ H^+ \Rightarrow 2\ NH_4^+ + 16\ ADP + 16\ P_i + H_2$$

Sixteen molecules of ATP are needed to split each N_2 molecule into two ions, and concurrently three electrons (e^-) are transferred to each of the two N ions formed. Two ammonium (NH_4^+) cations are formed when the unpaired electrons on each N ion are balanced by hydrogen ions (H^+) from the interior of the bacteroid.

The nitrogenase enzyme also catalyzes the reduction of two hydrogen ions to H_2 (gas) during N_2 reduction in many legume-bacteria symbioses. In species like soybean (Glycine max (L.) Merr.), the H_2 loss to the atmosphere consumes 4 ATP and may reduce substantially the efficiency of N_2 fixation. Certain strains of root-nodule bacteria have an uptake hydrogenase enzyme to internally recycle the H_2 to the reactant side of the foregoing equation, with a consequent improvement of about 12 percent in efficiency of N_2 fixation.

Dependence Upon Photosynthesis

The ATP required to split the N_2 molecule and the electrons required for reduction of N_2 to ammonium are both produced during the respiration of photosynthate in the mitochondria of the bacteroid. Fixation of 100 kg of N_2 as NH_3 requires the metabolic energy needed for production of 1500 kg of plant dry matter (LaRue and Patterson, 1982). While the energy cost of N_2 fixation as photosynthate is considerable, there is no direct evidence that N_2 fixation restricts legume productivity by diversion of dry matter to the nodules. Non N_2 fixing legumes adequately nourished with fertilizer N yield similarly to their N_2 fixing counterparts.

COMPARISONS AMONG SPECIES

Many of the estimates of N_2 fixation quoted in older literature, some as high as 600 kg N/ha/growing season for alfalfa, were obtained by assuming that all of the N in the legume was provided by nodule activity. Most of these estimates were erroneous because the investigators failed to realize that legumes derive only part of their N requirements from symbiotic fixation. Indeed, legumes preferentially use N from carryover fertilizer or from decomposition of soil organic matter before commencing symbiotic fixation.

The proportion of legume N derived from nodule activity varies with species, with stage of growth, and with the N status of the soil. For perennial legumes like alfalfa, red clover (Trifolium pratense L.), and birdsfoot trefoil (Lotus corniculatus L.), nodule activity usually is lowest in the spring and fall, and highest during midseason (Table 1). The low level of N fixation early in the season compared with midseason is thought to be attributable to higher levels of soil N from mineralization early in the season, which inhibit nodule formation and functioning until plant uptake exhausts soil solution nitrate levels to low values. Reduction in N_2 fixation in fall probably is due to the onset of plant dormancy and cooler soil temperatures. Nitrogen fixation of an annual legume like soybeans also is strongly dependent upon the N supplying power of the soil (Table 1). Depending upon species, growth stage, and environment, the proportion of total N in the crop grown in fertile midwestern soils which is symbiotically derived may vary from less than 30 to over 80 percent.

Table 1. The percentage of nitrogen in hay, pasture and grain legumes derived from symbiotic fixation in root nodules.*

	Seeding year harvests				1st production year harvests				
Species	I	II	III	Mean	I	II	III	IV	Mean
	---------- Nitrogen from Symbiosis (%) -----------								
Alfalfa	49	81	58	63	62	34	36	29	40
Red Clover	51	79	65	65	9	59	37	-	35
Birdsfoot Trefoil	27	67	25	40	0	61	28	-	30
Soybean¶	-	-	-	28	-	-	-	-	-
Soybean§	-	-	-	52	-	-	-	-	-

*Derived from data in Heichel et al. (1981), Ham (1978), Vasilas (1981).
¶Grown at site with high available soil nitrogen.
§Grown at site with low available soil nitrogen.

The most reliable and quantitative field measurements of N_2 fixation capability of hay and pasture legumes have arisen from use of the "difference" method or the nitrogen-15 stable isotope tracer method. With the first method, the difference between the N content of the legume (soil plus symbiotic N) and the nonlegume (soil N only) represents the legume N from symbiotic fixation. The nitrogen-15 stable isotope method relies on labeling the organic matter of the soil in which the legume is growing with the mass 15 isotope of N, which is scarce (0.4%) in the soil and atmosphere compared with the abundant (99.6%) mass 14 isotope. Extent of dilution of the mass 15 N isotope tracer in the legume by the mass 14 isotope in comparison with the nonlegume values is a measure of N_2 fixation. Both the difference and isotope tracer methods are adapted to use in field crop communities, integrate crop performance over several weeks of the growing season between samplings, and facilitate representative sampling.

Nitrogen fixation by hay and pasture legumes varies among geographic locations as well as among species at the same location (Table 2), with values of 150 to 200 kg N/ha/growing season being common. Within a location (e.g. Kentucky, Minnesota) N_2 fixation of alfalfa generally exceeds that of red clover, with white clover (Trifolium repens L.), or birdsfoot trefoil ranking third. Many hay and pasture legumes fix 15 to 25 kg N per metric ton of herbage or hay. The more productive the species, the more N_2 is fixed. It is not unusual for seasonal N_2 fixation of alfalfa or red clover to be double that of soybeans. This difference is largely attributable to the longer growing season of the perennial legume, its persistant, long-lived nodules, and to its greater reliance upon N_2 fixation compared with soil N.

MANAGEMENT OF NITROGEN FIXATION

The producer has several opportunities to influence the amount of N_2 fixed by hay and pasture legumes, principally by inoculation to insure the formation of root nodules, and by managing crop rotations and pastures for maximum return of symbiotic N_2 to the soil. Proper soil fertility also is important, but its treatment is beyond the scope of this presentation.

Inoculation

Inoculation is the practice of adding effective nodule bacteria to legume seed before planting to assure adequate nodulation and promote

Table 2. Comparison among species of rates of nitrogen fixation of legume crop communities on a land area basis.

Crop	Rate (kg N/ha/ growing season)	Location	Method[¶]
Alfalfa	212	Lexington, KY	Difference (Kentucky bluegrass)
	148	Rosemount, MN	N-15 isotope (Reed canarygrass)
	193	Rosemount, MN	N-15 isotope (Reed canarygrass)
White clover	128	Lexington, KY	Difference (Kentucky bluegrass
Red clover	154	Lexington, KY	Difference (Kentucky bluegrass)
	84-149	Rosemount, MN	N-15 isotope (Reed canarygrass)
Suberrranean clover	58-183	Hopland, CA	N-15 isotope (Soft chess)
Birdsfoot trefoil	58-115	Rosemount, MN	N-15 isotope (Reed canarygrass)
Soybean	15-84	Ames, IA	Difference (non-nodulating isoline)
	43-146	Mead, NE	N-15 isotope (non-nodulating isoline)
	76-152	Rosemount, MN	N-15 isotope (non-nodulating isoline)

¶The non-nitrogen fixing control species is shown in parentheses.

adequate N_2 fixation by the crop. Traditionally, it is done by coating the seed at planting with a water-based slurry consisting of a commercial preparation of bacteria mixed with a peat carrier enriched with sugars, gums, and complex polysaccharides to provide nutrition, adhesion, and protection. One to 4 g inoculum/kg of seed is a typical application.

In many localities where the same hay or pasture legume has been grown regularly for several years, inoculation of seed is not necessary because the soil contains a sufficiently large population of nodule bacteria to fulfill the requirements of the crop. There are other localities, however, where nodule bacteria do not persist because of infertile soil or stressful environmental conditions, and regular inoculation is necessary. Seedsmen are increasingly merchandizing preinoculated hay and pasture legume seed, especially alfalfa, on which the nodule-forming bacteria are encapsulated in a seed coating. The seed coating adds only 1 to 2 percent to the total cost of the seed, so it is relatively inexpensive insurance to assure that effective nodule bacteria are present.

If preinoculated seed of a legume species are not available, the following criteria should govern the decision to inoculate: 1) inoculate when the particular legume species has not been grown on a site within the past 5 years. Thus, inoculation is advised when a legume is first interseeded into an unimproved grass pasture or established stand of trees, when the legume follows several years of nonlegumes, or when it displaces an unrelated legume previously grown on the field. 2) Inoculate when previous experience on a field or soil type shows that frequent inoculation is necessary to prevent development of N deficiency symptoms. 3) Inoculate when soils have been disturbed or reconstituted as in the case of mine spoils, or when they are strongly acidic, alkaline, or prone to major nutrient deficiences. In these cases, a major program of fertilization with macro- or micronutrients probably will be required for good plant growth and persistence of nodule bacteria.

Forage Harvest

Removal of herbage by harvesting or grazing may decrease temporarily the N_2 fixation capability of hay and pasture legumes. In legumes like alfalfa that have indeterminant nodules, herbage removal causes a temporary senescence of the older bacteroids near the base of the nodule. This may be due to the decrease in the supply of photosynthate or other growth factors from the shoot. Nitrogen fixation capability decreases to a minimum within 1 to 2 days of harvest and commences recovery with onset of shoot regrowth. There is little evidence of substantial nodule death and sloughing as occurs after harvest of other legumes such as white clover. Nevertheless, the harvest-induced loss of nodule activity might lead to the conclusion that N_2 fixation would be greater with fewer harvests. On the contrary, the potential for N_2 fixation is much greater in alfalfa that is periodically harvested than in unharvested alfalfa (Cralle and Heichel, 1981). This may be due to prevention of flowering and to the development of young regrowth with a higher level of photosynthetic activity than that of old leaves. The general principle is that a management scheme that maximizes herbage productivity and stand persistence in a particular locality also will maximize N_2 fixation by the crop.

Root and nodule death after herbage removal is comparatively greater in white clover, subterranean clover (Trifolium subterraneum L.), and birdsfoot trefoil than in alfalfa. Since reinfection of roots and formation of new nodules would be necessary on these species before N_2 fixation could recover after harvest, fixation of white and subterranean clover, and birdsfoot trefoil is frequently less than in alfalfa (Table 2). Other factors undoubtedly are involved. However, the nodule and root senescence accounts for more N transfer from white and subterranean clover to a companion grass than occurs from alfalfa.

Crop Rotations

In legume-grass pastures and in legume-nonlegume rotations, the amount of fertilizer N that can be displaced by legume N depends upon (a) the quantity of N_2 fixed by the legume, (b) the quantity of legume residues returned to the soil, (c) the proportion of N in the residues derived from symbiotic fixation, and (d) the rate at which the N in the legume residues becomes available to the succeeding nonlegume crop (Heichel and Barnes, 1983). Although hay and pasture legumes may fix large quantities of N by symbiosis (Table 2), only part of it is typically returned to the soil for use by a succeeding crop. This is because a portion of the symbiotically fixed N_2 is removed from the land when the legume is grazed or harvested with the rest remaining in unharvested roots and crowns. The N available for incorporation into the soil depends upon the time of season when incorporation occurs, and the proportion of the plant that is N-rich herbage compared with the relatively N-poor roots and crowns. The N budget in Table 3 exemplifies the N enrichment of the soil that is possible when alfalfa is incorporated by either fall or late summer plowing in the seeding year (Heichel et al., 1981).

Table 3. Nitrogen budget for seeding year alfalfa showing the allocation of symbiotically fixed and soil-derived nitrogen among plant parts, and the input of nitrogen to the soil with two management options.§

	Seeding Year Harvests		
	First (7/12)	Second (8/30)	Third (10/20)
Herbage Yield (kg DM/ha)	3505	3054	1156
Total Nitrogen Yield (herbage and crown and roots) (kg N/ha)	118	127	59
Total Nitrogen Fixed (kg N/ha)	57	102	34
Herbage	52	74	22
Roots and crown	5	28	12
Nitrogen From Soil	61	25	25
Herbage	54	18	16
Roots and crown	7	7	9
Management Options			
Plow 10-20			
N input/harvest (kg/ha)	-49	+10	+34
Cumulative N input (kg/ha)	-49	-39	-5
Plow 8-30			
N input/harvest (kg/ha)	-49	+102	-
Cumulative N input (kg/ha)	-49	+53	-

§Adapted from Heichel et al. (1981)

If two herbage harvests are taken during the seeding year, followed by herbage regrowth before plowing on October 20, the early season N deficit is nearly all replaced by late season N_2 fixation so that only a small net loss of 5 kg N/ha occurred. In contrast, removal of one herbage harvest followed by a plowdown of lush regrowth on August 30 allows a net input of 53 kg N/ha. In succeeding production years, similar considerations might allow a net return of more than 100 kg N/ha from symbiotic fixation to the soil. Clearly, the benefits gained from substituting a hay or pasture legume for N fertilizer in a crop rotation will depend on how the legume is managed. If the legume is managed for maximum hay production (typified by three to four harvests annually in the north central U.S.), little net N input to the soil may occur if regrowth is sparce. If a lush stand of herbage regrowth is incorporated, however, the prospect of a net N addition to the cropping system is enhanced.

Nitrogen Transfer in Crop Communities

Nitrogen transfer can occur between neighboring plants by exchange of N between legume roots and nonlegume roots. This is thought to occur through mineralization of root exudates or of sloughed-off or dead nodules or small roots during the growing season. Nitrogen transfer also occurs by decomposition of litter or of large plant organs, or by herbivore ingestion of herbage from one area and excretion onto a different area.

Intercropping of forage legumes and nonlegumes is extensively practiced to provide herbage for grazing animals and hay or silage for ruminants. In these crop communities, the evidence for N transfer is more persuasive than in other circumstances. Simpson (1976) found that under a regime of periodic mowings over 3 years in which all clippings were removed, the cumulative N transfer of legume N to orchardgrass (*Dactylis glomerata* L.) was 20 percent for subterranean clover, 6 percent for white clover, and 3 percent for alfalfa. An additional 21, 34, and 21 percent of subterranean clover, white clover, and alfalfa N, respectively, was retained in the soil. Substantial transfer of N from white clover to ryegrass (Lolium rigidum L.) also has been reported (Broadbent et al., 1982). The differences between species apparently are attributable to senescence of nodules, roots, and stolons of clovers after harvest. Comparatively little root or nodule turnover occurs after harvest of alfalfa (Vance et al., 1979; Cralle and Heichel, 1981).

In mixed legume-grass swards, substantial transfer of legume N to the companion grass may occur through the excretion of grazing animals. Large losses of legume N through volatilization of ammonia from urine also may occur from these systems (Ball and Kenney, 1983). However, sufficient nitrate-N from mineralization of excreta and plant tissues may accumulate in clover-grass swards to suppress N_2 fixation of the legume (Hoglund and Brock, 1978). Under these conditions knowledge of the entire N cycle of the sward including N export in animal products is necessary to manage the legume-grass community for maximum N_2 fixation.

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