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DISTRIBUTION AND CYCLING OF ^{15}N -LABELED UREA
IN A KENTUCKY BLUEGRASS TURF

presented by

Eric David Miltner

has been accepted towards fulfillment
of the requirements for

Ph.D. degree in Crop and Soil Sciences

Bruce Branham
Major professor

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**DISTRIBUTION AND CYCLING OF ^{15}N -LABELED UREA
IN A KENTUCKY BLUEGRASS TURF**

By

Eric David Miltner

A DISSERTATION

**Submitted to
Michigan State University
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ABSTRACT

DISTRIBUTION AND CYCLING OF ^{15}N -LABELED UREA IN A KENTUCKY BLUEGRASS TURF

By

Eric David Miltner

The fate of ^{15}N -labeled urea applied to Kentucky bluegrass (*Poa pratensis* L.) turf was studied using intact monolith lysimeters. Soil type was a Marlette fine sandy loam (fine-loamy, mixed mesic Glossoboric Hapludalfs). Lysimeters were used only for the collection of leachate. PVC microplots were installed for destructive soil sampling. Urea was applied at a rate of $196 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ in five equal applications of $39.2 \text{ kg N ha}^{-1}$, based on two application schedules. The 'Spring' treatment was fertilized at approximately 38 day intervals from late April through late September. The 'Fall' treatment was fertilized from early June through early November. In 1991 only, the April and November applications were made with ^{15}N -labeled urea (25 atom % excess). For the Spring treatment, 35% of the LFN was harvested in clippings over two years. Approximately 30% of LFN was recovered from thatch 18 days after treatment (DAT). This value remained constant for the next year, then declined. Only 8% of the LFN was recovered from soil 18 DAT. This increased to 14% two years after application. Over the two year period, LFN in leachate totaled 0.09 kg ha^{-1} . For the Fall treatment, 38% of the LFN was harvested in clippings over two years. Eighteen days after the November application 62% of the LFN was recovered from thatch. This value declined to 35% by the following June. LFN in soil increased from 12% to 25% over two years. Leachate LFN totaled 0.07 kg ha^{-1} over the two year period. Volatile

losses of N were suspected for both treatments, but a subsequent experiment to measure this was inconclusive.

Two years following application of LFN, soils were incubated to determine N mineralization rates. Over 50 days, 46.1 kg N ha⁻¹ was mineralized from the surface 10 cm of soil, of which 0.40 kg ha⁻¹ was LFN. Addition of N as either NH₄⁺ or grass clippings induced a priming effect on the turnover of soil N.

The results of these experiments showed that the thatch layer and microbial biomass played extremely important roles in immobilization of LFN. Mineralization of organic N provides a significant source of N available for plant uptake. Application of fertilizer N to turfgrass results in very little leaching potential, even when applied in the late Fall.

To Laura, Ella, and Zelda
for all of your love, understanding, and friendship

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INTRODUCTION

Turfgrass management is of major economic importance in the United States. As of 1988 in Michigan alone, approximately 1.2 billion hectares were occupied with turfgrass with an annual maintenance cost of \$1 billion. Of this, approximately \$24 million is spent on fertilizers, not including the amount spent by homeowners (Michigan Turfgrass Foundation, 1989). Nitrogen is the fertilizer nutrient required in the greatest amount (Beard, 1973). The fate of applied fertilizer nitrogen has both economic and environmental impacts. Based on the figures given above, inefficient utilization of fertilizer N can obviously result in excessive fertilizer expenditures. Perhaps a greater impact lies in the ultimate destination of the nitrogen not utilized by plants. The obvious intent of fertilizer application is for plant uptake and use in growth and development. However, plants must compete with other organisms in the ecosystem. Microorganisms in particular play an important role in immobilizing nitrogen and making it unavailable for immediate plant uptake. The nitrogen is eventually released, but residence time in the microbial biomass can keep it unavailable for weeks, and it can be immobilized for even longer periods in more complex forms of soil organic matter. Inorganic nitrogen can be held in exchangeable forms on soil particles and eventually released into the soil solution. From the soil solution, it may be lost to the atmosphere through either volatilization or denitrification. It may be removed from the system by surface runoff, or can be lost by deep percolation and eventual entry into aquifers. This can result in a significant health hazard to animals and humans if concentrations become high enough. The Environmental Protection Agency has established a limit of 10 mg NO_3^- -N L^{-1} in drinking water supplies (U.S. Environmental Protection Agency, 1976). Leaching of fertilizer nitrogen into

ground water supplies has become a topic of much attention in recent years, and the turfgrass industry has received considerable scrutiny because of the perception that nitrogen is over-applied to turfgrass, which is sometimes viewed as an unnecessary crop with no real value to society. There are, however, many benefits of turfgrass, including insulation from noise, protection from glare, increased percolation of precipitation and less surface runoff of water, softening of the ground surface for recreational use, cooling of the ground surface, and aesthetic benefits, as well as many others. These many benefits provided by turfgrasses justifies their continued cultivation and future research that refines management practices and defines the environmental impact of these practices. The objectives of this research were twofold:

- 1) to examine the fate of fertilizer nitrogen applied to turfgrass,
including the partitioning of this nitrogen into different pools in the
turfgrass system
- 2) to compare the leaching potential of fertilizer nitrogen applied in the
Spring (April) and the late Fall (November).

The answers to these questions will help us to understand both nitrogen use efficiency and the environmental impacts of fertilizer nitrogen use in turf.

CHAPTER I

DISTRIBUTION OF SPRING AND FALL APPLIED ^{15}N -LABELED UREA IN A KENTUCKY BLUEGRASS TURF

LITERATURE REVIEW

A thorough review of the literature concerning the fate of nitrogen in turfgrass was recently authored by Petrovic (1990). The review presented here will not restate all of the literature included in the above published review, but will concentrate largely on the use of ^{15}N in monitoring fertilizer nitrogen fate. The reader is referred to Petrovic (1990) for a broader discussion. The present review will cover nitrogen dynamics throughout the turfgrass community starting with the plant and moving downward through thatch and soil, and will conclude with information on losses of nitrogen from the turfgrass community through runoff, leaching, and gaseous transformations.

Plant Uptake

Studies of nitrogen uptake by turfgrasses are scarce. However, in several experiments Bowman and colleagues have shown potential for very rapid uptake and transport of N. Bowman *et al.* (1989b) foliarly applied 50 kg N ha⁻¹ as ^{15}N -labeled ammonium sulfate to field grown Kentucky bluegrass, followed by 0.3 cm irrigation, and recovered 75% of the labeled fertilizer N in plant shoots five days after application. The application was made in August 1985 and the turf had received no fertilizer nitrogen since the previous November (50 kg ha⁻¹), and was in an N-deficient state as described by the authors. Studying plants grown in solution culture, Bowman *et al.* (1989a)

found that perennial ryegrass (*Lolium perenne* L.) removed nitrate-N from solution at a rate equivalent to 63 kg N ha⁻¹ in 50 hours and 90 kg N ha⁻¹ in 96 hours. Uptake of ammonium-N was even greater: 50 kg N ha⁻¹ in 38 hours and 128 kg N ha⁻¹ in 96 hours. These plants had been previously starved of N for periods between one and four weeks. For turf that was maintained continuously with nitrogen, uptake was approximately 50% of the N-deprived turf for both NO₃⁻ and NH₄⁺. Nitrogen uptake by Kentucky bluegrass (*Poa pratensis* L.) was 50 and 77 kg N ha⁻¹ from NO₃⁻ and NH₄⁺ sources, respectively, compared to 88 and 114 kg N ha⁻¹ for perennial ryegrass over a four day period. Increases in root dry weight were noted for plants grown in the absence of N for one week or more, and the authors conclude that this was partially responsible for the increased uptake in the deficient turf. They also observed that NO₃⁻-N uptake was reduced in perennial ryegrass grown in +N solution culture by mowing between 30 min. and four days prior to application, while uptake by plants receiving no N for 14 days prior to additional fertilization was not affected by mowing.

Nitrogen uptake by a mixed stand of Kentucky bluegrass and red fescue (*Festuca rubra* L.) grown on a sandy loam soil under more typical management practices was quite different (Starr and DeRoo, 1981). Over three years, total nitrogen removed in clippings in plots where clippings were not returned averaged 95 kg ha⁻¹, an amount equivalent to 50% of the fertilizer N applied. Where clippings were returned after mowing, harvested N averaged 137 kg ha⁻¹ (73%). Total nitrogen and fertilizer nitrogen uptake and dry matter accumulation was rapid for a period of approximately three weeks following application. Following this period, nitrogen uptake rates were relatively constant at approximately 0.44 and 0.23 kg N ha⁻¹ day⁻¹ where clippings were returned and removed, respectively. Use of ¹⁵N-labeled ammonium sulfate

resulted in total fertilizer N uptake of approximately 30 kg ha⁻¹ (one-third of total application) during a 120 day period following a May application and 20 kg ha⁻¹ following a September application, regardless of clipping management. Most of this uptake occurred during the first 30 days. Uptake rates of fertilizer nitrogen during the steady state period (after 30 days) were very low, 0.05 and 0.04 kg N ha⁻¹ day⁻¹ where clippings were returned and removed.

Studying perennial ryegrass under pasture conditions, Bristow *et al.* (1987) applied ¹⁵N-ammonium nitrate at a rate of 60 kg N ha⁻¹. They observed recoveries in herbage of 33, 49, and 55% for the periods 28, 111, and 370 days after application. Inclusion of stubble raised these recoveries to 53, 54, and 56%. Similar first-year recoveries in perennial ryegrass herbage of 46 to 53% of applied ¹⁵N-calcium nitrate were observed by Dowdell and Webster (1980, 1984). Recoveries over a three to five year period were 52 to 60%.

Consistent levels of approximately one-third of applied fertilizer nitrogen were recovered in soft chess (*Bromus mollis* L.) herbage over a three year period when the application was made in October. This remained consistent over different application schedules of 100 kg N ha⁻¹ applied once or for three consecutive years, or for a single application of 500 kg N ha⁻¹. When the fertilizer was applied in February at rates of 100 kg N ha⁻¹ either once or for three consecutive years, recovery increased to approximately 59% (Jones *et al.*, 1977). For subterranean clover (*Trifolium subterraneum* L.) recoveries from October fertilizations of 100 kg N ha⁻¹ one time and for three consecutive years, and 500 kg N ha⁻¹ one time were 11, 15, and 17%, respectively. Herbage recovery of February applied fertilizer N approached 50%. For crested wheatgrass [*Agropyron desertorum* (Fisch. ex Link) Schult.] fertilizer N recovery in plant tops was approximately 30% in the first year following

application (Power and Legg, 1984). An additional 15% was recovered in plant roots. Over a five year period approximately 40% was recovered in the herbage. Seventy five percent of the total ^{15}N recovered in the herbage was recovered in the first year. Rekhi *et al.* (1982) applied ^{15}N -labeled urea to rice at a rate of 180 kg N ha^{-1} in either a single application or in three equal applications of 60 kg ha^{-1} . Recovery in grain and straw was 22 and 42% for the single and split applications, respectively.

The literature illustrates wide variability in the potential for uptake and transport to shoots of fertilizer nitrogen. Important variables include species, nitrogen source, application rate, and management practices. Potential uptake rates are very high, but in actual practice utilization is generally 60% or less.

The Thatch Environment

Thatch has been defined as "a tightly intermingled layer of dead and living stems and roots that develops between the zone of green vegetation and the soil surface" (Beard, 1973). Mat is a mixture of soil and organic material, generally occurring between the thatch layer and soil surface. Operationally, it is difficult to distinguish discrete thatch and mat layers and the boundary of mat and soil, generally leading to inclusion of mat with the thatch layer. This region often serves as an important component of the turfgrass growth medium (Hurto *et al.*, 1980; Ledebauer and Skogley, 1967). Thatch possesses high porosity, allowing for water and nutrient influx and air circulation, and does possess some water holding capacity, although this is generally low due to the high percentage of macropores (Hurto *et al.*, 1980). The fact that plant crowns and roots can reside in the thatch layer has long been established (Ledebauer and Skogley, 1967). The existence of enzymatic activity (Torello and Wehner, 1983) and active microbial populations (Mancino *et al.*, 1993; Mancino *et al.*,

1988;) in the thatch layer has been documented. Because thatch does include living plant tissue including crowns, rhizomes, stolons, roots, and probably soil (depending on sampling technique), it is difficult to distinguish whether nitrogen in the thatch layer is actually in living plant parts, plant material that has senesced since actively taking up the N, microbial biomass components, or non-living organic or inorganic components of the thatch. This can make interpretation of this data difficult.

Thatch can act as a significant source and sink for fertilizer nitrogen. Starr and DeRoo (1981) found a bluegrass/fescue thatch to have a total nitrogen content equivalent to 280 to 510 kg N ha⁻¹ where clippings were removed and returned, respectively. Immobilization in the thatch of 38 and 42 kg ha⁻¹ of labeled fertilizer N (180 kg N ha⁻¹ applied) was measured during one growing season. In extracted Kentucky bluegrass cores in the laboratory, under suction to simulate leaching, 46% of N applied as urea and 67% of N applied as IBDU remained in the thatch after 15 days (Nelson *et al.*, 1980). Thirty minutes after application of either calcium nitrate or ammonium sulfate to Kentucky bluegrass, Bowman *et al.* (1989b) recovered approximately 29% of the nitrate-N and 46% of the ammonium-N in the thatch. Of the NH₄⁺-N recovered, 53% was soluble and 47% was exchangeable, as determined by saturation extraction with deionized water and KCl extraction respectively. The application was made in 0.2 cm water and followed with 0.3 cm irrigation. An additional 49% of NO₃⁻-N and 40% of NH₄⁺-N was found in the underlying soil. Total recovery was 82 and 89%, and declined rapidly by four hours after application. By four days after application, almost no NO₃⁻-N or NH₄⁺-N was recoverable by KCl extraction. The authors attribute the amount not recovered to biological immobilization.

Data on the role of thatch in nitrogen fate is scarce. The ability of thatch to immobilize large percentages of applied fertilizer nitrogen has been demonstrated. However, the disposition (live plant material, microbial biomass, non-living organic matter, soil) of that nitrogen in the thatch is unclear. The developed thatch layer is unique to stands of perennial grasses, and not all species of grass are prone to develop thatch layers. Generally only those with secondary lateral shoots are affected (Turgeon, 1991). The experiments reviewed on pasture grasses utilized species not prone to thatch accumulation, and so cannot contribute data on this subject.

Soil Residence and Transformation

Starr and DeRoo (1981) demonstrated that although uptake of fertilizer N was rapid in the first 30 days following application, most of the nitrogen taken up after that time was from other sources. Of the 180 kg N ha⁻¹ applied as ¹⁵N, 26 and 37 kg ha⁻¹ were immobilized in soil organic matter and 38 and 42 kg ha⁻¹ were immobilized in thatch where clippings were removed and returned, respectively. After the initial 30 day period, nitrogen uptake rates ranged from 0.23 to 0.44 kg ha⁻¹ day⁻¹ where clippings were removed and returned. Uptake of ¹⁵N-labeled fertilizer N was 0.04 and 0.05 kg ha⁻¹ day⁻¹. They also determined a quasi-constant rate of uptake of 0.075 kg N ha⁻¹ day⁻¹ from clippings returned during mowing. Clearly, the majority of the N uptake was from N mineralized from soil or thatch. Over the entire growing season, the sources of nitrogen recovered in clippings were as follows: approximately one-third derived from fertilizer N, approximately one-third from soil N, and approximately one-third from clippings returned. Of the N derived from clippings, approximately one-third was from clippings returned in the current year, and the remainder from clippings returned in the previous two years.

These previous two year's clippings constitute a source analogous to soil N: that is, a long-term mineralizable source. The importance of clipping management in nitrogen utilization by turf is obvious. Over four years of the experiment, soil N in plots where clippings were returned increased 32% over unfertilized controls and 45% over fertilized plots where clippings were removed.

Increases in NO_3^- concentrations in both a fine sandy loam and a sand beneath a Kentucky bluegrass turf were observed during July and August by Rieke and Ellis (1974). The increase was seen not only in plots fertilized in May, but also where no fertilizer N had been applied. This was attributed to mineralization of soil nitrogen. Geron *et al.* (1993) filled lysimeter containers with a silt loam soil which had been excavated and mixed in equal amounts of A, B, and C horizon material soil to simulate commercial construction practices. Seven months later Kentucky bluegrass was established from both sod and seed. During the first nine months following establishment, NO_3^- -N concentrations in percolate averaged approximately 20 mg L^{-1} . During the next year, percolate concentration averaged approximately 3 mg N L^{-1} . The authors attribute the early high levels to a flush of mineralization of soil N following disturbance. These studies indicate that immobilized soil nitrogen can become an important source of N upon mineralization. This can have an impact both on plant utilization and groundwater contamination.

Bristow *et al.* (1987) observed the dynamic nature of fertilizer nitrogen transformations in the soil in a perennial ryegrass pasture. Two days after application of ^{15}N -labeled ammonium nitrate, 45 and 37% of the fertilizer N was accounted for as soil mineral N and microbial biomass N, respectively. After ten days, these levels had dropped to 20 and 13%. Soil organic matter contained 4% of the fertilizer N at this time. After 28 days, soil mineral N,

microbial biomass, and soil organic matter contained 3, 3, and 8% of the fertilizer N, respectively. During this time, cumulative amounts of fertilizer N in above ground plant tissue were 11, 37, and 52%. A portion of the fertilizer N was immobilized briefly by microbial biomass but was re-mineralized quickly and taken up by the plants.

Three years after an October application of 100 kg N ha⁻¹ as ¹⁵N-labeled ammonium sulfate to soft chess, 54 kg fertilizer N ha⁻¹ remained in the soil (Jones *et al.*, 1977). Where the same rate was applied in three consecutive years, 183 kg N ha⁻¹ remained in the soil. When the fertilizer was applied in February, soil immobilization accounted for 24 and 102 kg N ha⁻¹. Greater amounts were removed by the plants from the February applications.

Power and Legg (1984) found that between one and five years after application of ¹⁵N labeled ammonium nitrate 25 to 40% of the fertilizer N remained in the soil. There was a trend of decreasing levels of soil ¹⁵N over time, indicating mineralization. They also observed that 15% of the applied N was recovered in roots in the year of application. After three years, root N accounted for 5% of the labeled fertilizer. They concluded application of N fertilizer to a perennial grassland results in immobilization of significant amounts of that nitrogen, primarily in plant roots and microbial biomass, and that this immobilized N is then recycled in the system. Rekhi *et al.* (1982) found approximately 40% of applied ¹⁵N urea in the soil following rice and wheat crops. In the following season, 1.5 to 3% of the amount applied was taken up as a result of mineralization.

Similar relative amounts of applied fertilizer N were recovered in soil following application to Coastal bermudagrass (*Cynodon dactylon* L.) but the dynamics of the remaining nitrogen was quite different than that observed by Power and Legg. Of the 40% of the applied N (560 kg ha⁻¹) remaining in the

soil, 12.5% was in plant roots, 6% in inorganic forms, 21% was immobilized in organic forms, and 0.5% existed as exchangeable NH_4^+ (Kissel and Smith, 1978). In the following year, 17% of the applied N (almost one-half of what remained in the soil) was recovered in the forage. Even if all root N was transported into herbage, an additional 5% (28 kg ha^{-1}) of the applied N was mineralized and taken up by the plants. The actual amount is probably greater than this.

Comparing the fate of ^{15}N anhydrous ammonia in soils cropped with perennial ryegrass or uncropped, Chalk and Keeney (1975) recovered approximately 22%, 1%, and 0.5% of the applied N in organic, fixed NH_4^+ , and NO_3^- forms in the cropped soil. In the fallow soil, approximately 9%, 2%, and 60-70% were recovered in these same forms. Plants play an important role immobilizing nitrogen. The authors offered no explanation of the high NO_3^- levels in the fallow soil, but it appears that nitrification occurred readily but without plant uptake immobilization was limited. Unrecovered ^{15}N in the fallow soil was thought to be lost through biological and chemo-denitrification.

The above studies utilizing ^{15}N labeled fertilizers illustrate that the majority of fertilizer N uptake by plants occurs in the first growing season, and in fact the first few weeks after application. Much of the remaining N in the soil is present in organic forms, either as microbial biomass or plant material. This immobilized nitrogen is mineralized and again made available to plants, but at relatively low rates in succeeding years. Efficiency of fertilizer nitrogen use is therefore low, but unused nitrogen is mostly immobilized and not susceptible to off-site transport.

Nitrogen Losses in Runoff

Morton *et al.* (1988) found very little potential for loss of fertilizer N in runoff from a Kentucky bluegrass - red fescue lawn. The soil on the site was a sandy loam with a high infiltration rate. They observed only two events over a two year period when runoff occurred. The first was caused by rainfall on snow-covered, frozen ground, and the other occurred following a 12.5 cm rainfall when the soil was already very wet. Inorganic N concentrations during these events were 1.1 to 4.2 mg L⁻¹. Losses by runoff were less than 7% of the total N lost in the aqueous phase.

On bermudagrass golf greens overseeded with perennial ryegrass, appreciable concentrations of N in runoff were observed, varying with nitrogen source, timing, and precipitation (Brown *et al.*, 1977). When ammonium nitrate was applied in February to turf receiving 1 cm irrigation on alternate days, N concentrations in runoff were fairly constant at approximately 10 mg L⁻¹, except when approximately 30 mg L⁻¹ was detected following a 3 cm rainfall event 25 days after application. When applied in September, a detection of approximately 60 mg N L⁻¹ was made 10 days after application during a daily irrigation cycle. Runoff was detected on limited occasions only from organic sources of N (ureaformaldehyde, Milorganite) when applied in the summer or fall, but none from ureaformaldehyde when applied in winter. In a separate experiment, runoff losses were reported only from greens constructed of sandy loam soil, and not from greens constructed of sand-based mixtures (Brown *et al.*, 1982). Losses were greater from inorganic sources (NH₄NO₃) than from organic sources (Milorganite, IBDU).

Chichester (1977) compared nitrogen losses through runoff and leaching under several different cropping systems in lysimeters. In one system, Kentucky bluegrass maintained as a pasture was grown in lysimeters in a silt

loam soil on a 23% slope and received an average of $148 \text{ kg N ha}^{-1} \text{ yr}^{-1}$. In another, corn was grown in a silt loam soil with 13% slope in a conventional tillage system and received a yearly average of 219 kg N ha^{-1} . A third set of lysimeters containing silt loam on a 6% slope was cropped to minimum tillage corn which received a yearly average of 255 kg N ha^{-1} . The plots were unirrigated but received very similar amounts of precipitation. Over four years, the bluegrass plots averaged 0.5 cm runoff loss per year, the conventional corn plots averaged 2.6 cm, and the minimum till corn averaged 0.9 cm. The bluegrass plots lost an average of $1.1 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ as inorganic N in runoff water. The conventional corn lost $3.5 \text{ kg N ha}^{-1} \text{ yr}^{-1}$, and the minimum till corn lost $1.9 \text{ kg N ha}^{-1} \text{ yr}^{-1}$. Loss of soil in runoff was $5200 \text{ kg ha}^{-1} \text{ yr}^{-1}$ carrying with it $10 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ for conventional corn, $35 \text{ kg ha}^{-1} \text{ yr}^{-1}$ with less than $0.1 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ for minimum till corn, and less than $5 \text{ kg ha}^{-1} \text{ yr}^{-1}$ transporting less than $0.1 \text{ kg ha}^{-1} \text{ yr}^{-1}$ for the bluegrass pasture. Clearly the perennial pasture grass conserves not only nitrogen, but also water and soil. The pasture system is more efficient than the conservation system for corn, and losses from the grass are less even from a much more severe slope.

Although N losses from turf through runoff have been reported, they generally occur only in isolated events triggered by high precipitation. The turfgrass environment is generally regarded to be one of low runoff potential due to the presence of thatch, which impedes lateral flow, and a dense root zone near the soil surface which increases permeability. The perennial nature of turfgrass stands and pasture grasses probably leads to development of similar environments. This system allows for very little loss of water, soil, or nitrogen through runoff.

Losses through Deep Percolation

Percolation of fertilizer nitrogen through the soil and into aquifers is the area of greatest environmental concern in the nitrogen fate puzzle. Nitrate-N is considered a pollutant of drinking water, and the Environmental Protection Agency has established a threshold limit of $10 \text{ mg L}^{-1} \text{ NO}_3^- \text{-N}$ ($45 \text{ mg L}^{-1} \text{ NO}_3^-$) (USEPA, 1976). The turfgrass industry has received considerable scrutiny in this area from the popular press. Turfgrass researchers have been examining this problem for over 25 years. Soil texture, nitrogen source and rate, and irrigation and precipitation levels all play important roles in determining leachable nitrogen.

Soil type effects:

Following a single application of 290 kg N ha^{-1} as NH_4NO_3 on May 1 to Kentucky bluegrass growing on a fine sandy loam, Rieke and Ellis (1974) observed a trend towards some downward movement of NO_3^- into late June. On May 15 soil $\text{NO}_3^- \text{-N}$ concentrations were 44.2, 16.4, and 10.2 mg L^{-1} at depths of 0-15, 15-30, and 30-45 cm, respectively. On May 29 concentrations at these depths were 41.1, 32.1, and 15.0 mg L^{-1} . By June 24, concentrations had decreased at each of these depths. From May 1 through June 24, $\text{NO}_3^- \text{-N}$ concentrations in the 45-60 cm depth increased from 5.8 to 10.9 mg L^{-1} . On a sand soil three weeks following application of 390 kg N ha^{-1} , concentrations of $\text{NO}_3^- \text{-N}$ in the 0-15, 15-30, and 30-45 cm depths were 46.0, 37.1, and 15.8 mg L^{-1} . Two weeks later, concentrations were 8.4, 30.1, and 19.0 mg L^{-1} at each depth, respectively. In the 45-60 cm depth, concentrations increased from 5.7 to 16.0 mg L^{-1} between May 1 and June 19. In the second year of the study, significant downward movement of NO_3^- was seen again on the sandy soil.

Nitrate concentrations in drainage water for sand-based greens were observed to be higher than for sand-soil mixtures or sandy loam soil greens for a variety of nitrogen sources (Brown, *et al.*, 1982, 1977). They observed leachate concentrations as high as 326 mg $\text{NO}_3^- \text{ L}^{-1}$ from sand greens, 314 mg $\text{NO}_3^- \text{ L}^{-1}$ from mixed greens, and 180 mg $\text{NO}_3^- \text{ L}^{-1}$ from soil greens following single applications of 163 kg N ha^{-1} as NH_4NO_3 (Brown *et al.*, 1982). Mitchell *et al.* (1978), studying NO_3^- leaching from golf greens using eight different soil mixtures, proposed that differences in leachate NO_3^- concentration might be due more to the amount of total N in the soil than to soil texture. Mean annual NO_3^- concentrations in percolate from a sandy loam soil in Connecticut supporting a bluegrass-fescue lawn were 4 mg N L^{-1} or less under a variety of fertilization rates and irrigation schedules (Morton *et al.*, 1988). Over a two year period, Starr and DeRoo (1981) found no increase in groundwater NO_3^- -N concentrations (2.0 mg N L^{-1}) beneath fertilized sandy loam plots as compared to samples collected upstream (1.8 mg N L^{-1}). After fertilizing the Kentucky bluegrass lawn with ^{15}N labeled ammonium sulfate, ^{15}N was detected in percolate only one time, but total concentration was near background levels. Morton *et al.* (1988) and Starr and DeRoo (1981) concluded that under management practices common to home lawns, the risk of groundwater contamination from fertilizer nitrogen is extremely low. As a basis for comparison, data of Weil *et al.* (1990) from Maryland illustrates groundwater nitrate levels under other planting systems. Sampling wells placed in farmers' fields indicated mean NO_3^- -N levels in groundwater under manured fields of corn and soybean of 27 mg N L^{-1} and 15 mg N L^{-1} in unmanured fields. In hardwood and pine forests, NO_3^- -N levels averaged 0.15 mg L^{-1} .

Nitrogen source effects:

Rieke and Ellis (1974) observed downward movement of ammonium nitrate through a fine sandy loam and a sand, but no downward movement of Milorganite or ureaformaldehyde on these soils, or IBDU on the sand. On a sand green, Brown *et al.* (1982) found that nitrate leaching was the greatest from NH_4NO_3 , intermediate from 12-12-12, and the least from Milorganite and IBDU. Similar results were observed on sand/soil mixture and sandy loam greens, except that the magnitude of N leached was smaller. They also saw a small flush of NO_3^- in the leachate from Milorganite between 20 and 40 days after application on the sand and sand/soil mixture greens. Snyder *et al.* (1984) found that more N was lost in leachate from ammonium nitrate than sulfur coated urea when applied to bermudagrass (*Cynodon dactylon* X *C. transvaalensis*) on a sandy soil with surface applications. It should be noted that NH_4NO_3 was applied every two months and SCU was applied twice each month, both at a rate of $50 \text{ kg N ha}^{-1} \text{ month}^{-1}$. However, injection of the same monthly rate of N as NH_4NO_3 through the irrigation system in small increments produced the smallest amount of N loss through leaching. Nelson *et al.* (1980) found that 54% of applied urea and 15% of applied IBDU leached from intact turf and soil cores in the laboratory following application of 295 kg N ha^{-1} . The amount of IBDU retained in the thatch layer was much greater than the amount of urea, while similar amounts of each source were retained in the soil. Over a 15 week period of laboratory incubation and leaching, Bredakis and Steckel (1963) found that urea and ammonium sulfate yielded the greatest amount of leachable nitrogen, followed by castor pumice and then Milorganite, Uramite, and nitroform.

Irrigation and precipitation effects:

Studying the effects of irrigation and N rate on NO_3^- leaching from a Kentucky bluegrass/red fescue lawn on a sandy loam soil, Morton *et al.* (1988) applied 97 or 244 kg N ha⁻¹ as an equal mixture of urea and ureaformaldehyde and irrigated at one of two rates: either to prevent moisture stress (low rate) or at a rate of 3.75 cm wk⁻¹ (overwatered). Where no fertilizer was applied, 1.9 and 2.8 kg N ha⁻¹ was lost through leaching (mean concentrations 0.5 and 0.4 mg N L⁻¹). At the low rate of fertility, losses were 3.0 and 13.7 kg N ha⁻¹ (0.9 and 1.8 mg N L⁻¹). At the high N rate, losses were 4.9 and 31.9 kg N ha⁻¹ (1.2 and 4.0 mg N L⁻¹). This data illustrates clearly that overwatering produces unnecessary losses of nitrogen through leaching. However, even at the high rate of fertilization, NO_3^- concentration remained well below the EPA threshold limit.

Snyder *et al.* (1984) compared nitrate leaching under daily irrigation to replace evapotranspiration and an automated system which irrigated as determined by soil moisture status. Fertilizer was applied as ammonium nitrate (two month intervals) or sulfur coated urea (bimonthly) to the surface or as ammonium nitrate through the irrigation system at the time of each irrigation (fertigation). The rate was 50 kg N ha⁻¹ month⁻¹ for each treatment. The automated irrigation system produced much lower nitrogen leaching losses across all fertilization treatments. Daily irrigation resulted in overwatering and higher nitrate leaching. Fertigation yielded less nitrate leaching than conventional granular applications. Similar results were obtained when comparing daily fertilization through an irrigation system with surface fertilization every three weeks in conjunction with daily irrigation (Snyder *et al.*, 1980). The authors collected leachate during three periods characterized by different environmental conditions. When daily at approximately 2.5 times

ET, more N was leached from the conventional fertilization plots. During another period when irrigation was less than ET but rainfall compensated for the difference, conventional fertilization again produced more N leaching. When irrigation and precipitation approximated ET, leaching losses between the treatments did not differ. These studies indicate that metering fertilizer nitrogen in small increments through the irrigation system as an effective way of limiting losses by leaching. This holds true even when over irrigating, presumably because the smaller amounts of N applied at one time are cycled within the soil are rapidly immobilized.

Webster and Dowdell (1984) grew perennial ryegrass in lysimeters under pasture conditions in clay loam and silt loam soils. Under conditions of natural precipitation, 7.3 and 3.7% of the applied ^{15}N -labeled calcium nitrate was recovered in the leachate from the clay loam and silt loam soils, respectively. Supplemental irrigation to provide 120% of mean annual precipitation yielded leaching recoveries of 8.0 and 3.8%. In a third treatment, precipitation was withheld for four weeks prior to fertilizer application. Following fertilization, irrigation in an amount equivalent to the precipitation withheld from the plots was applied over a two week period. Percolate recoveries increased to 8.2 and 17.9%, respectively.

Establishment method effects:

Method of establishment of turf can also have an impact on nitrate leaching (Geron *et al.*, 1993). Kentucky bluegrass established from seed on a mixed silt loam soil yielded greater NO_3^- -N concentrations in leachate (15.9 mg L^{-1}) than sodded bluegrass (9.5 mg L^{-1}) during the first three months after establishment. There was no significant difference during the next three months, but for the subsequent 15 months the sodded plots yielded greater

concentrations than the seeded plots (7.9 and 2.9 mg N L⁻¹, respectively). Greater root mass in seeded plots at that time was given as an explanation. During the first nine months following establishment, average NO₃⁻-N concentration in the leachate was approximately 20 mg L⁻¹ averaged for both establishment methods. For the next year, average concentrations were approximately 3 mg L⁻¹. The authors attribute this to mineralization following soil disturbance. They caution that construction and establishment may be a high risk time for nitrate leaching.

Nitrate leaching from other grass crops:

When ammonium sulfate was applied to soft chess at 100 kg N ha⁻¹ in October, 16 kg N ha⁻¹ was recovered in leachate over three years (Jones *et al.*, 1977). When the same rate was applied for three consecutive years, the total loss was 29 kg N ha⁻¹. A single application of 500 kg N ha⁻¹ produced 68 kg N ha⁻¹ in the percolate over three years. For a both a single February application of 100 kg N ha⁻¹ and three annual applications of the same amount, 3 kg N ha⁻¹ was detected in leachate. Single and repeated applications of 100 kg N ha⁻¹ yr⁻¹ and a single application of 500 kg N ha⁻¹ yr⁻¹ in October to subterranean clover yielded leaching losses of 27, 68, and 106 kg N ha⁻¹. February rates of 100 kg N ha⁻¹ in a single application or repeated produced leaching of 9 and 17 kg N ha⁻¹. Timing appears to be a very critical factor in both of these cropping systems.

In bluegrass and corn systems, Chichester (1977) found mean inorganic nitrogen concentrations in lysimeter drainage from bluegrass pasture over a four year period to be 1.6 mg N L⁻¹. Total loss of N was 5.8 kg ha⁻¹yr⁻¹. For conventional and minimum tillage corn, concentrations were 50 and 39 mg N L⁻¹ and total losses through leaching were 167 and 149 kg N ha⁻¹ yr⁻¹. Low and

Armitage (1970) compared nitrogen leaching from meadow fescue (*Festuca elatior*), white clover (*Trifolium repens*), and fallow soil in intact monolith lysimeters (sandy loam soil). Over a three year period, total N recovered in percolate was 3, 132, and 269 kg N ha⁻¹ for the grass, clover, and fallow lysimeters, respectively. Total N in rainfall during this time was 39 kg ha⁻¹. The perennial pasture grass was very effective in mining available nitrogen from the soil. Dowdell and Webster (1980), growing forage perennial ryegrass in lysimeters containing loamy sand soil, found that 2 - 5% of applied ¹⁵N-calcium nitrate (400 kg N ha⁻¹ yr⁻¹ application rate) was recovered in leachate in the first year. This was 60 - 70% of the total N in the leachate. Mean NO₃⁻-N concentrations in three lysimeters were 7, 4, and 16 mg N L⁻¹. After the first year, 0.1% or less of the applied ¹⁵N was recovered in leachate in each successive year. Over three years, ¹⁵N labeled fertilizer comprised 45 - 60% of the total N in the percolate.

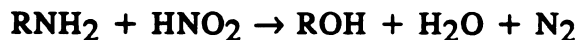
The literature indicates that nitrate leaching can be widely variable dependent upon a number of factors, including soil texture, nitrogen rate and source, and irrigation and precipitation. Soil texture is an important factor (Brown *et al.*, 1982, 1977; Mitchell *et al.*, 1978; Morton *et al.*, 1988; Rieke and Ellis, 1974; Starr and DeRoo, 1981), but is usually dictated either by location or by use, as in the case of golf course putting greens. Good management practices must be employed to limit nitrogen losses to groundwater. Slow release sources provide less potential for loss than easily soluble sources (Bredakis and Steckel, 1963; Brown, *et al.*, 1982; Nelson *et al.*, 1980; Rieke and Ellis, 1974; Snyder *et al.*, 1984;), but soluble sources can be used if applied in reasonable quantities and watered judiciously. Frequent application of small amounts is an effective method to limit leaching losses

(Snyder *et al.*, 1984). Over irrigation can create a high potential for leaching, especially in sandy soils (Morton *et al.*, 1988; Snyder *et al.*, 1984). Managers must make the correct decisions in managing fertilizer applications in order to conserve fertilizer nitrogen and protect the groundwater supply.

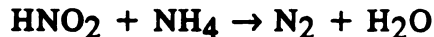
Gaseous Losses: Denitrification and Volatility

Denitrification is the stepwise reduction of NO_3^- to N_2 , which can either be mediated biologically or occur through a number of chemical reactions (chemodenitrification). Several pathways are possible by which chemodenitrification may occur. Examples include (Paul and Clark, 1989)

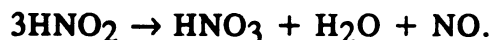
a) the reaction of α -amino groups with nitrite:



b) the reaction of nitrite with ammonium:



c) and spontaneous decomposition of nitrous acid:



Biological denitrification is an enzymatic process mediated by soil microorganisms. The general reaction sequence is:



There is some uncertainty as to whether or not NO is an intermediate in the process. Both N_2O and N_2 can be terminal products and escape into the atmosphere. The entire denitrification sequence requires more than one species of organism to reach completion. The reduction of nitrate to nitrite is mediated by many different bacteria. The subsequent steps are mediated by a much more restricted group (Paul and Clark, 1989).

Presence of nitrate, limited oxygen availability, an available carbon source, and presence of denitrifying bacteria are all required for denitrification

to occur. Conditions enhancing denitrification include high nitrate availability, high organic matter content, high soil water content, near neutral soil pH, and soil temperatures between 5 and 75°C (Paul and Clark, 1989).

Mancino *et al.* (1988) have been the only authors to publish data on direct measurements of denitrification from turfgrass. They studied the effects of soil texture, soil moisture content, and soil temperature on denitrification from a Kentucky bluegrass sod using the acetylene reduction technique. For silt and silt loam soil types, only 0.1 and 0.4% of N applied as KNO₃ (45 kg N ha⁻¹) was recovered as N₂O when soil was at 80% saturation at 22°C for 10 days. Above 80% saturation, denitrification losses increased, with a maximum of 5.4 and 2.2% loss from the silt and silt loam soils, respectively. For the silt soil at a soil water content of 75% saturation, denitrification losses increased linearly with temperature between 22 and 30°C from 0.02 to 0.11% of applied N. Maximum losses from the silt and silt loam soil were 94 and 46% of applied N over 10 days when the soils were at 100% saturation and the temperature was 30°C.

Oxidation status of Kentucky bluegrass thatch and underlying silty clay loam soil was measured under conditions of no irrigation, daily irrigation (0.6 cm day⁻¹), and continuous irrigation (0.6 to 1.2 cm hr⁻¹) on plots with either natural drainage or drainage impaired by a plastic sheet at a 6 cm depth (Thompson, *et al.*, 1983). Over seven days, non-irrigated thatch and soil remained well-aerated under both drainage regimes. Naturally drained plots irrigated daily remained well aerated, but under continuous irrigation low oxygen content after three days indicated reducing conditions. Impaired drainage created low oxygen concentrations and reducing conditions in the thatch after one and three days for the continuously and daily irrigated plots, respectively. Reduced oxygen content of the underlying soils was measured at

one and seven days for each of these treatments. Temperatures in the thatch were as high as 36°C at midday. This data indicates that conditions for denitrification could exist where poorly drained soils are present and irrigation is applied daily or more frequently. These types of conditions probably occur with regularity on selected turfgrass sites.

Rekhi *et al.* (1982) made direct measurements NO, N₂O, and N₂ for 70 days following application of ¹⁵N labeled urea to flooded rice. Loss as N₂O or NO was 0.0001% of applied N. Loss as N₂ was 7.2% and 4.8% of 180 kg N ha⁻¹ applied at one time or split in three equal applications.

The subject of volatility is addressed in detail in Chapter 2. Losses of N from turf due to volatility have been measured directly by several researchers (Bowman *et al.*, 1987; Wesely *et al.*, 1987; Torello *et al.*, 1983; Nelson *et al.*, 1980; Volk, 1959). The activity of the enzyme urease in turf has also been documented (Torello and Wehner, 1983).

Many researchers conducting mass balance experiments with ¹⁵N have estimated total loss through gaseous evolution of N by attributing non-recovered ¹⁵N to denitrification and/or volatility. Starr and DeRoo (1981) attributed losses from 24 to 36% of ammonium sulfate applied to Kentucky bluegrass to denitrification and volatility. A loss of 47% of 500 kg N ha⁻¹ applied to clover in the fall was attributed to these two mechanisms by Jones *et al.* (1977)

Late Fall Nitrogen Fertilization

Fertilization of cool season turfgrasses in the late fall (early November in Michigan) has been a recommended practice for a number of years, primarily due to early spring green up and positive growth response, without a large flush of growth as is often seen with an early spring application. This practice has received some attention and criticism from scientists and industry professionals.

It is sometimes perceived that there must be increased risk of groundwater contamination by nitrates if N is being applied after the time that most plant growth has slowed or stopped. One of the objectives of this experiment was to assess this risk.

Wilkinson and Duff (1972) studied color and growth response, cold tolerance, and chlorophyll content of Kentucky bluegrass to nitrogen fertilization throughout the fall. Ammonium nitrate was applied to separate plots at a rate of 100 kg N ha⁻¹ on either October 1, October 15, November 1, November 15, December 1, or December 15. Application on November 1 or earlier resulted in enhanced green up and chlorophyll content throughout the fall and winter. Fertilization on or after November 15 did not result in a substantial fall color response, but these plants had higher chlorophyll contents in mid-April during the spring green up period. Plants fertilized on October 1 or on or after December 1 had adequate cold tolerance, but fertilization between October 15 and November 15 reduced cold tolerance. Incorporation of N at this time apparently delayed the hardening process. Fertilization on October 1 resulted in increased growth in the fall. Later applications did not produce increased growth, but growth rates declined more slowly than the unfertilized control. In the spring, plants which received fertilizer later in the fall displayed greater growth rates. Based on these results, fertilization in early November would appear to produce the greatest benefits: enhanced color but no increased growth in the fall, and enhanced chlorophyll content and growth response in the spring. Some cold tolerance may be sacrificed, however. These responses are probably largely dependent on temperature, and so may vary with location and year.

Response of 'Kentucky 31' tall fescue (*Festuca arundinacea* Schreb.) and 'Cohansey' and 'Pencross' creeping bentgrasses (*Agrostis palustris* Huds.) to

nitrogen fertilization in the fall and winter were studied in Virginia (Powell *et al.*, 1967). Ammonium nitrate was applied in single applications at a rate of 50 kg N ha⁻¹ at the following times: October; October and December; October, December, and February; and October through February, monthly. It was also applied in January only and monthly from October through February at 100 kg N ha⁻¹ month⁻¹. Ureaformaldehyde was applied either in October only or in October, December, and February at a total rate of 500 kg N ha⁻¹. Both species responded similarly in color and growth response. Each additional increment of soluble N resulted in improved color between November and May. For the single October application, however, turf was brown by January. A single application of 100 kg N ha⁻¹ in January produced a rapid color response from brown to green. None of the treatments resulted in increased top growth between December and March. Greater clipping yields resulted beginning in March in response to increasing rates of soluble N. Yield from ureaformaldehyde treated plots responded more slowly in March, but yield was similar to the high rate soluble N plots by May. Carbohydrate concentrations in stems were higher than in leaves from October through February. After February, concentrations in leaves were as high or higher than in stems. Carbohydrate concentration was generally higher in low N treatments. Photosynthetic rates of the Cohansey bentgrass were measured on four dates between January and March. Turf fertilized with higher rates of N showed higher net photosynthesis.

Hanson and Juska (1961) measured root, rhizome, crown, and top growth of 'Merion' Kentucky bluegrass between October and May in response to nitrogen fertilization in the fall and winter. Root plus rhizome weights of untreated controls increased 4.4 g plot⁻¹ between October and May. Plots receiving 147 kg N ha⁻¹ as NH₄NO₃ in September or September and October

showed additional increases of 0.8 and 2.2 g plot⁻¹ respectively. These figures include losses of 2.6 and 1.9 g plot⁻¹ compared to the control during May. Therefore, the balance at the end of April was additional growth of 3.4 and 4.1 g plot⁻¹ (increases of 57 and 68% as compared to the control). Increases in top plus crown weights during this period were 52.9 g plot⁻¹ for the unfertilized check, and an additional 7.0 and 17.0 g plot⁻¹ for September and September and October fertilized turf. There were also decreases in May for top and crown growth. The balance at the end of April was 14.9 and 21.2 g plot⁻¹ additional growth (89 and 126% increases).

These three experiments demonstrate that metabolic activity in cool season turfgrass is maintained through the winter months. Although rates and timing of fertilizer applications were not the same as current recommended practices, the potential of late fall fertilization to stimulate root development and spring recovery is clear. Concerns over the fate of unutilized nitrogen applied at this time need to be addressed. Geron *et al.* (1993) examined this issue in relation to establishment methods of Kentucky bluegrass and nitrogen source and application timing effects. Between the summer of 1989 and the fall of 1990 no significant differences were detected in NO₃⁻-N concentrations in percolate from plots receiving 72.75 kg N ha⁻¹ in November as compared to plots receiving no November N (equivalent annual rates of 218 kg N ha⁻¹). However, during December of 1990 and January 1991 November fertilized plots had a significantly higher NO₃⁻-N concentration in the percolate (3.37 vs. 2.39 mg N L⁻¹). The paper does not present data after this time. During the winter and spring of 1990, concentrations were 14.07 and 1.10 mg N L⁻¹ for plots not receiving no November N and 15.81 and 1.52 mg N L⁻¹ for those receiving N in November, but these differences were not significant. It should be noted that these plots were established in May of 1989. High NO₃⁻-N

concentrations during the first winter were due largely to mineralization of nitrogen following disturbance of the soil in the lysimeter filling process. In addition, leaching characteristics of this system may be different than those of established mature turf.

Chalk and Keeney (1975) applied ^{15}N anhydrous ammonia to pots seeded to ryegrass or left fallow under simulated fall and spring conditions. Recovery in plants and soil were very similar for both treatments on two of three soils tested. Total recovery averaged 96% of applied ^{15}N for both spring and fall treatments averaged over these two soils. For the third soil, recovery was 97% for the spring treatment and 89% for the fall treatment. Losses from this particular soil were 40 to 50% when fallow, and the authors attribute this to denitrification. They explain that denitrification might be higher following freeze-thaw cycles due to increased availability of organic matter serving as an energy source for denitrifying bacteria.

MATERIALS AND METHODS

Lysimeter Construction

Four intact monolith drainage lysimeters were constructed at the Hancock Turfgrass Research Center at Michigan State University to study nutrient and pesticide leaching. The soil type at the site is a Marlette fine sandy loam (fine-loamy, mixed mesic Glossoboric Hapludalfs) and had been in turfgrass for six years prior to construction of the lysimeters and was planted to corn prior to that. Two of the lysimeters, constructed in 1989 and 1990, were constructed with a bare soil surface following removal of the existing sod. The other two, built in 1991, were constructed with newly established turfgrass intact. The lysimeters are constructed of grade 304 stainless steel 0.5 cm thick and are cylindrical in shape, 1.14 m in diameter (1m^2 surface area) and 1.2 m deep. The bottoms of the lysimeters have a 3% slope so that leachate collects on one side where a drainage tube was later installed. To install each lysimeter, a soil monolith was excavated by hand in depth increments of approximately 20 cm, so that the diameter of the monolith was slightly greater than the internal diameter of the container. After the first excavation, the open-ended lysimeter container was placed over the monolith and downward pressure was applied with a backhoe to slide the container over the monolith. Excavation and enclosure of the soil core continued incrementally until the container was filled and the soil surface was flush with the top edge of the container. The soil core was excavated for an additional 15 cm so that it extended below the bottom of the container. This exposed portion of the column was then excavated by hand from two sides of the lysimeter, working inward toward the center of the core. When approximately 20 to 25 cm of soil was removed from each side, steel H-beams were placed under the cores,

oriented parallel to each other. A sheet of steel slightly wider than the lysimeter was then placed on top of these beams and was pushed with a backhoe so that it slid between the beams and the lysimeter core, severing the core from the soil below. A round piece of 1.8 cm thick plywood, cut to fit the lysimeter, was placed on top, the lysimeter was wrapped with chains, and the entire core, including wood cover, steel sheet, and H-beams were lifted from the excavation site using a small truck crane. The lysimeters were designed with a pair of protruding axles on the outer surface on opposite sides at the center of mass of the core. This allowed the lysimeters to be easily inverted when lifted from the excavation pit. Once inverted, the core was unwrapped and the steel beams and plate were removed, revealing the bottom soil surface of the lysimeter core. Approximately 3 cm of soil was removed with a small hand shovel and this void was then filled with 1 - 2 cm pea gravel that had been rinsed in 1% sodium hypochlorite and water. The stainless steel bottom was then welded in place, a hole was drilled, and a stainless steel drain tube approximately 1.5 cm diameter and 10 cm long was welded into place. The core was then reinverted.

Manhole structures had been constructed of 0.32 cm steel around a steel beam framework. An access/work area measuring 1.14m wide x 1.35m long x 1.53m tall with a top entry hatch door was designed with a platform extending from one side that the lysimeter core rested upon. The top surface of this platform had a 3% slope to match the bottom of the lysimeter. When the core was set on the platform, the top edge of the core was flush with the soil surface and the bottom of the core was approximately 0.6m above the floor of the manhole allowing for placement of a sampling vessel. The manhole was set into a different hole from which the cores were extracted. Cores were not removed from the same site as their final destination because of a concern over

soil disturbance in the area of construction. The two locations were within 10m of each other and were of the same soil type. After placing the cores on the manhole structures, the two units were welded together along all external contact areas to create a sealed manhole area with access to the lysimeter for sampling. Excavation holes were then backfilled, matching soil types for each distinct layer of the profile.

The lysimeters were installed in a 18m x 36m plot area reserved for all future lysimeter studies. In May 1990 the entire area was sprayed with glyphosate, prepared for seeding, and in June was seeded to 'Adelphi' Kentucky bluegrass at a rate of 49 kg ha⁻¹. Fertilizer (12-12-12) was applied at a rate of 49 hg N ha⁻¹ prior to seeding. Additional fertilizer was applied at a rate of 24.5 kg N ha⁻¹ on June 15 (as 12-12-12) and on July 11, July 24, August 7, August 21, and August 27 as urea. Due to a high soil seed bank of annual bluegrass (*Poa annua* L.) and establishment of the desired species at a less than optimum time, the stand of turf that developed was of poor quality. In September the area was again renovated with glyphosate and sodded with a blend of Adelphi, 'Nassau', and 'Nugget' Kentucky bluegrasses (equal proportions by weight at seeding). Urea was applied at a rate of 24.5 hg N ha⁻¹ on September 14 and 27. Following establishment a thin piece of sheet metal 360 cm long and 5 cm wide was installed inside the interior edge of each lysimeter to create a slight vertical extension of the lysimeter containers. This piece extended approximately 1.25 cm above the soil surface. This prevented the turf from both inside and outside the lysimeters from crossing the lysimeter edges, and also prevented the possibility of surface runoff from the lysimeters.

In March 1991 microplots for soil sampling were installed adjacent to the lysimeters. Use of confined microplots reduces the area to which ¹⁵N-labeled fertilizer must be applied because lateral movement is not a concern. This was

necessary in order to conserve ^{15}N . The microplots were constructed of 20 cm diameter PVC, 60 cm in length. One edge of the microplots was beveled to limit soil resistance at the leading edge during installation. They were installed by pushing directly into the soil with a hydraulic cylinder unit constructed especially for this purpose and mounted to a tractor. The thatch was first cut with a knife around the inside edge of the microplot because it provided a great deal of resistance against initial penetration of the PVC tube. The microplots remained open at the bottom after installation. This method preserved the native soil structure of the microplots and the surrounding area. A total of 64 microplots were installed in a randomized complete block layout with four replicates.

In June of 1991 construction on the second pair of lysimeters started and was identical procedurally to the first set, except that the established sod was left in place. Complete enclosure of the cores and attachment of bottoms was completed on August 1 and lysimeters were in place and the holes were backfilled by September 12. These lysimeters were placed in the holes created by excavation of the original set of cores near the center of the plot area. These holes had been previously refilled but the same site was re-excavated so that the area where soil was disturbed was minimized. This maximized the area of the plot where native soil structure was preserved. These lysimeters were completed in June.

Experimental Design

The experiment was initiated in April 1991 with fertilizer application to the two completed lysimeters. There were two treatments in the study, defined by fertilizer application timing. Both treatments received a total of $196 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ as urea, applied in five equal applications of $39.2 \text{ kg N ha}^{-1}$. The

'Spring' treatment received its first application in late April and succeeding applications at approximately 38 day intervals, the final application being made in late September. The schedule of application for the 'Fall' treatment was similar except that there was no April fertilization and there was an additional application made in early November. Exact dates for all fertilizations in 1991 are shown in Table 1. The April and November applications were made with ^{15}N -labeled urea (24.9613 atom % excess in April, 25.2283 atom % excess in November). Use of this stable isotope of nitrogen allowed for discrimination of those particular fertilizer applications from all other N in the environment. For 1992 and 1993 fertilizer application dates were similar to those in 1991. The ^{15}N -labeled material was applied in 1991 only.

The ^{15}N -labeled fertilizer was applied by hand as a solution to each lysimeter and microplot in 0.05 cm water. For the microplots this was dispensed from a 125 ml polypropylene bottle which had five holes drilled in the top. Lysimeter fertilization was performed with a plastic watering can. Fertilization of each individual plot was immediately followed with irrigation with an additional 0.45 cm water applied from the same container to ensure that all of the fertilizer solution was rinsed from the container. After all lysimeters and microplots were fertilized and irrigated, each plot was covered individually with a piece of plywood and surrounding border areas were fertilized with non-labeled fertilizer. This border fertilization and all other applications of non-enriched fertilizer to microplots and lysimeters were made with a commercial boom sprayer utilizing flood-jet nozzles at a rate of 561 L ha^{-1} (60 gal A $^{-1}$). This was immediately followed with 0.5 cm irrigation.

Soil samples were collected from the surrounding plot area annually, submitted to the Michigan State University Soil Testing Laboratory for analysis, and fertilized with potassium based on recommendations developed for

Table 1. Fertilization dates for Spring and Fall treatments in 1991.

<u>Spring</u>	<u>Fall</u>
April 26 †	
June 4	June 4
July 12	July 12
August 19	August 19
September 27	September 27
	November 8 †

† These applications included ^{15}N labelled urea.
 All applications were at a rate of $39.2 \text{ kg N ha}^{-1}$.

turfgrass in Michigan. Annual rates of 49 and 98 kg K₂O ha⁻¹ were applied in 1992 and 1993, respectively. Application of fertilizer phosphorus was not necessary. Irrigation was applied as necessary to maintain a high quality turf. A concurrent study investigating vertical mobility of common turfgrass pesticides was conducted in the lysimeters. Several pesticide applications were made and are recorded in Table 2. Pesticides applied to lysimeters were also applied to corresponding microplots. Additional pesticides were applied sparingly as needed to manage weeds and diseases.

Sample Collection and Analysis

Leachate was collected from the lysimeters in 19 L glass jars. These jars sat within 76 L plastic basins to catch overflow in the case of very large drainage events. Jars were emptied when approximately 1/2 full, usually every 7 to 10 days, but sometimes more or less frequently depending on precipitation patterns. Total mass of water was recorded and approximately 100 ml was saved in a 125 ml polypropylene bottle and frozen until analysis.

Clippings were collected from all plots approximately weekly throughout the growing season as dictated by growth rate. A pair of manual hand clippers was used to cut the grass while holding a hand-held vacuum against the clippers in order to collect clippings as quantitatively as possible. Clippings were dried at 65°C for 72 hr then ground to pass an 80 mesh screen prior to analysis.

Microplots were excavated periodically for soil nitrogen analysis. Sampling dates for each treatment are given in Table 3. This sample schedule was designed so that for each treatment four sampling dates occurred in the first year following ¹⁵N application and two sampling dates occurred in the second year. For both treatments, the first sample was taken 18 days after application and the final sample was removed approximately two years (748 days) after

Table 2. Schedule of pesticides applied to lysimeters 1 and 2 (Spring treatment) and 3 and 4 (Fall treatment).

<u>Date</u>	<u>Pesticide</u>	<u>Application rate kg a.i. ha⁻¹</u>	<u>Lysimeters</u>
8/12/91	isazofos	2.24	1 & 2
8/21/91	chlorothalonil	9.56	1 & 2
9/17/91	dicamba	0.12	1 & 2
9/17/91	2, 4-D	1.14	1 & 2
5/3/92	fenarimol	0.76	1 & 2
6/18/92	propiconazole	0.84	1 & 2
7/21/92	triadimefon	1.53	1 & 2
7/21/92	metalaxyl	1.53	3 & 4
7/21/92	chlorothalonil	9.56	3 & 4
8/5/92	metalaxyl	1.53	1 & 2
8/5/92	chlorothalonil	9.56	3 & 4
8/13/92	metalaxyl	1.53	3 & 4
8/20/92	chlorothalonil	9.56	3 & 4
9/4/92	chlorothalonil	9.56	3 & 4
9/4/92	metalaxyl	1.53	3 & 4

Table 3. Microplot sampling dates for Spring and Fall treatments.

<u>Date</u>	<u>Treatment Sampled</u>
May 14, 1991	Spring, Fall
June 21, 1991	Spring
October 14, 1991	Spring
November 26, 1991	Spring, Fall
May 26, 1992	Spring, Fall
June 29, 1992	Fall
September 17, 1992	Fall
November 30, 1992	Spring, Fall
May 14, 1993	Spring, Fall
November 30, 1993	Fall

application. The sample collected in May 1991 for the Fall treatment was used for background N and ^{15}N levels.

Samples were collected by first excavating the microplots intact. A 1.9 cm diameter soil probe was used to collect a sample from the 60 - 100 cm depth removing five core samples from directly below the microplot. After transport to a laboratory, each PVC microplot was split open longitudinally to expose the soil core within. The top segment of the core was separated from the rest of the core just below the thatch layer using a kitchen knife. Verdure was defined as all green plant material above the thatch layer, and was removed using scissors. From the remainder of the excised section, soil was scraped away until a layer of rhizomes was reached. These rhizomes defined the bottom of the thatch layer. Soil removed during this operation was returned to the top of the remainder of the core. Verdure and thatch samples were dried at 65°C for 72 hr. Because of different grinding methods, thatch samples were separated into organic and soil components by hand massaging. Verdure and thatch organic matter, as this material was labeled, were ground to pass an 80 mesh screen with a Wiley mill. Thatch soil was prepared for total N and ^{15}N analysis by pulverizing with a rolling pin until it was a fine powder.

The remainder of the soil core was sectioned by depth into the following increments: 0 - 5 cm; 5 - 10 cm; 10 - 20 cm; 20 - 40 cm; 40 - 60 cm. Three subsamples were then removed from each increment. One was put into a soil sample tin and dried at 65°C for 72 hr for soil moisture determination and nitrogen analysis. Soils were not dried at 110°C because of the possibility of organic matter decomposition at this temperature (Gardner, 1986). Another subsample was stored at 5° for 2 - 5 days for microbial biomass determination. The third subsample was frozen at -25°C .

Inorganic nitrogen (NO_3^- , NH_4^+) was determined first by extracting duplicate samples of dry soil with 1N KCl (5:1 v/w). Nitrate (Lachat Instruments) and ammonium (Lachat Instruments) were determined by flow injection analysis on a Lachat QuikChem autoanalyzer (Lachat Instruments, Milwaukee, WI). Following this analysis, inorganic N was converted for ^{15}N analysis by the diffusion method of Brooks *et al.* (1989). A subsample of the dried soil was prepared for total N and ^{15}N analysis by pulverizing as described above.

Microbial biomass was determined for each depth increment below the thatch layer by the chloroform fumigation-incubation method (CFIM) (Jenkinson and Powlson, 1976) using 25g dry weight equivalent of field moist soil. Evolved CO_2 was trapped in 2N NaOH and titrated with 1.5 N HCl. Non-fumigated samples were also incubated but this control value was not used in the calculations (Voroney and Paul, 1984). Samples were prepared for biomass N and ^{15}N analysis by extraction of fumigated samples with 1N KCl followed by flow injection analysis and diffusion of NH_4^+ (Brooks *et al.*, 1989). Pre-fumigation NH_4^+ was accounted for by subtraction of NH_4^+ measured above as inorganic soil N.

Concentrations of NO_3^- and NH_4^+ in leachate were determined by flow injection analysis. Samples were prepared for ^{15}N determination by the method of Brooks *et al.* (1989). Due to very low NO_3^- and NH_4^+ concentrations and inadequate volume for separate diffusions of NO_3^- and NH_4^+ , a single diffusion for both species was performed and ^{15}N analysis of leachate was for $\text{NO}_3^- + \text{NH}_4^+$.

Total nitrogen content of clippings, verdure, thatch organic matter, thatch soil, and soil samples, and ^{15}N enrichment of these samples as well as all diffusion samples was determined using a Europa Scientific Roboprep C-N

Biological Sample Converter and Tracermass mass spectrometer (Europa Scientific USA, Cincinnati, OH).

The microplot data was analyzed statistically as a split-plot in time design (Steel and Torrie, 1980), with time serving as main plots and depth as subplots. In analysis of total N and labeled fertilizer N recovery, "depths" included clippings, verdure, thatch, and soil (total of all soil depths). Clipping data was totaled to include all clipping collection dates up to the time of the respective soil sampling time. Concentration of N and ^{15}N in the soil by depth was analyzed in a similar manner in order to determine downward movement of N through the soil profile. Single degree of freedom comparisons were made contrasting 0 - 5 cm vs. 5 - 10 cm, (0 - 5) + (5 - 10) cm vs. (10 - 20) + (20 - 40) + (40 - 60) cm, and 10 - 20 cm vs. (20 - 40) + (40 - 60) cm depths. Because of the time difference in application of ^{15}N labeled fertilizer, differences in distribution of the ^{15}N , at least in the short-term, seemed obvious. In addition, the scheduling of ^{15}N applications and soil sampling dates resulted in unequal time intervals between treatment application and soil sampling for the two fertilization schedules. For these reasons, fertilizer application timing was not included as a variable in the analysis. In this sense, the two treatments were analyzed as separate experiments. The exception to this is analyses of the first and last samplings following ^{15}N applications, which occurred 18 and 348 days following application for both treatments, in which direct comparisons were made. Statistical analyses were conducted using PC-SAS v. 6.04 (SAS Institute, Cary NC).

RESULTS AND DISCUSSION

The overall analyses of variance for LFN (labeled fertilizer nitrogen) recovery by depth for each fertilization schedule in a split-plot design are shown in Tables 4 and 5. Four "depths" were included in this analysis: clippings, verdure, thatch, and soil. Leachate N was not included because the amounts were extremely low, LFN values being several orders of magnitude less than in other components. Because of significant time x depth interactions for both treatments, further analyses of N content at each depth over time were conducted. Thatch and soil N were segmented into different components for detailed analyses. The results will be presented in order of the depths designated above: clippings, verdure, thatch, and soil. Treatment of leachate data will follow.

Clipping Yield and Clipping and Verdure N

Clippings:

Clipping yield closely followed growing degree day (GDD) accumulation (10° C base, through November 30) for the first month of the growing season in 1991 (Figure 1). For the remainder of the year however GDD accumulated at a faster rate than did clipping yield. In 1992, which was a cooler year, clipping yield outpaced GDD accumulation throughout the growing season for the Spring treatment (Figure 1). Total GDD were 1617 in 1991 and 1165 in 1992, while clipping yields were 3668 and 4925 kg ha⁻¹, respectively. The turf exhibited greater growth under cooler temperature conditions. For the Fall treatment in 1992 clipping yield accumulated rapidly as compared to GDD early in the season, but then tapered off (Figure 2). In 1993 rates of accumulation of GDD and clipping yield were similar throughout the year. For the Fall

Table 4. Analysis of Variance of Labeled Fertilizer Nitrogen recovered for Spring treatment for a split-plot design.

<u>Source</u>	<u>df</u>	<u>Sum of Squares</u>	<u>Mean Square</u>	<u>F</u>
Time	6	80.09	13.35	2.14
Replication	3	7.69	2.56	0.41
Error (a) (Time x Rep)	18	112.53	6.25	
Depth	3	778.97	259.69	59.65**
Time x Depth	18	1336.35	74.24	17.06**
Error (b)	63	274.23	4.35	
Total	111	2589.86		

** Significant at P = 0.01.

Table 5. Analysis of Variance of Labeled Fertilizer Nitrogen recovered for Fall treatment for a split-plot design.

<u>Source</u>	<u>df</u>	<u>Sum of Squares</u>	<u>Mean Square</u>	<u>F</u>
Time	6	194.18	32.36	4.45**
Replication	3	10.01	3.34	0.46
Error (a) (Time x Rep)	18	130.87	7.27	
Depth	3	1246.98	415.66	60.30**
Time x Depth	18	2315.98	128.67	18.67**
Error (b)	62	427.38	6.89	
Total	110	4339.71		

** Significant at P = 0.01.

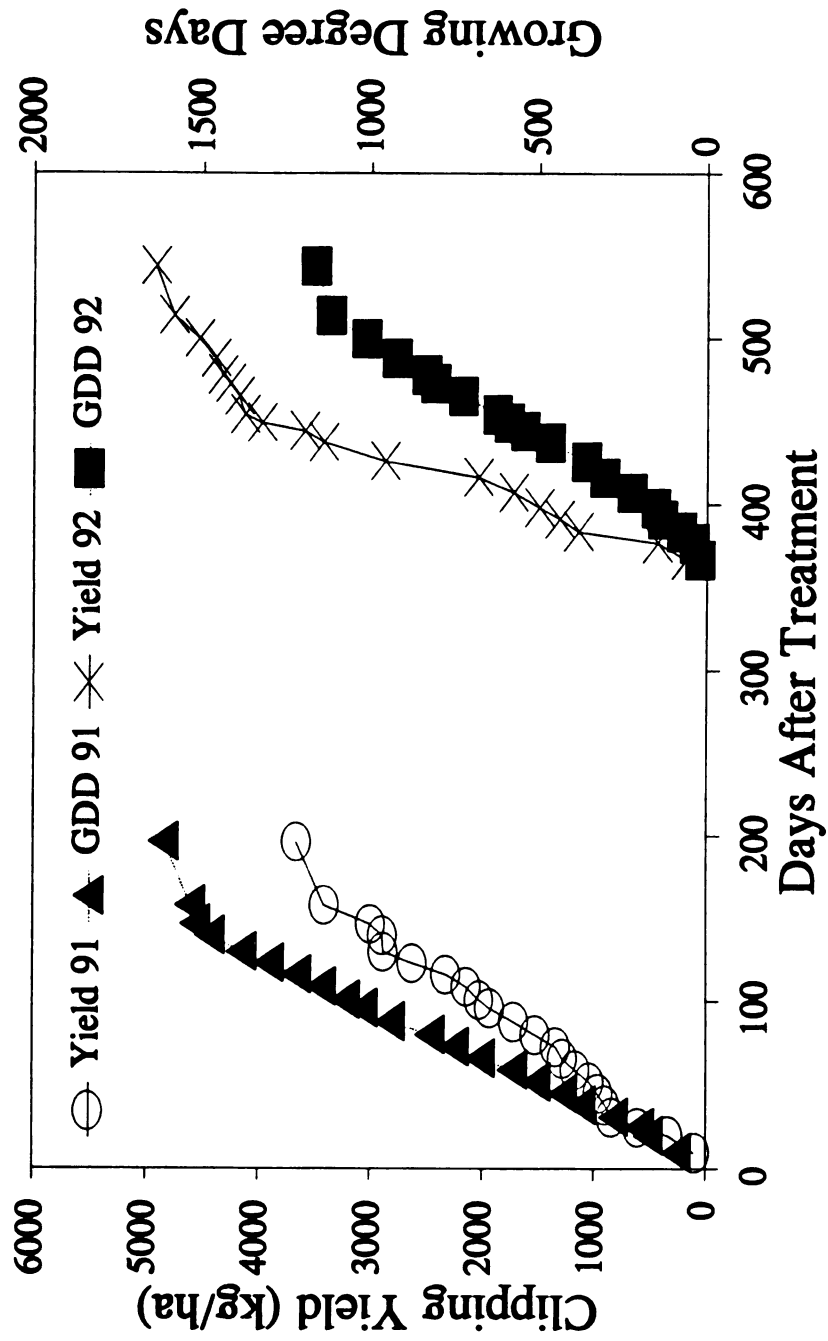


Figure 1. Growing degree days and cumulative clipping yields for Spring treatment during 1991 and 1992.

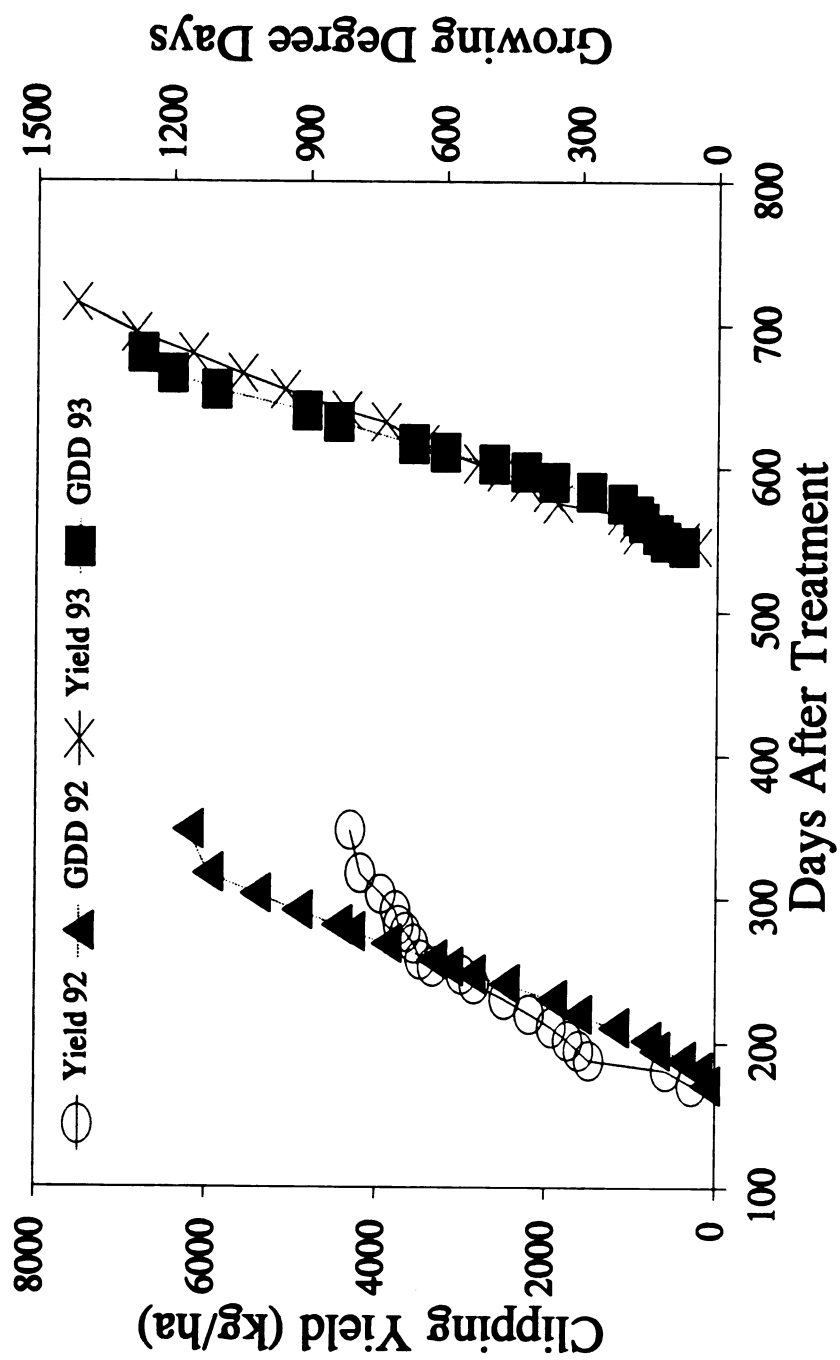


Figure 2. Growing degree days and cumulative clipping yields for Fall treatment during 1992 and 1993.

treatment 1993 was a warmer year than 1992 (1165 GDD in 1992, 1268 GDD in 1993). In 1993 GDD was calculated only through September 30. GDD through September 30 in 1992 was 1121. Clipping yields for the Fall treatment were 4310 and 7542 kg ha⁻¹ in 1992 and 1993, respectively. Clipping yields followed similar patterns during the two years early in the season, but accumulation rates slowed in 1992 and were maintained in 1993. Because GDD was roughly the same in these two years it is difficult to attribute this difference in clipping yield to temperature effects alone.

Cumulative clipping yield and cumulative nitrogen removed in microplot clippings over the duration of the experiment are shown in Figure 3 for the Spring treatment and Figure 4 for the Fall treatment. Clipping yield and nitrogen content followed the same patterns within each treatment. Both treatments showed similar trends in dry matter production and N content over time. Flushes of growth and N content occurred for short periods during the spring after which rates tapered off and were more gradual through most of the growing season, with very little growth and uptake during winter months. Total clipping yield over two years for the Spring treatment was 9220 kg ha⁻¹, containing 276 kg N ha⁻¹ (mean of 3.0% N). The Fall treatment yielded 12,102 kg ha⁻¹ in clipping weight containing 349 kg N ha⁻¹ (mean of 2.9% N). Spring treatment clippings were collected from May to October in 1991 and 1992, while Fall treatment clippings were harvested during 1992 and 1993. Because of these timing differences it is unclear whether yield differences were due to treatment or environment.

Content of total nitrogen and labeled fertilizer nitrogen (LFN) in clippings is broken down on an annual basis for both treatments in Figures 5 through 8. Following fertilization on April 26, 7.0 kg LFN ha⁻¹ were removed in clippings in the first 31 days (Figure 5). This was 18% of the LFN applied

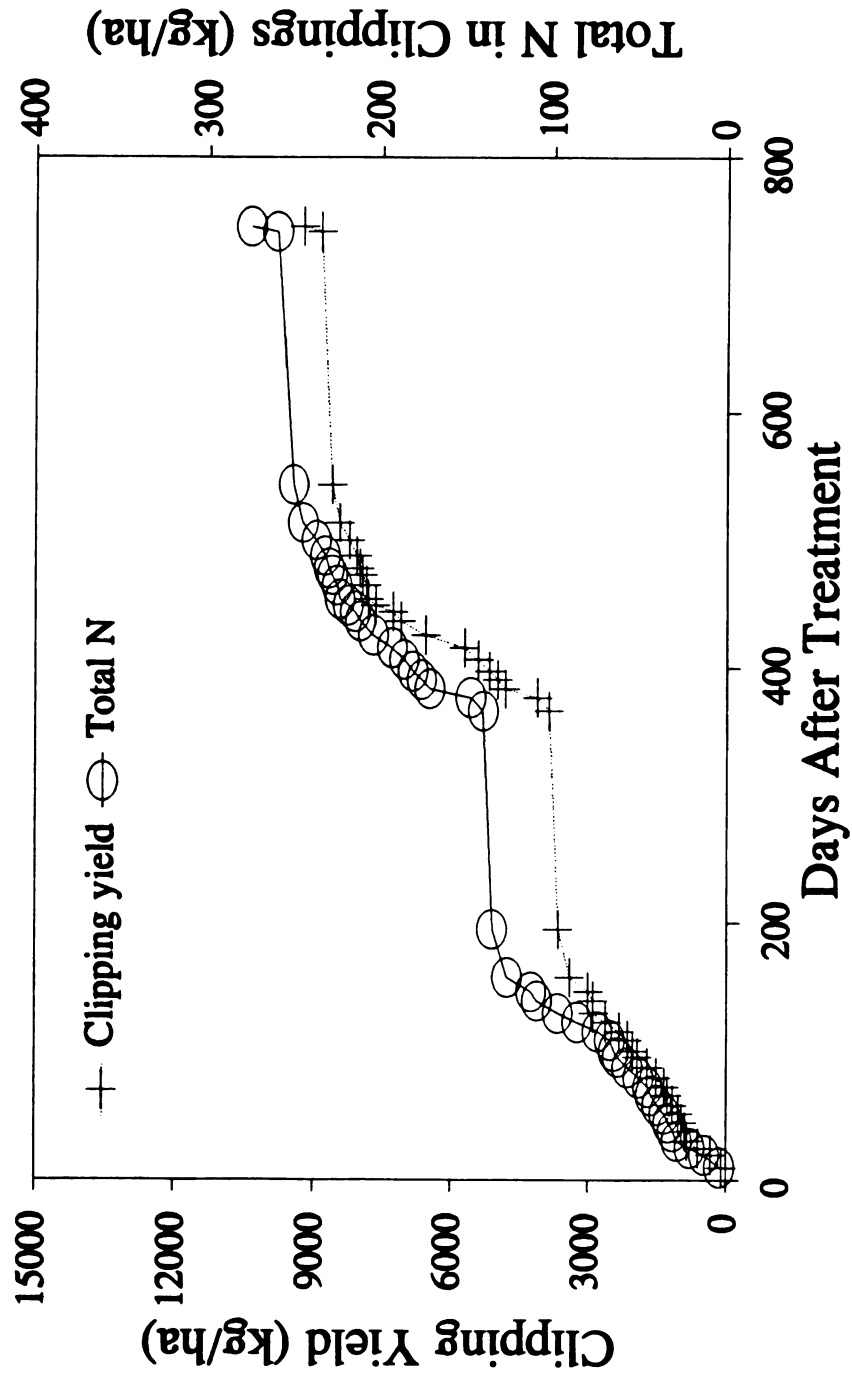


Figure 3. Cumulative clipping yield and total nitrogen harvested in clippings from microplots receiving the Spring treatment.

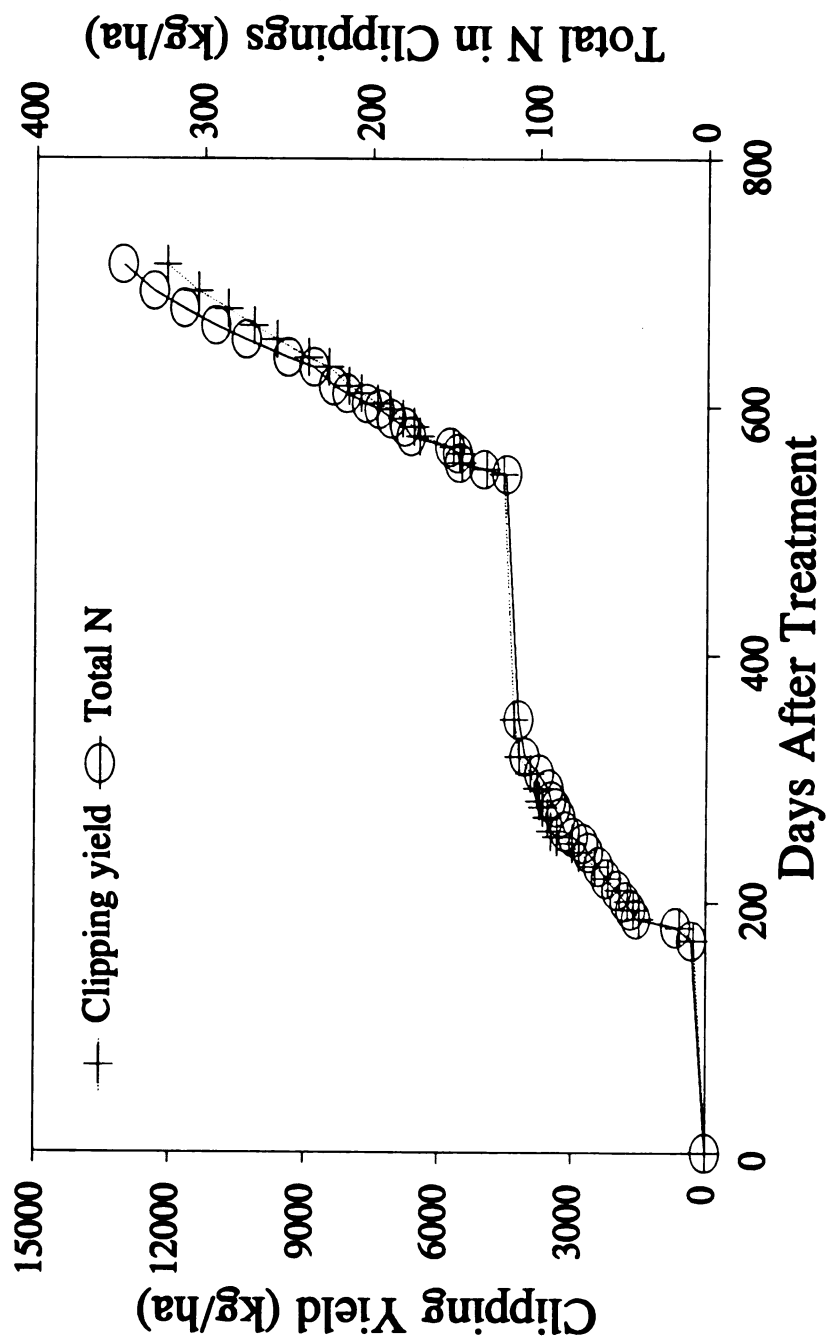


Figure 4. Cumulative clipping yield and total nitrogen harvested in clippings from microplots receiving the Fall treatment.

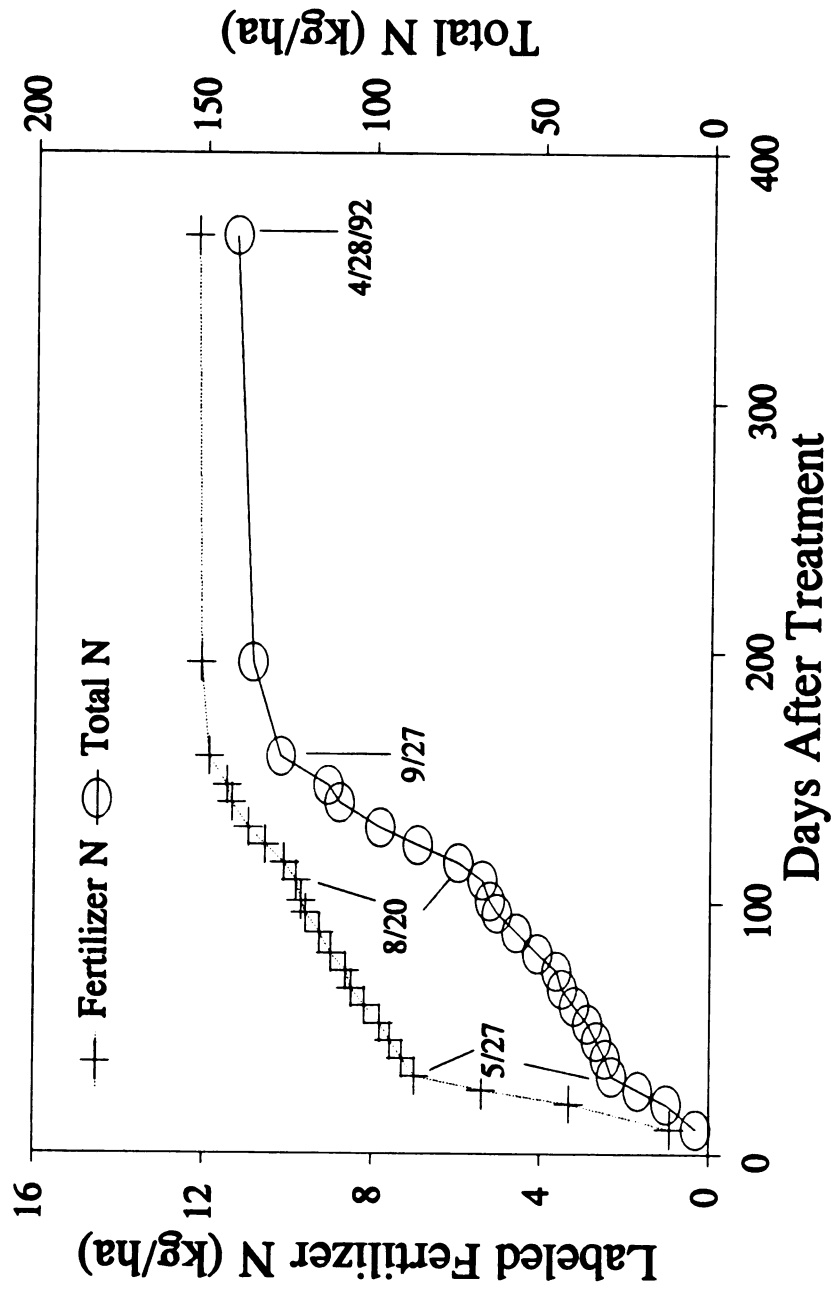


Figure 5. Cumulative Labeled Fertilizer Nitrogen and Total Nitrogen harvested in clippings from May 6, 1991 through April 28, 1992 from microplots receiving the Spring treatment.

and 50% of the total amount harvested in clippings for the Spring treatment. Following this period of rapid uptake (average rate of $0.23 \text{ kg LFN ha}^{-1}\text{day}^{-1}$), rates tapered off but remained steady throughout the summer and early fall. The shape of the curve of LFN content between 31 and 158 DAT (days after treatment) appears to be linear, but the curve was analyzed in two segments due to the increased rate of total N uptake apparent following 116 DAT. This change was associated with a fertilizer application made on August 19 (115 DAT). Mean daily uptake of LFN from 31 through 116 DAT was 0.036 kg ha^{-1} , and from 116 through 154 DAT was 0.043 kg ha^{-1} . Total N uptake rates for these periods were 0.54 and $1.26 \text{ kg N ha}^{-1}\text{day}^{-1}$. Some of this large increase in non-labeled N measured in the clippings was presumably fertilizer N from the August application, but uptake of additional LFN was also influenced, indicating a priming effect on mineralization of ^{15}N incorporated into soil organic matter. Applications made on June 4, July 12, and September 27 were not followed by clear plant responses in N content. In fact, very little additional N was harvested in clippings following the September fertilization. In the first 31 days following fertilization, LFN accounted for 25% of the total N recovered in the clippings. By November 8 (day 196) LFN harvested in clippings totaled 12.1 kg ha^{-1} , 31% of the total amount applied, and 9% of the total N in clippings.

During the second growing season (1992), three different phases of nitrogen accumulation, characterized by decreasing mean daily uptake rates, were observed for Spring fertilized plots (Figure 6). Uptake was rapid from 367 DAT (April 28) through 384 DAT (May 15), with total N harvested in clippings averaging $1.8 \text{ kg ha}^{-1} \text{ day}^{-1}$, and LFN averaging $0.035 \text{ kg ha}^{-1} \text{ day}^{-1}$. Following this period, uptake rates decreased, yielding two relatively steady periods of uptake. Once again, increases in uptake following fertilization were

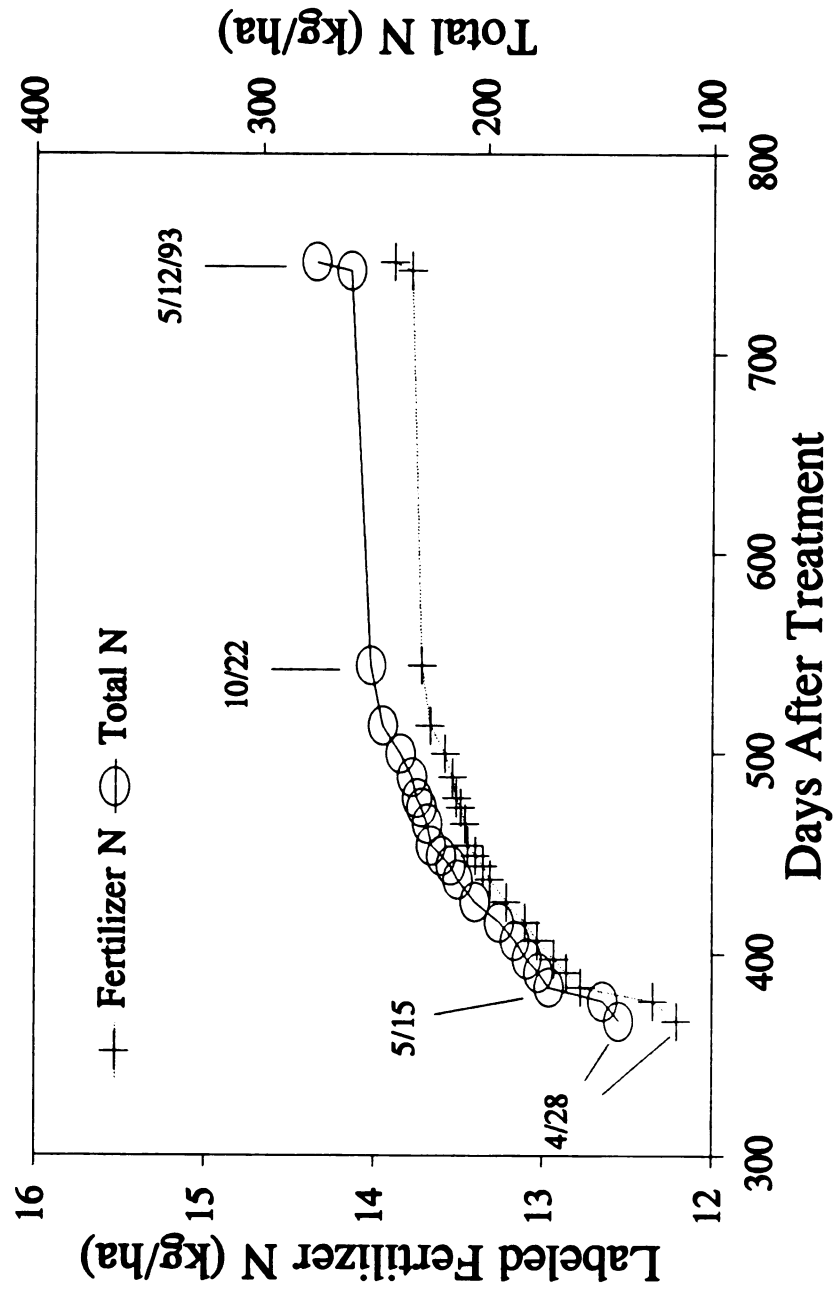


Figure 6. Cumulative Labeled Fertilizer Nitrogen and Total Nitrogen harvested in clippings from April 28, 1992 through May 12, 1993 from microplots receiving the Spring treatment.

not generally observed, with the exception of the April application. Very little additional uptake occurred through the fall and winter. The following spring, a pulse of increased ^{15}N and total N was observed as in previous springs, but microplot clipping sampling for this treatment ended at this time due to excavation of the last set of microplots. Total uptake of LFN over the two year period was 13.9 kg ha^{-1} (35% of that applied), while a total of $276 \text{ kg total N ha}^{-1}$ was recovered.

Following application of LFN urea in November 1991, 1.8 kg ha^{-1} of LFN was recovered in clippings on April 28, 1992 (171 DAT), the first mowing date (Figure 7). A period of rapid uptake ensued through 188 DAT (May 15), with mean uptake rates of $0.36 \text{ kg LFN ha}^{-1} \text{ day}^{-1}$ and $1.92 \text{ kg total N ha}^{-1} \text{ day}^{-1}$. Total uptake of LFN was 8.0 kg ha^{-1} by this time, 20% of the applied amount and 53% of the total amount eventually harvested in clippings. Nitrogen accumulation in clippings continued at decreasing rates throughout the growing season. By 348 DAT (October 22) $12.4 \text{ kg labeled fertilizer N ha}^{-1}$ had been harvested in clippings (32% of applied amount).

The next spring, rapid N accumulation occurred again in the clippings until 555 DAT (May 19) (Figure 8). Fertilizer was applied on May 26 (562 DAT), and contrary to the more gradual uptake observed in the Spring treatment following fertilization in May of 1992, uptake continued at a relatively high rate throughout the growing season. Clipping harvests indicated mean daily rates of $0.014 \text{ kg LFN ha}^{-1}$ and $1.38 \text{ kg total N ha}^{-1}$ for the Fall treatment. Rates during a similar time period for the Spring treatment the preceding year were $0.008 \text{ kg LFN ha}^{-1} \text{ day}^{-1}$ and $0.66 \text{ kg total N ha}^{-1} \text{ day}^{-1}$. A direct comparison of total N uptake from lysimeters for both treatments from June 9 to October 7, 1993 showed greater rates of uptake for the Fall treatment than the Spring treatment (0.86 and $0.34 \text{ kg N ha}^{-1} \text{ day}^{-1}$), indicating a

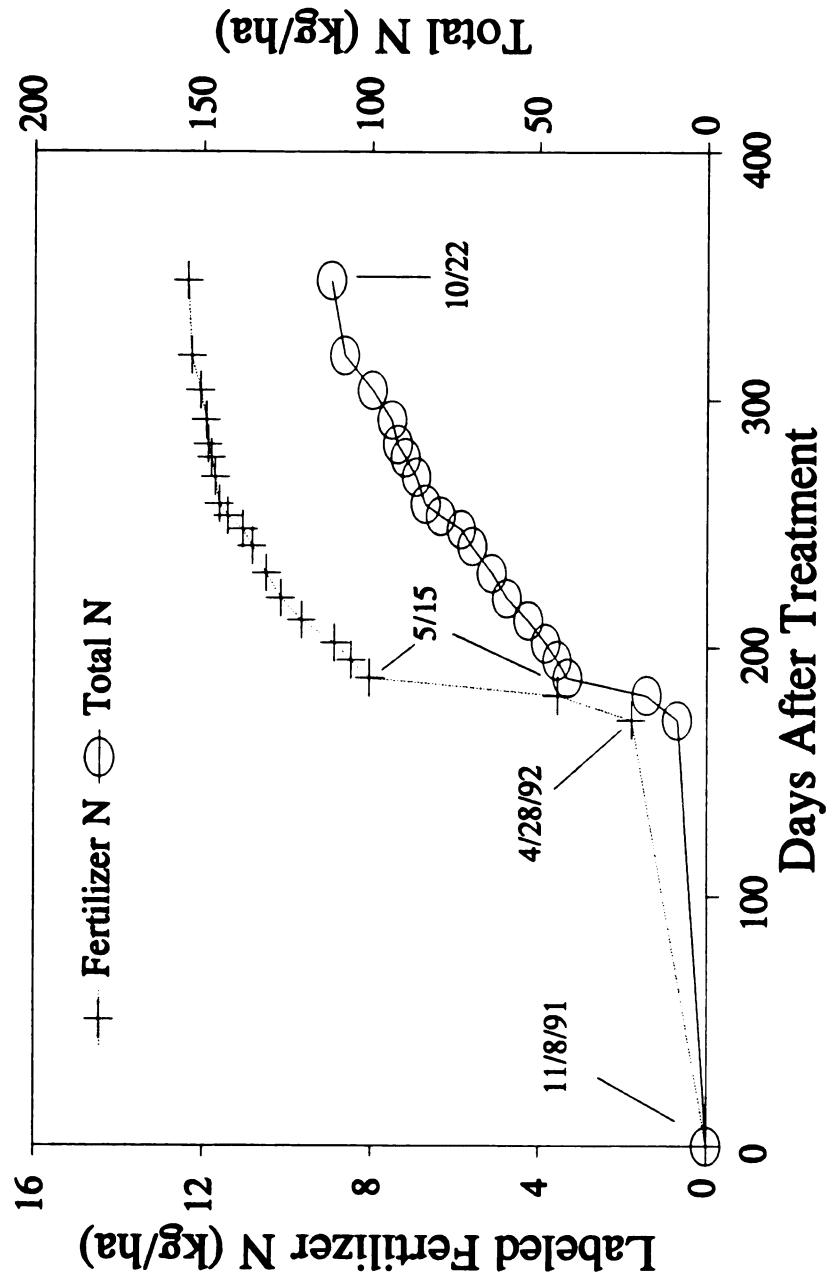


Figure 7. Cumulative Labeled Fertilizer Nitrogen and Total Nitrogen harvested in clippings from November 8, 1991 through October 22, 1992. from microplots receiving the Fall treatment

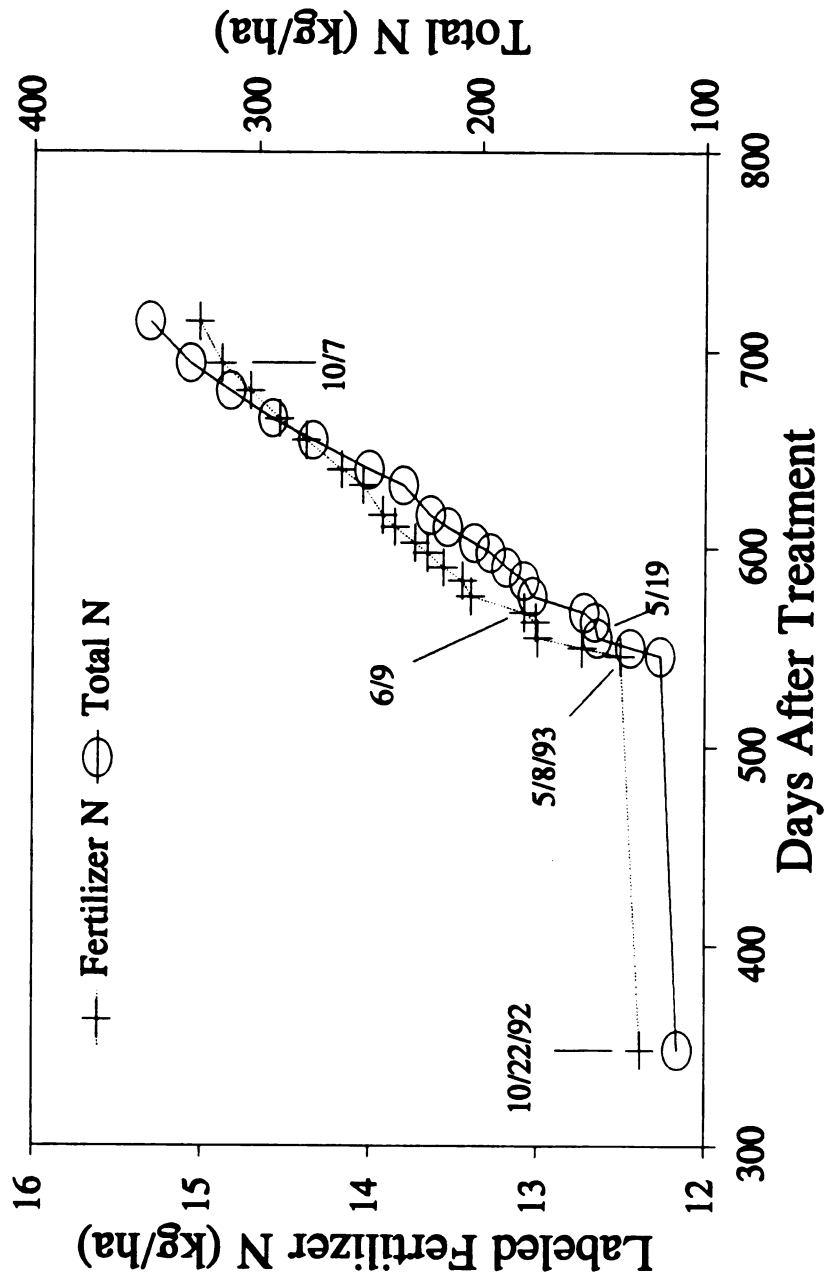


Figure 8. Cumulative Labeled Fertilizer Nitrogen and Total Nitrogen harvested in clippings from October 22, 1992 through October 28, 1993 from microplots receiving the Fall treatment.

treatment effect. There may have been an environment effect as well, due to different growing seasons.

Total nitrogen harvested in clippings during two years was much greater in the present study (276 and 349 kg ha⁻¹ for the Spring and Fall treatments, respectively) than that observed by Starr and DeRoo (1981) (95 kg ha⁻¹) over a three year period. Expressed as percentage of fertilizer applied, these amounts are 70% and 89% in the present study and 50% from Starr and DeRoo (1981). Starr and DeRoo (1981) observed approximately 30% and 20% uptake of LFN in the first 120 days following application of nitrogen in May and September, respectively. In the present study, these values were 26% and 34% for April and November applications. The measurement for this November application occurred during the following spring. Calculated mean daily uptake rates of fertilizer N as measured in clippings during a 'steady state' period following initial surges in uptake were very similar in this study and in the work of Starr and DeRoo (1981), 0.04 - 0.05 kg N ha⁻¹ day⁻¹. Uptake rates of total nitrogen, however, were at least twice as high in this experiment, resulting in much greater total N uptake over the entire course of the study.

Measurement of uptake varies widely in the literature. Power and Legg (1984) observed uptake of 30% of applied LFN in one growing season by crested wheatgrass, which was similar to the findings of the present study. However, Bowman *et al.* (1989b) observed much higher uptake rates by nitrogen deficient Kentucky bluegrass in the field (12.5 to 19.25 kg N ha⁻¹ day⁻¹ vs. maximum rates of 1.92 kg total N ha⁻¹ day⁻¹ in the present study). Bowman *et al.* (1989a) also observed uptake as high as 32 kg N ha⁻¹ day⁻¹ by perennial ryegrass in solution culture. Other researchers have shown that perennial ryegrass pastures exhibited approximately 50% greater uptake of LFN in herbage as compared to the turf in this study (Bristow *et al.*, 1987; Dowdell

and Webster, 1980, 1984). This may be at least partially explained, however, by greater uptake rates by perennial ryegrass as compared to Kentucky bluegrass as measured by Bowman *et al.* (1989b) in solution culture.

Verdure:

The total amount of LFN harvested in the verdure decreased at each sampling date for both the Spring (Figure 9) and Fall (Figure 10) treatments, as would be expected as N was removed in clippings with each successive mowing. The decreases in verdure LFN were associated with increases in clipping LFN, indicating transport upward within the shoots to the leaf blades, predominantly during the first growing season following application. However, the amount of total shoot LFN (plotted as "clippings + verdure") did not change significantly throughout the study for the Spring treatment, and differences that occurred for the Fall treatment showed no trend towards a general increase or decrease. This indicates that after the first microplot sampling (18 DAT for both treatments) there was no significant transport of additional LFN to above ground plant tissue.

Bristow *et al.* (1987) observed very similar results in a perennial ryegrass pasture. Between 16 and 310 DAT of LFN as NH_4NO_3 , total recovery in herbage (cut above 5 cm) and stubble (soil surface to 5 cm) varied little (49.9% to 55.8% of the applied LFN).

Nitrogen in Thatch

Thatch was found to be a very important sink of fertilizer nitrogen and also a large pool of total nitrogen. The amount of total N in the thatch was not significantly changed over time with the regular addition of fertilizer under either fertilization schedule (Tables 6 and 7). There were fluctuations in the

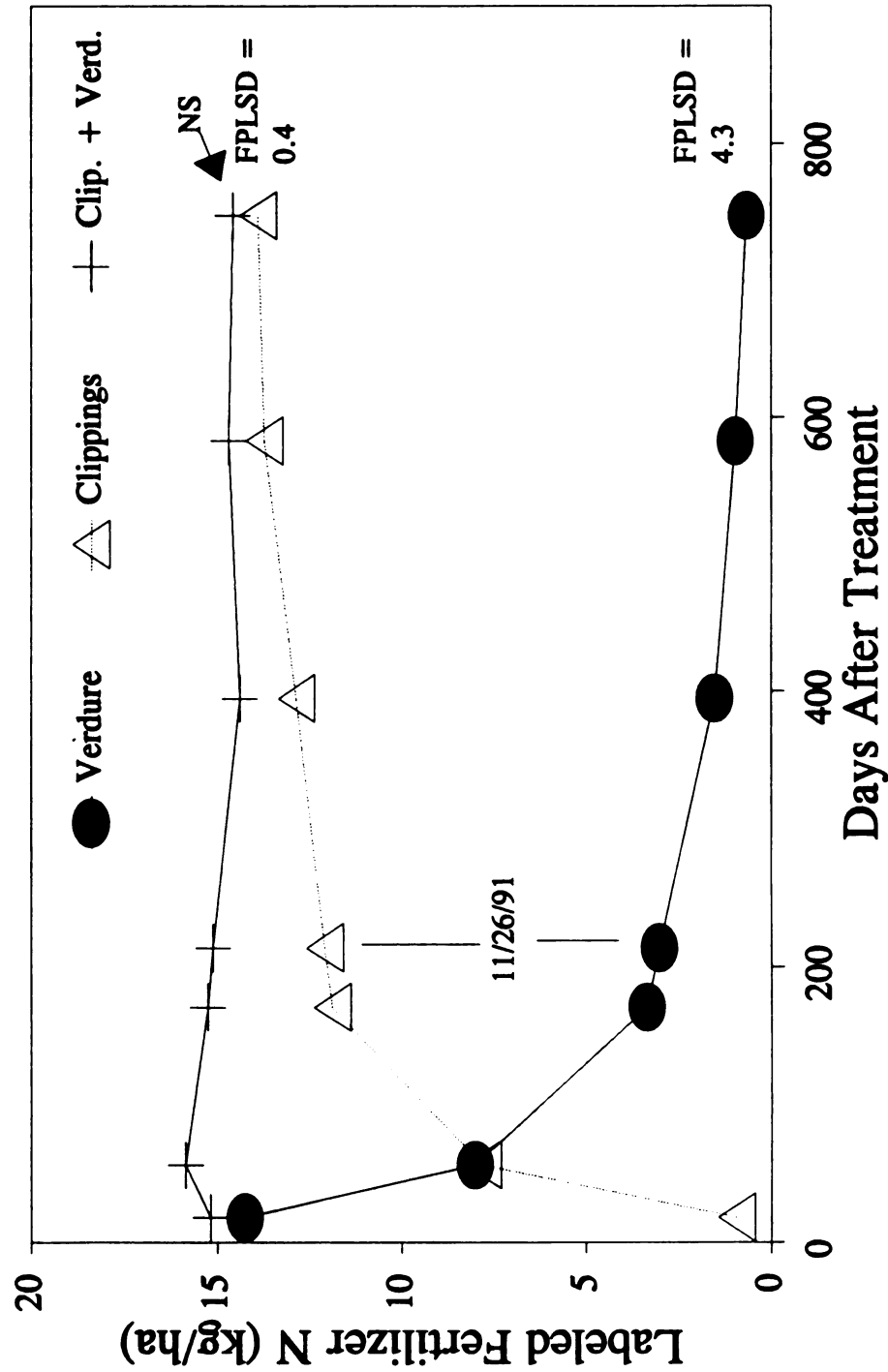


Figure 9. Labeled Fertilizer Nitrogen recovered in verdure, cumulative clippings, and verdure plus cumulative clippings at each sample date from microplots receiving the Spring treatment.

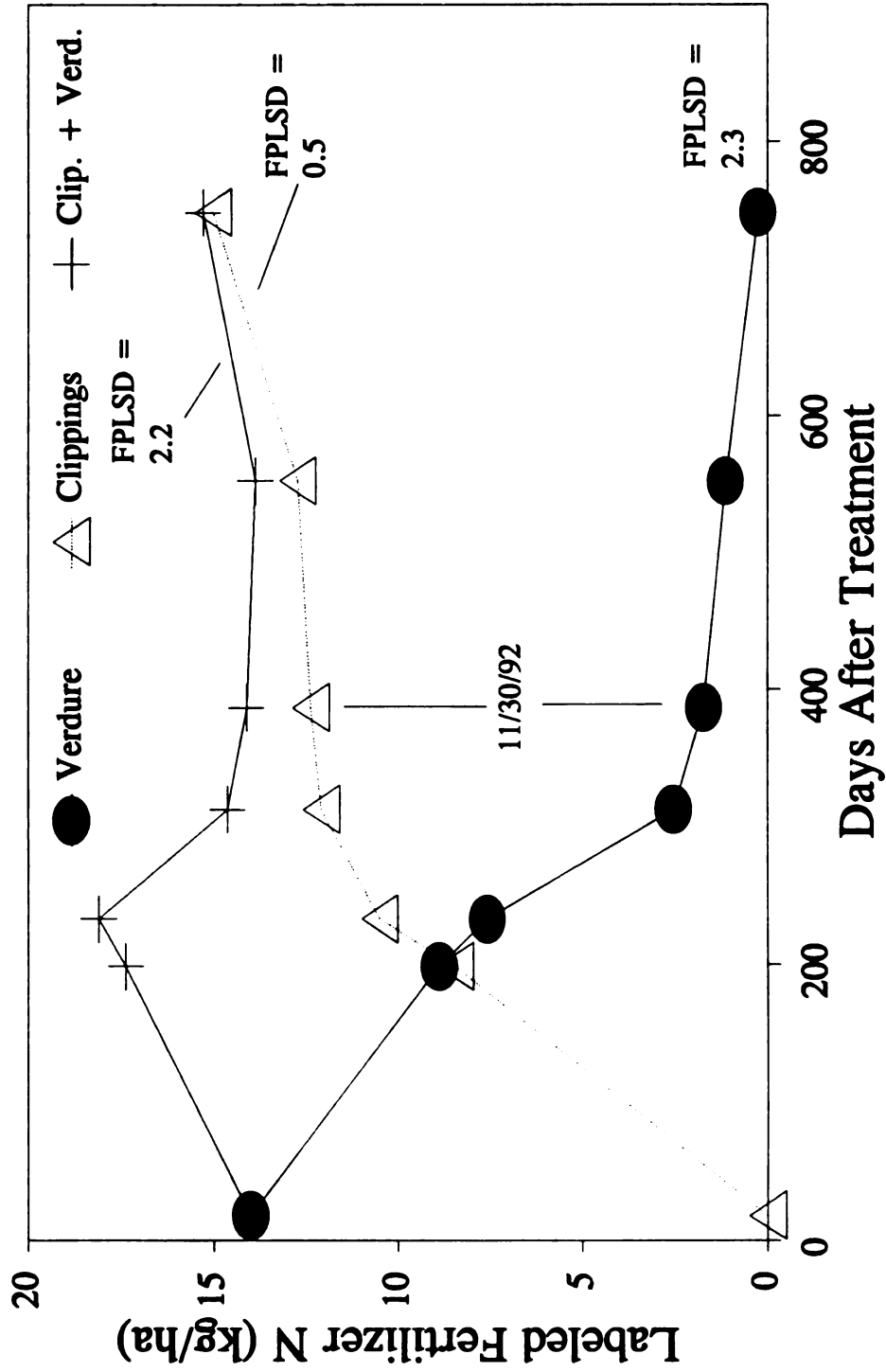


Figure 10. Labeled Fertilizer Nitrogen recovered in verdure, cumulative clippings, and verdure plus cumulative clippings at each sample date from microplots receiving the Fall treatment.

amount of total N in the organic portions of thatch, but these fluctuations showed no regular trend over time. A large proportion of the total N in thatch was present in the soil component. Averaged for all samples, thatch was 89% soil by dry weight.

Fertilizer N in the thatch layer was a much more labile pool as compared to total N. Following April application, 31% of the LFN was found in thatch 18 DAT (Table 6). This amount remained fairly constant for the next year, with the exception of the sample taken on October 14. The 32% of LFN found in the thatch in November 1991 was considerably higher than the 19% reported by Starr and DeRoo (1981). Between May and November of 1992 LFN in thatch decreased significantly, presumably due to mineralization and subsequent transport deeper into the soil, or loss through volatility. Plant uptake was shown previously not to be a factor later than 18 DAT. Examination of the data over the two year period shows an apparent trend in decrease of LFN in the organic component and an associated increase in the soil component (with the exception of the final sample with respect to soil LFN). Thatch is extremely heterogeneous, and this N could be present in living plant material (crowns, roots, rhizomes), incorporated into plant material that senesced since the time of uptake, adsorbed to previously senesced plant material, incorporated into microbial biomass in either the organic or soil components of the sample, or could be present in the soil component as organic, exchangeable, fixed, or soluble inorganic N. Because the technique used for thatch separation segregated components based on particle density and size, the shift in LFN from organic to soil components indicates breakdown of the organic material into smaller components which migrated downward into the soil portion of the thatch layer or were small enough to be included in the soil portion in the screening process. This breakdown could have included complete

Table 6. Total Nitrogen and Labeled Fertilizer Nitrogen content of thatch, including organic and soil components, for each sampling date for Spring treatment.

<u>Date</u>	Total Nitrogen			Labeled Fertilizer Nitrogen		
	<u>Organic</u>	<u>Soil</u>	<u>Total</u>	<u>Organic</u>	<u>Soil</u>	<u>Total</u>
	----- kg ha ⁻¹ -----					
5/14/91	110	367	477	8.8	3.4	12.1
6/21/91	112	250	673	8.7	3.6	12.2
10/14/91	81	393	475	4.1	3.4	7.4
11/26/91	170	628	798	6.8	5.8	12.5
5/26/92	201	417	618	7.4	6.4	13.7
11/30/92	78	523	601	1.4	6.9	8.4
5/14/93	136	235	371	2.0	3.3	5.2
FPLSD (0.05)	65	NS	NS	2.8	2.4	4.0

mineralization of thatch organic material or a more partial breakdown of large pieces of organic material into smaller ones, and was probably a combination of the two.

Recovery of 62% of LFN from the thatch layer occurred 18 DAT following application in November (Table 7). By the following May this had not changed significantly. By June 29 there was a significant decrease in both total thatch LFN and LFN in the organic component compared to the May sample, and soil LFN increased compared to the November 1991 sample. These trends were maintained throughout the study. Breakdown of LFN in the organic component and transfer to the soil component occurred in a similar manner as for the Spring treatment. This breakdown cycle occurred in 1992 for both treatments, even though April LFN was applied in 1991. It is unclear why thatch LFN remained stable throughout the summer following application for this Spring treatment.

The total amount of LFN in thatch shortly after application of the Fall treatment was striking. This difference in early recovery from the thatch accounted for most of the difference in total recovery between the two treatments. As thatch LFN decreased in the Fall treatment over time, so did total recovery. Most of the LFN in thatch from the Fall treatment in the November 1991 sampling was in the organic component. Large amounts of LFN were probably not in live plant material in the thatch layer because recoveries in clippings and verdure were similar for the two treatments during the first spring following application. If November-fertilized plants were storing this additional LFN, it would be expected to result in increased LFN content in clippings of these plants as compared to those fertilized in the spring. Root tissue could account for some of the additional thatch LFN in the Fall treatment. Some other organic pool was also probably acting as a short-

Table 7. Total Nitrogen and Labeled Fertilizer Nitrogen content of thatch, including organic and soil components, for each sampling date for Fall treatment.

<u>Date</u>	Total Nitrogen		Labeled Fertilizer Nitrogen			
	<u>Organic</u>	<u>Soil</u>	<u>Total</u>	<u>Organic</u>	<u>Soil</u>	<u>Total</u>
	----- kg ha ⁻¹ -----					
11/26/91	152	495	647	20.7	3.6	24.3
5/26/92	217	403	620	17.6	4.4	21.9
6/29/92	96	450	546	7.0	6.9	13.9
9/17/92	55	381	437	2.3	7.3	9.6
11/30/92	64	458	522	2.2	7.7	9.9
5/14/93	323	261	584	5.0	3.6	8.6
11/30/93	152	449	601	1.8	4.9	6.7
FPLSD (0.05)	169	NS	NS	5.5	2.9	7.1

term storage reservoir over the winter for LFN. Significant losses of thatch LFN occurred with the onset of warmer temperatures. Root turnover would probably not be rapid enough to account for all of the loss. The differences in early recovery from the thatch between Spring and Fall treatments, significant rapid loss of LFN from the thatch for the Fall treatment, and total recovery data (presented later) indicate the possibility of gaseous losses of N from the thatch layer.

Soil Nitrogen

Soil N and ^{15}N were partitioned into inorganic, biomass, organic, and total N pools in each of the sampling depths of the soil profile. Total, inorganic, and biomass N were directly measured and organic N was calculated by subtracting inorganic from total N. Biomass carbon was also measured. Samples from the top five depths (to 60 cm total depth) only were used in statistical analysis. These samples were contained within the microplots at the time of excavation. The sixth sample (60 - 100 cm) was collected from below the microplots with a soil probe, and it was found upon analysis that this method resulted in contamination of the sample. This data, therefore, was not used. Complications were encountered in measuring biomass N. In the biomass determination, NH_4^+ from a non-fumigated, non-incubated control sample is subtracted from the amount of NH_4^+ detected in a chloroform-fumigated, incubated sample, resulting in NH_4^+ released due to fumigation. In the soil from this experiment NH_4^+ levels in non-fumigated samples remained relatively high at depths below 10 cm. The combination of immobilization of liberated NH_4^+ in fumigated samples and high control NH_4^+ resulted in negative numbers when this calculation was performed for a large number of the samples below the 10 cm depth. The result was negative

values for biomass N. Data was examined for approximately 50 samples from the 0 - 5 and 5 - 10 cm increments where this problem did not occur and a biomass C:N ratio of 5.7:1 was calculated. This ratio was used for all samples to estimate biomass N from biomass C. For soil sample 8, removed in November 1992, a large number of base traps used to trap CO₂ in the incubation process became unusable due to evaporation. Reliable numbers could not be obtained, so there is no data for biomass C or N from this sample. Tables 8 and 9 present summaries of the statistical analyses of biomass C and soil N and ¹⁵N pools for the Spring and Fall treatments, respectively. Time by Depth interactions were seen for all parameters except biomass C and N in the Spring treatment, where depth only was a significant factor. Pursuant to these interactions biomass C, N, and ¹⁵N and the relationships between inorganic and organic N and ¹⁵N were examined in greater detail.

Biomass C and N:

In Tables 10 and 11 concentrations of biomass C, N, and ¹⁵N at each depth for each sampling date are given. In this analysis means for each parameter at each depth were compared over time to distinguish long-term trends in biomass activity. Values for C and N follow identical trends due to the constant ratio used to estimate biomass N. For this reason, explicit discussion of biomass N will be avoided, but can be implied from biomass C trends. For the April fertilized treatment there were no significant differences for any parameter at any depth (Table 10). There appeared to be a trend towards increasing biomass C over time, especially during the first year. This held true at all depths throughout the profile. Almost all of the ¹⁵N in biomass was confined to the upper 10 cm of the profile, although there were trace amounts in the 10 to 40 cm depth. No ¹⁵N was detected in biomass below 40

Table 8. Summary of split-plot analysis of variance for concentration of biomass carbon and total N and ^{15}N ($\mu\text{g g}^{-1}$) in various soil pools for Spring treatment.

<u>Soil Pool</u>	-----Source of Variation-----			
	<u>Time</u>	<u>Replication</u>	<u>Depth</u>	<u>Time*Depth</u>
Biomass C			**	
Biomass N			**	
Inorganic N	**	*	**	*
Organic N	**	**	**	*
Total N	**	**	**	*
Biomass ^{15}N		**	**	*
Inorganic ^{15}N	**		**	**
Organic ^{15}N	*		**	**
Total ^{15}N	*		**	**

*, ** Significant at $P = 0.05$ and 0.01 , respectively.

Table 9. Summary of split-plot analysis of variance for concentration of biomass carbon and total N and ^{15}N ($\mu\text{g g}^{-1}$) in various soil pools for Fall treatment.

<u>Soil Pool</u>	<u>-----Source of Variation-----</u>			
	<u>Time</u>	<u>Replication</u>	<u>Depth</u>	<u>Time*Depth</u>
Biomass C	**		**	**
Biomass N	**		**	**
Inorganic N	**		**	**
Organic N	*	**	**	*
Total N	**	**	**	*
Biomass ^{15}N	**		**	**
Inorganic ^{15}N	*		**	**
Organic ^{15}N	**		**	**
Total ^{15}N	**		**	**

*, ** Significant at $P = 0.05$ and 0.01 , respectively.

Table 10. Concentrations of biomass C, N, and ^{15}N ($\mu\text{g g}^{-1}$) by depth for each sampling date for Spring treatment.

Depth (cm)		-----1991-----				1992	1993	FPLSD (0.05)
		<u>5/14</u>	<u>6/21</u>	<u>10/14</u>	<u>11/26</u>	<u>5/26</u>	<u>5/14</u>	
0-5	C	424	367	737	609	503	717	NS
	N	74	64	127	109	90	126	NS
	^{15}N	0.64	0.40	0.68	0.53	0.42	0.38	NS
5-10	C	370	241	275	374	312	609	NS
	N	65	42	47	67	56	107	NS
	^{15}N	0.08	0.08	0.10	0.14	0.11	0.14	NS
10-20	C	245	217	135	327	460	220	NS
	N	43	38	23	58	29	39	NS
	^{15}N	0.03	0.01	0.03	0.04	0.03	0.02	NS
20-40	C	90	213	150	193	211	96	NS
	N	16	37	26	34	38	17	NS
	^{15}N	0.01	0	0.01	0.02	0.02	0	NS
40-60	C	87	207	0	210	63	105	NS
	N	15	36	0	38	11	18	NS
	^{15}N	0	0	0	0	0	0	NS

NS = not significant

cm at any time. In the 0 to 5 cm and 5 to 10 depths, the data shows that the biomass maintained an active role in the cycling of the applied ^{15}N over the entire two years of the study. Between May and November of 1991 the percentage of ^{15}N in biomass as compared to total biomass N in the upper 0 to 5 cm decreased from 0.86 to 0.48%, while it increased from 0.12 to 0.21% in the 5 to 10 cm depth. This indicates downward movement of the ^{15}N over this time. This movement could occur either as inorganic N or in senesced roots followed by incorporation into microbial biomass. After November of 1991 these percentages decreased at both depths.

For fall fertilized turf, microbial biomass populations appeared to decrease over the first year following application (Table 11). This was not significant in the 0 to 5 cm depth, but was significant at 5 to 10 cm. This is in contrast to the trend observed for the Spring treatment. In May of 1993 biomass levels increased and were significantly higher than in the previous June and September for the two upper depths. Measured biomass C was very low for the final sampling date throughout the profile, although this was significant only at the two upper depths. Because these values were so much lower than any others measured, including those from a similar time of year in 1991, it is suspected that the 1993 values are not a real effect but are due to some procedural error. However, a systemic error should have affected all samples equally, but values for lower depth samples are not entirely inconsistent with data from earlier dates. The reason for these inconsistencies is not clear.

Biomass ^{15}N decreased significantly in the top soil depth between November 1991 (shortly after application) and the following May. If these values are converted to kg LFN ha^{-1} , this equates to a decrease from 2.15 to $1.07 \text{ kg LFN ha}^{-1}$, a release of approximately 1 kg LFN. Differences in biomass ^{15}N during 1992 were not significant, but a significant decrease

Table 11. Concentrations of biomass C, N, and ^{15}N ($\mu\text{g g}^{-1}$) by depth for each sampling date for Fall treatment.

Depth (cm)		1991	-----1992-----				-----1993-----		FPLSD (0.05)
		<u>11/26</u>	<u>5/26</u>	<u>6/29</u>	<u>9/17</u>	<u>5/14</u>	<u>11/30</u>		
0-5	C	703	614	426	486	929	60	364	
	N	126	110	75	85	163	10	60	
	¹⁵ N	0.90	0.45	0.28	0.36	0.50	0.03	0.33	
5-10	C	468	342	153	171	302	58	114	
	N	83	61	27	30	53	10	19	
	¹⁵ N	0.15	0.11	0.05	0.04	0.10	0.02	NS	
10-20	C	273	212	100	207	258	76	NS	
	N	49	38	18	36	45	13	NS	
	¹⁵ N	0	0.03	0.01	0.03	.03	0.01	NS	
20-40	C	250	107	179	72	322	81	NS	
	N	45	19	31	13	56	14	NS	
	¹⁵ N	0	0	0	0	0.01	0	NS	
40-60	C	148	145	112	117	71	98	NS	
	N	26	26	32	21	12	17	NS	
	¹⁵ N	0	0	0	0	0	0	NS	

NS = not significant

occurred between May and November of 1993. This decrease was tied to the low biomass C measurements in the November sample. Similarly to the Spring treatment, percent of ^{15}N in biomass as compared to total biomass N decreased during the first year after application in the 0 to 5 cm depth, from 0.71% in November 1991 to 0.42% in September of 1992, and continued to decrease through 1993. In contrast to the Spring treatment, an increasing trend did not occur in the second depth (0.18%, 0.18%, 0.13%, and 0.19% in 11/91, 6/92, 9/92, and 5/93, respectively).

Calculations of the percentage of ^{15}N as compared to total N in biomass raise an interesting point. Using the 0 to 5 cm depth from the Spring treatment as an example, 0.86% of biomass N was ^{15}N 18 days after ^{15}N application. Thirty eight days later, and 17 days after an application of an equivalent amount of non-labeled N, the value decreased to 0.63%. In October, following two additional non-labeled N applications, the value was 0.54%. If the biomass was utilizing the applied N similarly from all applications, dilutions in the percentage of ^{15}N in the biomass would be expected with each subsequent application. This was not seen however. It appears that either the microbial biomass is not as active in the cycling of fertilizer N applied during the summer as compared to that applied in the spring, or there is preferential cycling of ^{15}N over ^{14}N . This interpretation should be viewed with caution, however. Biomass ^{15}N values are estimates, calculated based on constant biomass C:N ratios as described. In addition, total amounts of ^{15}N were extremely small. Considering the importance of the microbial biomass in nitrogen cycling, further investigation of this effect is warranted.

Relationships between inorganic and organic N and N mobility:

Inorganic N made up approximately 2% of the total soil N in the profile in the spring treated plots in May 1991 (Table 12). Of the 98% of the N in organic forms, approximately 7% was present as microbial biomass. From LFN, 14% and 17% of the ^{15}N found in the upper two depths was in inorganic forms. Below 10 cm all ^{15}N detected was in organic forms. Of the organic ^{15}N , 75% or more was present as microbial biomass. One year later, distribution of total N was similar (Table 13). Of the LFN applied one year previously, between 0 and 11% of that recovered in various soil depths was in inorganic forms. Of the organic ^{15}N measured (89 to 100% of total), 24 to 38% was in microbial biomass. Two years after initiation of the study, 4% of the ^{15}N applied was in inorganic forms, and 17 to 24% of organic ^{15}N was in microbial biomass (Table 14).

For the Fall treatment, trends in total N were similar to the Spring treatment. Inorganic N averaged 2% of total N in the first fall (Table 15) and 1% (Tables 16 and 17) thereafter. Biomass N was 9% and 8% of organic N in 1991 and 1992, respectively (Tables 15 and 16). Because of problems with biomass determination in the November 1993 sample as discussed previously, data for this pool for 1993 is not reliable.

Eighteen DAT of LFN in November, an average of 11% of the ^{15}N was present in inorganic forms. Of the organic ^{15}N present in the top two depths, 80 to 100% was in microbial biomass (Table 15). One year following the late fall application, 0 to 7% of the ^{15}N was in inorganic forms, and 14 to 43% of organic ^{15}N was microbial biomass (Table 16). Two years after application, 98 to 100% of ^{15}N present in soil was in organic forms (Table 17).

For both fertilization timings, the importance of microbial biomass in immobilizing LFN that reaches the soil is clear. The biomass comprised 6 to

Table 12. Inorganic, biomass, organic, and total N and ¹⁵N concentrations by depth for the Spring treatment, sampled on May 14, 1991.

Depth (cm)	Total N (μg g ⁻¹)			¹⁵ N (μg g ⁻¹)		
	Inorganic	Biomass	Organic	Inorganic	Biomass	Organic
0-5	28	74	1251	0.13	0.65	0.79
5-10	17	65	842	0.02	0.08	0.10
10-20	15	43	711	0	0.03	0.04
20-40	6	16	195	0	0.01	0.01
40-60	4	15	156	0	0	0
Contrast:						
0-5 vs. 5-10				**	**	**
0-10 vs. 10-60				**	**	**
10-20 vs. 20-60						

*,** Significant at P = 0.05 and 0.01, respectively.

Table 13. Inorganic, biomass, organic, and total N and ¹⁵N concentrations by depth for the Spring treatment, sampled on May 26, 1992.

Depth (cm)	-----Total N (μg g ⁻¹)-----				----- ¹⁵ N (μg g ⁻¹)-----			
	<u>Inorganic</u>	<u>Biomass</u>	<u>Organic</u>	<u>Total</u>	<u>Inorganic</u>	<u>Biomass</u>	<u>Organic</u>	<u>Total</u>
0-5	11	90	1518	1529	0.03	0.41	1.74	1.77
5-10	7	56	1072	1079	0.02	0.11	0.39	0.41
10-20	6	29	697	703	0.01	0.03	0.08	0.09
20-40	2	38	438	440	0	0.02	0.07	0.07
40-60	3	11	176	180	0.01	0	0.01	0.02
Contrast:								
0-5 vs. 5-10					**	**	**	**
0-10 vs. 10-60					**	**	**	**
10-20 vs. 20-60					*			

*, ** Significant at P = 0.05 and 0.01, respectively.

Table 14. Inorganic, biomass, organic, and total N and ¹⁵N concentrations by depth for the Spring treatment, sampled on May 14, 1993.

Depth (cm)	Total N (µg g ⁻¹)			Total	¹⁵ N (µg g ⁻¹)			Total
	Inorganic	Biomass	Organic		Inorganic	Biomass	Organic	
0-5	9	126	1526	1535	0.03	0.38	2.22	2.25
5-10	7	107	973	980	0.01	0.13	0.54	0.55
10-20	7	39	750	756	0.01	0.02	0.10	0.11
20-40	3	17	254	257	0	0	0	0
40-60	2	18	129	131	0	0	0	0
Contrast:								
0-5 vs. 5-10					**	**	**	**
0-10 vs. 10-60					**	**	**	**
10-20 vs. 20-60					**	**	**	**

*, ** Significant at P = 0.05 and 0.01, respectively.

Table 15. Inorganic, biomass, organic, and total N and ¹⁵N concentrations by depth for the Fall treatment, sampled on November 26, 1991.

Depth (cm)	-----Total N (μg g ⁻¹)-----				----- ¹⁵ N (μg g ⁻¹)-----			
	<u>Inorganic</u>	<u>Biomass</u>	<u>Organic</u>	<u>Total</u>	<u>Inorganic</u>	<u>Biomass</u>	<u>Organic</u>	<u>Total</u>
0-5	31	126	1961	1992	0.12	0.9	1.11	1.23
5-10	17	84	1193	1210	0.04	0.15	0.15	0.19
10-20	17	49	1022	1039	0.01	0.01	0.02	0.04
20-40	10	45	291	301	0	0	0.03	0.03
40-60	6	26	184	190	0	0	0.05	0.05
Contrast:								
0-5 vs. 5-10					**	**	**	**
0-10 vs. 10-60					**	**	**	**
10-20 vs. 20-60					**	**	**	**

*, ** Significant at P = 0.05 and 0.01, respectively.

Table 16. Inorganic, biomass, organic, and total N and ¹⁵N concentrations by depth for the Fall treatment, sampled on September 17, 1992.

Depth (cm)	-----Total N (µg g ⁻¹)-----				----- ¹⁵ N (µg g ⁻¹)-----			
	<u>Inorganic</u>	<u>Biomass</u>	<u>Organic</u>	<u>Total</u>	<u>Inorganic</u>	<u>Biomass</u>	<u>Organic</u>	<u>Total</u>
0-5	18	82	1360	1378	0.07	0.36	1.68	1.75
5-10	9	30	885	894	0.02	0.04	0.28	0.30
10-20	6	36	712	718	0	0.03	0.07	0.08
20-40	3	13	256	260	0	0	0	0
40-60	1	21	155	156	0	0	0	0
Contrast:								
0-5 vs. 5-10					**	**	**	***
0-10 vs. 10-60					**	**	**	**
10-20 vs. 20-60							**	**

*** Significant at P = 0.05 and 0.01, respectively.

Table 17. Inorganic, biomass, organic, and total N and ¹⁵N concentrations by depth for the Fall treatment, sampled on November 30, 1993.

Depth (cm)	-----Total N (µg g ⁻¹)-----				----- ¹⁵ N (µg g ⁻¹)-----			
	<u>Inorganic</u>	<u>Biomass</u>	<u>Organic</u>	<u>Total</u>	<u>Inorganic</u>	<u>Biomass</u>	<u>Organic</u>	<u>Total</u>
0-5	17	10	1983	2000	0.05	0.03	2.23	2.28
5-10	8	10	1146	1153	0.01	0.02	0.55	0.56
10-20	4	13	811	815	0	0.01	0.12	0.13
20-40	2	14	194	196	0	0	0.06	0.06
40-60	4	17	157	161	0	0	0	0
Contrast:								76
0-5 vs. 5-10					**		**	**
0-10 vs. 10-60					**	**	**	**
10-20 vs. 20-60								

*, ** Significant at P = 0.05 and 0.01, respectively.

9% of the immobilized total N, but shortly following fertilizer application the biomass immobilized at least 75% of the LFN that reached the soil. Although a relatively high percentage of inorganic LFN was present as compared to total N in inorganic forms, most of the LFN had been protected from leaching through rapid incorporation into organic forms. Total organic N was present in more stable forms, but LFN was also converted to less labile forms over the two years of the experiment. Because fertilizer N was present in inorganic forms to a larger extent than total N, fertilization could lead to increased leaching potential. However, the total amount of N introduced to the soil from a single fertilizer application in this study was only about 0.07% of total N. The impact of a single fertilizer application on environmental quality would seem to be insignificant. The use of ^{15}N , however allows for accurate tracing of this small addition through the soil profile.

Tables 12 through 17 also show single degree of freedom orthogonal contrasts for comparison of mobility potentials of ^{15}N pools over time. As an example, if a difference exists between two adjacent soil depths at Time 1 but no longer exists at Time 2, the data would probably indicate downward movement from the upper to the lower layer over this time period. If a lack of difference later becomes a significant difference, it could indicate that N has moved out of the lower layer of the pair of interest into the depth below. Analysis of ^{15}N concentrations for the Spring treatment indicates potential vertical movement of LFN. In 1991, there was no inorganic ^{15}N below 10 cm (Table 12). In May of 1992, inorganic ^{15}N was present in the 10 to 20 cm depth (Table 13). The statistics indicate a significant increase in this depth as compared to 20 to 60 cm. In May of 1993 this relationship was unchanged, and it is encouraging that there was no ^{15}N below 20 cm (Table 14). However, because inorganic N is very labile in soils this may not be the best indicator of

potential leaching. Examination of organic ^{15}N data may provide additional information on the potential for long-term vertical mobility. Between May of 1991 (Table 12) and May of 1992 (Table 13) total ^{15}N and organic ^{15}N levels increased throughout the profile, presumably due to movement of LFN downward from the thatch layer. Biomass levels remained consistent at these depths. The increase in ^{15}N at lower depths may have been due to root production. Statistically no change in the relationships between depths occurred because increases occurred at all depths. In May of 1993 total and organic ^{15}N were no longer detected below 20 cm. There was therefore a significant difference between the 10 to 20 and 20 to 60 cm depths for biomass, organic, and total ^{15}N . Since the previous year, ^{15}N had either been transported upward in plants or mineralized and moved downward out of the sampling area.

For the Fall treatment, inorganic ^{15}N was found in the 10 to 20 cm depth in November 1991 (Table 15) but not below 10 cm after that time. In 1991, organic and total ^{15}N were greater in the 20 to 60 cm depth than the 10 to 20 cm depth. In September 1992 ^{15}N was not detected below 20 cm (Table 16). As for the Spring treatment this ^{15}N was either transported upward in plants or mineralized and moved through the soil. In November 1993 organic ^{15}N was again detected in the 20 to 40 cm increment. This caused non-significance in the contrast of 10 to 20 cm vs. 20 to 60 cm, indicating significant downward movement in the organic form since September 1992.

Although vertical mobility of LFN is indicated by this data, involvement of organic material indicates cycling of fertilizer N through plant material before potentially being lost to deep soil depths. This is in sharp contrast to the perceived notion of fertilizer rapidly flushing through a system, presumably in soluble forms. Consider data from May 1992 for the Spring treatment (Table

13) and November 1993 for the Fall (Table 17), which represent the greatest concentrations of LFN below the 10 cm depth for each treatment. If all of the LFN were mineralized and leached, this would total 2.10 and 1.68 kg LFN ha⁻¹ for the Spring and Fall treatments, respectively. These values represent 5% and 4% of the amounts applied. This is a very liberal estimate considering the nature of the nitrogen cycle and the fact that significant rooting exists to depths of 20 cm or more. The extremely small amounts of ¹⁵N involved illustrate the power of ¹⁵N in nitrogen cycling research.

Table 18 presents data on LFN in inorganic, biomass, and organic pools as well as total soil LFN on a mass balance basis for each sample date. The total amount of LFN in the soil shortly after application was only 8% and 12% of the total applied for April and November applications, respectively. Very little was present in mobile inorganic forms. Microbial biomass played an important role in immobilization. Over time the amount of LFN in the soil increased for both treatments, but remained predominantly in organic forms. The proportion of organic ¹⁵N present as microbial biomass decreased with time, indicating incorporation into more stable forms. This immobilization pattern is positive from an environmental standpoint, in that organic forms of N, even in the relatively labile microbial biomass pool, are relatively immobile. From a fertilizer utilization point of view, it appears that fertilizer N that enters the soil is readily immobilized and is therefore unavailable for immediate uptake. Much of the non-biomass organic ¹⁵N however is probably plant root material, and represents utilized LFN. As roots senesce they enter the soil organic matter cycle, which then constitutes a significant source of the applied fertilizer N. Investigation of the dynamics of this soil organic nitrogen pool and its mineralization would provide greater understanding of fertilizer nitrogen utilization in turf.

Table 18. Recovery of Labeled Fertilizer Nitrogen in various soil pools for each sampling date.

<u>Treatment</u>	<u>Date</u>	<u>Inorganic</u>	<u>Biomass</u>	<u>Organic</u>	<u>Total</u>
-----kg LFN ha ⁻¹ -----					
Spring	5/14/91	0.44	2.22	2.73	3.16
	6/21/91	0.12	1.48	4.19	4.31
	10/14/91	0.22	2.16	5.94	6.16
	11/26/91	0.23	2.00	6.51	6.74
	5/26/92	0.31	1.92	7.64	7.95
	11/30/92	0.25	-----	6.31	6.56
	5/14/93	0.09	0.99	5.26	5.34
Fall	11/26/91	0.47	2.65	4.31	4.77
	5/26/92	0.28	1.78	3.48	3.76
	6/29/92	0.12	0.99	2.64	2.76
	9/17/92	0.28	1.28	6.02	6.31
	11/30/92	0.33	-----	5.76	6.10
	5/14/93	0.16	2.02	8.63	8.79
	11/30/93	0.21	0.22	9.76	9.96

Leaching of Nitrogen

The cumulative water balance for the entire time of the study is shown in Figure 11. Between April 26, 1991 and December 23, 1993 a total of 277 cm of precipitation and irrigation fell on the lysimeters. Precipitation accounted for 221 cm of this, and irrigation totaled 56 cm. A total of 137 cm of water was collected as leachate from the Spring treatment, and 108 cm was collected from the Fall treatment. Collection of leachate from the Fall treatment did not begin until November 8, 1991. Subtracting out the period from April 26 to November 8 for the Spring treatment to equalize collection intervals, the total amount of leachate was 125 cm. Total leachate amounts were not significantly different between the two treatments regardless of which Spring value was used. Table 19 shows flow weighted means of N as NO_3^- and NH_4^+ , collected in the leachate. There were no significant differences between treatments for any of these measurements. Brown *et al.* (1982) also reported NH_4^+ present in leachate from golf greens. Soil mixtures with greater proportions of "soil" as compared to sand produced higher proportions of NH_4^+ . Flow-weighted mean NO_3^- -N concentrations were well below the EPA threshold limit of 10 mg L^{-1} . These findings agree with the conclusions of Starr and DeRoo (1981) and Morton *et al.* (1988) who stated that the potential for groundwater contamination resulting from fertilization of lawn turf is negligible.

All four lysimeters showed elevated levels of NO_3^- -N at the initiation of the study. For Lysimeters 1 and 2 (Spring treatment), concentrations above 1 mg L^{-1} occurred until September 3 and July 22, respectively (Figure 12). July 22 was the only day during this period for both lysimeters when ^{15}N enrichments in the leachate were above background (0.00065 and 0.00015 atom % excess for lysimeters 1 and 2, respectively). For the majority of the study

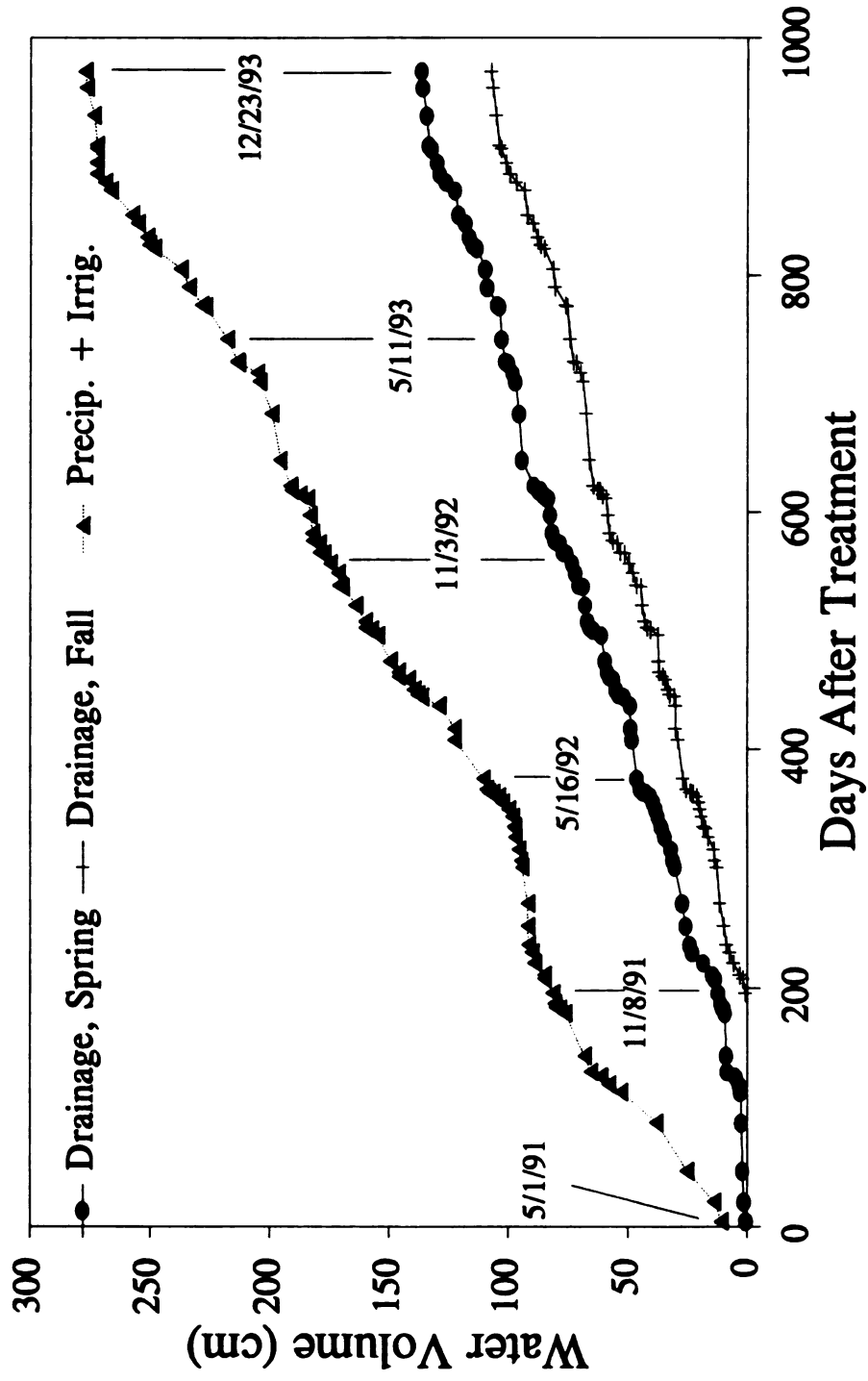


Figure 11. Cumulative drainage and precipitation plus irrigation from lysimeters .

Table 19. Flow-weighted means of nitrate and ammonium N concentrations in leachate from Spring and Fall treatments.

<u>Treatment</u>	<u>Nitrate-N</u>	<u>Ammonium-N</u>
	-----mg L ⁻¹ -----	
Spring	0.31	0.12
Fall	0.63	0.14

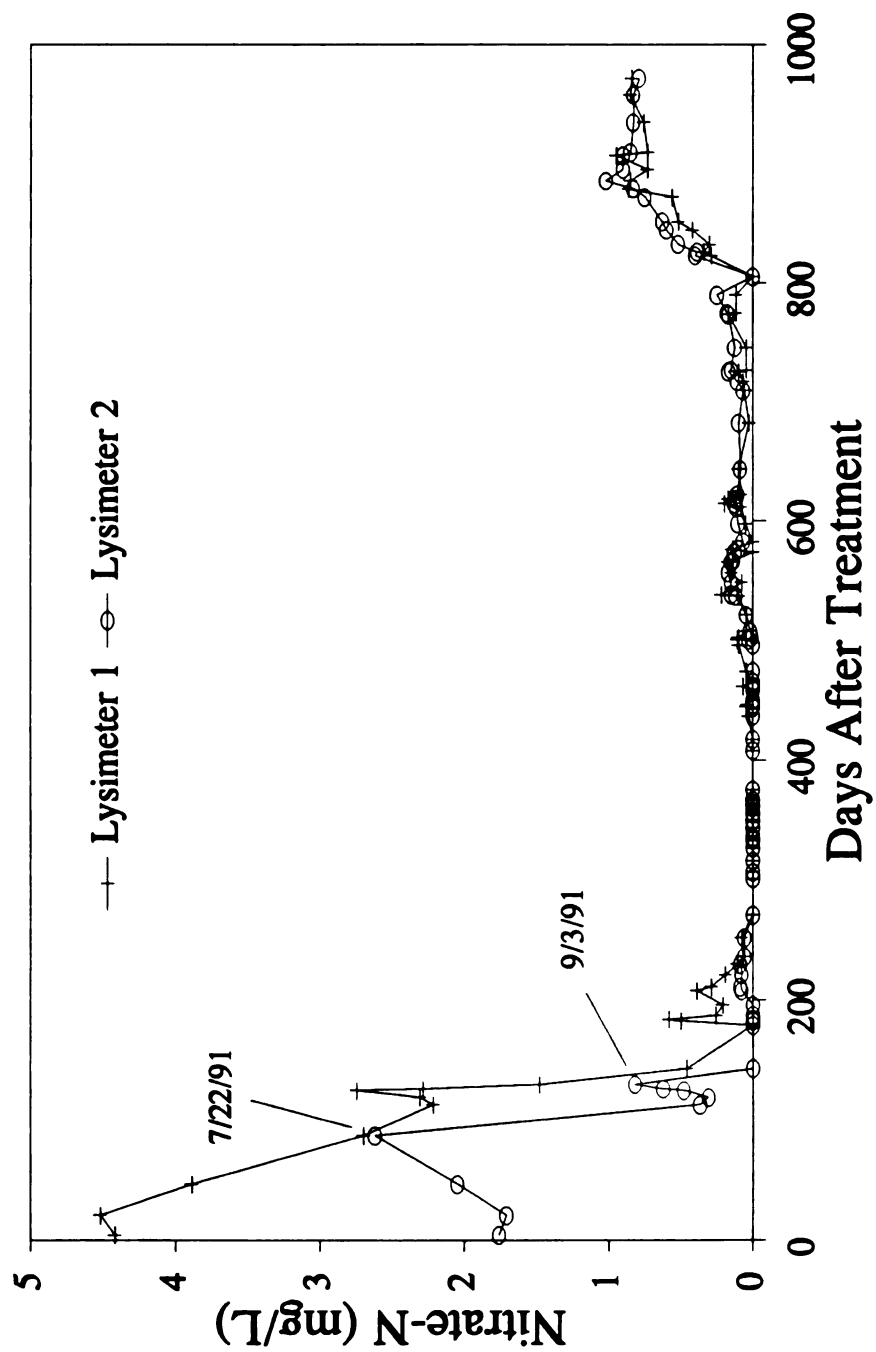


Figure 12. Nitrate-N concentration in leachate for each sample collected for lysimeters receiving the Spring treatment.

NO_3^- -N concentrations were well below 1 mg L^{-1} . In the fall of 1993 concentrations began to increase slightly to approach 1 mg L^{-1} .

For lysimeters receiving the Fall treatment concentration above 1 mg L^{-1} occurred during the first month of the study, but declined throughout the winter and spring (Figure 13). During this first month, there was only one occasion when ^{15}N enrichment was above background (0.00320 atom % excess, Lysimeter 4, 12/3/91). From June 8 to July 14 1992 concentrations near or above 1 mg L^{-1} were detected in Lysimeter 4. NO_3^- -N concentrations were in general slightly higher in leachate from the Fall treatment than the Spring treatment during much of the study. Beginning in the fall of 1993 NO_3^- -N levels were elevated in leachate from the Fall treatment as well. The elevated concentrations of NO_3^- -N which occurred for all lysimeters in the early samples were probably due to enhanced mineralization caused by soil disturbance during construction of the lysimeters. This effect was also noted by Dowdell and Webster (1980) and Geron *et al.* (1983).

The total amount of nitrogen collected in leachate from lysimeters receiving the Spring treatment was $5.77 \text{ kg N ha}^{-1}$ (Figure 14). Total LFN in leachate was 0.09 kg ha^{-1} , which was 0.23% of the amount applied and 1.6% of the total N in leachate. For the Fall treatment total N in leachate was 8.30 kg ha^{-1} (Figure 15). LFN contributed $0.07 \text{ kg N ha}^{-1}$, or 0.18% of the amount applied and 0.8% of the total N leached. There were no significant differences in total N or LFN in leachate between treatments. The vast majority of the LFN leached for both treatments occurred in a few isolated incidents, as is evident in Figures 12 and 13. Details on sample composition for these events appears in Table 20. Note that the data in Table 20 is for individual lysimeters, and that in Figures 12 and 13 are for means of two lysimeters. The

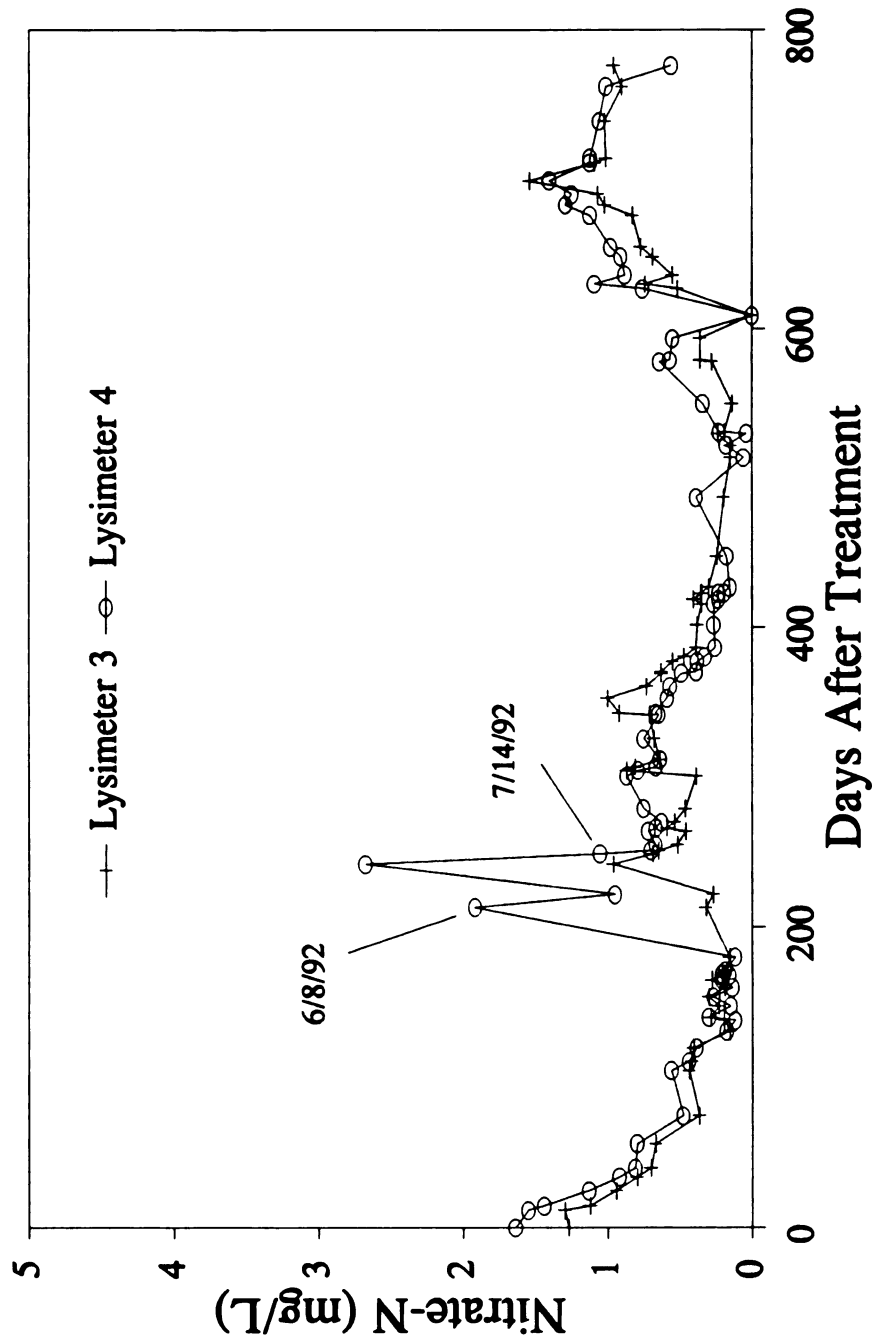


Figure 13. Nitrate-N concentration in leachate for each sample collected for lysimeters receiving the Fall treatment.

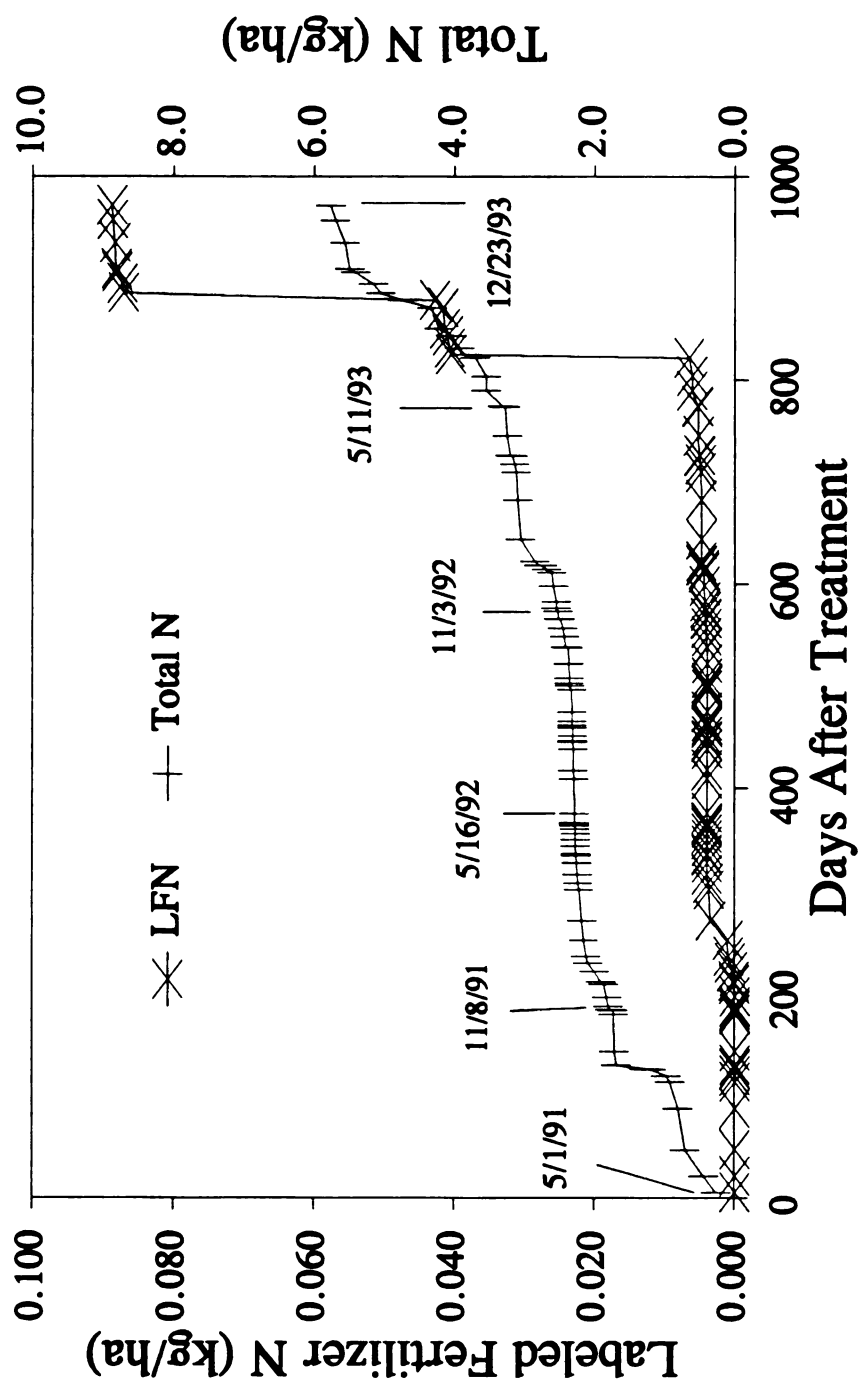


Figure 14. Cumulative Labeled Fertilizer Nitrogen and Total Nitrogen in leachate from lysimeters receiving the Spring treatment.

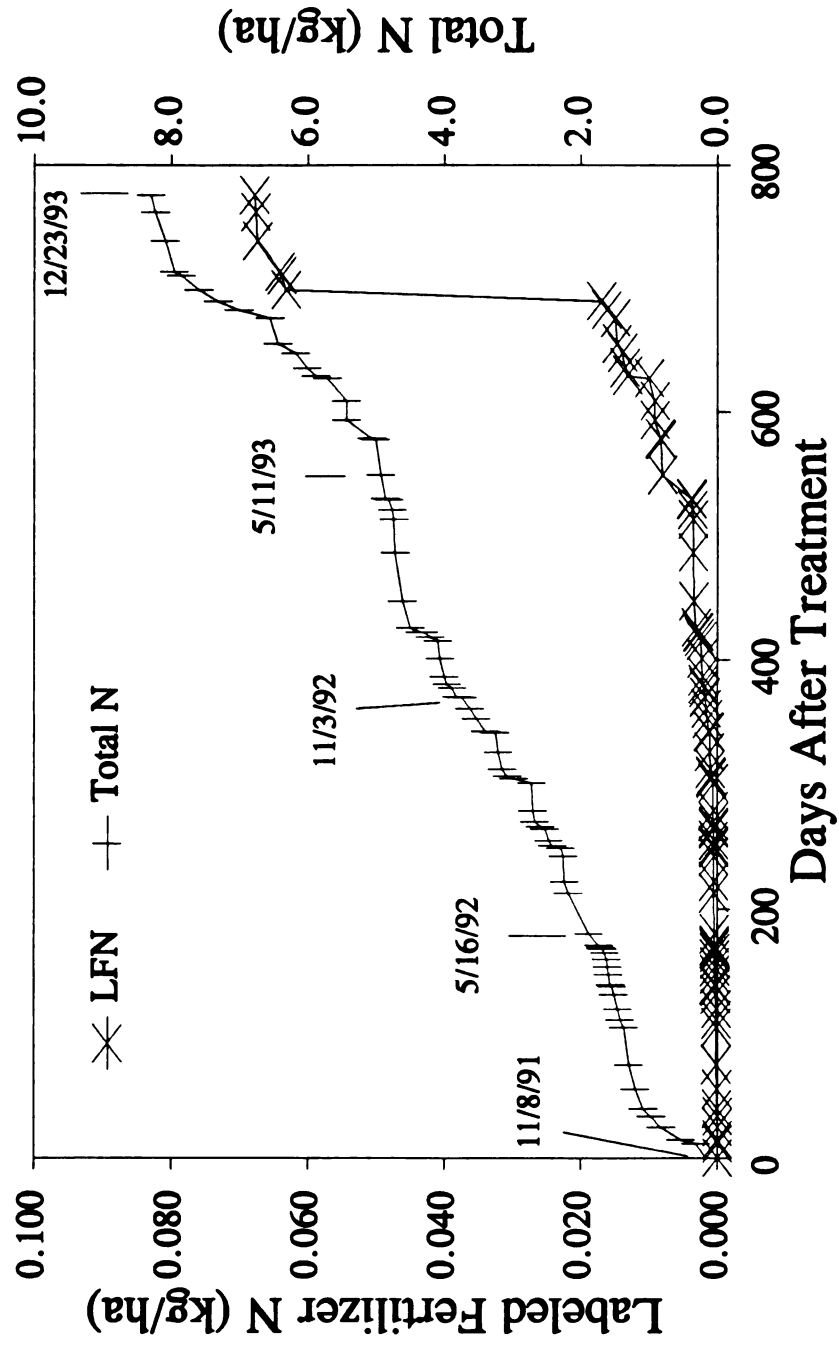


Figure 15. Cumulative Labeled Fertilizer Nitrogen and Total Nitrogen in leachate from lysimeters receiving the Fall treatment.

magnitude of these leaching events as represented in the figures is, therefore, one-half of that as presented in Table 20.

Precision of ^{15}N content in leachate should be noted. Background ^{15}N enrichment of drainage waters were calculated from a mean of 22 samples collected prior to application of LFN. The mean and standard deviation for atom % ^{15}N were 0.37121 and 0.00439, respectively. Atom % excess of each sample was calculated based on this background measurement. In most samples, ^{15}N enrichment was near background levels. In some cases, conversion to atom % excess resulted in negative values. For the purpose of mass balance calculations, these were set to zero, because it is impossible to have "negative leaching." Therefore, total values of LFN in leachate were probably inflated. Consider the early ^{15}N detection from the lysimeters receiving the Spring treatment. The value of 0.00320 atom % excess in this sample was actually less than the SD (0.00439) of the atom % of the background samples. This raises the question of whether or not this "detection" of ^{15}N was real.

Mass Balance

Mass balances for total N for both treatments on the first and last microplot sampling dates are given in Table 21. The data column for the first date includes N from fertilizer and an estimate of N from atmospheric deposition over the two year period covered by the samplings. Totals of initial N amounts plus added N compared to final N amounts indicate whether N is being gained or lost by the system. For the Spring treatment two data sets are included for initial N. The first data is total N measurements from microplots sampled for the Spring treatment in May 1991 following addition of the first fertilizer application. Comparison of this data with data from May 1993

Table 20. Water volume, inorganic N concentration, atom % excess, and total amount of LFN leached for selected samples.

<u>Treatment</u>	<u>Lysimeter</u>	<u>Date</u>	<u>Volume</u> -----cm-----	<u>Inorganic N</u> ----mg L ⁻¹ ----	<u>Atom % Excess</u>	<u>Total LFN</u> ---kg ha ⁻¹ ---
Spring	2	7/30/93	1.96	1.04	8.26307	0.067
Spring	1	9/28/92	2.36	1.20	4.23911	0.048
Fall	3	10/7/93	1.58	1.98	7.41761	0.092

Table 21. Mass balance of total nitrogen for Spring and Fall treatments at initial and final microplot sampling dates.

<u>Source</u>	<u>Spring Treatment</u>		<u>Fall Treatment</u>	
	<u>5/14/91</u>	<u>5/14/91†</u>	<u>11/26/91</u>	<u>11/30/93</u>
	-----kg N ha ⁻¹ -----			
Clippings	3.9	3.6	0	349
Verdure	60	38	93	45
Thatch	477	492	648	601
Soil	3683	4891	5470	4918
Leachate	0.3	0.3	0	8
Fertilizer N	396	435	396	
Atmospheric deposition	26	26	26	
Total	4646	5886	6633	5926

indicates an increase in total nitrogen of 324 kg ha^{-1} in addition to the amount added from fertilizer and atmospheric deposition. Total soil N in this sample was much lower than total soil N on all other sampling dates for both treatments. Because of this, the second set of data from May 1991, derived from non-fertilized microplots harvested at the same time, was included in Table 21. Comparing this data with that from 1993, decreases in total N in thatch and soil were partially offset by the increase of N in clippings and a small amount recovered in the leachate, but an overall decrease of 862 kg N ha^{-1} resulted. This was equivalent to 15% of the initial plus added N and was approximately double the amount of N added as fertilizer. Previous data has shown that approximately 20% of the fertilizer N may have been lost through volatility, but this would account for only about 87 kg N ha^{-1} of the loss calculated here. The remainder of the N was probably lost through denitrification.

Results for the Fall treatment were similar. Losses of N occurred from the soil and thatch while increases were seen in clipping N and leachate. An overall loss of 707 kg N ha^{-1} , or 11% of the total amount, was observed. A loss of 20% of fertilizer N would account for 79 kg ha^{-1} of the total loss calculated here, but most of the loss is probably attributable to denitrification.

The overall analyses of variance for total LFN recovery were presented previously (Tables 4 and 5). Total recoveries of LFN from each canopy increment at each sampling appear in Tables 22 and 23 for the Spring and Fall treatments, respectively. Cumulative recovery in clippings at each sample date increased and recovery in verdure decreased correspondingly for both treatments. Thatch was an important sink for LFN. Over 30% of Spring applied LFN was recovered from thatch 18 DAT (Table 22). A relatively stable amount was retained in thatch throughout the first year for the Spring

Table 22. Recovery of Labeled Fertilizer Nitrogen from each canopy increment and total percent recovery at each sampling date for the Spring treatment.

<u>Date</u>	<u>Clippings</u>	<u>Verdure</u>	<u>Thatch</u>	<u>Soil</u>	<u>Leachate</u>	<u>Total</u>	<u>% Recovery</u>
-----kg LFN ha ⁻¹ -----							
5/14/91	0.94	14.25	12.15	3.16	0.000	30.50	78
6/21/91	7.83	8.02	12.24	4.31	0.000	32.40	83
10/14/91	11.89	3.36	7.43	6.16	0.000	28.84	74
11/26/91	12.09	3.03	12.54	6.74	0.000	34.39	88
5/26/92	12.86	1.53	13.72	7.95	0.004	36.06	92
11/30/92	13.72	0.97	8.38	6.56	0.004	29.63	76
5/14/93	13.89	0.68	5.23	5.34	0.005	25.14	64
FPLSD	0.44	4.27	3.97	2.81	NS	NS	

treatment. During the second year thatch LFN levels decreased significantly, coinciding with a trend of decreasing total recovery. Recovery from soil increased significantly between May and October 1991, and remained significantly higher through November 1992. Total recovery did not differ significantly over time, ranging from 64 to 92%. This was similar to the range in recovery reported by Starr and DeRoo (1981) of 64 to 76%.

Over 62% of the Fall applied LFN was recovered from thatch at 18 DAT (Table 23). Recovery from thatch was similar in the May 1992 then decreased sharply and significantly by the end of June 1992. For the remainder of the study there was a trend towards decreasing thatch LFN, and in November of 1993 the amount was significantly lower than in June of 1992. Soil LFN tended to increase while thatch LFN was decreasing. The largest decrease in thatch LFN was not associated with an increase in the soil, but rather coincided with a significant decrease in total recovery. Total recovery from the November 1991 and May 1992 samples was significantly greater than from all other dates, but the latter dates were not different from one another.

Decreases in thatch LFN from both treatments were usually associated with increases in soil LFN, but not in equivalent amounts. The loss from thatch was also related to decreasing total recovery. It was suspected that loss of LFN in gaseous forms was occurring. This was first suspected following harvest of the first sample for the Spring treatment, which resulted in total recovery of only 78%. The apparent loss from the Fall treatment over six months after application was surprising. It is unknown whether this was due to volatility or denitrification, but the rapid rate of loss would favor volatility.

As described previously, the timing of LFN applications and soil sampling generally precluded direct comparisons between treatments. However, there were two sample dates which coincided in DAT for the two

Table 23. Recovery of Labeled Fertilizer Nitrogen from each canopy increment and total percent recovery at each sampling date for the Fall treatment.

<u>Date</u>	<u>Clippings</u>	<u>Verdure</u>	<u>Thatch</u>	<u>Soil</u>	<u>Leachate</u>	<u>Total</u>	<u>% Recovery</u>
-----kg LFN ha ⁻¹ -----							
11/26/91	0	14.01	24.28	4.77	0.000	43.1	109
5/26/92	8.49	8.9	21.93	3.76	0.000	43.1	109
6/29/92	10.5	7.59	13.88	2.76	0.001	34.7	89
9/17/92	12.09	2.55	9.57	6.31	0.001	30	76
11/30/92	12.38	1.74	9.93	6.1	0.002	30.1	77
5/14/93	12.73	1.15	8.58	8.79	0.008	31.2	80
11/30/93	15.02	0.27	6.69	9.96	0.067	31.9	81
FPLSD	0.58	2.29	7.07	2.93	NS	8.28	

LFN applications, and direct comparisons were made for these times. For the Spring treatment, the May 1991 and May 1993 samples were 18 and 748 DAT, respectively. For the Fall treatment, the November 1991 and November 1993 samples coincided. This provided an opportunity to examine the disposition of LFN after equal time intervals for the two treatments. Comparisons of differential responses of treatments over time were not of primary interest, and so time was not included as a factor. Data for each sample time was analyzed separately, and effects of treatment and depth were examined. As before, the depth factor represented clippings, verdure, thatch, soil, and leachate. For both sample times, treatment x depth interactions were significant ($P = 0.01$ and $P = 0.05$ for times 18 and 748, respectively). Comparisons were then made between treatments at each depth (Table 24). Totals of all depths within each treatment were analyzed separately and are included in Table 24.

The significant difference in clipping harvest at 18 DAT is meaningless because clippings were not harvested during this period for the Fall treatment. Content of LFN in verdure was not different. This was a surprising result because transport of this much N into shoots of fall fertilized turf was not expected. The difference in fertilizer N in thatch was great and coincided with a difference in total recovery. These were encouraging results after the suspected volatile loss from the April application. However, this difference in thatch LFN was apparently equalized the following spring when significant LFN loss from thatch occurred for the Fall treatment. Over the two year period, more LFN was recovered from clippings of late fall fertilized turf. More LFN was also present in the soil for the Fall treatment, and total recovery was significantly higher. The higher level in the soil could raise concern about potential groundwater contamination, but soil ^{15}N was present predominantly in organic forms which are relatively immobile. This higher soil LFN level

Table 24. Comparisons of Labeled Fertilizer Nitrogen recovery in each canopy increment at 18 and 748 days after treatment.

<u>Days After Treatment</u>	<u>Canopy Location</u>	<u>Treatment</u>	
		<u>Spring</u>	<u>Fall</u>
		-----kg LFN ha ⁻¹ -----	
18	Clippings	0.94	0**
	Verdure	14.25	14.01
	Thatch	12.15	24.28*
	Soil	3.16	4.77
	Leachate	0.00	0.00
	Total	30.50	43.06**
748	Clippings	13.89	15.02*
	Verdure	0.68	0.27
	Thatch	5.23	6.69
	Soil	5.34	9.96*
	Leachate	0.01	0.07**
	Total	25.15	32.01**

*,** Means within a row significantly different at P = 0.05 and 0.01, respectively.

may actually indicate greater fertilizer use efficiency by the late fall fertilized turf. Leachate LFN was also greater for the Fall treatment than the Spring treatment at 748 DAT. As was shown in Figure 14 however, leachate LFN increased in the Spring lysimeters during the fall of 1993 (after 800 DAT), and was not significantly different from the Fall treatment in December when data compilation was completed. This parameter was more dependent on season than DAT. The comparisons at 748 DAT should be viewed with some caution. Between November 1992 and May 1993 total recovery for the Spring treatment decreased by 12% (Table 22). The reason for this is not known, but this probably had an effect on the statistical comparison. Recovery in November 1992 was 76% for the Spring treatment, which is comparable to the 81% recovery for the Fall treatment in November of 1993. The differences detected between treatments at 748 DAT may not be true treatment differences.

CONCLUSIONS

Under both fertilization schedules more than 35% of applied LFN was removed in clippings over a two year period, the majority of that occurring in the first growing season after application. Application of nitrogen in the late fall yielded greater uptake of LFN as well as greater total N uptake and higher clipping yield. Thatch played an important role in storage of fertilizer nitrogen on both short-term and long-term scales, but may also be an environment that is conducive to gaseous losses of nitrogen. Initially following application, only 8 and 12% of the LFN applied in April and November, respectively, was found in the soil. These numbers increased to 14 and 25% over two years. Soil microbial biomass played an important role in early immobilization of this applied nitrogen. Only small proportions of this N were present as inorganic N and so potential mobility of the soil LFN pool was low. Mean flow-weighted NO_3^- concentrations in leachate were 0.31 and 0.63 mg N L⁻¹ for the Spring and Fall treatment, respectively. Total LFN recovered in leachate was approximately 0.2% of the amount applied over two years. Leachate will continue to be monitored due to the detections of higher N concentrations in the Fall of 1993.

This study indicates that potential for groundwater contamination due to nitrogenous fertilizer application to turfgrass is minimal. Application of N in early November, an agronomically favorable practice, does not pose an increased risk as compared to an April application. In fact, utilization of late fall applied N may be more efficient.

Questions posed by the results of this research include the potential for losses through volatility and denitrification, the role of the thatch layer in storage and cycling of nitrogen, and immobilization and mineralization

potentials of turfgrass soils. Research in these areas would more fully describe the nitrogen cycle in the turfgrass ecosystem.

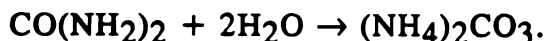
CHAPTER II
IRRIGATION AND NITROGEN SOURCE EFFECTS
ON RECOVERY OF ^{15}N -LABELED FERTILIZER
APPLIED TO TURFGRASS

LITERATURE REVIEW

Ammonia volatilization is the process by which NH_4^+ -N is converted to gaseous NH_3 and lost to the atmosphere. The general reaction is:



and requires an aqueous alkaline environment as the reaction indicates (Tisdale and Nelson, 1975). In order for loss of nitrogen to occur by volatilization, it first must be available in the NH_4^+ form. Ammonium can be supplied either from mineralization of N from soil organic sources, application of NH_4^+ containing fertilizers, or application of fertilizers containing other N sources which are subsequently converted to NH_4^+ . One of the most common forms of nitrogenous fertilizer is urea $[\text{CO}(\text{NH}_2)_2]$ which is hydrolyzed in the soil by the following reaction:



This reaction is mediated by the urease enzyme. Ammonium carbonate is not stable and dissociates yielding NH_4^+ and CO_3^{2-} , which consumes H^+ from soil solution, driving pH up. Therefore, even in neutral or slightly acidic soils, microenvironments surrounding urea particles or droplets can become alkaline, resulting in good conditions for volatility.

Urease Activity

Overrein and Moe (1967) incubated two soils (Chalmers silt loam, pH 6.5, O.M. 4.5%; Plainfield sand, pH 5.7, O.M. 1.2%) with urea at rates of 224, 448, and 896 kg N ha⁻¹ and studied the effects of several different environmental parameters on ammonia volatilization. They found the rate of urea hydrolysis in the Chalmers silt loam at 24% moisture to increase linearly with application rate at 28°C. At 4°C the response was linear for the first two rates, but hydrolysis dropped off for the high rate of urea, presumably due to more limited activity of urease at the lower temperature and an effective saturation of the enzyme. At 28°C rates of hydrolysis were approximately 110, 220, and 450 kg N ha⁻¹ day⁻¹ from the 224, 448, and 896 kg N ha⁻¹ application rates. Volk (1966) observed almost no hydrolysis of urea when applied to air dry soils incubated in the laboratory. In the field, hydrolysis and volatile loss was greatly reduced on dry soils as compared to moist soils.

Urease activity in a Kentucky bluegrass thatch was approximately 25 times higher than in underlying soil (Torello and Wehner, 1983). Activity in thatch of dormant turf was approximately 40% of that of actively growing turf. Ten days of greenhouse incubation increased the activity of the dormant thatch urease by about 40%. Activity was almost twice as great in the upper 1 cm of a 2 cm thatch layer as compared to the lower 1 cm. There was also great variability between two different sampling locations. Bluegrass leaves also had high urease activity, approximately 18 times greater than the soil. Bowman *et al.* (1987) found similar trends in urease activity in Kentucky bluegrass shoots, thatch, and soil, although the magnitude of activity was not as great (15 times greater in thatch compared to soil). Shoot and thatch tissue had comparable levels of activity on a dry weight basis, but activity in the thatch was double that in plant shoots when expressed on an area basis. Most of the urease

activity (96%) in plant shoots was in sheath tissue (including dead and senescing tissue) as opposed to leaf blades. The top 1 cm of soil had five times the urease activity of the 1 - 3 cm layer.

Effect of Nitrogen Rate

Ammonia volatilization rates from a Chalmers silt loam following application of 224, 448, and 896 kg N ha⁻¹ as urea were measured each day for five days, and were found to increase curvilinearly (Overrein and Moe, 1967). A greater proportion of NH₄⁺ was bound to soil at low application rates than high rates. The highest rates of volatilization, occurring on day two, were approximately 0.5, 2, and 9 kg N ha⁻¹ day⁻¹ for the three fertilizer rates. Total volatile losses over five days were 0.7, 1.0, and 1.8% of the applied N. Volk (1959) noted a similar effect when pelletized urea was applied to four different warm season turfgrasses. Percent lost through volatilization averaged 13.6, 18.0, and 20.6 for application rates of 28, 56, and 112 kg N ha⁻¹, respectively. Simpson and Melsted (1962) observed that approximately 30% of N applied as urea was lost by volatility from a bluegrass forage sod over 10 days when applied at 56, 112, and 168 kg N ha⁻¹. Total loss increased with rate of application for a number of grass crops tested. Wesely *et al.* (1987) also found that total N loss increased with increasing application rates, but the percent N lost remained relatively constant. For application rates of 17 and 34 kg N ha⁻¹, total losses over four days were 35 and 31% of the applied N.

Effect of Nitrogen Source

Volatile losses of N from ammonium sulfate, urea, and ammonium nitrate applied to Meloland clay loam (calcareous, pH 8.0, moisture content 75% of field capacity at time of application) were 25, 16, and 11% of applied

N over 70 days (Martin and Chapman, 1951). Loss from NH_4NO_3 was low because only one-half of the N was in the NH_4^+ form at the time of application. Applied to four different sandy loam soils of pH 7.7, 7.5, 7.1, and 6.7, loss of N from ammonium sulfate was 14, 19, 2, and 4%. Losses of urea-N were 14, 16, 18, and 36%. Losses of NH_4NO_3 -N were 7, 7, 1, and 1%. Volk (1959) measured volatile losses from pelletized urea averaging 30% from four turfgrasses in the field, as compared to 0.3% loss from NH_4NO_3 . Soil pH was not given for this particular experiment, but other soils in this paper ranged in pH from 4.4 to 6.7. The only significant loss of ammonium sulfate-N occurred from a Perrine marl with a pH of 7.8. In the above studies, application of ammonium salts to alkaline soils resulted in volatile loss, but application to neutral or acidic soils resulted in very little loss. Urea applied to acidic, neutral, or alkaline soils resulted in volatile loss.

Torello *et al.* (1983) studied the effects of different urea sources and application techniques on volatilization from a Kentucky bluegrass turf grown on a silt loam soil with pH 6.4. Applied at 293 kg N ha^{-1} , significantly more urea-N volatilized (10.3%) than N from two sulfur coated urea sources (2.3 and 1.1%). Sulfur coated urea with a dissolution rate of 37.5% produced greater volatile loss than formulations with 27.2 and 18.0% solubility (0.7, 0.2, and 0.2% lost, respectively; application rate of 98 kg N ha^{-1}). Comparing two ureaformaldehyde products formulated for application through a spraying system, they found that the formulation with 50% free urea yielded greater volatile loss than the product with 35% free urea (4.5 vs. 3.2%, application rate of 49 kg N ha^{-1}). In a fourth experiment, greater loss was measured from urea applied as a solution (4.6%) than when applied in a prilled form. This was explained by the fact that the solution application remained primarily on leaf blades, where it was subject to high urease activity (Torello and Wehner,

1983) and the lack of exchange sites provided no protection against volatile loss as would occur in the soil (Volk, 1959). Interestingly, Titko *et al.* (1987) observed the opposite effect. Greater loss occurred from granular urea than spray applied urea. Temperature and spray solution volumes were essentially the same in both experiments [24°C , 1629 L ha^{-1} (Torello *et al.*, 1983); 22.2°C , 1630 L ha^{-1} (Titko *et al.*, 1987)], but N rates were different (49 hg ha^{-1} and 73 kg ha^{-1} , respectively). Torello *et al.* (1983) noted that roughly equivalent amounts were lost from spray applied urea and ureaformaldehyde combination products. The UF components were not hydrolyzed on leaf tissue, and they concluded that volatile loss was from the urea components only.

Effect of Temperature

Increases in volatile loss of N from ammonium sulfate, urea, and ammonium nitrate with increasing temperature were observed by Martin and Chapman (1951). They attributed this to greater soil drying. Volk (1959) also noted this temperature effect. Titko *et al.* (1987) observed significantly higher loss from both granular and dissolved urea from Kentucky bluegrass at 22.2°C as compared to 10°C , but no further significant increase at 32.2°C .

Effect of Moisture

Martin and Chapman (1951) concluded that initial soil moisture content had no effect on total volatile losses, although they stated the reason for the temperature effect they observed was a greater degree of soil drying. This reasoning seems to be contradictory. Titko *et al.* (1987) subjected turf to alternate wetting and drying cycles every 24 hours, and maintaining constant relative humidity of air passing through the system ($68 \pm 4\%$), found greater loss from both dissolved and granular urea as compared to plots not subject to

the wetting/drying cycles. Martin and Chapman (1951) also saw additional loss with each increment of a wetting/drying cycle. Wesely *et al.* (1987) observed that the greatest rates of volatility occurred at times of drying of moist leaf tissue. Titko *et al.* (1987) observed lower volatile losses from turf maintained at 68% relative humidity compared to 31% relative humidity.

Irrigation of turf with 1 cm of water during the first hour following application of either granular or solution urea reduced volatile loss significantly. Bowman *et al.* (1987) applied 50 kg N ha⁻¹ as urea in 0.2 cm of water to Kentucky bluegrass followed by irrigation amounts of 0, 0.5, 1.0, 2.0, and 4.0 cm. With no irrigation, 25% of the applied N was lost in the first 24 hours. Increasing depths of irrigation decreased losses over this period to approximately 7, 4, 2, and 2%. When urea was applied in 0.05 cm of water with no supplemental irrigation, loss was lower than for the 0.2 cm depth of application (18% vs. 25%). If no supplemental irrigation was applied following application in 0.2 cm of water, 21, 47, and 8% of the applied N was recovered from shoots, thatch, and soil (0 - 3 cm depth). When 0.5 and 1 cm of irrigation was applied, 5, 26, and 26% and 1, 25, and 27% were recovered from these environments, respectively. From application in 0.05 cm, 75, 24, and 1% was recovered from shoots, thatch, and soil. The vast majority of the N recovered in the soil in all cases was in the upper 1 cm. Nitrogen not recovered was attributed to biological immobilization. Although apparent distribution in the thatch layer for the 0.5 and 1.0 cm treatments was the same, the lower loss rate from the 1 cm treatment was probably attributed to deeper incorporation within the thatch layer where urease activity is lower (Torello and Wehner, 1983).

Effect of Crop

Simpson and Melsted (1962) found that volatility losses from a bluegrass sod were considerably higher than from other grass crops. Hydrolysis began immediately and rates were high for the first two to three days for all crops. Volatility was measured in bluegrass on the first day, but for all other crops there was a delay of 2 - 3 days before the onset of N evolution. Bluegrass samples were taken from the field, whereas all other crops were planted from seed in the greenhouse. The authors attribute the difference in results to the organic litter in the bluegrass sod.

Volatile losses of fertilizer N from turfgrasses have been well-documented. Bowman *et al.* (1987) and Torello and Wehner (1983) have shown that urease activity in turfgrass thatch is much higher than the soil below. Elevated temperatures have been shown to contribute to volatility (Martin and Chapman, 1951; Titko *et al.*, 1987; Volk, 1959), as well as drying conditions (Martin and Chapman, 1951; Titko *et al.*, 1987; Wesely *et al.*, 1987). These three factors combine to make the thatch layer an area of high volatility potential. Some researchers have shown that higher percentages of N are lost through volatility as application rate increases (Overrein and Moe, 1967; Volk, 1959). Others have observed increased volatile loss with increased rate, but no marginal increases in loss (Simpson and Melsted, 1962; Wesely *et al.*, 1987). Urea exhibits higher volatility rates as compared to modified urea or nitrate sources (Martin and Chapman, 1951; Torello *et al.*, 1983; Volk, 1959). Irrigation immediately following application also reduces losses (Bowman *et al.*, 1987). Although ammonia volatility can be a source of loss of fertilizer N applied to turf, choice of the correct fertilizer source, application

during cooler and/or drier times of the day, and proper irrigation following application should limit such losses.

MATERIALS AND METHODS

Recoveries in the range of 75% to 90% of spring applied urea and recoveries in excess of 100% for fall applied urea in the experiment in Chapter I indicated that substantial losses of urea-N may have occurred through ammonia volatility under spring conditions. This experiment was designed to account for possible volatile losses under these conditions and determine the effect of irrigation on volatile losses of ammonia in the cool humid climate of Michigan.

In May of 1993 60 microplots 30 cm in depth were constructed of 20 cm diameter PVC pipe. These microplots were beveled on one end and installed in the same manner as those in the previous experiment. Soil and turf conditions were as described in Chapter I. Three fertilizer treatments were applied to these microplots in a randomized complete block layout with four replicates. Two treatments consisted of fertilization with ^{15}N -labeled urea (19.1991 atom % excess) at a rate of $38.0 \text{ kg N ha}^{-1}$. One of the urea treatments received a total of 0.5 cm irrigation with the application (UL) and the other received 2.0 cm (UH). Bowman *et al.* (1987) observed a reduction of volatile loss from 7% to 2% using these irrigation practices. The third treatment was fertilized with ^{15}N -labeled potassium nitrate (KNO_3) (19.4546 atom % excess) at a rate of $37.2 \text{ kg N ha}^{-1}$ watered-in with 0.5 cm water. Presumably, there should be no volatile loss of N from KNO_3 , at least in the short term. Comparison of KNO_3 and UL treatments should illustrate the potential loss due to use of urea, and comparison of UL with UH should illustrate the effect of irrigation. All treatments were applied in 0.05 cm water from 125 ml polypropylene bottles and immediately watered-in with the appropriate amount of water as described

in Chapter I. Treatments were applied on June 11, 1993. Air temperature was 24°C, wind was from the east at 13 km h⁻¹, and relative humidity was 53%.

Five designated sampling times for the experiment were 1 hr, 4 days, 20 days, 40 days, and 80 days after treatment. Bowman *et al.* (1987) found that volatile losses of N from urea under similar conditions occurred primarily in the first 12 hours after application, with slight additional losses occurring up until 72 hours. Volk (1966) also observed that the majority of total loss due to volatility occurred within 72 hours, and Wesely *et al.* (1987) found that volatilization rates peaked within 48 hours after application. It was hypothesized that the largest loss in this experiment would occur between 1 hour and 3 days after application. At each of these times one microplot for each sample in each replication was excavated and processed as described in Chapter I. Exceptions were that soil depth increments extended to 20 cm only, and only one soil subsample was saved from each depth, to be dried and ground for analysis. All plant tissue and soil samples were ground as before and analyzed for total N and ¹⁵N enrichment on a Europa Scientific Roboprep C-N Biological Sample Converter and Tracermass Mass Spectrometer. Data from the experiment in Chapter I indicated very little downward fertilizer N movement. It was assumed that ¹⁵N not recovered in plant and soil samples from within the microplots was lost to the atmosphere. Therefore, volatile losses of ¹⁵N were implied by difference.

Microplots designated for excavation at 1 hr after treatment were partially excavated before hand because of time constraints. Soil was removed from around each microplot, leaving the enclosed core standing freely but still attached to the soil below. After application of 2 cm irrigation to designated treatments, water was seen draining out of the bottoms of three of the four exposed microplots receiving this treatment. This drainage appeared to be from

the edges of the cores, and movement may have been primarily by preferential flow down the tube walls.

Data was converted to percent recovery since the application rates were slightly different for the two sources, and analyzed as a split-plot with treatment as main plot and "depth" as sub-plot for each sample time. Once again, depths were comprised of clippings, verdure, thatch, and soil (total of all depths). Analysis was conducted with PC-SAS v. 6.04 (SAS Institute, Cary, NC).

RESULTS AND DISCUSSION

There were significant treatment by depth interaction effects on percent LFN recovery at 1 hr and 20 and 40 days after treatment (Table 25). Data was further analyzed to separate treatment differences at each canopy level on each sampling date (Table 26). One hour after application there was significantly more LFN recovered from soil from the urea high irrigation treatment (UH) than from the urea low irrigation (UL) or potassium nitrate (K) treatments, indicating deeper placement as a result of increased irrigation. Flow of irrigation water from the bottoms of the partially excavated microplots receiving the UH treatment did not result in significant additional losses of LFN. Less than quantitative recovery of LFN is indicative of procedural errors, but the lack of significant differences between treatments indicate that these occurred uniformly across treatments. Lack of differences in total recoveries between all treatments and the similarity in location of LFN between UL and K treatments shows that there was no significant volatile loss of N from urea as compared to KNO_3 . Bowman *et al.* (1987) observed less than 1% volatile loss of N 1 hr following application of urea followed with 0.5 cm irrigation. Although total recovery was much less in their study (57%), Bowman *et al.* (1987) also found that 56% of the amount of LFN recovered 1 hr after application was in the thatch layer. This was very similar to the 53% of the total recovered in the 0.5 cm irrigation treatments in the present study.

Samples collected four days after treatment yielded recoveries well in excess of 100% for the UL and K treatments (Table 26). Recovery for the UH treatment (85.3%) was significantly lower than UL and K, but similar to total recovery for UH in the 1 hr sample. Total recoveries for UL and K treatments

Table 25. Summary of split plot analyses of variance at each sampling time.

<u>Source of Variation</u>	<u>Sample time</u>			
	<u>1 hr</u>	<u>4 days</u>	<u>20 days</u>	<u>40 days</u>
Treatment		**		
Replication				
Depth	**	**	**	**
Treatment x Depth	**		§	§

§, ** Significant at $P = 0.10$ and 0.01 , respectively.

Table 26. Percent recovery of LFN in each canopy level at each sampling time.

<u>Time</u>	<u>Treatment</u>	<u>Clippings</u>	<u>Verdure</u>	<u>Thatch</u>	<u>Soil</u>	<u>Total</u>
1 hr	UL	0	29.1	46.4	11.5	87.0
	K	0	29.7	46.5	12.4	87.6
	UH	0	16.9	38.4	30.0	85.3
	FPLSD (0.05)	NS	NS	NS	13.1	NS
4 days	UL	0	51.8	62.7	11.8	128.5
	K	0	46.9	57.7	15.4	119.9
	UH	0	28.4	37.7	19.2	85.3
	FPLSD (0.05)	NS	12.7	NS	NS	22.6
20 days	UL	16.7	24.9	42.6	5.4	83.3
	K	25.4	24.1	36.8	4.4	85.9
	UH	13.7	15.1	31.2	13.2	73.1
	FPLSD (0.05)	6.8	NS	NS	2.8	NS
40 days	UL	24.7	22.4	33.2	8.7	88.9
	K	35.1	20.2	27.2	6.2	88.8
	UH	20.6	15.1	24.7	12.2	72.7
	FPLSD (0.05)	6.6	4.4	NS	NS	NS

were between 83 and 89% for all sample times except 4 days. Large increases in LFN were seen in verdure and to a lesser extent thatch, but the reason for the overall discrepancy is unclear.

At 20 and 40 DAT, total recoveries were not significantly different between treatments. Recovery of LFN in clippings was greater for K than UL and UH at both times. LFN in verdure was significantly higher for UL and K than UH at 40 DAT and also appeared to be higher at 20 DAT, but this difference was not significant. Recovery from soil was higher for UH than UL and K at 20 and 40 DAT. This data indicates that deeper placement of fertilizer N due to irrigation moved the fertilizer past the zone of greatest plant uptake.

The percentage of LFN in the thatch remained high throughout the study, but did appear to decrease over time within each treatment (disregarding the 4 day sample because of the high recovery). The decreases in thatch LFN were accompanied by increases in the amount recovered in clippings + verdure, indicating either uptake of fertilizer N from the thatch or transport of plant LFN within the thatch layer upward in the plant.

The potential for volatile losses of N from turfgrass has been well documented (Volk, 1959; Nelson *et al.*, 1980; Torello and Wehner, 1983; Nelson *et al.*, 1980; Torello *et al.*, 1983; Bowman *et al.*, 1987; Titko *et al.*, 1987; Wesely *et al.*, 1987). In addition, the thatch layer has been described as an environment conducive to volatilization. Although data in this experiment indicates that a large portion of applied fertilizer nitrogen is resident in thatch immediately after application, decreases in LFN from thatch could be accounted for through LFN harvested in plant tissue above the thatch layer. Under the conditions of the present experiment, volatile losses of N were not a significant factor.

Based on the results of this experiment, no conclusions can be made concerning non-recovered LFN in the experiment in Chapter I. Although volatile losses were suspected in that experiment, the present data shows that non-quantitative recovery can occur even with a fertilizer source not prone to volatility. However, volatility was not measured directly in the present experiment, which may be a contributing factor in these results. Further investigation into the nature of thatch and its ability to immobilize N, especially following fall applications, and subsequent losses of N from thatch during warming periods, are warranted.

CHAPTER III

MINERALIZATION AND TURNOVER OF SOIL NITROGEN IN A TURFGRASS COMMUNITY

LITERATURE REVIEW

Prediction of Mineralization Rates

Soil organic matter constitutes a major reserve of nitrogen that can contribute to agricultural productivity. Estimating mineralization rates and availability of this nitrogen can lead to a better understanding of the plant-soil system and more efficient use of inputs. Stanford and Smith (1972) conducted fundamental research in use of long-term incubation of soils to construct estimates of N mineralization potential. Studying 39 different soils incubated and leached intermittently to remove mineralized N over a period of 30 weeks, they formulated the following model for estimating N mineralization potential:

$$N_m = N_o * (1-e^{-kt})$$

where

N_m = total mineralized N in time t

N_o = potential N mineralization

k = mineralization rate constant.

They found this model to have the greatest utility when applied to data for incubations from weeks 2 through 30. When the higher mineralization rates observed in the first two weeks were included, the fit of the model was not as accurate. For 29 soils included in the analysis, the average rate of mineralization was 0.054 wk^{-1} (5.4% of mineralizable N released per week).

They concluded that total mineralization potential could be most accurately predicted from the sum of mineralization in the first two weeks plus the model prediction for the long-term release of N.

Molina *et al.* (1980) reexamined the data of Stanford and Smith (1972) and found that the following two pool model for nitrogen mineralization provided a more accurate estimate:

$$Y = S * (1 - e^{-ht}) + (1-S) * (1 - e^{-kt})$$

where

Y = cumulative mineralization at time t

S = a fraction of potentially mineralizable N

h = mineralization rate for fraction S

k = mineralization rate for fraction (1 - S)

t = time.

This model implies the existence of two different pools of mineralizable soil N which are released at different rates. Based on the data, they calculated a labile pool (S = 15.8% of total N) which decomposed at rate $h = 1.131 \text{ wk}^{-1}$ and a more resistant pool ($1 - S = 84.2\%$) with a decomposition rate of $k = 0.0424 \text{ wk}^{-1}$. By non-linear least squares (NLLS) analysis it was determined that this model provided a more accurate explanation of the data. Smith *et al.* (1980) also determined that NLSS was a more precise test of fit as compared to least squares analysis of log-transformed data for the single exponential model.

Deans *et al.* (1986) also employed the two pool model of Molina *et al.* (1980), and further concluded that the single model underestimated N_0 (total mineralization) and overestimated the rate of decomposition k. A simplified version of the two pool model was constructed by Bonde *et al.* (1988):

$$N_m = N_a * (1 - e^{-ht}) + Ct$$

where

N_m = total N mineralized in time t

N_a = total labile mineralized N ($S * N_o$)

Ct = linear expression representing slope of mineralization curve of resistant pool.

For incubations of fallow soils and soils cropped to cereals with either no added fertilizer, added fertilizer, or added manure, mineralization rates were best described by the simplified model. The authors proposed that a constant amount of mineralization could occur when microbial populations reach some maximum level and equilibrium is established, and substrate is in abundant supply. Where the cropped soil was amended with straw, the full two pool model provided the best fit.

Boyle and Paul (1989) also employed the two pool model to describe N mineralization from sewage sludge amended plots. They found the period of mineralization of the labile fraction to be 11 weeks, with mineralization of the recalcitrant fraction predominating after that time.

A number of possible limitations to the use of the incubation method have been suggested. Bremner (1965) questioned the practices of pre-leaching the soil, using soil amendments such as vermiculite or sand to enhance leaching characteristics, the use of nutrient solutions to extract mineralized N, and using suction to equilibrate soil moisture content prior to incubation. Each of these methods was used by Stanford and Smith (1972) in their original work. Smith *et al.*, (1985) noted that organic N was removed with inorganic N when soils were leached with $CaCl_2$, but changing the volume or concentration of the extractant did not significantly affect this. Failure to account for partially mineralized N or removal of substrate could lead to errors in estimating mineralization potential. Juma and Paul (1984) explored a variety of extraction procedures, including autoclaving and different chemical extractants. They

concluded that any one extractant could not account for all sources of mineralizable N, and that further work in this area could lead to better discrimination between various mineralizable N pools.

Mineralization Characteristics of Some Soils

Mineralization potential and rate constants vary with methodology and soils. Stanford and Smith (1972) reported mineralization potentials for various soils ranging from 18 to 358 mg kg⁻¹. Based on percent of total soil N, the range was 4.6 to 40.6%. Mineralization rate constants ranged from 0.035 to 0.095 wk⁻¹, with a weighted mean of 0.054 wk⁻¹ for 29 of the soils. Smith *et al.* (1980), using the single pool model, reported N₀ values between 65 and 92 mg kg⁻¹ and rate constants between 0.124 and 0.240 wk⁻¹ for three different soils. Fitting the data of Stanford and Smith (1972) to the two pool model, Molina *et al.*, (1980) determined rate constants of 1.131 for the labile component (15.8% of N₀) and 0.0424 for the more resistant component (84.2% of N₀). Deans *et al.* (1986) fit data from several researchers for soils from a number of states to the single and two pool models. Based on the double model, mineralization potentials ranged from 76 to 881 mg kg⁻¹. Labile fractions comprised between 1 and 25% of total mineralizable N. Rate constants for the labile components ranged from 0.29 to 3.50 wk⁻¹, and from 0.004 to 0.069 wk⁻¹ for resistant fractions.

Nordmeyer and Richter (1985) found that addition of organic amendments to incubated soils altered the N mineralization dynamics. Addition of 6000 and 12000 Mg leaves ha⁻¹ (190 and 380 kg N ha⁻¹) from sugar beet (*Beta vulgaris*) prompted turnover and increased mineralization in an amount approximately equivalent to the amount of added N. Approximately 2/3 of the added material was quickly decomposable, and the remaining 1/3 was more

stable. Rates of decomposition of the labile soil N were reduced while rates for more stable N were increased in response to the addition of leaf material. Following addition of wheat straw (*Triticum aestivum*), immobilization rates increased for 30 days compared to the control. Mineralization rates were greater than immobilization, resulting in slight net mineralization for amended soils during this period, but net mineralization was greater in untreated soils for 100 days as compared to soils receiving 900 Mg straw ha⁻¹. For soils amended with twice the amount of straw, net mineralized N remained lower than the control for the entire 150 day incubation.

Boyle and Paul (1989) found total mineralization and mineralization rates increased in response to addition of sewage sludge to soil. Rates were 0.010, 0.013, and 0.020 wk⁻¹ for soil receiving 0, 45, and 180 Mg sludge ha⁻¹yr⁻¹ for 8 years. Sludge was not applied for three years prior to the incubation experiment, and barley (*Hordeum vulgare* L.) was grown in the field each year. These rates do not reference either labile or stable fractions, but apply to the entire soil N pool. Additional quantities of 266 and 458 mg N kg⁻¹ were mineralized in treated soil as compared to the control. They also observed a decrease in microbial biomass of over 50% in the first 20 weeks of the incubation followed by stabilization. If a C:N ratio of 7:1 is assumed, N mineralized from microbial biomass represented approximately 8% of total mineralized N averaged for all treatments.

Bonde *et al.* (1988) also saw increased mineralization due to added N sources. Mineralizable N over a 40 week incubation was 93 ug g⁻¹ for fallow soil. Soil cropped to cereals with no additional N mineralized 107 ug g⁻¹. Additions of calcium nitrate (80 kg N ha⁻¹ yr⁻¹), calcium nitrate plus straw (80 kg N ha⁻¹, 1800 kg C ha⁻¹), and calcium nitrate plus manure (80 kg N ha⁻¹, 1800 kg C ha⁻¹) to cropped soils resulted in mineralization of 114, 167, and

165 ug g⁻¹. Between 6.2 and 8.8% of the total N pool was mineralized. Reductions in the size of the microbial biomass pool were also reported. At 4, 9, and 47 weeks of the incubation, the biomass pool was 36%, 23%, and 8% of its initial size. Biomass N was reduced to 9% of the initial amount at 47 weeks. A model, analogous in form to the model for N mineralization, was developed to describe the decline in biomass:

$$B_t = B_a * (e^{-ht}) + B_r * (e^{-kt})$$

where

B_t = microbial biomass at time t

B_a = labile biomass

B_r = resistant biomass

h = rate constant, labile biomass

k = rate constant, resistant biomass.

Biomass N in the soil ranged from 3.9 to 6.8% of the total N. Of the N mineralized, 55 to 89% was from biomass.

In contrast, Juma and Paul (1984) determined that 15 to 25% of N mineralized in 12 weeks was from microbial biomass. Due to computational differences, this number can be recalculated as 6 to 10% in order to be compared to data of Bonde, *et al.* (1988). Muramoto *et al.* (1982) measured mineralization flushes from oven dried (70°C) and air dried soil and found that 77% and 55% of the mineralized N was biomass N. They observed a strong linear relationship between decline in biomass C and increase in mineralized N, in a ratio of approximately 10:1.

The literature indicates that mineralization potentials and rates vary widely among soils. The nature of labile and resistant components of organic N has not been completely defined, and values for mineralizable biomass N, considered generally to be labile, are vastly different. Aside from these

difficulties and inconsistencies, mineralizable N remains a very important subject. Its estimation and/or measurement can provide very useful information in the understanding of the nitrogen cycle.

Determinations of mineralizable nitrogen from turfgrass soils as measured by laboratory incubation procedures were not found in the literature. Starr and DeRoo (1981) observed increases in soil nitrogen due to fertilization and clipping management practices, and predicted that as much as 50% of this added N would be mineralizable. By their estimates, an additional 40 kg ha⁻¹ of N could be available for plant growth. Several authors have recognized the importance of mineralization and its potential impact on turf growth and environmental quality (Starr and DeRoo, 1981; Mitchell *et al.*, 1978; Geron *et al.* (1993). The objectives of this experiment were to measure mineralizable N from soil with a turfgrass cover. The effects of added fertilizer and leaf clippings on turnover of soil N were also studied.

MATERIALS AND METHODS

Two laboratory incubation experiments were conducted to study the dynamics of nitrogen cycling in a soil beneath a Kentucky bluegrass turf. The first experiment was designed to assess the potential for mineralization of N from soil over 180 days. The second experiment was designed to study the effects of added N sources on short-term cycling of N.

Long-term Incubation (LTI) Experiment

A variation of the long term incubation experiment of Stanford and Smith (1972) was initiated in August of 1993. The soil used was from the 0-5 cm and 5-10 cm depths of microplots harvested in May 1993 from the experiment described in Chapter I. Only plots which had received ^{15}N -labeled urea ($39.2 \text{ kg N ha}^{-1}$, 24.9613 atom % excess ^{15}N) in April of 1991 were used. Samples to be analyzed on twelve dates were weighed out with four replicates for each date and each depth. For each sample, approximately 12g of field moist soil was weighed into a 50 ml glass beaker. The beaker was covered with Parafilm to prevent moisture loss and placed into a 120 ml plastic specimen cup (Premium Plastics, Inc., Chicago, IL). Three holes were punched in the lid of the cup using a dissecting needle. A thirteenth set of samples, consisting of approximately 30g of soil, was also prepared. This set was placed into 1 L glass canning jars for trapping of CO_2 for measurement of microbial biomass. All samples were placed in a controlled environment chamber which remained at 24°C , and the samples were not exposed to light. Twelve sample times of 0, 1, 3, 10, 20, 30, 50, 70, 90, 120, 150, and 180 days after treatment (DAT) were designated, and at each of these times four

soil samples for each depth were removed for analysis. In addition, at each sample time, base traps of 2 ml of 2N NaOH, contained in glass scintillation vials, were placed into the glass jars. At each subsequent sample date the traps in the jars were removed and capped and new traps replaced them.

The beakers containing the soil samples were removed from specimen cups and placed in an oven at 65°C for 48 hr to dry the soil. Soil was then extracted with 1N KCl (5:1 v/w) and analyzed for NO_3^- -N and NH_4^+ -N by flow injection analysis with a Lachat QuikChem autoanalyzer (Lachat Instruments, Milwaukee, WI). This was followed by diffusion and trapping of N (Brooks, *et al.*, 19) for measurement of ^{15}N enrichment of each N species. Separate diffusions were conducted for NH_4^+ and NO_3^- due to the different volumes needed to yield appropriate amounts of total N for analysis. Enrichment was measured with a Europa Scientific TracerMass mass spectrometer (Europa Scientific USA, Cincinnati, OH). Base traps were titrated with 1 N HCl. All ^{15}N data is reported after correction for background ^{15}N in untreated soil.

Mineralization-Immobilization-Turnover (MIT) Experiment

This 30 day incubation experiment utilized the 0-5 cm increment of the soil described above. Samples were prepared by the same procedure and sample dates were identical. In this study, three treatments based on addition of supplemental N prior to incubation, were used. The first treatment (check) received no supplemental N. The same experimental units were utilized for this treatment and the corresponding samples in the LTI study. The second treatment (NH_4^+ -N) consisted of addition of 18.5 $\mu\text{g/g}$ of supplemental N as $(\text{NH}_4)_2\text{SO}_4$. This rate was equivalent to a field application rate of 40 kg N ha^{-1} . The third treatment (Clipping N) received an equivalent amount of

supplemental N applied as Kentucky bluegrass grass clippings. Samples were processed and analyzed as described for the LTI experiment.

RESULTS AND DISCUSSION

Long-term Incubation (LTI) Experiment

Samples collected at day 50 during the incubation had greatly elevated levels of NH_4^+ , approximately 12 and 30 times greater than surrounding samples in the 0-5 and 5-10 cm depths, respectively. Concentrations of ^{15}N in these samples were not elevated. Contamination of these samples in the lab following incubation was suspected, and they were not included in the analysis for NH_4^+ . Nitrate-N levels appeared to be consistent with surrounding samples and were used in the analysis.

Cumulative concentrations of total and ^{15}N -labeled NH_4^+ and NO_3^- for the 0-5 cm depth are shown in Figure 16. For the first 10 days there were few changes in N levels in the soil. Rapid mineralization occurred during the 10 to 50 day period, as evidenced by increasing NO_3^- concentrations. After 50 days, there was a slight decrease in both total and labeled NO_3^- concentrations. Because these values are cumulative, this indicates that either previously mineralized N was becoming immobilized, or that denitrification was occurring. Use of parafilm on the beakers to retain moisture probably also precluded O_2 diffusion, resulting in anaerobiosis and subsequent denitrification after 50 days. From 0 to 50 days, the net NO_3^- and $^{15}\text{NO}_3^-$ accumulations (final concentration - initial concentration) were 33.5 and 0.128 $\mu\text{g g}^{-1}$, respectively. By 180 days these values had decreased to 29.3 and 0.113 $\mu\text{g g}^{-1}$. On a field area basis, this equates to mineralization of 23.5 kg N ha^{-1} during the first 50 days. Mineralized nitrogen from the LFN applied two years previously totaled 0.36 kg N ha^{-1} . Concentrations of NH_4^+ -N remained relatively stable for the first thirty days, then declined for the remainder of the incubation. Over the course of the experiment, NH_4^+ -N decreased from 11.6 to 3.2 $\mu\text{g g}^{-1}$, and

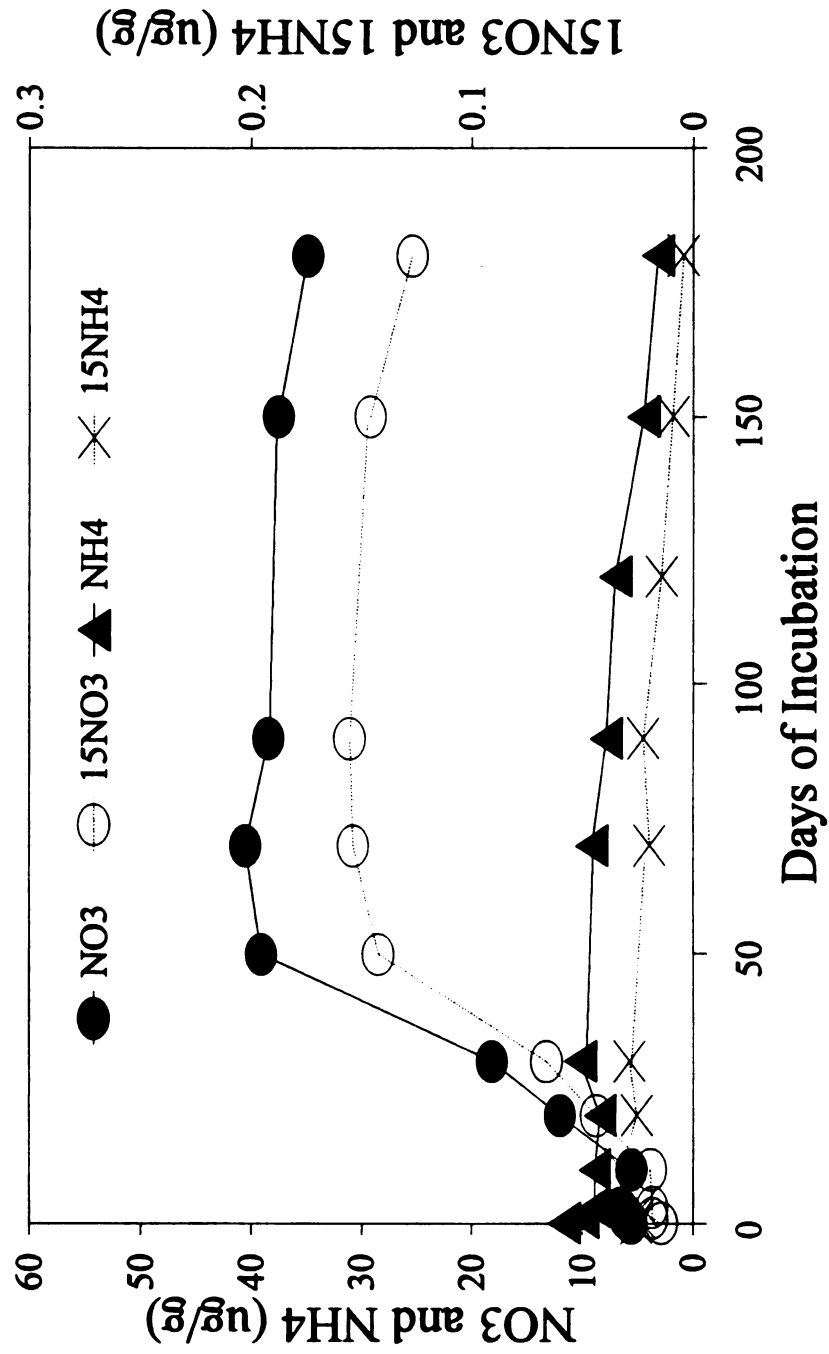


Figure 16. Nitrate-N and ammonium-N concentrations in soil from the 0 - 5 cm depth during a 180 day incubation.

$^{15}\text{NH}_4^+$ decreased from 0.026 to 0.004 $\mu\text{g g}^{-1}$. This coincided with the decrease in NO_3^- concentrations and indicates nitrification followed by denitrification.

Figure 17 shows that mineralization rates had peaked by 30 days in the 5-10 cm depth, as compared to a 50 day mineralization flush in the upper depth. Decreases in both NO_3^- and $^{15}\text{NO}_3^-$ between 30 and 50 days again indicates probable denitrification. Additional accumulations of NO_3^- continued from days 50 through 180. Net NO_3^- accumulations were 10.0 and 18.4 $\mu\text{g g}^{-1}$ at 30 and 180 days, respectively. Associated $^{15}\text{NO}_3^-$ levels were 0.019 and 0.036 $\mu\text{g g}^{-1}$, respectively. On a field area basis this equates to 8.1 and 14.8 kg N ha^{-1} over 30 and 180 days. Of the labeled fertilizer N applied, 0.060 and 0.116 kg N ha^{-1} were mineralized. The pattern of nitrification of NH_4^+ was similar to that in the upper depth. Concentrations of total NH_4^+ decreased from 5.3 $\mu\text{g g}^{-1}$ to 2.4 $\mu\text{g g}^{-1}$ over the entire incubation, while $^{15}\text{NH}_4^+$ decreased from 0.004 to 0.001 $\mu\text{g g}^{-1}$.

In the upper depth, total NO_3^- increased by a factor of 6 over 180 days, while $^{15}\text{NO}_3^-$ increased by a factor of 9. In the lower depth, these ratios were 3 and 4 respectively. The ^{15}N was in a more labile fraction as compared to total N. This is in agreement with data from Chapter I (Table 13), which shows 6% of total N and 23% of ^{15}N present in microbial biomass in the 0-5 cm depth. Long-term incubation of soil from the 5-10 cm depth yielded nearly equal amounts of net NO_3^- and $^{15}\text{NO}_3^-$ accumulation during the 0 to 30 day and 30 to 180 day periods. This is in contrast to data from the 0-5 cm depth, in which net immobilization occurred after 50 days. The recalcitrant fraction of organic matter in the lower depth is apparently more available than the recalcitrant fraction in the upper depth. This data is inconclusive, however, because denitrification probably masked the true mineralization rates.

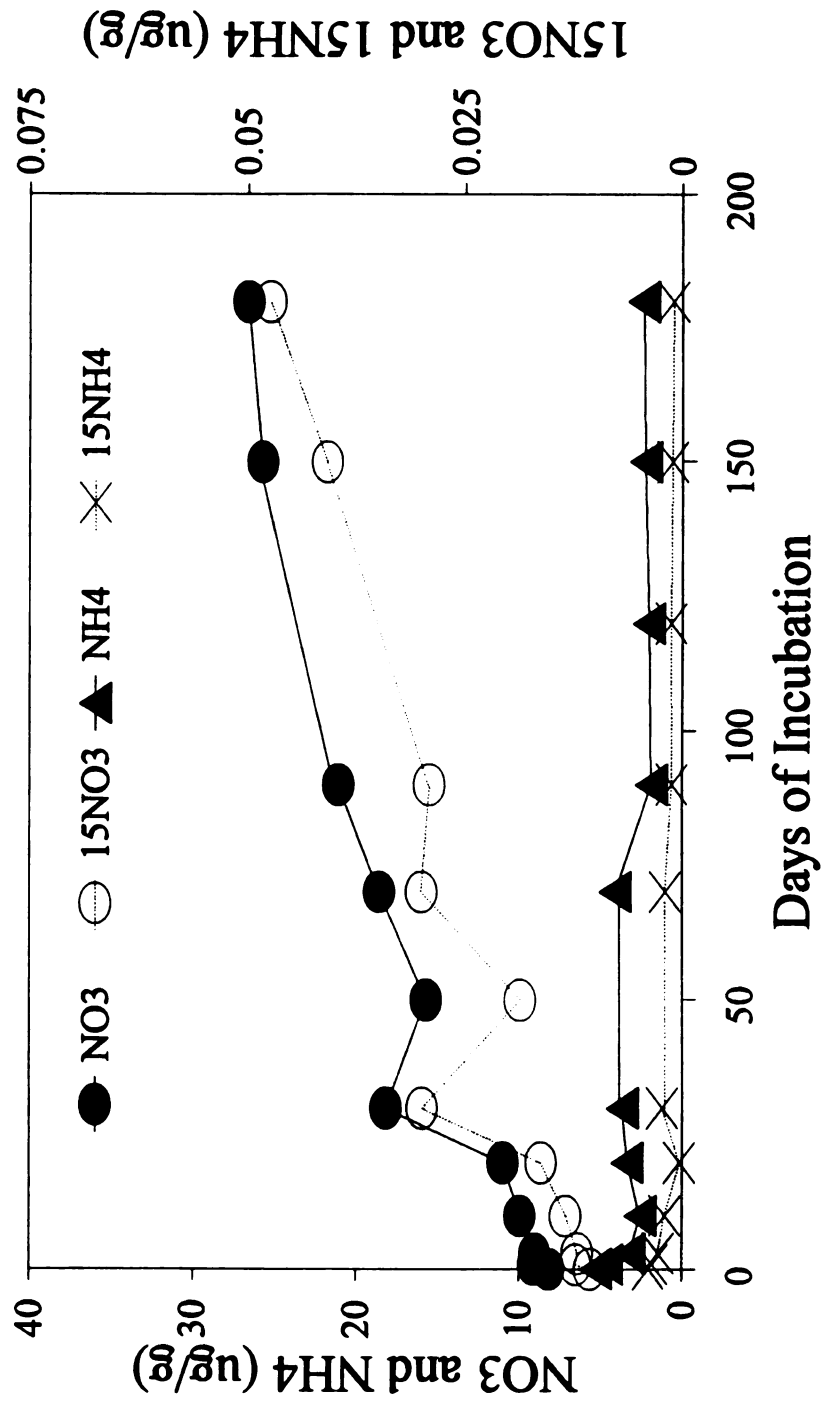


Figure 17. Nitrate-N and ammonium-N concentrations in soil from the 5 - 10 cm depth during a 180 day incubation.

Net mineralization of soil organic material in this soil resulted in the release of $41.6 \text{ kg N ha}^{-1}$ in the first 50 days of incubation. During this period $0.40 \text{ kg N ha}^{-1}$ of the LFN applied two years previously was mineralized. This was equivalent to 1.7% and 5.6% of the total N and fertilizer N, respectively, present in the soil. The fertilizer N mineralized also represents approximately 1% of the amount of LFN applied. Typical fertilizer N applications to turf are in the range of 24.5 to $49 \text{ kg N ha}^{-1} \text{ month}^{-1}$, and so a contribution of $25 \text{ kg N ha}^{-1} \text{ month}^{-1}$ from mineralization, as seen in this experiment, would be a significant source of N relative to fertilizer N additions.

Mineralization-Immobilization-Turnover (MIT) Experiment

Total concentrations of $(\text{NO}_3^- + \text{NH}_4^+)$ extracted from soil for each treatment at each time are shown in Figure 18. The curves were corrected for the amount of inorganic N present at time 0 (after addition of supplemental N). The resultant curves show the total inorganic N released through mineralization during the incubation. In the check treatment there was a lag in mineralization for the first 10 days. Between 10 and 30 DAT $13.9 \mu\text{g g}^{-1}$ of total N was mineralized, resulting in net mineralization of $11.2 \mu\text{g g}^{-1}$ over 30 days. This was equivalent to 8.1 kg N ha^{-1} on a field area basis. Addition of NH_4^+ -N stimulated N turnover almost immediately. During the 30 day incubation $41.6 \mu\text{g g}^{-1}$ of inorganic N accumulated, equivalent to $30.1 \text{ kg N ha}^{-1}$. Response to addition of grass clippings was intermediate to the other two treatments. A total of $23.1 \mu\text{g g}^{-1}$ or 16.7 kg ha^{-1} of inorganic N was released during the incubation. Addition of supplemental N stimulated mineralization of soil N as compared to the unamended check. The response to NH_4^+ -N was greater than the response to organic N due to the high availability of the NH_4^+ -N source.

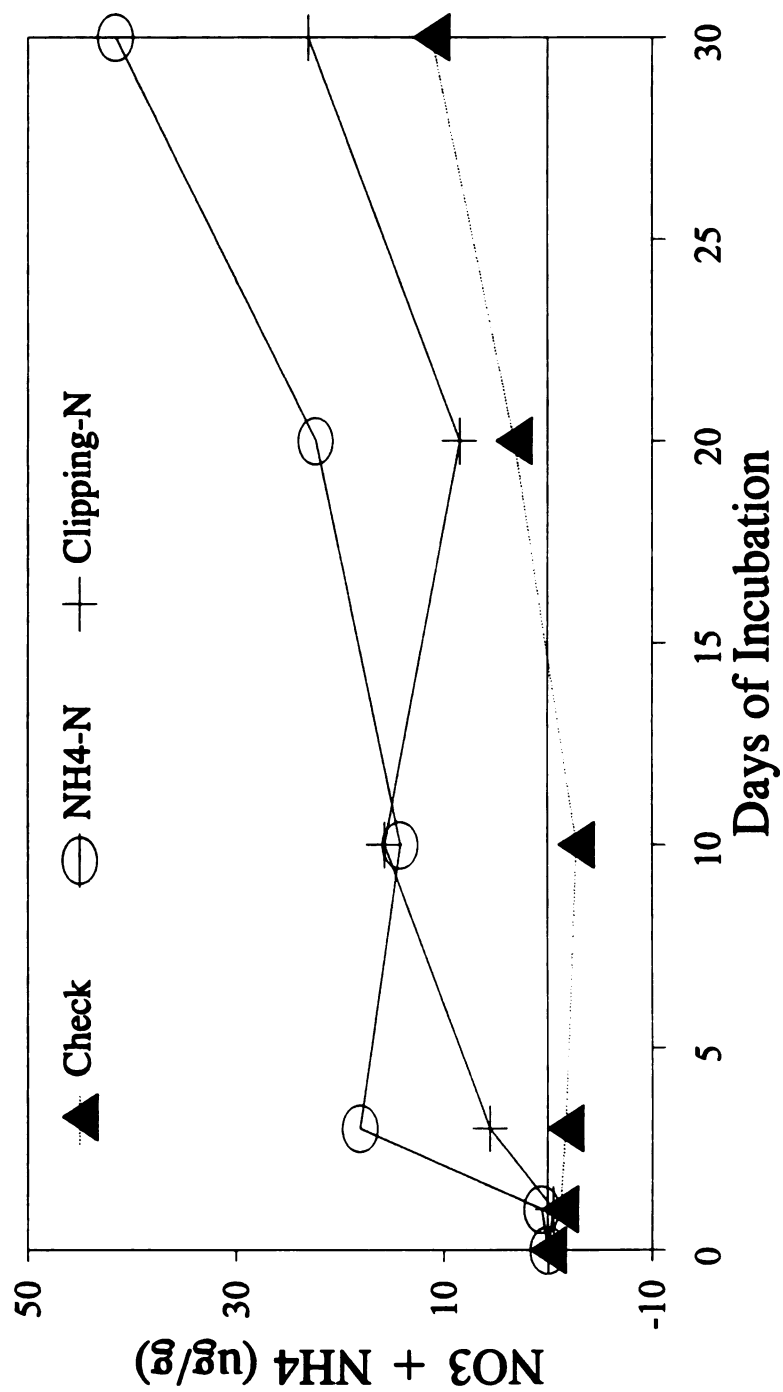


Figure 18. Total nitrate-N + ammonium-N accumulation in soils during a 30 day incubation.

Total concentrations of $^{15}\text{NO}_3^-$ -N accumulated during the 30 day incubations are shown in Figure 19. With no supplemental N added, a net of $0.051 \mu\text{g g}^{-1}$ of $^{15}\text{NO}_3^-$ -N was released by mineralization. This represents 2.3% of the total organic ^{15}N in the soil at the beginning of the incubation and 0.14 kg ha^{-1} of the LFN applied two years previously. Figure 20 presents the $^{15}\text{NO}_3^-$ -N released through mineralization from the amended soils corrected for the amount mineralized from the check treatment at each time, resulting in net ^{15}N mineralization due to addition of supplemental N. More $^{15}\text{NO}_3^-$ accumulated in N-amended soils than in the check treatment over the 30 day incubation. Following supplemental NH_4^+ -N an additional $0.065 \mu\text{g g}^{-1}$ of $^{15}\text{NO}_3^-$ -N was released through mineralization compared to the check. Following addition of grass clippings the additional $^{15}\text{NO}_3^-$ -N mineralized was $0.023 \mu\text{g g}^{-1}$. This additional mineralization of labeled soil N in response to N addition compared to the check treatment indicated a priming effect on soil microbial activity. The priming effects of NH_4^+ -N and clipping-N resulted in additional mineralization of 2.9% and 1.0% of the soil ^{15}N , respectively. On a field area basis total mineralization of the LFN applied two years previously was 0.32 kg ha^{-1} and 0.23 kg ha^{-1} in the NH_4^+ -N and clipping-N amended soils, respectively.

Summary

These experiments indicate that soil organic matter can be a substantial source of N in soils with a turf cover. Inorganic N is made available through mineralization processes, and this effect is stimulated by addition of supplemental N through either synthetic fertilizers or plant materials. Nitrogen from a single fertilizer application two years prior to the incubation experiments was found to be mineralized at faster rates as compared to the bulk

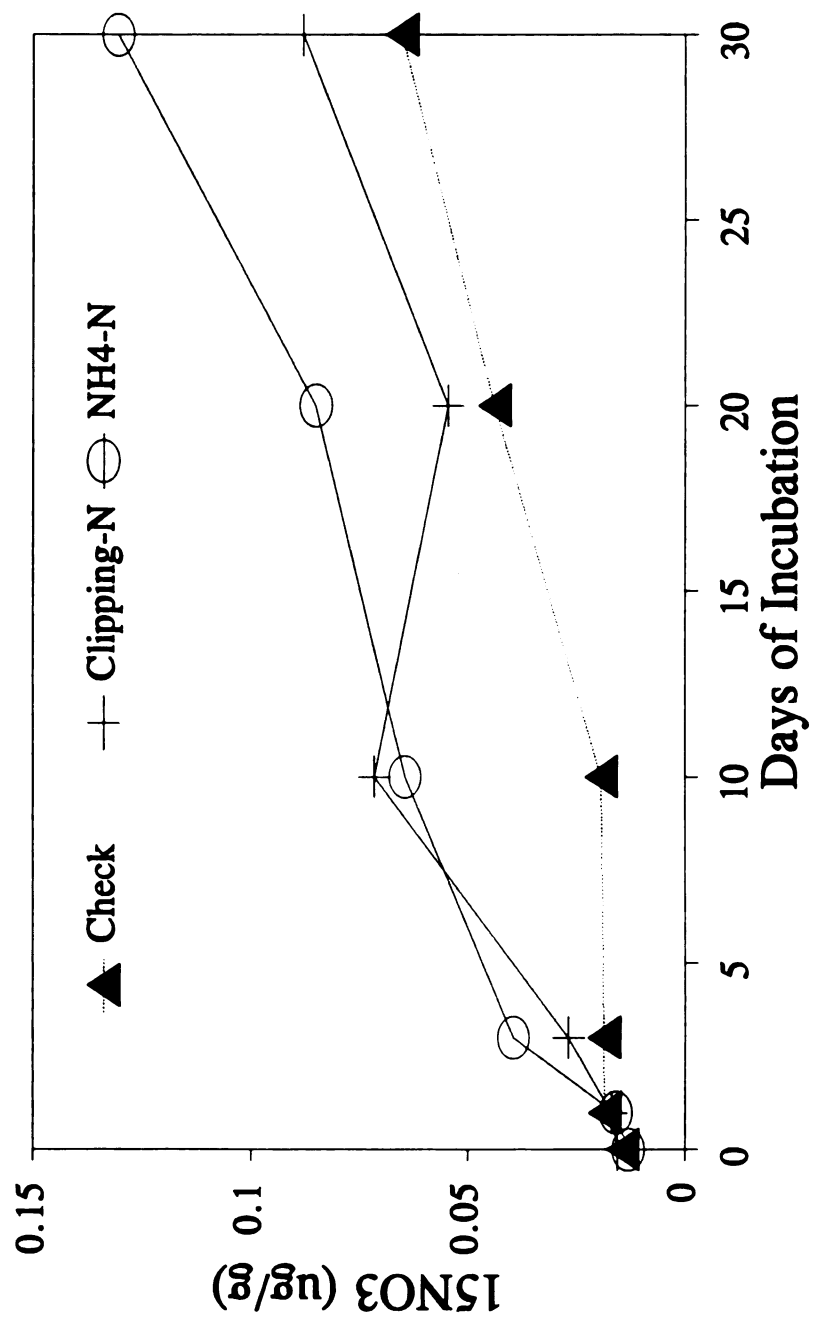


Figure 19. Total mineralization of ^{15}N from unamended and N-amended soils during a 30 day incubation.

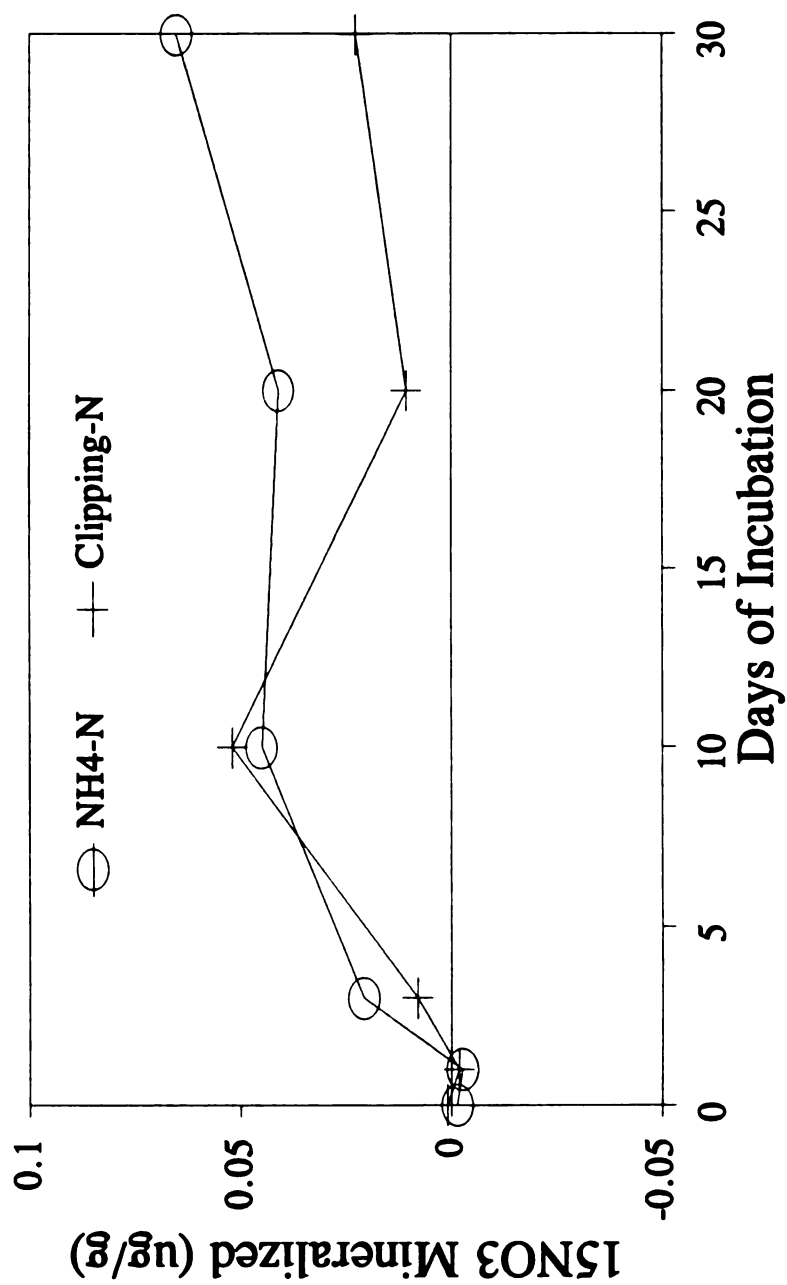


Figure 20. Mineralization of ^{15}N from $\text{NH}_4^+ \text{-N}$ and clipping-N amended soils during a 30 day incubation, corrected for mineralization from unamended check.

soil N, indicating its presence in the more labile fraction of soil organic matter. Nitrogen was mineralized most rapidly during the first 30 to 50 days of these experiments, but continued throughout the 180 day incubations. Anaerobic conditions and denitrification may have occurred after 50 days in the long term incubation experiment.

These experiments indicate that the benefits from fertilizer application are in excess to the amount of N applied. Although applied N may be quickly immobilized, this stimulates release of organic N in excess of the amount applied. This is an especially important consideration in clipping management. Return of clippings to turf not only alleviates the disposal problem, but enhances microbial activity and associated nitrogen and carbon cycling.

APPENDIX

APPENDIX

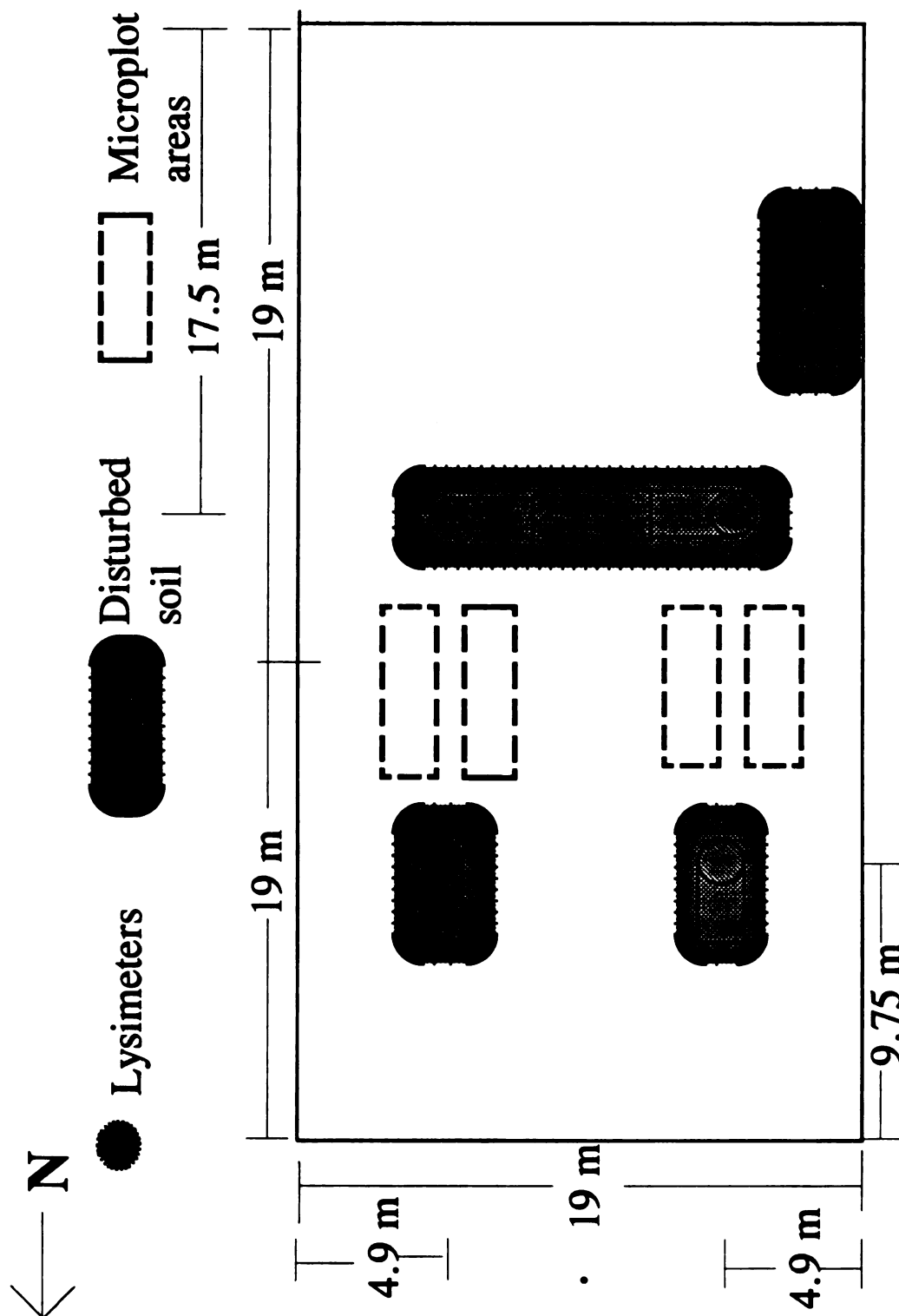


Figure 21. Diagram of lysimeter core (shaded portion) and access area, side view.

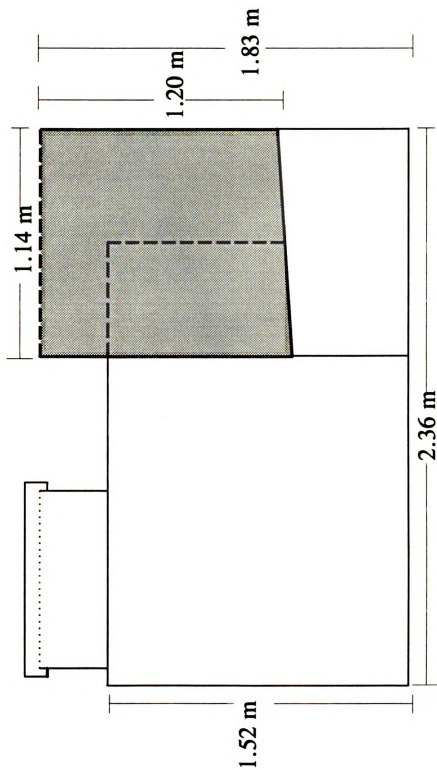


Figure 22. Map of lysimeter plot area including locations of lysimeters, microplots, and areas of disturbed soil.

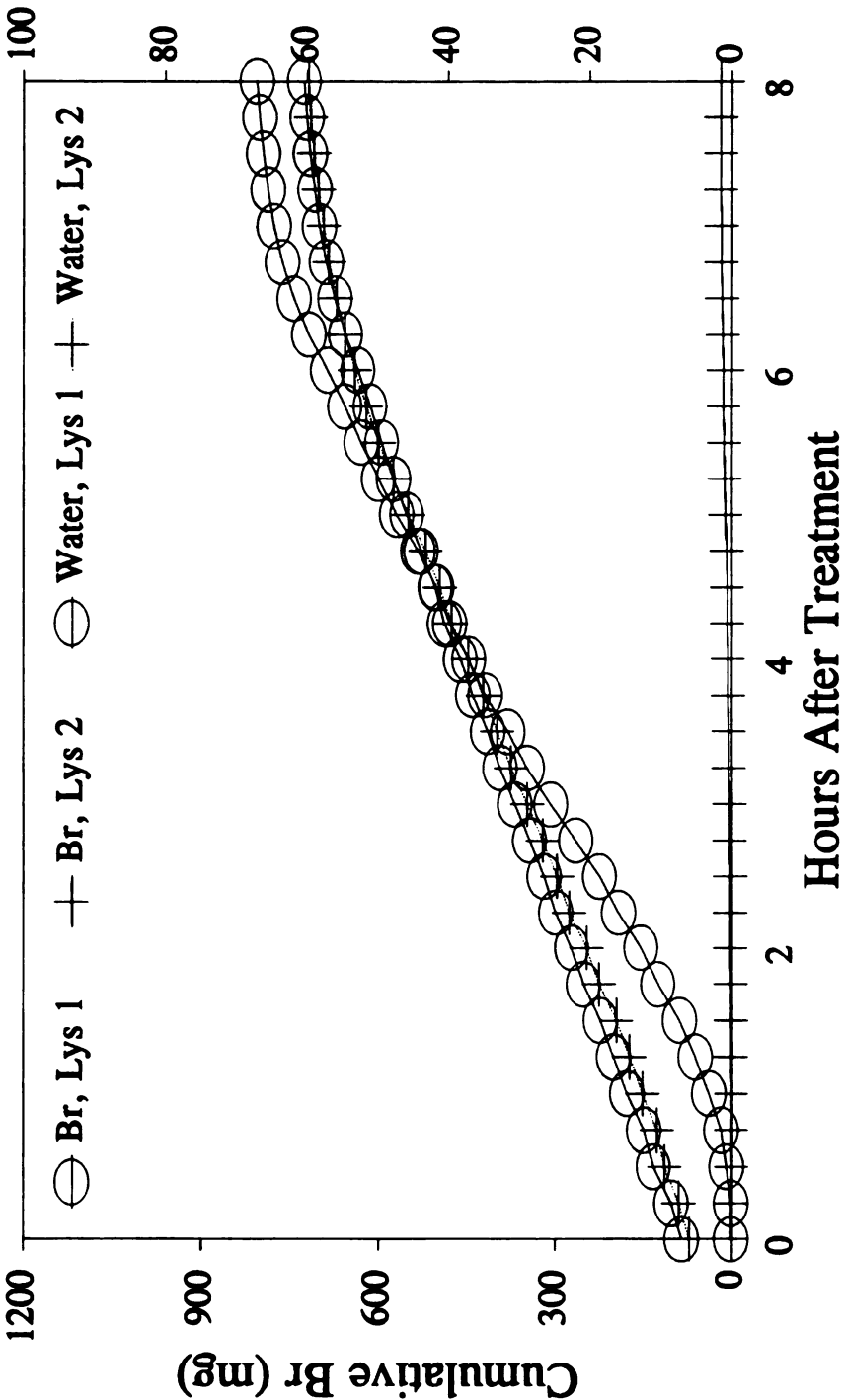


Figure 23. Breakthrough curves for water and bromide applied over surface of Lysimeters 1 and 2 under conditions of saturated flow.

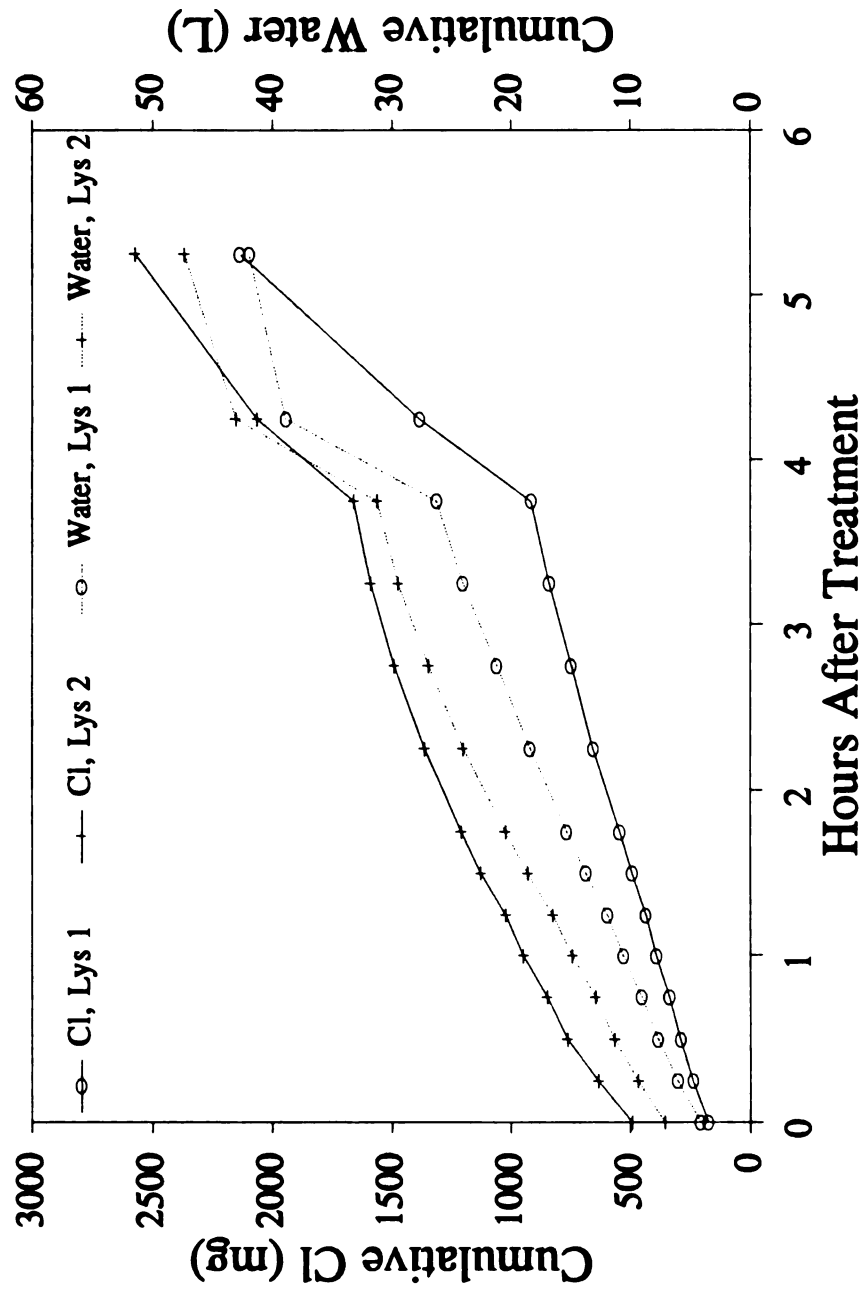


Figure 24. Breakthrough curves for water and chloride applied around interior edges of Lysimeters 1 and 2 under conditions of saturated flow.

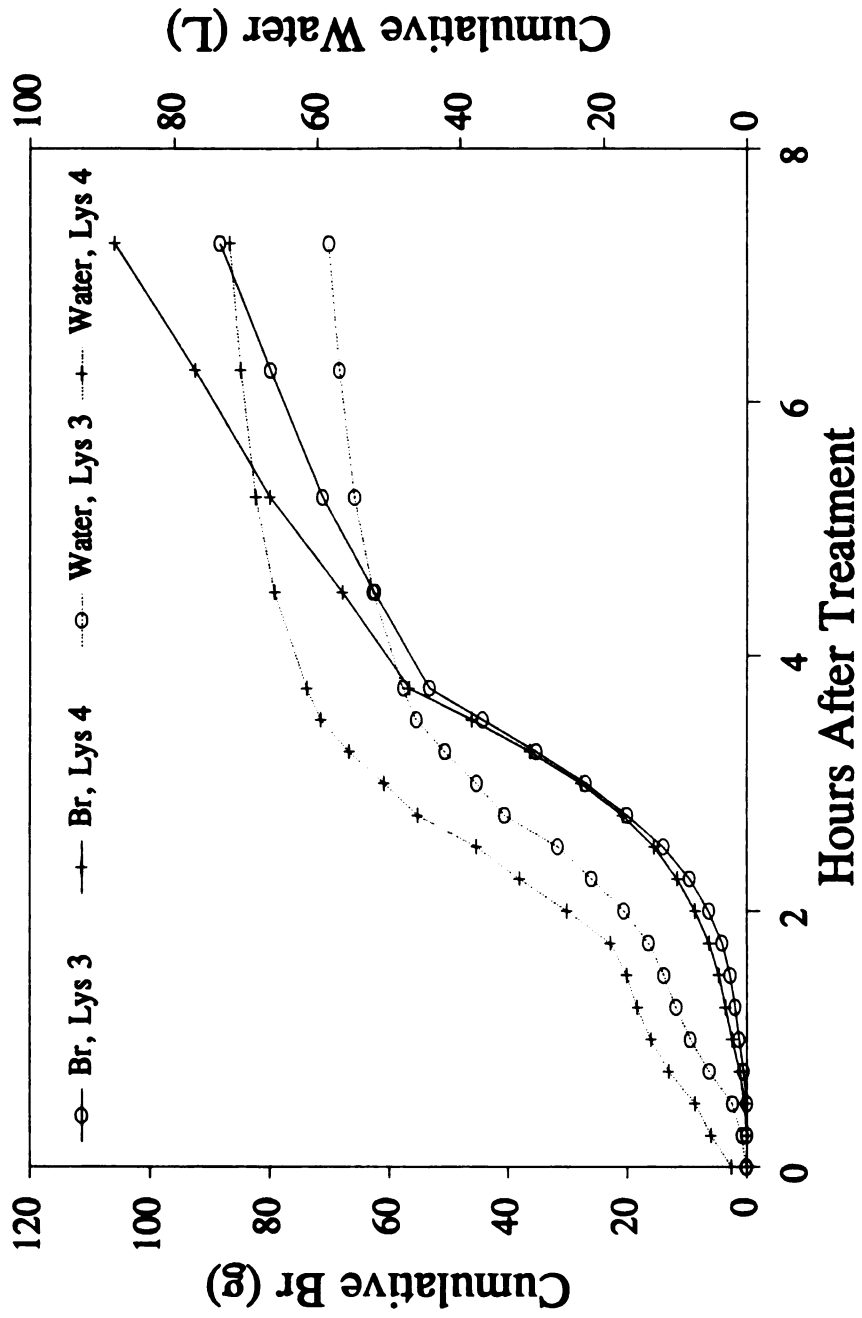


Figure 25. Breakthrough curves for water and bromide applied over surface of Lysimeters 3 and 4 under conditions of saturated flow.

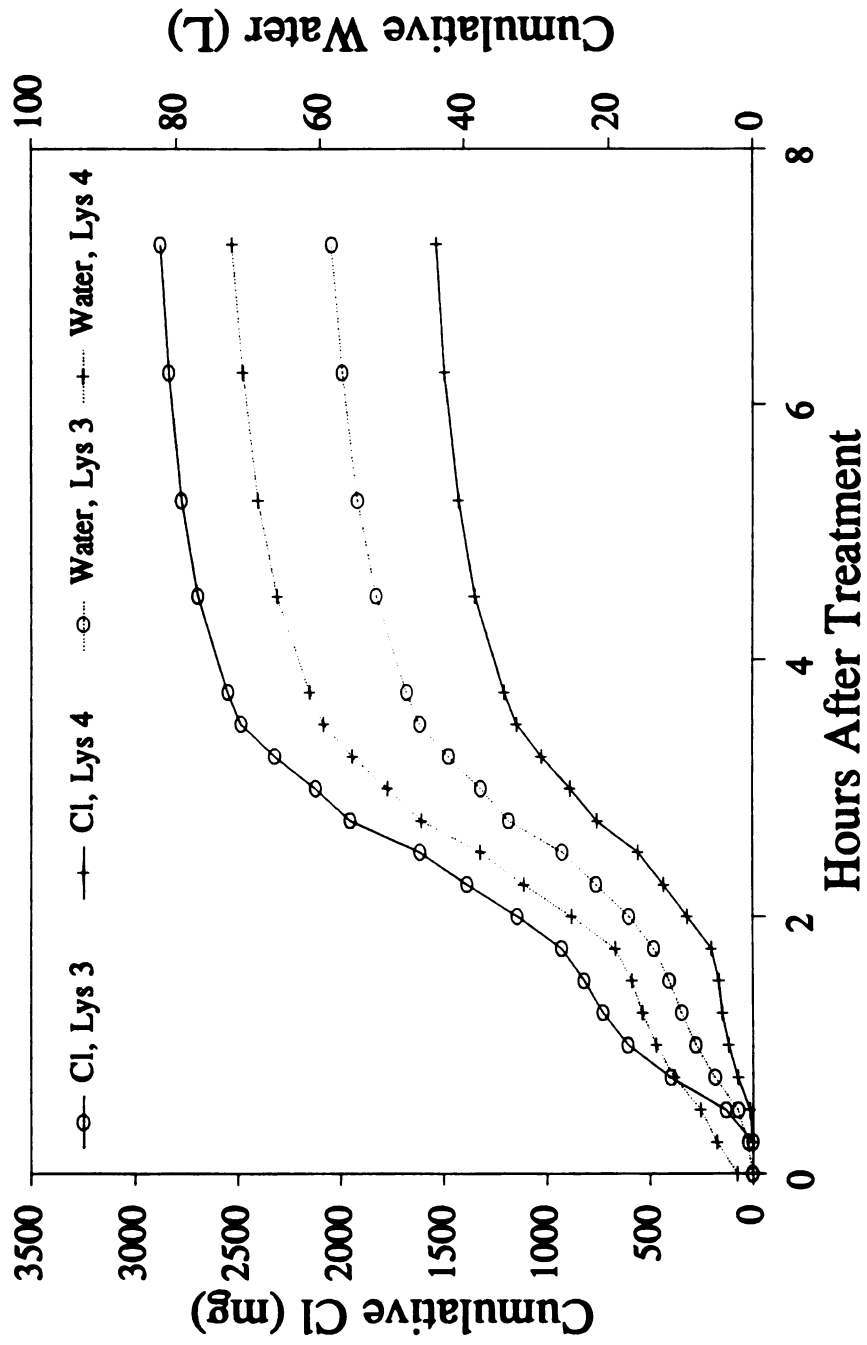


Figure 26. Breakthrough curves for water and chloride applied around interior edges of Lysimeters 3 and 4 under conditions of saturated flow.

Table 27. Atom % excess ^{15}N in each sampling increment at selected sample dates for the Spring treatment.

<u>Source</u>	<u>5/14/91</u>	<u>11/26/91</u>	<u>5/26/92</u>	<u>11/30/92</u>	<u>5/14/93</u>
Clippings	5.8107	0.5799	0.4305	0.2527	0.1663
Verdure	5.4821	0.6947	0.5876	0.2445	0.2105
Thatch:					
organic matter	1.9676	0.9999	0.9080	0.4563	0.3711
soil	0.2084	0.2791	0.3823	0.3302	0.3454
Soil:					
0 - 5 cm	0.0722	0.0806	0.1148	0.1161	0.1492
5 - 10 cm	0.0138	0.0358	0.0379	0.0511	0.0561
10 - 20 cm	0.0055	0.0098	0.0205	0.0139	0.0146
20 - 40 cm	0.0036	0.0145	0.0196	0.0117	0.0005
40 - 60 cm	0.0015	0.0192	0.0103	0.0000	0.0000

Table 28. Atom % excess ^{15}N in each sampling increment at selected sample dates for the Fall treatment.

Source	<u>11/26/91</u>	<u>5/26/92</u>	<u>11/30/92</u>	<u>5/14/93</u>	<u>11/30/93</u>
Clippings		3.6425	0.5658	0.4259	0.1645
Verdure	3.7893	3.5684	0.5039	0.3759	0.1480
Thatch:					
organic matter	3.4441	2.0198	0.8595	0.4797	0.2964
soil	0.1834	0.2733	0.4241	0.3476	0.2685
Soil:					
0 - 5 cm	0.0628	0.0539	0.1115	0.1362	0.1145
5 - 10 cm	0.0165	0.0201	0.0503	0.0406	0.0467
10 - 20 cm	0.0042	0.0095	0.0230	0.0148	0.0155
20 - 40 cm	0.0107	0.0095	0.0003	0.0043	0.0248
40 - 60 cm	0.0305	0.0167	0.0000	0.0000	0.0000

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