# FATE OF N MINERALIZED FROM $^{15}$N-LABELED ALFALFA IN NO-TILL INTERCROPPED CORN

By

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## ABSTRACT

### FATE OF N MINERALIZED FROM $^{15}N$-LABELED ALFALFA IN NO-TILL INTERCROPPED CORN

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This study examined the contribution of legume N to no-till corn intercropped with alfalfa. Two greenhouse experiments were conducted to measure the transfer of $^{15}N$ from labeled alfalfa tops and intact roots in an undisturbed system (Experiment 1). A second greenhouse experiment examined the availability of $^{15}N$ from labeled tops only.

Four treatments were applied in Experiment 1 and one treatment for Experiment 2. These treatments included herbicide + cutting, cutting only (Experiment 1 and 2), cutting (- residue) and herbicide only. The herbicide + cutting treatment provided greater uptake and recovery of N by corn compared to the treatments with either cutting or herbicide. When alfalfa regrowth was suppressed by both cutting and herbicide, corn recovered 12% of the alfalfa N but with only cutting, N recovery by corn was reduced to 4%. Most

of the $^{15}N$ applied in this system remained in the soil organic and inorganic pool.

Results from the second greenhouse experiment revealed no significant differences due to time in the treatment (cutting only). Rather, the results serve as a useful index to discern between the availability of N from various sources. Of the total 4% recovered by corn, alfalfa tops contributed 1% of the $^{15}N$ to corn tops, soil contributed 2.7% and roots 0.3%. This study suggests that alfalfa root N contribution from alfalfa was not significant in this intercropped system.

The final results of both experiments suggest that N immobilization is an important process in intercropping and no-tillage systems. The degree of legume suppression is the key paraameter that controls the availability of legume N to the second crop.

For Elizabeth and Shirley,

my best friends.

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# CHAPTER I

# INTRODUCTION

The value of legumes in cropping systems has been long known. It was recognized centuries ago when Virgil in 30 B.C. made the following observation:

> After the harvest let fallow fields lie at rest in succeeding years...Then you reaped the legume with shaking pod, the vetch and the lupine, sow your wheat or spelt.

Virgil obviously understood the value of legumes and advocated their use in cropping systems. After the decline of Rome, the practice of crop rotation, including leguminous crops, was lost for many centuries (Ripley, 1940). Probably the first intensive field work done in regard to rotations was that of Daubeney's (1845) in England, who grew oats, tobacco, flax, potatoes, beans and clover, continously and in rotations for 10 years (Ripley, 1940). He observed that crops grew better in rotation than in a monocrop system. Rotation experiments were also conducted at the Agricultural Experiment Station in Urbana, Illinois during the 1800's. The Morrow plots established in 1876 showed more legume crops in rotations raised both soil organic matter and N content (Anonymous, 1957). In the 1900's, scientists

spoke of the nitrogen benefits. Lyon (1936) observed gains in soil nitrogen after 10 years in which legumes were rotated with barley and rye. In similar studies Lyon and Bizzell (1936) reported increases in the protein percentage of timothy grass grown with alfalfa compared to timothy grass grown alone. Although the nitrogen benefits from legumes were seen, the picture for legume use in cropping systems became bleak during World War II when fertilizer production began.

With surging oil prices in the 1970's, interest was rekindled in legumes as a nitrogen source. Nitrogen fertilizer constitutes about 50% of the energy required for no-tillage corn production and about 30% of the total production costs (Martin and Touchton, 1983). Legumes may not only be economically valuable but use of perennial legume rotations in no-tillage systems have reduced erosion and improved water infiltration (Meisinger, 1984). Despite periods of declining use, the legume has once again become a valuable resource in today's modern agricultural systems.

## Rotation Systems

Legumes are grown with either one or two of the principle multiple cropping patterns: sequential cropping or intercropping (Andrews and Kassam, 1976). Sequential cropping is growing two or more crops in sequence on the same field per year. The succeeding

crop is planted after the preceding crop has been harvested. Crop intensification is only in time dimension and there is no intercrop competition (Andrews and Kassam, 1976). Intercropping, which was the primary interest of this research, is simultaneous growth of two or more crops on the same field. Crop intensification is both in time and space dimensions and there is crop competition during all or part of crop growth (Andrews and Kassam, 1976). There are several types of intercropping systems: mixed, row, strip, and relay intercropping. The goal of intercropping systems is utilizing extra time and spatial arrangements of companion crops with possible benefits of one crop to the other. This benefit, of course, depends on management.

The interaction of components in a mixture depends upon the proportion of interplant contacts between individuals of different components (Trenbath, 1976). In intercropping systems, plants share factors like light, temperature, nutrients, carbon dioxide, and water. Varying growth response depends on distribution of these factors in the soil system. For instance, in a strip intercropping system, most of the early interference and competition will be between plants of the same component. Plants in the strips will differ little from those of a sole crop. Gradually, however, starting at the interface between

strips, competition will develop between plants of different components and within component competition will be added to between component competition (Trenbath, 1976).

Light and $CO_2$

When crops are mixed, the photosynthetic canopy of one crop is set higher than those of another, the taller canopy intercepts more light. If soil conditions are non-limiting and crops are still vegetative, photosynthesis and growth rates of their canopies are nearly proportional to the radiation which they intercept (Trenbath, 1976). When small and large seeds of subterranean clover (Trifolium subterranean) produced a sward, plants from the small seeds were suppressed and obtained only 2% of the incident light after 82 days (Black, 1958). This is because in mixed intercrops where soil conditions are non-limiting and competition is only for light, slight differences in height, even early in growth, can lead to strong competition effects. Even during grain-filling in cereals, a height difference can profoundly affect the grain yield of shorter plants, mostly by reducing grain-size (Trenbath, 1976). This effect is reflected in the leaf area index (LAI). LAI is the leaf surface area over a given amount of land area. For example, one unit of LAI of prostate-leaved white clover (T. repens) absorbed 50% of the incident light

whereas the same LAI of erected-leaved perennial rye grass (Lolium perenne) absorbed only 26% (Brougham, 1958).

Shading is also an important component in the intercropping system. Usually shading by taller crop plants generally reduces photosynthesis rates in the lower canopy; however, if shading is not too intense plants in the shaded canopy will continue to grow and will adapt to lower light levels. This may, however, be complicated by multiple limiting factors. Short plants in dense monocrops will be light-stressed. This leads to carbon stress and, ultimately, low root/shoot ratios. Small roots, in turn, compete less effectively for soil factors than its shoot compete for light, and so the shoot becomes still less efficient through water and nutrient deficiency.

Water and Nutrients

Competition for water and nutrients is common in intercropping systems and has a profound effect upon the less competitive crop. First, roots of less competitive crops may develop less compared to more competitive crops, affecting absorption of soil nutrients and water. However, when water and nutrients are limited, the less competitive crop may develop adaptive features like increased root suction (Gardner, 1960), greater exudation of substances able to mobilize deficient nutrients (Brown and Ambler,

1973), or increased root elongation in the case of nitrogen deficiency (Bosemark, 1954). Compensatory root activity is often shown by part of the root system when another part of the root system is in a depleted soil zone. On the other hand, quite a different effect may be seen instead of the adaptive features. Competition between two plant species for water may lead to wilting and growth depression due to water stress. In the case of nutrients, visible symptoms of mineral deficiency and physiological impairment may be apparent (Salter and Goode, 1967; Donald, 1958).

Temperature

Intercropping systems which leave surface residues have two properties which tend to change the soil temperature compared with a bare soil. First, the color of the residue is usually, although not always, lighter than the soil (G.W. Thomas, 1986). When this is true, incoming radiation tends to be reflected more than it would from the surface of the soil itself. Second, the plant residue acts as an insulator and heat produced by solation does not reach the soil (G.W. Thomas, 1986). Therefore, the untilled soil temperature is usually lower than in a tilled soil.

The apparent advantages or disadvantages of this is seasonal and geographically related. The cooler

temperatures under residue systems often require later planting dates of subsequent crops because of poor seed germination. On the other hand, in the United States' southern regions or tropics, this may be beneficial because bare surface soil may reach high temperatures (50°C), damaging crop seedlings (G.W. Thomas, 1986).

## No-Tillage in Intercropping Systems

Intercropping using no-tillage techniques is an important concept in agriculture today. It was given little attention until the late 1940's when plant growth regulators were introduced and selective herbicides developed. With these two developments, intercropping became a more practical consideration for growing multiple crops without tilling the soil. In 1974, the United States Department of Agriculture (USDA) estimated that the amount of crop land in the U.S. under no-tillage agriculture was 2.23 million hectares, and that 62 million hectares or 45% of the total cropland will be under no-tillage by the year 2000 (Phillips et al., 1984). At least 65% of the seven major annual crops (corn, soybeans, sorghum, wheat, oats, barley, and rye) will be grown by the no-tillage system by the year 2000, possibly 78% by the year 2010 (Phillips et al., 1984). Some of these changes have already been seen in Kentucky where no-tillage corn and soybeans rose from 44,000 ha in 1969

to 220,000 ha in 1978. Although intercropping has been more commonplace in the tropics, the apparent advantages and similar yield data to conventional tillage indicates that it may be feasible in many parts of the United States. The major advantages of the no-tillage systems are: 1) reduced soil erosion (wind and water); 2) reduced labor and costs; 3) reduced moisture loss commonly associated with conventional tillage; 4) production of multiple crops per year; 5) maintenance of soil structure; 6) time saved in planting the second and third crops.

Erosion Control

Erosion control is an attractive feature of no-tillage intercropping systems. The United States Department of Agriculture estimates that each year nearly 5 billion tons of soil wash or blow from farmland in the United States. This is equivalent to losing the full plow layer from 5 million acres (Plaster, 1985). No-tillage systems reduce this amount. Some studies showed that this reduction approaches 40-50% with adequate ground cover (Stein et al., 1986). Studies in the 1950s at Clemson University showed that vetch and rye mulch averaged 3.11 inches less water runoff per year, 2.38 tons/A less soil erosion per year and that yield was equal or greater than plowed unmulched corn (Hargrove, 1982). In a study conducted by Sturgul and Daniel (1986),

alfalfa was no-till planted after corn and runoff, sediment concentration, and soil loss produced by two erosive rainfalls were measured. The no-tillage treatments with residue lost an averaged of 0.085 tons soil/acre for the rainfalls compared to no-tillage without residue which had a loss of 0.54 tons/acre. In Hawaiian studies on maize yield and soil loss with conservation and conventional tillage practices, soil loss with Crotolaria as a cover crop was 32% compared to chisel and moldboard plowing which were 57% and 61%, respectively (Fahrney et al., 1987).

Reduced Labor Costs

The Kentucky Farm Business Analysis Report shows a 50% labor reduction in land preparation and planting in favor of no-tillage (Phillips, 1984). Furthermore, typical acreages of no-tillage crops grown by farmers are 2-320 hectares per person as compared to 0.8-160 hectares per person for conventional tillage on mechanized farms (Phillips, 1984).

Reduced Moisture Loss

Soil water is used more efficiently by plants in no-tillage because of decreased water evaporation from soil and increased water infiltration into the soil. Blevins et al. (1971) determined soil moisture under killed sod and conventional tillage planted to corn. These studies indicated 19 percent higher moisture in

no-tillage plots with the highest amounts at the 0-75 cm depth.

Disadvantages

Although there are several advantages, the no-tillage system is not without disadvantages. Insect populations and plant pathogens and resulting crop damage may be higher than in conventional tillage systems because of a more favorable habitat. Soil temperatures are lower in no-till systems creating problems for temperate zone crops. However, in the tropics, this may be an advantage.

Where plowing is not applied, weed control will be a greater problem in no-tillage or conservation systems. This has been consistently demonstrated by several researchers (Moomaw and Martin, 1976; Wicks and Fenster, 1971; Williams et al., 1971; and Worsham and White, 1987). Studies in Nebraska show that application timing and proper herbicide selection are two factors that must be considered in the management scheme (Moomaw and Martin, 1976; Moomaw and Robison, 1971). Weed yields were significantly higher with fall-applied herbicides and greater reduction was seen with spring-applied herbicides. Proper herbicide selection must be carefully considered; however, this depends on geographical location and crop combination. Often proper herbicide combinations work more effectively than a single herbicide (Worsham and

White, 1987). Phillips and Phillips (1984) suggested that with intercropping or sequential crop change, weed control may be more effective than monocropping in no-till systems.

Finally, greater management is required for success in no-tillage systems. Fertilizer use and timing as well as planting techniques require careful planning and management.

Nitrogen Management in No-Till Intercropping Systems

Fertilizers constitute about 78.4% of the total energy required for no-tillage corn production with nitrogen contributing 67.7% of the total energy (Hargrove, 1982). Escalating fertilizer costs and nitrate groundwater contamination, have renewed interest in alternate nitrogen sources.

The terrestrial nitrogen cycle is dynamic and very different processes predominate under different tillage systems. Nitrogen, once mineralized to ammonia, has several possible fates: 1) nitrification--ammonia is converted to nitrite then to nitrate by Nitroso-type sp. and Nitrobacter sp., respectively; 2) plant uptake; 3) immobilization--absorbed by microbes and assimilated into cell components; 4) leaching; 5) immobilization in clay lattices; 6) volatilization at high pH; 7) dissimilatory reduction to $NH_4^+$, and 8) denitrification--reduction of $NO_3^-$ to $N_2O$ or $N_2$.

These fates may be controlled by management practices. For instance, denitrification is a significant process in no-till versus plowed systems. Rice and Smith (1982) measured denitrification using intact cores on different soils and found more $N_2O$ produced under no-tillage than conventional-tillage soil. Ratios of no-till rates in the Maury silt loam to conventional tillage ranged from 1.5 to 77. Although the no-till soils had consistently higher denitrification activity, this does not necessarily implicate tillage per se in all cases. Rather, it relates more to higher moisture contents in no-till due to the residue cover. Studies of aerobic and anaerobic microbial populations by Linn and Doran (1984) in no-till and plowed soils further suggests that this is likely the case.

Surface soils from long-term tillage comparison experiments at six United States locations were characterized for aerobic and anaerobic microbial populations and denitrification potential using an in-situ acetylene inhibition technique. In no-till soils numbers of aerobic and anaerobic microorganisms in the surface (0-75 mm) averaged 1.55 to 1.41 and 1.27 to 1.31 times greater, respectively, than in surface-plowed soils. To further substantiate this finding, denitrifying activity, after irrigation, was greater in the no-till soil than in the conventionally

tilled soils at all locations. Again, as with Rice and Smith (1982), Linn and Doran (1984) suggest that the observed results are due to greater bulk density and water which acts to increase water-filled porosity and the potential for water to act as a barrier to the diffusion of oxygen through the soil profile.

Nitrogen Transfer

The literature concerning legume N transfer shows limited understanding and obvious conflict in conservation systems (no-till or intercropped) which is neither recent nor resolved. Early, researchers studied factors influencing nitrogen excretion from legume nodules. Virtanen, a Finnish scientist, led the path in investigating this phenomena. Virtanen et al.'s (1937) greenhouse and laboratory experiments demonstrated that nodules can excrete significant amounts of the total fixed nitrogen depending on which factor was limiting such as compatible strain/host combination or plant age. They also proposed and demonstrated that the extent of excretion is greater when the legume is grown in association with some non-legume, whose roots continually absorb nitrogen compounds excreted from the nodules, than when the legume grows alone. Wyss and Wilson (1941) reported negative results as well as positive results on nitrogeneous excretion. When legume growth was restricted, greater excretion was observed. Scholz

(1939) observed little benefit to the associated non-legume even though large quantities of excreted nitrogen were present in the substrate (Wyss and Wilson, 1941). He suggested that microorganisms in the soil immobilize the nitrogen as it is excreted and renders it unavailable.

Many field and greenhouse experiments have been conducted without resolving questions concerning nitrogen transfer. Vallis and colleagues (1967) conducted longer experiments compared to earlier studies to examine nitrogen uptake and transfer. After 9 weeks, the cumulative uptake of soil nitrogen by the lucerne was only 6% and 3% at 13 weeks. No significant transfer of unlabeled nitrogen from legume to grass was detected. Nitrogen transfer depends on legume management. Henzell and Vallis (1977), suggest that pasture legumes are weak competitors for mineral N uptake when grown with pasture grasses. The same assumption may not hold when legume and nonlegume crops are grown together. Several researchers have reported that in many cases the legume crop may provide a substantial portion, if not all, of the nitrogen fertilizer requirements (Triplett et al., 1979; Ebelhar et al., 1984). Mitchell and Teel (1977) reported that hairy vetch (Vicia villosa Roth.) and crimson clover produced grain yields comparable to those obtained by the

application of 112 kg N $ha^{-1}$. Baldock and Musgrave (1980) reported N fertilizer equivalent value of 86 kg N $ha^{-1}$ for alfalfa in an alfalfa-corn rotation. With the advent of $^{15}N$ methodologies, more accurate results are being obtained which suggest that transfer may not be as great as once thought.

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# CHAPTER II

## FATE OF N-MINERALIZED FROM $^{15}N$-LABELED ALFALFA IN NO-TILL INTERCROPPED CORN

### Introduction

Nitrogen management is one of the greatest challenges in producing corn (Zea mays L.) under no-till systems (Ebelhar et al., 1984). While the role of legumes in providing nitrogen to subsequent crops has been long recognized, quantifying the amount of legume nitrogen transferred to a subsequent crop has not been accurately assessed either in no-till or in intercrop systems. This has been hampered by lack of suitable methods. Those commonly used are total Kjeldahl nitrogen in legumes, calculating the fertilizer replacement value (the equivalent amount of inorganic fertilizer nitrogen required to produce a yield following a legume) and tracing $^{15}N$ from labeled legume residues. Of these methods, the $^{15}N$ tracer technique is most useful because it actually traces the $^{15}N$ released from legume residues and subsequent crop uptake.

Typically, in a no-till system the nitrogen contribution from $^{15}N$-labeled tops is measured but root nitrogen is not assessed. If root nitrogen is

assessed, then the soil and roots are disturbed and the roots are mixed into the soil, typifying a plowed system. **Therefore, the first objective was developing a system for measuring the nitrogen contribution from legume tops and intact roots in an undisturbed system (either no-till or intercropped).**

Many factors influencing the quantity of nitrogen transferred to subsequent crops: the amount of legume residue returned to the soil; the proportion of legume N derived from symbiotic activity; the availability of N from decomposing legumes for uptake by non-legumes. Underlying these factors, legume species, management practices, and environmental conditions play a significant role in legume N transfer. With forage legumes, significant N transfer occurs after the plant reaches maturity, or when the plant is stressed through shading, low temperatures, and repeated defoliation (Haystead and Marriott, 1978). Henzell and Vallis's (1977) proposed pathway summarizes the flow of N from legumes to other crops (Figure 1).

Studies have been done to compare rotation and non-rotation systems. The yields of corn grown in an alfalfa, oats rotation were compared with continuous corn on a Brookston clay soil (Bolton et al., 1976). The 2-year alfalfa sod system produced a significant yield increase over continuous corn and 1-year alfalfa sod system. Yields varied widely from season to

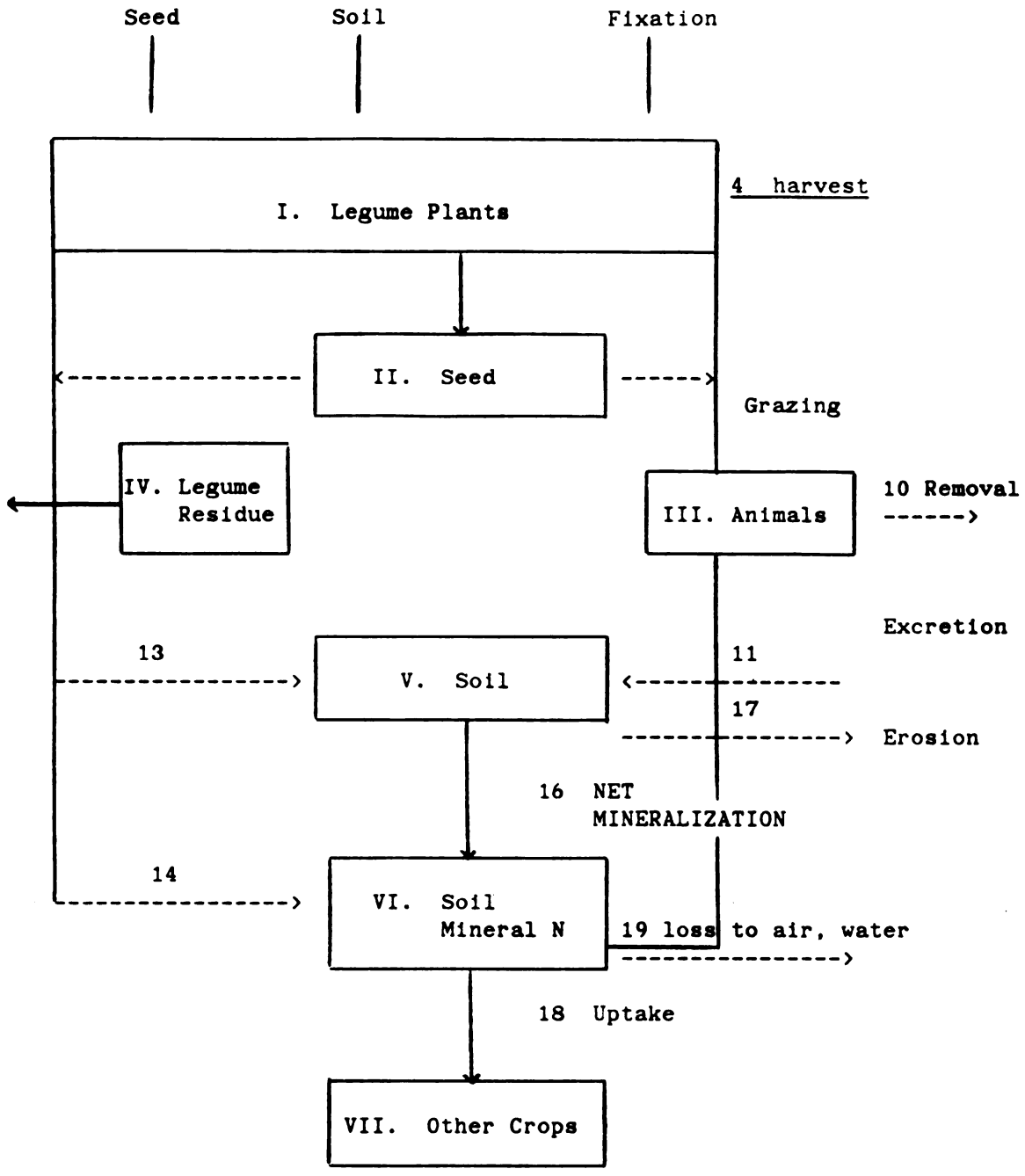


Figure 1. Pathways for flow of N from legumes to other crops

season according to moisture conditions, but always responded to alfalfa in the rotation, particularly where no fertilizer was applied.

Baldock and Musgrave (1980) suggest that the legume crop could completely supply the fertilizer nitrogen requirement of a subsequent crop without leading to a decrease in soil fertility. Two years of alfalfa (Medicago sativa L.) contributed 135 kg N $ha^{-1}$ to a subsequent crop which is adequate for seasonal growth. Further studies showed that estimates of nitrogen added to soil by a seeding-year alfalfa stand ranged from 31 to 151 lbs per acre. Boawn et al. (1963) found that significant N was available from alfalfa to produce 112 kg corn $ha^{-1}$ for two years without N fertilization and that N uptake from these plots was equivalent to the N uptake from plots receiving 224 kg N $ha^{-1}$. On the other hand, Ladd et al. (1981, 1983) incorporated $^{15}N$ labeled medic (Medicago littoralis L.) residue and, following an eight-month incubation, measured $^{15}N$ uptake by wheat (Triticum aestivum L.) for a range of soil types and environmental conditions. Wheat recovered 11-28% of the incorporated legume N. These percentages are significantly less than those reported by the other common methods, suggesting that legume N is overestimated. Schulz (1985) suggested that corn yields were not influenced by nitrogen from actively

growing legumes. Further $^{15}N$ studies also indicate that only 15 to 25% of the legume symbiotic N will be recovered by the first subsequent nonlegume crop and perhaps another 4% by the second. Harris and Hestermann (1987) found that whole corn plants recovered an average of 16.6% and 25.0% of $^{15}N$ from incorporated alfalfa residues at the East Lansing and Kellogg experiment stations, respectively. Greenhouse studies examining $^{15}N$ uptake in a Rhodesgrass/lucerne mixture indicated no significant transfer of legume N to grass (Vallis et al., 1967). These contradictory results show an evident need to better understand the N contribution of legumes in intercropping systems. Therefore, **the second objective was determining the quantity and proportion of alfalfa nitrogen recovered by intercropped corn from alfalfa tops and roots versus the proportion that goes to microbial biomass, alfalfa regrowth and soil mineral nitrogen (Figure 2).**

To establish nonlegumes (such as grain) in perennial legume intercropping systems, some treatment must be applied to control the actively growing plants. They can be chemically suppressed, mowed, or completely killed by herbicides. Schulz's (1985) data indicates that appreciable nitrogen release will only occur if the legume is killed or heavily suppressed. The small amounts of nitrogen released is likely caused by inadequate initial legume suppression due to

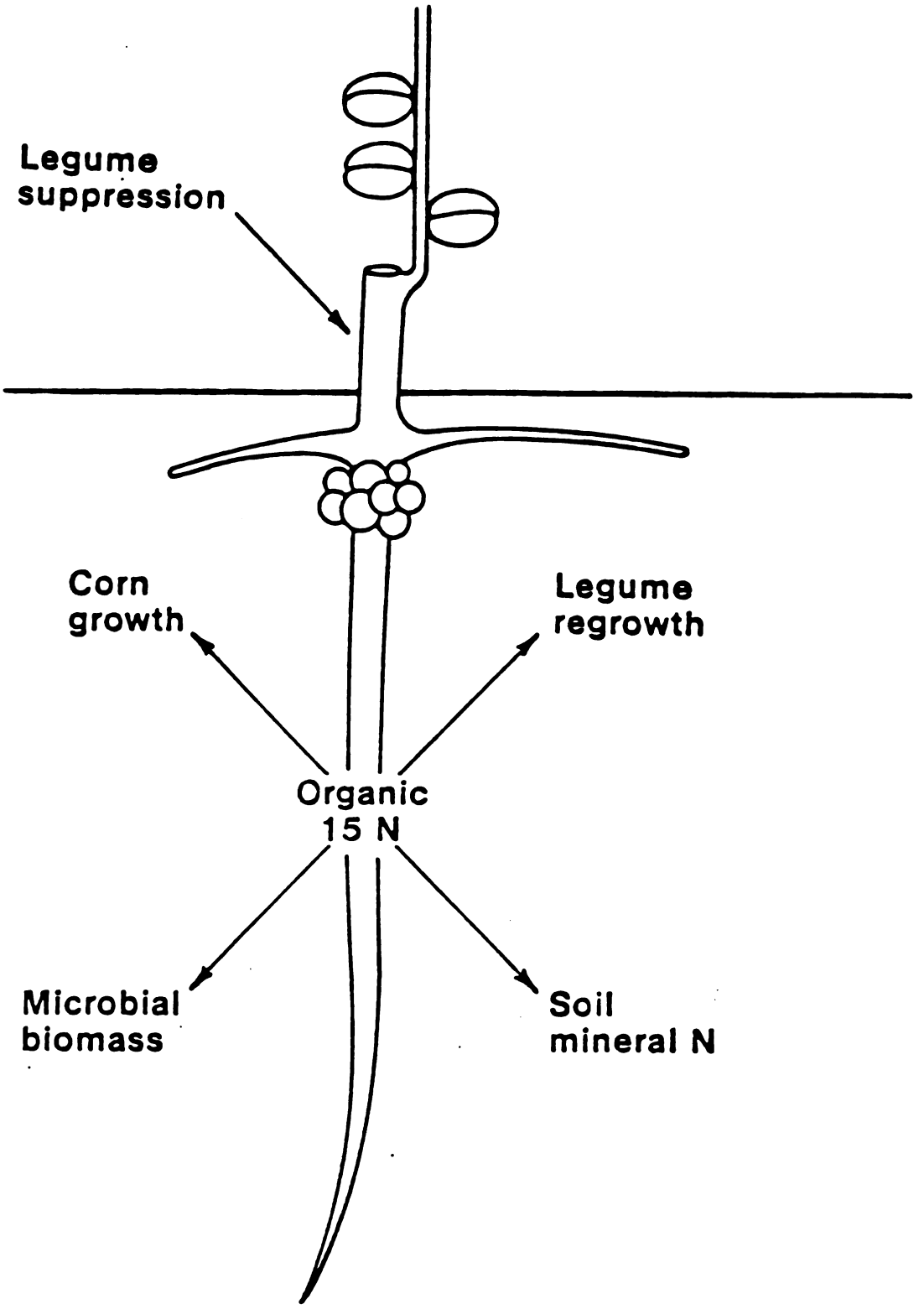


Figure 2. Potential fates of legume N ($^{15}N$-labeled) in intercropped soil following suppression of the legume crop.

inappropriate herbicide rates or adverse conditions resulting in poor seedling growth due to a dense canopy, and to the vigorous regrowth of plants such as alfalfa outcompeting the grain crop and "mining" the soil for nutrients and water. Minotti and Grubinger (1986) examined sweet corn yield response in a white clover intercrop using different suppression treatments. The suppression treatments included rotovation, mowing five times, and mowing two times. Yield responses were significantly larger in the rotovation treatment compared to the mowing treatment suggesting that the level of suppression may reduce legume competitiveness allowing the corn to become adequately established and produce greater yields.

Wells et al. (1979) reported a 4-year rotation study in which no-till corn first grew two years in killed grass sod then in killed red clover sod for the last two years of the rotation. Their results from zero-N treatments showed that the average yield of no-till corn grown in grass sod was 590 kg grain/ha compared to 834 kg grain/ha for no-tilled corn in red clover. In other studies no-till crop yields have been improved by killing the cover crop 10 to 20 days prior to planting summer crops (Martin and Touchton, 1983). The benefit of complete-killed treatments is seen in improved yields. However, the advantages of suppression treatments may be more promising in the

long run if the legume can be appropriately managed to not reduce grain yields. Suppression treatments eliminate annual establishment costs, can provide a high-fiber protein feed for livestock, preserve top soil better by reducing erosion and can be a dynamic recycling machinery for limited nutrients. **Therefore, the third objective was comparing and determining nitrogen transfer under suppression and complete-killed treatments at different intervals (2 and 4 months).**

## Materials and Methods

### A. Preliminary Experiment

To delineate the N contribution from suppressed alfalfa roots versus tops to corn plants, a method was needed for labeling the entire plant via the shoots. A preliminary experiment was performed to determine the most effective method for applying $^{15}N$-urea (99% enriched) to alfalfa while minimizing contamination of the underlying soil. Spraying or dipping the plants into the desired solution were acceptable foliar feeding procedures. The four treatments were spraying only, spraying plus Triton X-100, dipping only, dipping plus Triton X-100 and a control which was neither dipped or sprayed. Triton X-100 is a surfactant which permits better penetration of applied nutrients. The plants grew approximately four months in washed sand pots and $^{15}N$ solutions and were foliar-fed twice a week for two weeks beginning late in the growth stage. Immediately after harvesting, each plant part was washed. Samples were dried at 65$^{o}$C. After drying, they were ground through a 2mm mesh screen and analyzed for total N and $^{15}N$ content.

### B. Greenhouse Experiment

#### Experiment 1

A greenhouse experiment examined the contribution of $^{15}N$-labeled alfalfa shoots and roots to a subsequent corn crop. The experiment had three

treatments and six replications (Appendix 1 & 2). The treatments included: glyphosate only (complete-kill), glyphosate followed by cutting, and cutting only.

1. Soil Preparation

A Kalamazoo sandy loam (fine-loamy, mixed, mesic Typic Hapludalfs) was collected from Kellogg Biological Station, air dried, and passed through a 6mm sieve, and 5 kg of soil was weighed into each of 54 (20.3 cm diameter) pots. The general physical and chemical properties of the soil are presented in Table 1. A protection apparatus was designed to prevent $^{15}N$-urea solution from contaminating the soil (Appendix 3).

2. Culture Preparation

To prevent effective nitrogen-fixation and to ensure that foliar feeding was the major nitrogen source, the soil was inoculated with a competitive strain of ineffective Rhizobium. The infective but ineffective strain (Rhizobium meliloti #191) was obtained from Dr. P. Bottomley at Oregon State University. The R. meliloti was grown in a yeast extract/mannitol broth containing per L of distilled water: mannitol 10g, yeast extract 0.4 g, $K_2HPO_4$ 0.5 g, $MgSO_4*7H_2O$ 0.2 g, NaCl 0.1 g. The broth was autoclaved at 121$^oC$ for 15 min. The culture was incubated 3 days on a rotary shaker at room

Table 1. Soil Characteristics

| Soil Series | Classification | Vegetation | Texture | pH | Total N g $Kg^{-1}$ | Total C g $Kg^{-1}$ |
|---|---|---|---|---|---|---|
| Kalamazoo | Typic hapludalf | Corn-Alfalfa rotation | Sandy Loam | 7.34 | 2.00 | 14.10 |

temperature prior to inoculation. The soil was evenly dispersed and a sterile syringe was used to spread 50 ml of culture throughout the 5 kg of soil. In addition, seeds were presoaked approximately 4h in the R. meliloti culture before planting. The number of rhizobia added to soil was $10^8$org. $g^{-1}$ soil, adequate to outcompete an indigenous R. meliloti population of $10^6$org. $g^{-1}$ soil.

3. Legume Establishment in Protection Apparatus

A protection apparatus was designed to minimize soil contamination (Appendix 3). Holes were punched into rectangular sheets of plastic. A circular wire ring was designed to fit on top of the plastic to hold it firmly in each pot. Once the plastic sheets were firmly placed in each pot, seeds were planted into each hole. Twenty-five pre-soaked alfalfa (Medicago sativa var. Vernal) seeds were initially planted into each pot. Vernal alfalfa was chosen because it has a high level of winter hardiness, resistance to bacterial wilt and is currently grown on 25-30% of alfalfa acreage in the North Central States (Lowe et al., 1972). Due to the extremely high temperature in the greenhouse, a few pots were replanted. Cotton was placed around each plant to cover the holes in the plastic sheet after plants had grown two weeks. Alfalfa establishment and growth period was 13 weeks which included eight weeks for foliar feeding.

4. Nutrient Solution Preparation

A N-free nutrient solution was prepared containing: 0.5 M $K_2SO_4$, 1 M $MgSO_4$, 0.05 M $(H_2PO_4)_2$, 0.01 M $CaSO_4$, and standard micro-nutrients. Before planting, 200 ml of nutrient solution was added to each pot followed by 1500 ml of tap water. Plants were individually watered from the top through the holes in the plastic protection sheet using a plastic wash bottle before any foliar treatment. After foliar treatment began, plants were watered from the bottom by applying 500 ml of tap water to the pot holder.

5. Foliar Feeding to Obtain $^{15}$ N-labeled Alfalfa

Spraying was the method selected for foliar feeding. A hand sprayer was used to apply 99% (enriched) $^{15}N$ urea to the foliage twice a week after 18:00 h to reduce the chance of foliar burn (Harper 1984). Urea was selected as the nitrogen source because it has a lower salt index and is less likely to cause foliar burn than other nitrogen sources. Harper (1984) reported 80% or more of the urea-N was absorbed within 48h after application. Urea penetrates the cuticular membrane at a rate of 10-to-20 fold higher than other ions or compounds, and the penetration rate is independent of concentration (Franke, 1967). Tolerance of plant foliage to repeated applications is 1.92 g N $liter^{-1}$. Alfalfa plants tolerate 1.2 to 1.4 g N $liter^{-1}$ (Wittwer,

1967). In this experiment, 1.2 g N liter$^{-1}$ was used. The actual amounts and treatment periods are given in Table 2.

6. Treatments of the Established $^{15}$N-labeled Alfalfa

Three different treatments were applied after foliar labeling was complete. A schematic diagram of the treatment application and experimental time frame are presented in Appendix 4. Two levels of suppression and complete-kill were used as treatments. Suppression 1 was glyphosate application followed by cutting 4h later. Suppression 2 was cutting only. The alfalfa was cut 5.1 cm from the soil surface. This was done one week after the last foliar application. The shoots and roots were dried at 65$^{o}$C and 25g of dry top material containing 0.69g N was put on the 0.035m$^{2}$ soil surface. Corn (Zea mays L. variety GL 5922) was planted 5 days after cutting. The variety was chosen for its success in previous greenhouse experiments (seeds were obtained from E. Rossman, Michigan State University). The plants were thinned to one corn plant per pot approximately 7 days following planting. A second set of pots were grown for eight weeks, constituting time period 2, and then harvested. A third set of pots was grown for an additional 16 weeks constituting time period 3. These plants were harvested for final plant analyses.

Table 2. Spraying Treatment Calculations

| * Spraying Times | No. of $mls^{-1}$ | No. of pots | Total No. of mls | Amt. of stock soln. ml |
|---|---|---|---|---|
| week 1 | 5.00 | 54 | 270.00 | 21.60 |
| week 1 | 6.66 | 54 | 360.00 | 29.00 |
| week 2 | 8.32 | 54 | 449.30 | 36.00 |
| week 2 | 9.98 | 54 | 539.00 | 43.11 |
| week 3 | 11.64 | 54 | 629.00 | 50.30 |
| week 3 | 13.30 | 54 | 718.20 | 57.50 |
| week 4 | 14.96 | 54 | 808.00 | 65.00 |
| week 4 | 16.62 | 54 | 897.50 | 72.00 |
| week 5 | 18.28 | 54 | 987.30 | 79.00 |
| week 5 | 19.94 | 54 | 1077.00 | 86.13 |
| week 6 | 21.60 | 54 | 1166.40 | 93.31 |
| week 6 | 23.26 | 54 | 1256.04 | 100.50 |
| week 7 | 24.92 | 54 | 1346.00 | 108.00 |
| week 7 | 26.58 | 54 | 1435.32 | 115.00 |
| week 8 | 28.24 | 54 | 1525.00 | 122.00 |
| week 8 | 29.90 | 54 | 1615.00 | 129.17 |

Calculations:

1) No. of $mls^{-1}$ pot
   y = b + ax
   y = 5 + 1.66 (1)
   y = 6.66
   5 ml = 0 time - 1st week
   x = spray no.
   6.66 ml = time 1 or 2nd week

2) Total no. of mls
   5 x 54 = 270 $ml^{-1}$
   treatment period
   zero

3) mls of stock soln.
   Given:
   $\frac{1.2g}{1}$ Urea N = 20 mmole
   $15g^{-1}$1 (99% $^{15}N$ urea)
   = 0.015 $g^{-1}ml$
   Calculation:
   1) $1.2g^{-1}ml$ x 270 = .324g
   2) 0.015 g (Xml) = .324g
      X = 21.60 ml stock soln.

*Application time - spraying time after three weeks of plant growth.

Complete-kill was achieved by using glyphosate which slowly but permanently kills alfalfa. Corn was planted in each pot as described in suppression treatments. It was planted approximately 7 days after the herbicide treatment. The general scheme is shown in Appendix 5. Glyphosate was applied at a 2 1/2 percent concentration by hand spraying a set of six pots each.

During harvesting, plant height and leaf number was taken. Corn height was measured in centimeters by placing a meter stick at the base of the corn plant and reading the corresponding value. A total corn leaf number was recorded as well as a number for fully developed leaves.

7. Preparation of Plant and Soil Samples for Analyses

Plant Samples

Immediately after harvesting, each plant part was washed in water, dried at 65$^{o}$C for 2 days, weighed, then ground in a Wiley Mill 2 mm sieve screen and further ground in a circular bar grinder which produced a fine-powdered material for $^{15}N$ analyses.

Soil Samples: Soil samples were air-dried and subsampled for grinding on a circular bar grinder to produce a fine-powdered soil for $^{15}N$ analyses.

Leaf Area: A total leaf area was taken immediately after harvest where possible or samples were immediately placed in the refrigerator (4.0$^{o}$C).

The samples were analyzed the following day. Each corn leaf was carefully separated from the corn stalk and passed through a light sensing Licor leaf area machine which calculates the leaf area ($cm^2$) using the following formula:

$$LAI = \frac{\text{leaf surface area}}{\text{land area}}$$

8. Analytical Procedures

Kjeldahl N: Total and $^{15}$ N-labeled N

Total N analysis was determined on soil and plant samples by the salicylate method using a flow-injector analyzer (Lachat) after a standard Kjeldahl digestion (Bremner, 1965). Samples were digested in a block digestor at 360°C for 3 h with 3 ml of concentrated 5 M $H_2SO_4$ and Kjeltabs (1.5 g $K_2SO_4$ + 0.0075 g Se). After digestion, samples were cooled for no more than 10 min and diluted to 100 ml. Ammonium ($NH_4^+$) was determined colorimetrically by taking a 3 ml-aliquot for injection into a Lachat FIA.

The $^{15}N$ content of plant and soil materials was determined on a Tracermass continuous flow isotope mass spectrometer after conversion of sample N to $N_2$ by Dumas combustion on a Roboprep CN analyzer (Europa Scientific Ltd.) (Preston and Owens, 1983). Finely ground (< 125 µm) samples were weighed into tin cups (6 x 4 mm). These were fed by an autosampler into the combustion tube ($Cr_2O_3$ at 1000°C) of the CN analyzer.

Concurrent with sample introduction, the He carrier gas is replaced by a pulse of $O_2$. Flash combustion of the sample and tin cup raises the local temperature to about 1700°C. This and the highly oxidizing environment produces complete combustion of the sample. The reaction products are carried by the He gas stream through a reduction tube (Cu at 550°), water and $CO_2$ traps [$Mg(ClO_4)_6$ and 'carbosorb'] to a GC column (Carbosieve) which separates and resolves the $N_2$ into a peak. A splitter valve regulates the flow of a small portion of the effluent gas stream into a triple collector isotope ratio mass spectrometer via a capillary interface. A desktop computer controls the events during the sample cycle and acquires and stores data from the mass spectrometer.

Inorganic N: Soil samples were analyzed for inorganic N by extracting 20 g of moist soil with 100 ml 1 M KCl by shaking for 1 h and filtering with #18 Whatman filter paper. The extract was stored at 4°C until analyzed for $NH_4^+$ and $NO_3^-$. Nitrate and ammonium concentrations were determined colorimetrically (Keeney & Nelson, 1982) using the FIA. Concentrations were adjusted to a soil-dry weight basis.

Microbial Biomass C and N: Microbial C and N were determined using the modified procedure of Voroney and Paul (1983). Before fumigation soil

samples were adjusted to 25-30 g $H_2O$ 100 $g^{-1}$ soil, placed into mason jars (2L wide mouth), and preincubated 5 days at 25°C to obtain an active microbial population.

Two replicate soil samples (20g) were placed in 150 ml glass beakers or 130 ml plastic specimen cups; one was to be fumigated and the other unfumigated samples. Soils were fumigated for 24 h by ethanol-free $CH_3Cl$ in a 2 L glass vacuum dessicator lined with moistened paper towels, transferred to a clean dessicator, and all residual chloroform vapour removed by repeated evacuation. The samples were then placed in 2 L wide mouth mason jars containing a 100 ml plastic vial with 1 ml of 2 N NaOH to absorb evolved $CO_2$. Each mason jar was sealed with a fresh rubber lined lid and incubated for 10 days at 25°C.

Determination of $CO_2$ -C Evolved: At the end of the 10-day incubation, vials were removed from the mason jars and capped until analysis. The base trap was titrated with 0.1 M HCl to determine the volume of acid needed to decrease the pH of the solution from 13.00 to 7.00.

Microbial C Calculations: Microbial C was calculated from the amounts of $CO_2$-C evolved from fumigated and unfumigated sample during the 10-day incubation.

Three calculation methods were employed:

1. Microbial C = ($CO_2$-C evolved from fumigated-unfumigated soil/Kc [Jenkinson, 1966]).

2. Microbial C = $CO_2$-C evolved from fumigated soil/Kc (Voroney and Paul, 1984).

3. Modified version of 1 and 2 (Horton, 1987).

Kc is the fraction of microbial C mineralized over a 10-day incubation, and equals 0.41 at 22°C (Anderson and Domsch, 1978).

Microbial Biomass N: At the end of the 10-day incubation, soil samples were ammended with 100 ml of 1 M KCl and stored at 4°C and extracted as described for inorganic N. To determine the $^{15}N$ content, 20 ml of solution was placed into 100 ml specimen cups. To collect $^{15}N$, a chromel stainless wire holding a piece of fiberglass paper onto which 10 ul of 1 M HCl had been pipetted was placed into each specimen cup (modified procedure of Turner and Bergersen, 1980). Two milligrams of MgO and then Devarda's alloy was added to the solution to recover $NH_4^+$ and $NO_3^-$, respectively.

Microbial N Calculations: Microbial N was calculated from the net accumulation of exchangeable $NH_4^+N$ (exchangeable $NH_4^+$-N accumulated in the fumigated sample at the end of 10 days/Kn (Horton, 1987, personal communications).

Total Carbon: Duplicate samples were weighed into 25 x 200 mm Pyrex culture tubes; 15 mg plant

material or 150 mg for soil samples. One gram of potassium dichromate was added to each sample, mixed and 12 ml of a 3:2 mixture of 8M sulfuric/13.7 M phosphoric acid was added. A 2 ml 2M NaOH base trap tube was immediately placed in the tube and capped. These tubes were placed in a digestion block at 110°C for 2 h, then removed and stored overnight. The samples were then titrated using 0.1 M HCl with phenolphathalein solution as an indicator after precipitating the carbonate with $BaCl_2$.

## Results and Discussion

### A. Preliminary Experiment

In most treatments the roots received a small amount of $^{15}N$ (Table 3). There are two possible reasons why only a small amount reached the roots: 1) since alfalfa was nodulated, $N_2$ fixation may have retarded foliar absorption and translocation of the applied nitrogen, and 2) the spraying may have been at too late of a growth stage. With these two reasons in mind, I felt that foliar application was still a feasible means of translocation of labeled N to the roots.

All the dipping treatments were only slightly greater in $^{15}N$ content than the control. Dipping treatments which included Triton X-100 were slightly higher than dipping alone. An opposite effect was seen in the spraying only treatments which contained a greater percentage of $^{15}N$. The presence of the surfactant did not substantially increase the $^{15}N$ content in the plant, therefore, the surfactant was not included in the foliar application protocol for the main experiment. Because of a more practical pot design setup (Appendix 3), and slight differences between spraying and dipping treatments, spraying was the chosen method of application.

Table 3. Percent $^{15}N$ in Alfalfa after Foliar Application

| Treatment | Tops | Roots |
|---|---|---|
| | % $^{15}N$ | |
| *Control | 0.3730 | 0.3734 |
| Dippling Only | 0.3840 | 0.3772 |
| Dipping + Triton | 0.3937 | 0.3841 |
| Spraying Only | 0.7882 | 0.5783 |
| Spraying + Triton | 0.6489 | 0.3805 |

* no dipping or spraying

## Soil Contamination versus Root Turnover

Since one objective of the experiment was to minimize contamination of the soil, a protection apparatus was designed to prevent $^{15}N$ urea (99% enriched) from entering the soil (Appendix 3). The results of using this method, however, indicate that a high percentage of the $^{15}N$ urea solution still entered the soil by stem runoff. $^{15}N$ analyses were done for surface and the remaining soil portion for the time zero period. The surface soil contained an average of 0.625 atom % excess compared to the remaining portion of soil which contained an average of 0.410 atom % excess. Several assumptions and calculations have been made to help resolve how significant soil contamination was versus root turnover as the source of the surface soil N. Calculations are presented on the following page. The amount of soil $^{15}N$ due to root turnover and to soil contamination was calculated to be 10-15% and 85-90%, respectively. The soil contamination was greater than expected and several modifications would be needed in the experimental design to successfully evaluate N transfer from roots to the other compartments studied.

Calculations of $^{15}N$ add to produce (.625 atom%) $^{15}N$ at the end of foliar application period.

1.) $\frac{5000g \times .19\%}{100}$ N x $\frac{.37}{100}$ atom % $^{15}N$ = 0.035 g $^{15}N$ (amt. of $^{15}N$ at the beginning of the experiment)

2.) $\frac{5000g \times .21\%}{100}$ N x $\frac{.625}{100}$ atom % $^{15}N$ = 0.065 g $^{15}N$ (amt. of $^{15}N$ at the end of the experiment)

3.) 0.065 - 0.035 = 0.029 g $^{15}N$ amt. required to obtain a .625 $^{15}N$ enrichment

4.) 0.0024 g $^{15}N$ - 8.7 g of roots will produce this amt. of $^{15}N$

5.) Therefore, approx. .609 % of Roon N is needed to produce 0.029 g $^{15}N$ or 60.9 g roots.

Amt. due to Root turnover = approx. 10-15%.

Amt. due to Soil Contamination = approx. 85-90%

## B. Greenhouse Experiment 1

### General Physical Appearance of Plants throughout the growing period.

#### Alfalfa

After the foliar application period (8 weeks), six pots were harvested for analyses. The shoot portion was green and showed no nutrient deficiencies. This was the case throughout the growing period. The roots were practically devoid of nodules at time zero. The few nodules present were a dull white in color outside and located on the lateral roots where the ineffective Rhizobium inoculum may have been less numerous. The inside of the nodule was examined for leghemoglobin, which gives a red or pinkish pigmentation, indicating strain effectiveness. A dark brown or greenish color was observed inside the nodules indicating strain ineffectiveness.

#### Corn

The corn was green with no apparent nutrient deficiencies until after the two month harvest. The corn plants began to turn purple along the veins indicating a phosphorus deficiency. Nutrient solution was added to correct the deficiencies. Pesticide treatments were also used to combat any insect infestation problems. However, these measures did not sufficiently correct the problems. Watering the

plants only once a day as opposed to twice may have also contributed to the problem. Pot size (20.32 cm) may have contributed to the problem since the corn root growth was restrained. This was even further complicated by intercropping with alfalfa where competition was apparent.

Plant Growth

The differences between treatments were clearly reflected in corn plant growth. At two months, a plant height of 80.6 and 62.5 cm and plant biomass of 11.2 and 3.95 g was observed for herbicide + cutting treatment versus cutting only treatment (Figure 3). Greater plant growth was seen with the herbicide + cutting treatment where the alfalfa had been severely repressed.

Regrowth of alfalfa suppressed by cutting alone was vigorous, while regrowth of alfalfa treated with herbicide and cutting was delayed and reduced. The additional suppression of alfalfa growth by the herbicide treatment thus reduced competition between the corn and alfalfa regrowth, allowing the corn plants to recover much more of the mineralized N. These results were consistent with field research using chemical or mowing suppression treatments for grain establishment (Elkins et al., 1979; Minotti and Grubinger, 1987). Elkins et al. (1979) investigated the feasibility of chemically suppressing grass sod

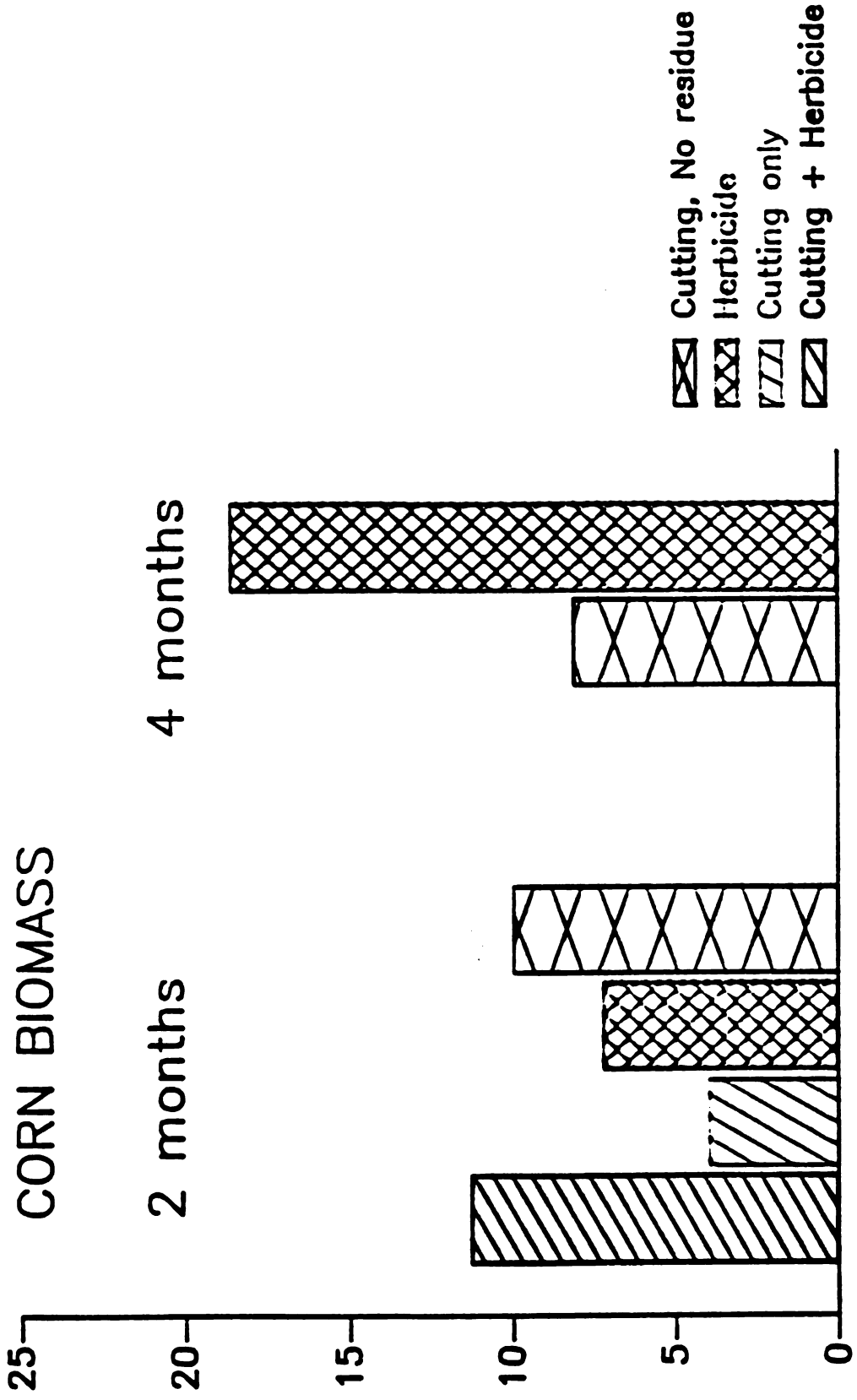


Figure 3. Plant biomass in different treatments over time.

for maize production. Maleic hydrazide was the best retardant and glyphosate in combination with atrazine was slightly less effective. It was possible to obtain good maize yields while maintaining at least 50% of the grass sod with little or no erosion observed.

At four months plant biomass as well as plant height was greater with herbicide only treatment, because there was no competition with alfalfa for available nutrients and moisture (Figure 3). However, at four months plant biomass and height (9.79 g and 75 cm, respectively) was slightly reduced compared to two (10.3 g and 89.6 cm, respectively) with the cutting only treatment (-residue) where greater competition is apparent.

Leaf Area

A pattern similar to that for corn biomass was observed for leaf area (Figure 4). At two months, a greater leaf area was seen in the herbicide + cutting treatment (5.63) compared to the cutting only treatment (2.85). The herbicide only treatments had more biomass due to less or no competition with alfalfa resulting in greater leaf area. For each crop there was an optimum total leaf surface per land area for optimum photosynthesis. The LAI is significant in determining appropriate seedling rates and planting patterns (Chapman and Carter, 1976). In pastures or

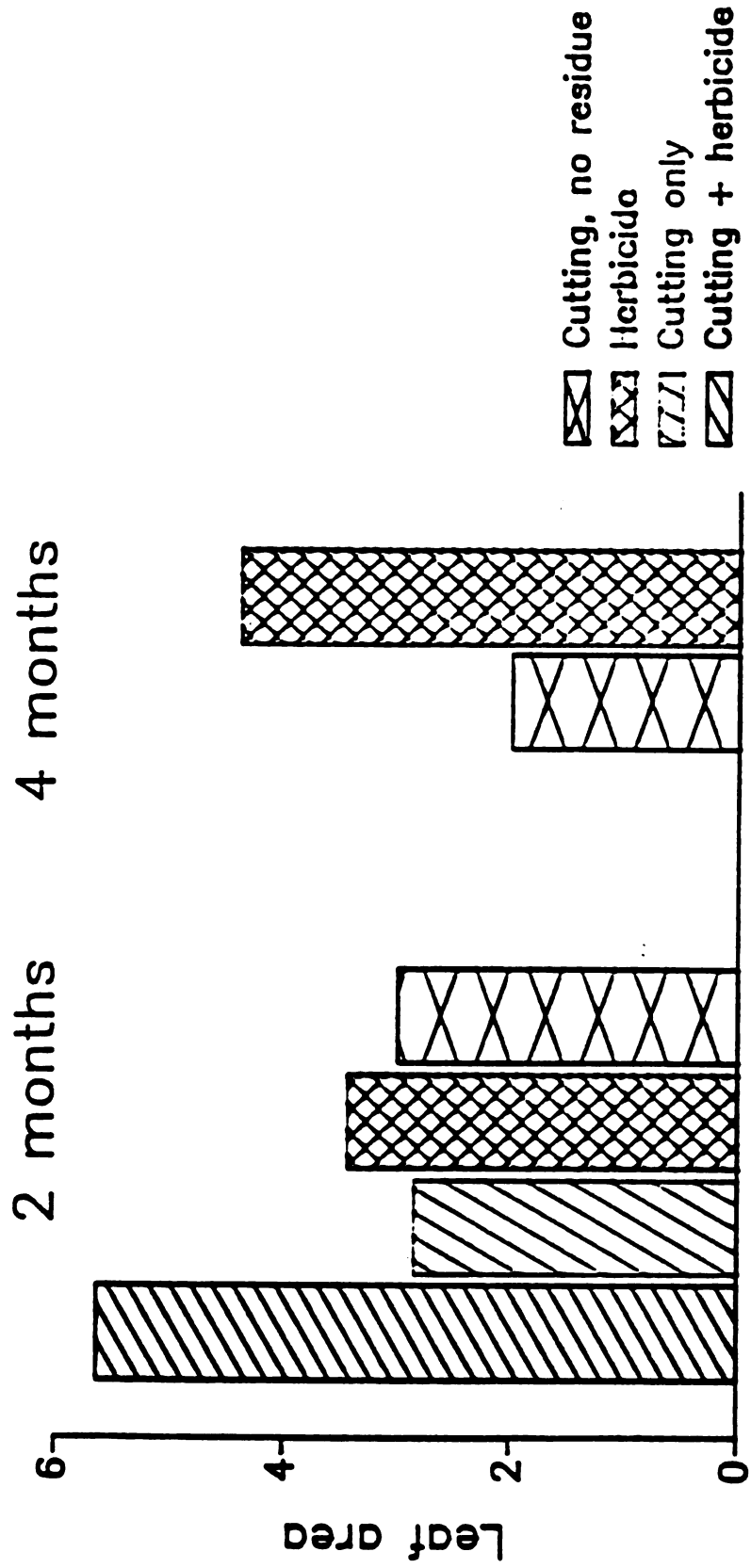


Figure 4. Leaf area for different treatments over time.

intercropping systems in which different species are mixed, this may be critical because competition for light may reduce yields. Corn leaf area is usually between 4-6 [Smucker (1986), personal communication] depending on development or management patterns. The greater leaf area here (5.63) is associated with management.

The extractable soil nitrate was high at time zero when only alfalfa was growing (Table 4). When corn was intercropped a decrease in nitrate was seen due to subsequent crop uptake. Although the results were more variable with ammonium, the trend is a decreasing amount of ammonium with time for the different treatments.

Percent Nitrogen Recovery and Uptake by the Subsequent Crop

Nitrogen loss from the alfalfa residues was 76% with cutting alone and 71% with herbicide + cutting treatment. Corn tops had significantly greater N uptake and recovery with herbicide + cutting as compared to the cutting only treatment (Table 5). When alfalfa regrowth was suppressed by both herbicide and cutting treatment, the corn plants recover 12% of the alfalfa N (24% of the N released). With cutting alone, the recovery of N by the corn fell to 4% of the total N [8% of the N released (Table 5)].

Table 4. Extractable $NO_3^-$ and $NH_4^+$ in soil over time in different treatments.

| Treatments | $NO_3^-$ 0 | 2 (months) | 4 | $NH_4^+$ 0 | 2 (months) | 4 |
|---|---|---|---|---|---|---|
| | | | $\mu g\ NO_3^- + NH_4^+N\ \mu g^{-1}$ | | | |
| Control | 8 | 4.1 | 3.4 | 1.36 | 2.13 | 0.51 |
| Herbicide & cutting | - | 4.2 | - | - | 2.48 | - |
| Cutting only | - | 5.6 | - | - | 2.10 | - |
| Cutting (-residue) | - | 2.6 | 3.8 | - | 2.69 | 0.80 |
| Herbicide only | - | 9.8 | 3.3 | - | 1.88 | 0.69 |

* Time after foliar application (8 weeks of plant growth).

Table 5. Nitrogen in corn tops and roots from nitrogen-15-labeled alfalfa at two different suppression levels at two months.

| Plant Part and Suppression | Total N (mg/kg) | Total N uptake (g/plant) | $^{15}N$ from Legume (g) | N Recovery (%) |
|---|---|---|---|---|
| Corn tops | | | | |
| Herbicide and cutting | 0.258(.043)+ | 0.185(.055)* | 0.0065(.002) | 10.8(2.94)* |
| Cutting only | 0.228(0.56) | 0.061(.030) | 0.002(.0008) | 3.41(1.39) |
| Corn roots | | | | |
| Herbicide and cutting | 0.0804(.0458) | 0.028(.020) | 0.0006(.0004) | 0.963(.703) |
| Cutting only | 0.0608(.0297) | 0.007(.004) | 0.0002(.0001) | 0.405(.213) |

+ Standard deviation shown in parentheses.

* N recovery and total N uptake greater in herbicide and cutting treatment than cutting only for corn tops at $P < 0.05$ using a paired t-test.

In the cutting only treatment, where the N source was alfalfa roots, nitrogen recovery was greater at two months compared to four months (Table 6). This effect can probably be attributed to alfalfa root turnover. The quantities of materials lost by roots vary with their age (Rovira and Davey, 1974). At two month cutting, young alfalfa roots release more N as compared to the four month cutting where roots are older, more suberized with higher lignin contents. Moori (1974) suggest that most of the rapidly released nitrogen in the root material was associated with fine roots.

A comparison of nitrogen in corn tops and roots from $^{15}N$-labeled alfalfa in complete-kill treatments did not indicate an increase in nitrogen over time (Table 7). A burst of nitrogen was probably initially released after complete-kill with slower decomposition as residues became depleted of readily decomposable nutrients. Parr and Papendick (1978) suggest that the chemical composition of most plants changes dramatically during their growing period. As the plant matures, its protein content, nitrogen, and water-soluble constituents steadily decreases, while the amount of hemicelluloses, cellulose, and lignin increases. This is further confirmed by the wide C:N ratio of the alfalfa residue and root material (18:1 and 45:1, respectively). Although the wide C:N ratio

Table 6. Nitrogen in corn tops and roots from nitrogen-15-labeled roots at the same suppression level at different time periods.

| Plant Part and Suppression | Total N (mg/kg) | Total N uptake (g/plant) | $^{15}N$ from Legume (g) | N Recovery (%) |
|---|---|---|---|---|
| Corn tops | | | | |
| Cutting (2 mths.) | 0.45(.10)+ | 0.030(.006) | 0.0003(.000) | 1.08(.283)* |
| Cutting (4 mths.) | 0.35(.01) | 0.031(.009) | 0.0002(.000) | 0.711(.166) |
| Corn roots | | | | |
| Cutting (2 mths.) | 0.028(.09)* | 0.0077(.004) | 0.0001(.000) | 0.57(.327)* |
| Cutting (4 mths.) | 0.016(.036) | 0.0018(.001) | 0.0000(.000) | 0.045(.025) |

+ Standard deviation shown in parentheses.

* N recovery and total N were greater in cutting treatment at two months than cutting only treatment at four months at $P < 0.05$ using a paired t-test.

Table 7. Nitrogen in corn tops and roots from nitrogen-15-labeled alfalfa in complete-kill (herbicide only - glyphosate 2 1/2%) treatments at different time periods.

| Plant Part and Suppression | Total N (mg/kg) | Total N uptake (g/plant) | $^{15}N$ from Legume (g) | N Recovery (%) |
|---|---|---|---|---|
| Corn tops | | | | |
| Herbicide only (2 mths.) | 0.73(.30)+ | 0.045(.033) | 0.0011(.001) | 3.15(3.5) |
| Herbicide only (4 mths.) | 0.36(.085) | 0.046(.022) | 0.0013(.001) | 3.75(1.72) |
| Corn roots | | | | |
| Herbicide only (2 mths.) | 0.36(.08) | 0.0073(.007) | 0.0002(.000) | 0.43(.59) |
| Herbicide only (4 mths.) | 0.192(.013) | 0.0060(.006) | 0.0002(.000) | 0.43(.52) |

+ Standard deviation shown in parentheses.

of the residue is not unusual, a narrower C:N ratio (14-15:1) is usually seen for alfalfa residues (Bruulsema and Christie, 1987). A wide C:N is usually associated with a slower decomposition rate (Parr and Papendick, 1978).

Since $N_2$-fixation had been depressed by an ineffective Rhizobium strain, the alfalfa root became a greater sink for N uptake from the soil throughout the growing period. This likely reduced the amount of nitrogen available for corn uptake.

At two months, a higher percentage of $^{15}N$ was measured in the soil for the herbicide only treatment (Figure 5). The higher $^{15}N$ remaining in the soil was due to growing the corn in a "killed mulch" as opposed to a "live mulch". In the "live mulch" where both crops were maintained, uptake was greater compared to a monocrop in a "killed mulch". A similar pattern was observed for four months.

A comparison of methods for estimating nitrogen transfer (Total N versus $^{15}N$ procedure) was examined for the two month treatments (Table 8). The total N procedure showed greater N quantities transferred to corn compared to the $^{15}N$ procedure. The $^{15}N$ procedure, however, gives the actual amount of N transferred from the legume to the subsequent crop. Similar results from field and greenhouse studies using $^{15}N$ techniques agree that total N or fertilizer

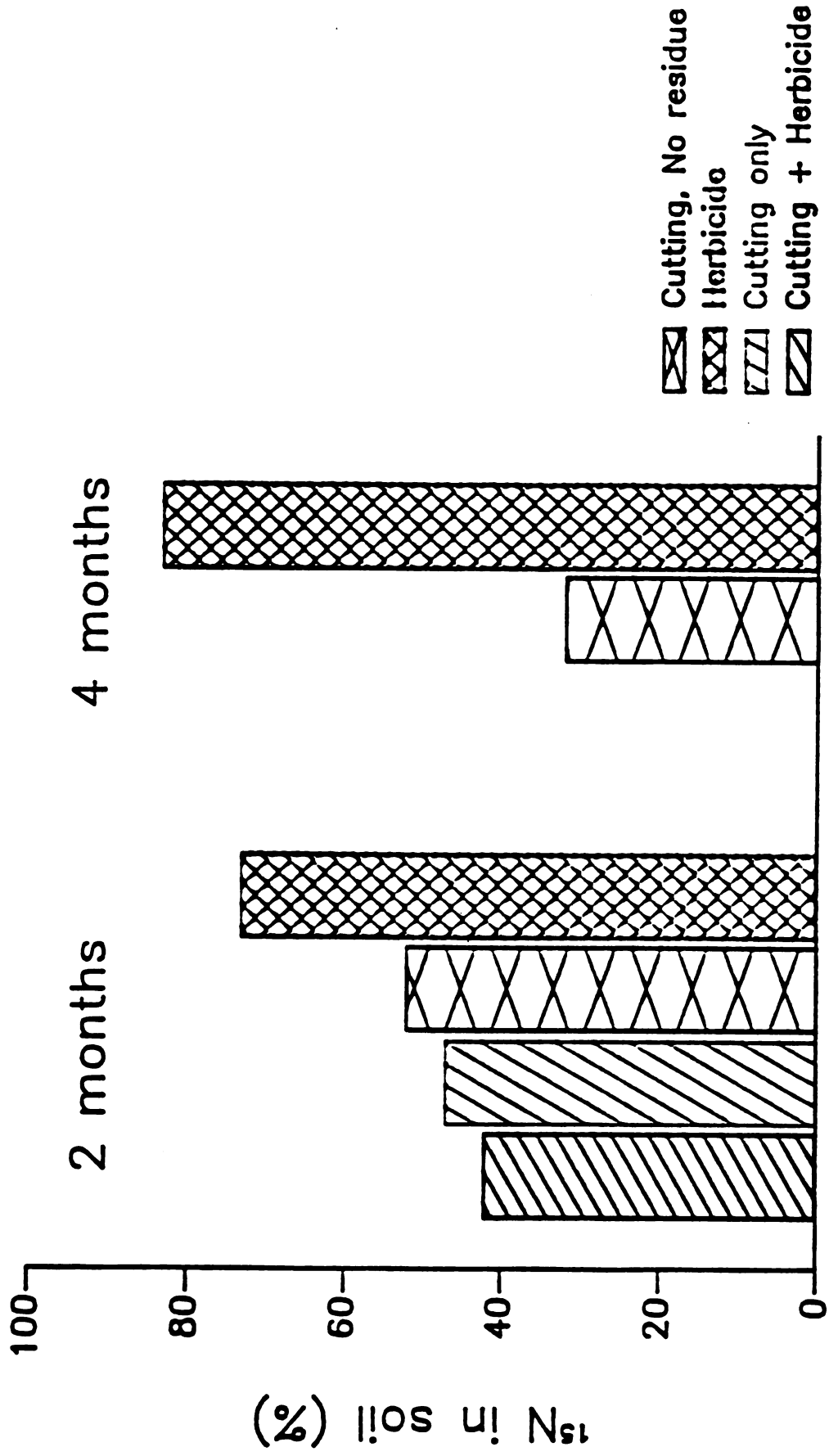


Figure 5. Percent $^{15}N$ in soil for different treatments over time.

Table 8. Comparison of methods for estimating nitrogen transfer from labeled alfalfa residues (total N versus $^{15}N$ procedures) to whole corn plants at two months.

| Treatments | Whole corn plants | |
|---|---|---|
| | Total N | $^{15}N$ |
| | g N | g $^{15}N$ |
| * Control | 0.0399 | 0.00054 |
| Herbicide + cutting | 0.2219+ | 0.00696 |
| Cutting Only | 0.0295 | 0.00184 |
| Herbicide Only | 0.0055 | 0.00064 |

* No added N.

+ Treatment - Control = X

equivalent tests overestimate the N transferred (Hesterman et al., 1987; Harris and Hesterman, 1987; Ladd et al., 1981; Haystead and Marriott, 1979). These studies indicate that at least 20-30% less is transferred using $^{15}N$ techniques. As $^{15}N$ is more widely used in similar studies, the controversy surrounding amounts of N transferred is likely to be resolved.

The microbial biomass carbon and nitrogen data (flushes) indicates the effects of management practices over time (Table 9). No significant differences were observed between C and N microbial biomass in the herbicide + cutting treatment versus the cutting only treatment. These results suggest that crop management did not play a major role in modifying the microbial community between these treatments. This is also reflected in similar C:N ratios (Table 9). However, in the cutting only treatments (-residue) a significant decrease in the C and N microbial biomass was observed possibly due to a lack of carbon material to stimulate a more active microbial population. A more interesting pattern was seen in the herbicide only treatment where the C and N flush was almost one-half of the herbicide + cutting treatment (Table 9). Although part of the difference seen was due to less surface residue and only corn growing, it was interesting to note that an increase

Table 9. Biomass carbon and nitrogen in different treatments over time.

| Treatments | $C_F$[1] (months) 2 | $C_F$[1] (months) 4 | $N_F$[2] (months) 2 | $N_F$[2] (months) 4 | $C_F$[1]/$N_F$[2] (months) 2 | $C_F$[1]/$N_F$[2] (months) 4 |
|---|---|---|---|---|---|---|
| | $\mu g\ g^{-1}$ soil | | $\mu g\ g^{-1}$ soil | | | |
| Control | 296±36* | 284±55 | 30±5 | 30±4 | 6±0.29 | 9±0.74 |
| Herbicide + cutting | 487±57 | - | 60±2 | - | 8±0.74 | - |
| Cutting only | 466±25 | - | 60±4 | - | 8±0.30 | - |
| Cutting (-residue) | 341±111 | 283±67 | 48±11 | 33±7 | 7±0.64 | 8±0.47 |
| Herbicide only | 192±14 | 314±59 | 30±2 | 34±5 | 7±0.76 | 9±1 |

[1] $CO_2$-C evolved during Chloroform Fumigation Incubation Method (CFIM).

[2] Net $NH_4^+$-N accumulated during CFIM.

* Mean ± Standard Error.

microbial biomass was seen at four months for both C and N. This result suggests that the herbicide may have depressed microbial activity initially.

Extractability ratio is defined as the fraction of applied N extracted over the fraction of native soil N extracted (Legg et. al., 1971). The extractability ratios were not significantly different with the exception of the 4 month control treatment (Table 10). Such a high extractability ratio value was not expected; however, most of the $^{15}N$ remained in the soil where only one crop was grown with no residue added.

A significant amount of $^{15}N$ was found in the biomass at four months (Table 10). This further confirms my theory that the small amount of $^{15}N$ recovery seen in the corn after four months was due to immobilization.

Up to 25% of the applied $^{15}N$ was found in the biomass (Table 10). It was either retained or slowly released over time as indicated by the herbicide only and cutting only treatment (Table 10).

When alfalfa residues are placed on the soil surface, a proportion of the available N is released into the soil system where it first cycled through the microbial biomass (1) (Figure 6). A proportion is used for cell synthesis and energy and a proportion is released (2) to the inorganic pool where several fates

Table 10. Microbial Biomass $^{15}N$ recovered in different treatments over time.

| Treatments | ER[1] | | | Biomass $^{15}N$ | | |
|---|---|---|---|---|---|---|
| | 0 | 2 (months) | 4 | 0 | 2 (months) | 4 |
| | | | | $\mu g/g^{-1}$ | | |
| Control | 10.2±2.9* | 5.8±1.2 | 36.4±7.2 | 0.24±.06 | 0.84±0.02 | 0.14±0.15 |
| Herbicide + cutting | - | 5±0.4 | - | - | 0.67±0.08 | - |
| Cutting only | - | 13±7.6 | - | - | 0.27±0.19 | - |
| Cutting (- residue) | - | 6.4±1.1 | 6.2±0.58 | - | 0.24±0.07 | 0.11±0.37 |
| Herbicide only | - | 4.9±0.2 | 5.58±0.91 | - | 0.35±0.08 | 0.60±0.18 |

[1] ER = Extractability Ratio.

* Mean ± Standard Error.

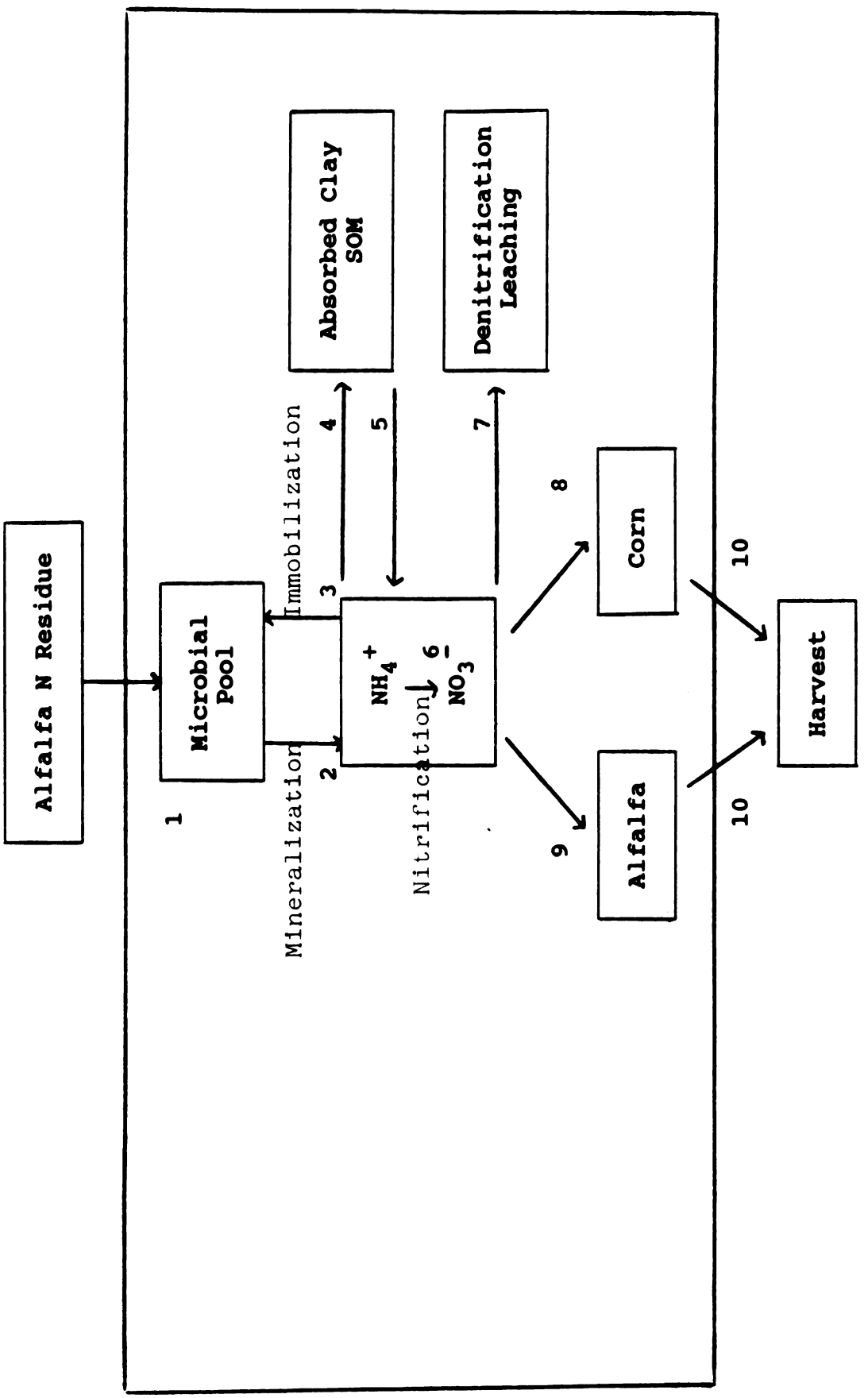


Figure 6. Diagram of the pools and flows of N important in this study in which $^{15}N$ added residues was traced to pools of corn, alfalfa, inorganic N and microbial biomass. The numbers refer to the processes discussed in the text.

are possible. It may be absorbed by clays (4) or stabilized with organic matter (5), or it may be immobilized by microorganisms (3). The ammonium form may be nitrified to nitrate (6) or taken up directly by plants. Once in nitrate form several important fates may occur which can have either a detrimental or beneficial (improved plant growth) effect. The detrimental effects are loss by leaching or denitrification (7). It has clearly been demonstrated that denitrification may account for up to 30% loss in fertilizer nitrogen in agricultural systems (Sextone et al., 1985). The question is: How important is this process in intercropped no-till systems? Rice and Smith (1982) and Linn and Doran (1984) have shown this process to be important in no-till systems where moisture and carbonaceous material are increased. However, I believe these losses are minimized in systems where the fate of nitrate is to both corn (8) and alfalfa (9). The major inefficiency in N recovery by the crops is due to retention of the N in the soil microbial biomass. Thus, the focus should be on how can the system be managed to minimize immobilization and stimulate N release for plant uptake. Varco (1987) suggests that the addition of fertilizer N in combination with alfalfa N decreased the quantity of N immobilized. From my data (Table 11) maximum recovery by corn was 12% even with stunted alfalfa (and some

Table 11. Balance sheet of $^{15}N$-labeled nitrogen in different treatments.

| Treatment | Corn | Alfalfa | Residues | Soil | Soil +M.B. | Total |
|---|---|---|---|---|---|---|
| | ----------% of $^{15}N$ added---------- | | | | | |
| Herbicide + cutting | 12 | 7 | 17 | | 42 | 78 |
| cutting | 4 | 5 | 20 | | 47 | 76 |
| Herbicide (2 mths) | 3.6 | - | 9.5 | | 73 | 86.1 |
| Herbicide (4 mths) | 4.2 | - | 11 | | 83 | 98.2 |
| Cutting (2 mths) | 1.6 | 7.8 | - | | 52 | 61.4 |
| Cutting (4 mths) | 0.8 | 2 | - | | 32 | 35 |

+ M.B. = microbial biomass.

$^{15}N$ contamination of the soil). Generally, 32-83% of the $^{15}N$ was tied-up in the soil fraction of which a small portion was contributed by the microbial biomass.

I believe the data from experiment 1 and experiment 2 (Chapter 3) is also useful in helping resolve the issue that legumes supply 50-100% of the nitrogen to subsequent crops (Baldock and Musgrave, 1980). Other $^{15}N$ studies agree that legume N supply to subsequent crops is small. The comparison of methods, balance sheet and $^{15}N$ recovery by corn (Chapter 3, Table 13) consistently shows that there seems to be an overestimation of the N supplied to new crops by legumes. However, it has been suggested that $^{15}N$ may likely underestimate the amount of N transferred because of the dilution effect in the microbial pool (Jansson and Persson, 1982). This may be important in systems where only short-term studies are done. It becomes less important over time (long-term experiments) and when organic pools are measured. Harris and Hesterman's (1987) $^{15}N$ field studies also show only 16-25% N transferred to subsequent crops and these experiments were done with residues incorporated into the soil.

The high amounts of nitrogen being credited to legume sources may be due to effects other than nitrogen. These effects are termed rotation effects.

Although these effects may be difficult to demonstrate, this may be an alternative explanation for the legume benefit.

The objective of experiment 1 was to see how much root N from the legume was released which may be very important in pasture systems and high root N leguminous species. The second goal was to foliarly label the legume without contaminating the soil to see exactly how nitrogen can be transferred, from the legume. Although contamination occurred the first objective could still be addressed. This is shown by the following calculation (next page). This calculation indicates that the root N contribution in my experimental cropping systems was probably insignificant; most of the label N in the corn was derived from the tops (as shown by experiment 2), or contaminated soil (experiment 1).

A similar study by Ledgard et al. (1985) examining transfer also showed that the amount of $^{15}N$ transferred in mixed crops was small. They labeled subterranean clover plants by immersing trifoliate leaves in $^{15}N$ solution for 3 days. The plants were trimmed and allowed to grow for an additional 29 and 36 days. The greenhouse study indicated that only 2.2% of the clover N had been transferred to ryegrass and no transfer was observed in the field. Soil contamination was not a problem given that the

Calculation using results from experiment 1 and 2 which shows that the corn-N derived from alfalfa root N was very small.

LS = labeled soil.

LR = labeled roots.

LT = labeled tops.

For cutting only with labeled residues.

Given: Experiment 1 = LT + LR + LS = 4% N recovered by corn

T = Total

Experiment 2 = LT = 1% N recovered by corn

1.) T - LT = 3%

4% - 1% = 3% contributed by labeled soil + labelled roots.

2.) Labeled soil was approximately 90% contaminated
∴ 2.7% label is due to soil.

3.) 0.3% labeled came from the roots.

labeling was by a short-term dipping. Their result is in agreement with my theory that only a small amount of N transfer occurs in mixed cropping systems. In the experimental pots the roots of both species were concentrated in the pot and any N released was likely reabsorbed by other alfalfa roots. This may very well have been the case, or the N that released was used for microbial growth.

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# CHAPTER III

## AVAILABILITY OF N-MINERALIZED FROM $^{15}N$-LABELED ALFALFA TOPS IN NO-TILL INTERCROPPED CORN

### Introduction

Legume quality as well as quantity may influence the amount of nitrogen transferred to subsequent crops. The proportion of N released during residue decomposition is governed by chemical composition of the residues with N content being most significant, and the manner in which the residues are returned to the soil (Henzell and Vallis, 1977).

As plant material decomposes, the chemical composition of the residue changes (Bartholomew, 1967; Power and Legg, 1978; Parr and Papendick, 1978; Voroney, 1983). Water soluble compounds such as sugars, organic acids and proteins are readily decomposed with hemicelluloses, cellulose and lignin being more resistant (Parr and Papendick, 1978).

With regard to nitrogen availability, in general, returning residues with an N content greater than approximately 1.5% usually enhances N availability. However, returning residues with less N reduces N availability the first few years after addition (Power and Legg, 1978). Voroney (1983) indicates that the

mineralized labeled N from $^{14}C$, $^{15}N$-labelled barley residues (2.5-2.7% N) was utilized with an efficiency ranging from 32 to 52% which was comparable to that reported for fertilizer applications.

Typically, residues are incorporated into the plow layer of the soil; however, soil erosion has become a major issue in many systems (Varco, et al. 1987; Bruce, et al., 1987) leading to new interest in alternative management practices. Surface placed residues may be a more useful alternative for maintaining long-term productive soils. The decomposition is obviously slower with a different microbial population influencing N cycling. Varco and associates (1987) compared incorporated and surface-applied alfalfa and vetch (Vicia villosa Roth.) decomposition and N release in no-tillage and conventional tillage plots. Incorporated vetch residues decomposed and released N at a greater rate than surface-applied vetch. Within 15 days, surface-applied alfalfa lost about 27% of its original weight. Fertilizer N addition with alfalfa N decreased the quantity of alfalfa N immobilized in the soil organic fraction by diluting the N pool which the microbes use. Varco et al. (1987) also suggests that even though much of the legume N is immobilized the potential N losses associated with fertilizer usage are less with legume N. Because of the many potential

fates of nitrogen and decomposition of surface-applied residues are poorly understood, this lead to Objective 4: **What is the availability of N mineralized from $^{15}$N-labeled alfalfa residues (tops) to no-till intercropped corn, versus the proportion that goes to microbial biomass, alfalfa regrowth and soil mineral nitrogen.**

## Materials and Methods

### Experiment 2

A second greenhouse experiment examined the contribution of $^{15}N$-labeled alfalfa shoots to a subsequent crop. The experimental treatment was alfalfa suppression (cutting only) at 2 and 4 months. The treatments were replicated six times.

#### 1. Soil Preparation

A Kalamazoo sandy loam (fine-loamy, mixed, mesic Typic Hapludalfs) was collected from Kellogg Biological Station and prepared as described in Chapter II.

#### 2. Residue Preparation

Previously grown labeled alfalfa shoots were periodically cut, dried and stored until the experiment began. The shoots contain a 6.2% $^{15}N$ label.

#### 3. Nutrient Solution and $^{15}N$ Solution Preparation

A N-free nutrient solution was prepared containing standard macro-micro nutrients as described in Experiment 1 (Section 3). A dilute (20 mml) concentration of 99% $^{15}N$ enriched urea was prepared and used to water the soil two weeks after planting the alfalfa.

4. Treatment of Established Legumes

Twelve pots of unlabeled alfalfa in unlabeled soil had been grown approximately 5 weeks before the suppression treatment (cutting only) was applied. The alfalfa was cut 5.1 cm from the soil surface. Previously dried shoots (15g) containing 0.40 N was placed on the 0.035 $m^2$ soil surface. Corn (Zea mays L. variety GL5922) was planted 5 days after cutting (see Section 6, Experiment 1). Six pots were harvested after the first right weeks and the last six were harvested after the second eight weeks.

5. Preparation of Plant and Soil Samples for Analysis and Analytical Procedures

Plant and soil samples were prepared and analyzed as described in Experiment 1 (Section 7 and 8).

## Results and Discussion

### General Physical Appearance of Plants Throughout the Growing Period

#### Alfalfa

The shoot portion was green and showed no nutrient deficiencies. This was the case throughout the growing period. The roots contained many nodules on the laterals as well as the taproot. An examination of the inside of the nodules revealed a pinkish or red color indicating an effective indigenous *Rhizobium* population.

#### Corn

Although the corn was given adequate nutrient solution, pests were a problem later (after 6 weeks) in the growing period (after 6 weeks). Watering once a day as opposed to twice a day may have contributed to the problem. Pesticide treatments were used to combat insect infestation; however, these measures did not sufficiently correct the problem.

### Plant Growth

The expected differences in plant growth over time were reflected in corn plant growth. A plant height of 87 and 99 cm and plant biomass of 12.9 and 17.7 g was observed for 2 and 4 month suppression, respectively.

The extractable soil nitrate and ammonium was high at two months (Table 12) and a decrease was seen at the four month sampling.

Percent Nitrogen Recovery and Uptake by the Subsequent Crop

Corn tops had greater N uptake and recovery with the four month suppression as compared to the two month suppression. However, the same pattern was not observed for corn roots; the lower N recovery at four months may have been due to a water deficit and less N taken up by older corn roots.

When comparing the nitrogen released from labeled residues (Experiment 2) versus labeled residues + roots + soil (Experiment 1) at two months (refer to Table 5, Experiment 1), slightly more than 25% of the $^{15}N$ was derived from labeled residues. This may likely be a slight underestimation since the comparison was based on 25 g dry material returned to the surface (Experiment 1) versus 15 g dry material (Experiment 2) returned to the soil surface. When one examines the quantity of $^{15}N$ recovered at four months (Table 13), about 25% more N was recovered in corn tops compared with 2 month recovery. The results further suggest that a portion of the nitrogen may be immobilized and slowly released over time. Varco et al. (1987) suggested that vetch immobilization can be two to three times greater than fertilizer N

Table 12. Extractable $NO_3^-$ and $NH_4^+$ in cutting only treatment over time.

| Treatment | $NO_3^-$ | $NH_4^+$ |
|---|---|---|
| | $\mu g\ Ng^{-1}$ | |
| Cutting only (2 months) | 22 | 1.2 |
| Cutting only (4 months) | 3.4 | 0.5 |

Table 13. Nitrogen in corn tops and roots from nitrogen-15-labeled alfalfa in suppression treatments at different time periods.

| Plant Part and Suppression | Total N (mg/kg) | Total N Uptake (g/plant) | $^{15}N$ from Legume g | N Recovery (%) |
|---|---|---|---|---|
| Corn tops | | | | |
| Cutting only (2 mths) | 0.51 | 0.04 | 0.0006 | 1.03 |
| Cutting only (4 mths) | 0.54 | 0.08 | 0.0012 | 2.07 |
| Corn Roots | | | | |
| Cutting only (2 mths) | 0.36 | 0.16 | 0.00013 | 0.21 |
| Cutting only (4 mths) | 0.50 | 0.008 | 0.00009 | 0.15 |

immobilization. This release may have been further stimulated by cutting at 2 months for the four month harvest. Often suppression of legumes stimulate sloughing of nodules and roots resulting in greater N availability for the nonleguminous crop when the active legume is less competitive.

The microbial biomass carbon and nitrogen flushes were not significantly different over time for cutting only treatments (Table 14). No differences were observed between the C/N ratio. It was consistent with values observed for the same treatment (Table 10).

A similar pattern was seen in the microbial $^{15}N$ biomass (Table 15). However, slightly less of the applied $^{15}N$ was recovered at four months suggesting that a small percentage of $^{15}N$ was mineralized over time.

Most short-term greenhouse studies on N transfer indicate only a small percentage, if any, nitrogen is transferred to subsequent crops (Vallis, 1967). In multiple cropping systems where no-tillage techniques are applied, management of legume N as well as fertilizer N become important for adequate yields as well as to minimize environmental concerns.

Table 14. Microbial biomass carbon and nitrogen in cutting only treatments over time.

| Treatments | $C_f$[1] (months) 2 | $C_f$[1] (months) 4 | $N_f$[2] (months) 2 | $N_f$[2] (months) 4 | $C_f/N_f$ (months) 2 | $C_f/N_f$ (months) 4 |
|---|---|---|---|---|---|---|
| Cutting only | 305±59 | 216±356 | 45±6 | 34±4 | 7±0.37 | 6±0.29 |

[1] $CO_2$-C evolved during Chloroform Fumigation Incubation Method (CFIM).

[2] Net $NH_4^+$-N accumulated during CFIM

* Mean ± Standard Error.

Table 15. Microbial biomass $^{15}N$ recovered in cutting only treatments over time.

| Treatments | ER[1] (months) 2 | ER[1] (months) 4 | Biomass $^{15}N$ µg/g (months) 2 | Biomass $^{15}N$ µg/g (months) 4 |
|---|---|---|---|---|
| Cutting only | 3.8±0.57 | 4.4±0.77 | 0.35±12 | 0.25±3 |

[1] ER = Extractability ratio.

* Mean ± Standard Error.

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# CHAPTER IV

# SUMMARY AND CONCLUSIONS

The results suggest that the level to which legume plants were suppressed in an intercrop system will determine the uptake and percent recovery of mineralized N in subsequent crops. This was clearly reflected in the growth of the corn plant and percent nitrogen recovered. Nitrogen recovered by the corn plant was greater in the herbicide treatment than the cutting only due to the contribution of tops and roots (Table 5). Although about 75% of the N in the alfalfa was lost from the residues, only a small proportion of this N was recovered by corn. Direct release of labeled N during degradation of the plant material by the microbial biomass would probably not occur. The N would, therefore, be accessible to the corn only after microbial turnover. Higher immediate recoveries of legume N would be likely if the C:N ratio of residues was narrow.

To fully understand legume N nitrogen transfer, the proposed method needs one major adjustment. This method requires reducing contamination due to stem runoff. It is likely that this problem can be corrected either by sealing the plastic holes with wax

or by changing foliar application procedure (tilting pots at an angle when applying the $^{15}N$ solution. The two suggestions in combination may be even more successful. A second approach may involve growing plants simultaneously in sand as well as field soil. This would allow one to extrapolate a more accurate amount released.

A third approach may be to transplant; however, this is tedious and time consuming and also it destroys the whole concept of an intact system (no-till).

Although soil contamination was a greater problem than expected, the major issues of root N contribution and how nitrogen is transferred from legumes could still be addressed.

# APPENDICES

Appendix 1. Experimental Design

| | 0 time | 2 months | 4 months |
|---|---|---|---|
| Control (no corn) | 18* | 12 | 13 |
| | 5 | 9 | 7 |
| | 14 | 2 | 8 |
| | 16 | 3 | 6 |
| | 10 | 11 | 4 |
| | 1 | 17 | 15 |
| Suppression (corn) | 35 | 25 | 27 |
| 1. Cutting down to 2 inches | 33 | 34 | 32 |
| 2. Cutting down to 2 inches and Glyphosate 1 1/2% | 31 | 29 | 20 |
| | 36 | 19 | 30 |
| | 26 | 28 | 23 |
| | 22 | 24 | 21 |
| Complete-kill (corn) | 48 | 40 | 43 |
| Glyphosate 2 1/2% | 52 | 38 | 45 |
| | 42 | 51 | 37 |
| | 46 | 54 | 47 |
| | 53 | 44 | 50 |
| | 49 | 41 | 39 |

* Numbers in the columns are randomized replicates.

Appendix 2. What actually happens at zero-time period?

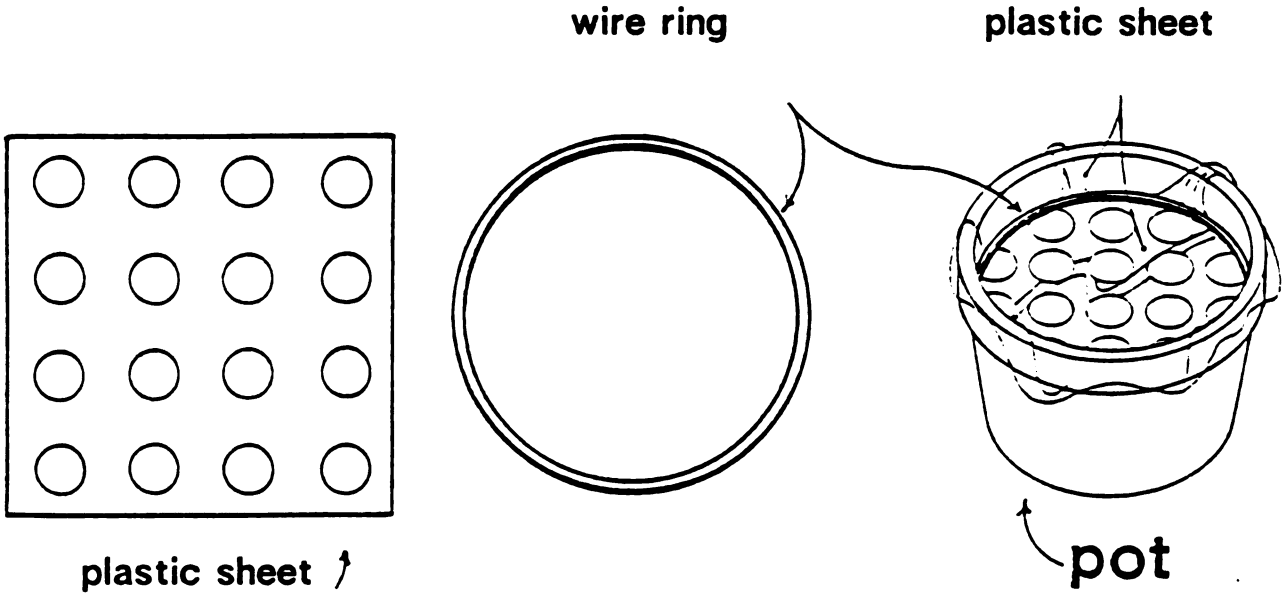
| | 2 months | 4 months |
|---|---|---|
| Control (no corn) | 12* | 13 |
| | 9 | 7 |
| | 2 | 8 |
| | 3 | 6 |
| | 11 | 4 |
| | 17 | 15 |

| | 1tops | ntops | ntops | 1tops |
|---|---|---|---|---|
| Suppression (corn) | 48 | 35 | 25 | 27 |
| | 52 | 33 | 34 | 32 |
| | 42 | 31 | 29 | 20 |
| | 46 | 36 | 19 | 30 |
| | 53 | 26 | 28 | 23 |
| | 49 | 22 | 24 | 21 |

| | | |
|---|---|---|
| Complete-kill (corn) | 40 | 43 |
| | 38 | 45 |
| | 51 | 37 |
| | 54 | 47 |
| | 44 | 50 |
| | 41 | 39 |

* Numbers in the columns are randomized replicates.

## Appendix 3. Design of Protection Apparatus



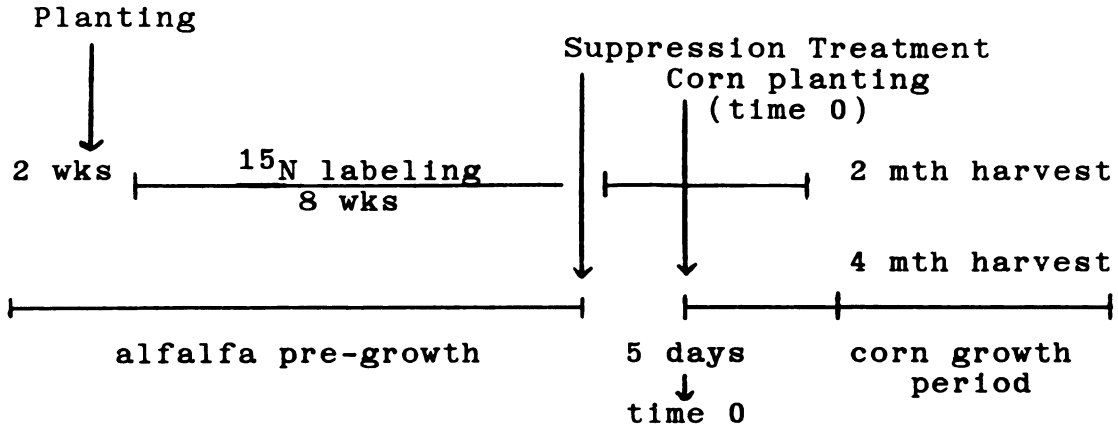

a. Holes were punched into each sheet of plastic.

b. A wire ring was designed to fit on the top of the plastic. The wire ring held the plastic sheet firmly to each pot.

c. Once the plastic sheets were firmly placed in each pot, seeds were planted into each hole.

d. After plants were two weeks old, cotton was placed around each plant to cover the holes.

Appendix 4. Schematic Diagram of Experimental Time Frame



Appendix 5. Fate of Nitrogen in No-Tilled Intercropping Systems