Fertilizer Uptake, Partitioning, and Recovery in Container-Grown Common Hackberry (*Celtis occidentalis*) Trees

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Abstract. Ammonium-nitrate (NH_4NO_3) double enriched with the ^{15}N isotope (1.5 atom %) was used to evaluate fertilizer N recovery, N partitioning, and aboveground N status in container-grown common hackberry (*Celtis occidentalis* L.) trees back-filled with native soil at Arlington, Wisconsin and Lisle, Illinois, U.S. Treatments consisted of 0, 1.42 g N tree⁻¹ (0.05 oz) and 4.27 g N tree⁻¹ (0.15 oz), the area equivalent of 0, 0.49, and 1.47 kg N 100 m⁻² (0, 1, and 3 lb N 1000 ft⁻²). Trees were harvested 14, 30, 60, and 90 days after fertilization. Fertilizer-induced changes in aboveground N status were significant only at the 4.27 g N tree⁻¹ (0.15 oz) treatment level. The amount of fertilizer N recovered in aboveground tissues increased with rate of application. Fertilizer N was preferentially partitioned to foliage and current season stem wood. The percentage of fertilizer recovered in aboveground tissues did not differ between the application rates, ranging from 15%–25% at Arlington, WI, and 5%–9% at Lisle, IL. Frost damage to the foliage at Lisle, IL may have resulted in location differences in aboveground biomass which affected fertilizer N uptake and recovery. These data suggest fertilizer N accumulated in nontarget sinks and/or were lost from the site of application at both rates of application.

Key Words. ANSI A-300; Fertilization; Landscape Trees; ^{15}N .

Applying nitrogen (N) based fertilizer to landscape trees has been a common arboricultural practice since the early 1900s. Early research demonstrated N limitations in urban landscapes with increased tree growth following the addition of N fertilizers (Wyman 1936; Neely et al. 1965; Smith 1978; Neely 1980). These growth-based studies and the resulting nutrient management recommendations were later supported by reports of reduced rates of N mineralization in urban soils resulting from declines in microbial activity (White and McDonnell 1988), reduced organic N inputs associated with tree litter collection and removal (Werner 2000), and the accumulation of heavy metals (Kandeler et al. 1996).

Observed reductions in fertilizer use efficiency (g N/g biomass) associated with step-wise increases in plant available N have indicated there is a physiological upper limit to the rate of application (Struve 1995; Rose 1999; Rose and Biernacka 1999). These findings suggest there is an increased potential for off-site losses or the partitioning of fertilizer N into nontarget sinks when rates of application exceed a plant's physiological need. In the late 1990s, landscape tree fertilization became a contentious environmental issue as annual N application rates consistent with tree growth studies were identified as potential nonpoint sources of surface and groundwater contamination (Perry and Hickman 1998; Miller 2003).

Nitrate (NO_3^-) has been reported to accumulate below the effective rooting depth in agricultural fields when fertilizer applications exceeded total demand for N within the area of application. The accumulated NO_3^- was susceptible to leaching during periods of saturated flow, with N losses proportional to the amount of surplus N (Masarik 2003). Similar increases in soil solution NO_3^- have been observed under landscape trees and turfgrass receiving chronic fertilizer N additions, suggesting N inputs exceeded the site's

physiological need and/or the ability to retain N and were susceptible to leaching (Werner 2000). The environmental ramifications associated with accumulations of fertilizer N in nontarget pools are serious, especially when application rates in excess of physiological need are applied on an annual basis (Perakis et al. 2005).

Concerns over ground and surface water contamination resulting from N fertilization of nursery trees (Colangelo and Brand 2001), fruit and citrus trees (Martinez et al. 2002; Scholberg et al. 2002), and turfgrass (Gross et al. 1990) resulted in the increased use of ^{15}N as an isotopic tag to quantify fertilizer N recovery and define partitioning patterns. For example, whole-plant uptake efficiency (total N uptake/amount of N applied) in production apple trees (*Malus sylvestris* var. *domestica* Borkh.) was calculated to be 22% of the recommended application rate (Nielsen et al. 2001). These findings prompted changes in water management practices so as to increase the soil residency time of the fertilizer during periods of peak N demand. Similarly, a mass balance approach was used to quantify recovery from a single application, 49 kg ha⁻¹ (43 lbs N acre⁻¹), of ^{15}N enriched ammonium sulfate in Kentucky bluegrass (*Poa pratensis* L.) and perennial ryegrass (*Lolium perenne* L.) (Engelsjord et al. 2004). Maximum fertilizer N recovery rates of 79% and 91% were observed two days after application for perennial ryegrass and Kentucky bluegrass, respectively. One year after treatment, fertilizer recovery rates from the soil and aboveground tissues were approximately 70%. The high recovery rates support claims that a judicious application of fertilizer N, 49 kg ha⁻¹ (43 lbs N acre⁻¹), is not prone to leaching.

Comparatively, the majority of landscape tree fertilization research has focused on stimulating aboveground growth through experimentation with fertilizer formulation and method of delivery (van de Werken 1981; van de Werken 1984), rate of application

(Smith 1978; Neely 1980), and season of application (Struve and Rose 1998; Rose 1999). Therefore, the objectives of this research were to quantify fertilizer recovery in aboveground tissues, describe fertilizer N partitioning patterns, and evaluate the effect of fertilization on the aboveground N status in common hackberry (*Celtis occidentalis* L.) using a ^{15}N enriched fertilizer at the upper and lower rate limits for landscape trees as proposed in the ANSI A-300 (2004) standards. Recovery rates were calculated several times over the course of a single growing season and native soil was used as the rooting media to better simulate landscape soils.

METHODS AND MATERIALS

Common hackberry, a tree native to eastern and central U.S. and Canada, was used to evaluate fertilizer partitioning and recovery in aboveground biomass at Arlington, Wisconsin and Lisle, Illinois, U.S. This riparian species reaches a height of 15–21 m (50–70 ft) at maturity, is tolerant of a wide range of soil types, pH, moisture regimes, and air pollution. As a result, common hackberry is used extensively as a street, park, and landscape tree (Dirr 1998).

The Lisle, IL site is located on the grounds of The Morton Arboretum (41°N–88°W) in northeastern Illinois. Mean growing season temperatures (April–September) range from 10–24°C (50–75°F) and the area averages 929 mm (36.6 in) of precipitation per year (Illinois State Water Survey 2002). The study site was located in a cleared field formerly consisting of mixed hardwoods. Soils in the study site are Oxyaquic Hapludalfs, dominated by Ozaukee silt loam (32% sand, 19% clay, 49% silt), a moderately well-drained soil consisting of a thin silt cap over sand, gravel, silt and clay glacial till (USDA 2000). Average pH, percent soil organic matter (% SOM), and cation exchange capacity (CEC) for the Lisle, IL study site were 6.9, 3.8% and 13 cmol_c kg⁻¹ soil, respectively. Concentrations of phosphorus (P), calcium (Ca), potassium (K), and magnesium (Mg), within the soil at Lisle, IL were 20, 1753, 159, and 458 mg L⁻¹ (ppm), respectively.

The Arlington Horticulture Research Station, Arlington, WI (43°N–89°W) study site is located in south-central Wisconsin. Mean growing season temperatures range from 14–21°C (57–70°F) and the area averages 833 mm (32.8 in) of precipitation per year (Wisconsin State Climatology Office 2002). The study site was formerly used for the production of agricultural crops. Soil in the study site is a Plano silt loam (Typic Argiudoll, 21% sand, 16% clay, 63% silt) that is moderately well-drained to well-drained, and possesses a silt cap approximately 102–152 cm (40–60 in) thick over a sand and gravel glacial till (USDA 1978). Average pH, % SOM, and CEC were 6.2, 3.0%, and 13 cmol_c kg⁻¹ soil, respectively. Additionally, concentrations of phosphorus (P), calcium (Ca), potassium (K), and magnesium (Mg), within the soil at Arlington, WI were 48.4, 1770, 164, and 436 mg L⁻¹ (ppm), respectively.

A randomized complete block design consisting of five blocks, three fertilizer treatment levels and four harvest periods was employed at each study location. On May 10 and May 16, 2002, sixty, 2.54 cm (1 in) caliper, container-grown, grafted trees from Bailey Nurseries, St. Paul, MN, were transplanted into 61 cm (24 in) diameter Root-Control™ bags (Root Control Inc., Oklahoma City, OK) and backfilled with native soil at Lisle, IL and Arlington, WI, respectively. Blocks were aligned in an east-to-west direction. Spacing between blocks and between trees within a block was 3.05 m (10 ft). Within each of the five blocks, there was one tree per treatment level and harvest period combination (twelve

trees per block). Treatments consisted of de-ionized water (0 g N), 1.42 g N (0.05 oz), and 4.27 g N (0.15 oz) per tree dissolved in 7.6 L (2 gal) of de-ionized water and four harvest periods (14, 30, 60 and 90 days after treatment). Fertilizer treatments were the area equivalent of 0, 0.49 and 1.47 kg N 100 m⁻² (0, 1 and 3 lb N 1000 ft⁻²), respectively. Ammonium-nitrate (NH₄NO₃) double enriched with the ^{15}N isotope (1.5 atom % for both the NH₄⁺ and NO₃⁻) was applied at the aforementioned rates on June 12 and June 20, 2002 at Arlington, WI, and Lisle, IL, respectively. Root zones were hand-weeded to eliminate competition for fertilizer N throughout the study and supplemental irrigation was provided, as needed, to ensure 2.54 cm of water per week.

A composite of five, 2.54 cm by 25.4 cm (10 in) soil cores obtained from the east and west end of each block at the time of transplanting were analyzed at the University of Wisconsin-Madison Soil and Plant Analysis Laboratory (Verona, WI) to determine soil pH (1:1 water), soil organic matter (% loss on ignition), P (Bray P1), K, Ca and Mg (1.0 N ammonium acetate), and CEC by summation of extracted K, Ca, and Mg.

Foliage (F), current season stem wood (Sc) consisting of the new terminal stem growth, stem wood (S), and roots (R) samples were collected at the time of transplanting to establish baseline total nitrogen and ^{15}N atom % values. At each harvest period, the entire aboveground biomass was separated into foliage, current season stem wood, and stem wood. Stem samples used in analysis consisted of all stem tissue above the graft union. Total root biomass was not determined due to an inability to consistently separate the roots from the container growing medium. Root samples used in analysis consisted of approximately 30–50 g (1.05–1.76 oz) of woody and nonwoody roots obtained equally from the lower, middle, and upper portions of the root system. Root samples were rinsed twice for approximately 5 s per rinse with de-ionized water.

Tissue samples were dried at 60°C (140°F) until processing. Total dry weight of aboveground tissues used in calculating fertilizer recovery was determined just prior to grinding in a Wiley mill (Thomas-Wiley Laboratory Mills, Philadelphia, PA) to pass a 1 mm (0.04 in) sieve. A homogenized 1 g (0.035 oz) subsample was pulverized in a Crescent “Wig-L-Bug” Dental Amalgamator (Crescent Dental Manufacturing, Chicago, IL) for 120 s using two, 5 mm (0.197 in) stainless steel ball bearings, at 5,000 rpm. Approximately 5 mg (0.0002 oz) of pulverized tissue were combusted at 1020°C (1868°F) in a Carlo-Erba Carbon : Nitrogen analyzer (Carlo Erba, Milan, Italy) interfaced with a Europa Scientific TracerMass mass spectrometer (Crew, Cheshire, United Kingdom). Analysis consisted of total N and atom % ^{15}N on a percent dry weight basis. Quality control was verified by duplicate analysis every 10–12 samples. The percentage of fertilizer N recovered was calculated according to methods reported by Kraimer et al. (2001).

$$\text{[Equation 1]} \quad \text{N Recovery} = \frac{\sum N(A - B)}{F(C - D)}$$

Where:

N = Weight of N(g) in a component tissue

A = ^{15}N % in fertilized tissue sample

B = ^{15}N % in tissue prior to fertilization

C = ^{15}N % in fertilizer

D = Natural abundance of ^{15}N which is 0.366% (International

Atomic Energy Agency 1983)

F = Grams of fertilizer

The percentage of nitrogen derived from fertilizer (NDFF) was adapted from equations developed by Hauck and Bremner (1976).

$$\text{[Equation 2]} \quad \text{NDFF} = \frac{A - B}{C - D}$$

Where:

A = ^{15}N % in fertilized tissue sample

B = ^{15}N % in tissue prior to fertilization

C = ^{15}N % in fertilizer

D = Natural abundance of ^{15}N which is 0.366%

Biomass weighted total N was calculated as follows:

$$\text{[Equation 3]} \quad \text{Weighted TN} = \sum (\% \text{ total biomass}_{F, Sc, S} \times \% \text{ TN}_{F, Sc, S})$$

Where:

F = foliage as a percent of total aboveground biomass (g)

Sc = current season stem wood as a percent of total aboveground biomass (g)

S = stem wood as a percent of total aboveground biomass (g)

Treatment effects within a harvest period were determined using mixed model ANOVA procedures in SAS (Littell et al. 2004). Application rate was the fixed effect and treatment within a block was a random effect. Scatter plots of residuals versus predicted values and the Kolmogorov-Smirnov test were used to test assumptions of normality. The log likelihood test was used to select the appropriate analysis model. Statistical analysis on the percentage of NDFF was performed on arcsin square root transformed data because many of the data sets did not meet the assumption of equal variances. Comparisons of treatment effects were made using Fisher's least significant difference test with $P \leq 0.05$. References to percent changes resulting from increases in the rate of application are based upon back-transformed values.

RESULTS AND DISCUSSION

Just prior to transplanting, a late spring frost did extensive damage to the foliage at Lisle, IL which resulted in a second flush of new foliage. Consequently, biomass partitioning (% of total aboveground dry weight) among the tissues was significantly different between the study site locations 14, 30, and 60 days after treatment. The percentage of total aboveground biomass accounted for by the foliage and current season stem wood at Arlington, WI was, on average, 84% and 78% higher than values observed at Lisle, IL during the 2002 growing season. The reduction in foliar biomass may have negatively affected fertilizer N acquisition and recovery at Lisle, IL. Similar results were observed by Kosola et al. (2001) who reported reduced uptake of NO_3^- and NH_4^+ in hybrid poplar (*Populus × canadensis* Moench. 'Eugenei') following defoliation by gypsy moth (*Lymantria dispar* L.).

Additionally, foliar frost damage may have reduced root proliferation and growth within the Root Control™ bags. Root initiation and expansion requires a carbon (C) substrate, with the primary source being carbohydrates produced during photosynthesis within the leaves. The flush of new foliage may have resulted in a demand for N that occurred after the fertilizer application date and/or

influenced carbohydrate allocation to the roots and subsequently, the capacity to acquire fertilizer N (Huett 1996; Kosola et al. 2001).

Impact of Fertilization on Total N Concentration

The rate of application significantly affected foliar total N concentration [N] 60 days after treatment and was borderline significant ($P = 0.052$) at 30 days at Arlington, WI and 14 days after treatment at Lisle, IL (Table 1). At Arlington, WI, the 4.27 g N application rate increased foliar N by 57% and 11%, relative to control trees and trees receiving 1.42 g N, respectively, at 30 days after treatment (Figure 1). Similarly, increases of 20% and 9% were observed 60 days after fertilization in Arlington, WI for 4.27 g N application rate. At Lisle, IL, foliar N at 14 days after treatment was 20% and 11% higher in trees receiving the 4.27 g N application rate, relative to control trees and trees receiving 1.42 g N, respectively. Increases in foliar N concentration have been shown to improve short-term photosynthetic efficiency (g C fixed m^{-2}) and net carbon assimilation, resulting in greater leaf area and enhanced aboveground biomass production (Gough et al. 2004). However, there is growing evidence to suggest there is a physiological threshold surrounding N accumulation in the foliage,

Figure 1. Total [N] in foliage of common hackberry at (A) Arlington, WI and (B) Lisle, IL in 2002. Symbols represent the mean ($n = 5$) $\pm$ the adjusted standard error. Asterisk indicates significant difference at $P \leq 0.05$.

whereupon excess N accumulation diminishes both the photosynthetic capacity and the activation state of Rubisco (Manter et al. 2005). The elevated concentration of N in the foliage of trees receiving the 4.27 g N application rate suggests an enhanced capacity for growth, although there is no published data to indicate if the observed concentrations approached a physiological threshold.

Despite the treatment-induced effects, foliar total [N] declined throughout the study period. Foliar total [N] values 90 days after fertilization at Lisle, IL were 41%, 35%, and 53% lower than values observed at 14 days for trees receiving 0, 1.42 g N, and 4.27 g N, respectively. Similarly, at Arlington, WI, foliar total [N] at 90 days for trees receiving 0, 1.42 g N, and 4.27 g N were 59%, 71%, and 59% lower than maximum values at 14 days. Seasonal declines in foliar total [N] are the product of N dilution resulting from an increase in leaf area due to cellular expansion, the accumulation of secondary metabolites associated with leaf maturation, and the export of N prior to leaf senescence (Escudero and Mediavilla 2003).

The rate of application did not affect total N in the current season stem wood at either location during any of the harvest periods. Maximum total [N] in current stem wood was observed prior to fertilization and decline substantially through 60 days after treatment (data not shown). At Arlington, WI, total [N] in the current season stem wood at 60 days was 36%, 28%, and 25% lower than pre-treatment values for trees receiving no fertilizer N, 1.42 g N, and 4.27 g N, respectively. At Lisle, IL, declines of 30%, 31%, and 42% were observed over the same period of time for current season stem wood. At both locations, total [N] in the current season stem wood increased between 60 and 90 days after treatment. Total [N] increased by 28%, 9%, and 17% between 60 and 90 days at Arlington, WI in trees receiving no fertilizer N, 1.42 g N, and 4.27 g N, respectively. At Lisle, IL, current season stem wood increased by 25%, 11%, and 19%, respectively, between 60 and 90 days after treatment.

The rate of application significantly affected total [N] in stem wood 90 days after treatment at Arlington, WI only. At 90 days, total [N] in stem wood of trees receiving the 4.27 g N application rate was 19% and 36% higher than trees receiving no fertilizer N and those receiving the 1.42 g N treatment, respectively (Figure 2). There was no difference between the control trees and trees receiving 1.42 g N. At Lisle, IL, the rate of application did not affect total [N] in stem wood tissues during any of the harvest periods. Independent of treatment level, total [N] in stem wood declined substantially at both locations during the period of time between pre-treatment sampling and the 14 days after fertilization harvest period. Such early season declines have been shown to be the result of the remobilization of stored N, primarily from perennial tissues, to support early season growth (Nielsen et al. 2001). Total [N] in stem wood at Lisle, IL was substantially and consistently lower than those observed at Arlington, WI, suggesting stored N may have been further depleted to support the second flush of foliage. These observations are consistent with findings surrounding changes in N partitioning and acquisition patterns following defoliation. In red oak (*Quercus rubra* L.) trees, Lovett and Tobiessen (1993) observed that the sources of N to support growth after defoliation included both acquiring soil derived N and remobilizing N stored from stem and root tissues.

The trend in the data for total [N] in current season stem wood and stem wood are consistent with theories surrounding the resorption and storage of N removed from the leaf prior to senescence. Gomez and Faurobert (2002) found that resorbed foliar N was

Figure 2. Total [N] in stem wood of common hackberry at (A) Arlington, WI, and (B) Lisle, IL in 2002. Symbols represent the mean ($n = 5$) $\pm$ adjusted standard error. Asterisk indicates significant difference at $P \leq 0.05$.

stored, to a great extent, as low molecular weight proteins within the radial parenchyma and phloem in the stem wood of peach (*Prunus persica* L. Batsch) trees. However, the extent of the increases in total [N] during the early stages of dormancy does not appear to be correlated to the rate of application. This lack of consistency among treatment levels may have been the result of N dilution associated with using the entire volume of the stem to prepare a sample for analysis or it may be an artifact associated with the 90 days sampling period. Werner (2008) observed continued and substantial increases in total [N] through 150 days after fertilization in a complimentary field study using common hackberry trees.

Total [N] in root tissues of trees receiving 1.42 g N was 55% and 31% higher than control trees 30 and 90 days after treatment, respectively, at Arlington, WI (Figure 3). During these same harvest periods, root total [N] in trees receiving 4.27 g N increased by 70% and 41%, respectively, over values observed in control trees. The observed increases in root total [N] of fertilized trees during these harvest periods suggest either a surplus of N that was stored in the roots and/or the incorporation of fertilizer N into the growth and development of new roots (Pregitzer et al. 1990). Hackberry's indeterminate growth pattern, the almost complete abscission of leaves at 90 days after treatment in Arlington, WI, and a continued decline in total [N] in the root tissues of control trees, suggests fertilization resulted in a surplus of

fertilizer N that was stored in root tissues during periods of low aboveground demand (i.e., cessation of active growth cycles).

At 14 days after treatment in Lisle, IL, total [N] in the root tissues of trees receiving 1.42 g N was 25% and 26% higher than values observed in trees receiving 4.27 g N and the control trees, respectively. The lack of significant difference among treatment levels during the remaining harvest periods at Lisle, IL may be attributed to a persistent need for N resulting from the second flush of foliage. This conclusion is supported by substantially lower pre-treatment total [N] values, relative to those observed at Arlington, WI, and a continued decline in total [N] throughout the study period for all treatment levels.

The rate of application significantly affected biomass weighted aboveground total [N] (Equation 3) 60 days after treatment at both Arlington, WI and Lisle, IL. At Arlington, WI, the biomass weighted aboveground total [N] at 60 days after treatment for trees receiving 4.27 g N was 22% greater than both the control trees and trees receiving the 1.42 g N treatment (data not shown). At 60 days in Lisle, IL, the biomass weighted aboveground total [N] in trees receiving 1.42 g N was 11% lower than values observed in control trees and 20% lower than trees receiving the 4.27 g N treatment. Previous studies have demon-

strated dormant season whole plant total N status in seedlings is positively correlated with future growth (Struve 1995; Cornelissen et al. 1997; Rose and Biernacka 1999). However, data from this study suggests fertilization had no significant effect on the whole plant [N] status upon entering dormancy (e.g., 90 days after treatment). The lack of treatment difference at 90 days or the inability to detect differences may, however, be the result of utilizing larger plants to study the effects of fertilization on whole plant [N] status. Larger plants typically possess greater percentages of metabolically inactive tissue relative to their total aboveground biomass (Meerts 2002). Nitrogen tends to accumulate in metabolically active sites within the tree, therefore, increases in the amount of inactive tissue relative to the whole plant biomass may have diluted the whole plant total [N] status.

N Partitioning

Foliage and stem wood were the largest N sinks (% of aboveground N) at both locations (Table 1; Table 2). At Arlington, WI, approximately 49%, 4%, and 46% of the N in aboveground tissues was contained in the foliage, current season stem wood, and stem wood, respectively. At Lisle, IL, the foliage, current season wood, and stem wood accounted for 40%, 4%, and 56% of the aboveground N, respectively. With the exceptions of foliage at 30 days after fertilization at Arlington, WI, and current season stem wood at 90 days at Lisle, IL, the rate of application did not affect the partitioning of N among the tissues. At 30 days, the percentage of N in foliar tissues receiving 4.27 g N was 21% and 11% greater than trees receiving 0 or 1.42 g N, respectively (data not shown). In Lisle, IL, 90 days after treatment, total N in the current season stem wood of trees receiving 4.27 g was 25% higher than values observed in control trees and 67% higher than trees receiving 1.42 g N. At both locations, stem wood accounted for an increasingly larger percentage of the aboveground N 60 and 90 days after treatment. These temporal shifts in partitioning patterns are associated with the retrieval and storage of N removed from leaves prior to abscission.

Nitrogen Derived from Fertilizer

Results involving nitrogen derived from fertilizer (NDFF), fertilizer N partitioning, and fertilizer recovery were based on increases in ^{15}N relative to baseline ^{15}N values. The observed increases in ^{15}N were the result of applying a fertilizer enriched with ^{15}N . Consequently, control trees are excluded from the computations and subsequent discussions.

At both locations, the percent NDFF (Equation 2) in aboveground tissues and roots increased significantly with increasing rates of application (Table 3). At both locations, the percent NDFF in tissues of trees receiving 4.27 g N was three times greater than values observed in trees receiving 1.42 g N (Table 4). Independent of application rate and location, the largest percent NDFF was found in aboveground tissues developed during the current growing season, specifically, the foliage and current season stem wood.

Trends in foliar NDFF differed between the two locations. At Arlington, WI, fertilizer N tended to accumulate in the foliage through 30 days after treatment, then declined. At Lisle, IL, the percent NDFF in the foliage generally declined, although there was slight increases in the percent NDFF between the 60 and 90 days harvest periods. The observed declines at Lisle, IL may be the result of ^{15}N dilution associated with a continued demand for N to support the second flush of foliage and/or leaf expansion and maturation.

Figure 3. Total [N] in root tissues of common hackberry at (A) Arlington, WI, and (B) Lisle, IL in 2002. Symbols represent the mean ($n = 5$) $\pm$ adjusted standard error. Asterisk indicates significant difference at $P \leq 0.05$.

Table 1. Analysis of Variance (ANOVA) *P*-values for the effect of application rate on total N concentration (N), weighted total (N), and N partitioning in common hackberry tissues at Arlington, WI, and Lisle, IL in 2002.

Parameter	Tissue Type	Arlington, WI Days after Fertilization				Lisle, IL Days after Fertilization			
		14	30	60	90	14	30	60	90
Total [N]	Foliage	0.144 ^z	0.052	<0.001	0.285	0.012	0.485	0.196	0.143
(% dry weight)	Current Stem Wood	0.845	0.093	0.100	0.229	0.098	0.787	0.750	0.449
	Stem Wood	0.859	0.804	0.679	0.037	0.101	0.661	0.079	0.641
	Root	0.058	0.001	0.263	0.002	0.021	0.165	0.607	0.656
Weighted Total [N]	All Aboveground	0.198	0.212	0.006	0.697	0.505	0.269	0.045	0.841
(% dry weight)	Tissues ^y								
N Partitioning	Foliage	0.670	0.005	0.747	0.939	0.189	0.947	0.929	0.983
	Current Stem Wood	0.677	0.813	0.229	0.804	0.242	0.840	0.793	0.049
	Stem Wood	0.095	0.061	0.362	0.908	0.263	0.923	0.930	0.955

^z All values are means of n = 5.^y Sum of percent N in foliage, current season stem wood, stem wood × percent aboveground biomass (dry weight) represented by each of these tissues.**Table 2. Total N partitioning, as a percentage of total N in all aboveground tissues, in common hackberry at Arlington, WI, and Lisle, IL in 2002.**

Trt (g)	Tissue Type	Arlington, WI Days after Fertilization				Lisle, IL Days after Fertilization			
		14	30	60	90	14	30	60	90
0	Foliage	51	47	47	6	38	43	38	20
	Current Stem Wood	4	4	5	8	4	4	4	5
	Stem Wood	45	49	48	86	58	53	58	75
1.42	Foliage	49	52	46	5	42	42	37	20
	Current Stem Wood	4	3	4	9	5	5	4	3
	Stem Wood	47	45	50	86	53	53	59	77
4.27	Foliage	54	55	48	4	36	44	36	20
	Current Stem Wood	4	3	6	9	6	4	4	5
	Stem Wood	42	42	47	87	58	52	60	75

Table 3. Analysis of Variance (ANOVA) *P*-values for the effect of 1.42 g N and 4.27 g N per tree on percent nitrogen derived from fertilizer (NDFF) and fertilizer N recovery in common hackberry tissues at Arlington, WI, and Lisle, IL in 2002.

Parameter	Tissue Type	Arlington, WI Days after Fertilization				Lisle, IL Days after Fertilization			
		14	30	60	90	14	30	60	90
% NDFF	Foliage	<0.001 ^z	<0.001	<0.001	0.003	0.002	0.004	<0.001	<0.001
	Current Stem Wood	<0.001	<0.001	<0.001	<0.001	0.002	0.004	<0.001	<0.001
	Stem Wood	<0.001	<0.001	<0.001	0.001	0.003	0.013	<0.001	0.002
	Root	<0.001	0.003	0.002	0.001	0.003	0.013	0.005	0.002
% Fertilizer Recovery	Above Ground	0.476	0.418	0.138	0.380 ^x	0.749	0.544	0.684	0.327 ^x
	Tissues ^y								

^z All values are means of n = 5.^y Sum of fertilizer recovery in foliage, current season stem wood, and stem wood.^x Sum of fertilizer recovery in remaining foliage, current season stem wood and stem wood.**Table 4. Percent nitrogen derived from fertilizer (NDFF) in common hackberry tissues at Arlington, WI, and Lisle, IL in 2002.**

Tissue	Trt (g)	Arlington, WI Days after Treatment				Lisle, IL Days after Treatment			
		14	30	60	90	14	30	60	90
Foliage	1.42	6.5 ^{zyx}	9.4	7.7	5.2	6.8	4.9	4.7	2.4
	4.27	16.7	24.7	20.1	18.0	19.7	18.3	12.4	14.3
Current Stem Wood	1.42	5.3	7.7	6.2	5.4	5.7	3.6	4.1	2.6
	4.27	16.7	20.9	14.7	16.9	15.6	13.8	9.4	11.2
Stem Wood	1.42	2.0	4.0	4.0	4.5	1.2	1.0	1.4	1.3
	4.27	6.1	13.0	11.9	13.7	4.3	4.1	4.1	5.4
Root	1.42	5.3	7.0	7.3	6.4	1.7	1.6	2.8	1.6
	4.27	15.2	18.6	18.1	14.2	4.4	4.1	5.5	6.9

^z Values are the adjusted means of n = 5.^y Back-transformed values.^x All treatment comparisons within a tissue type were significant at $P \leq 0.01$ for all harvest periods.

The percent NDFF in the current season stem wood of trees receiving the 1.42 g N application rate nearly mirrored the trends in foliar NDFF at both locations. At Arlington, WI, maximum NDFF in current season stem wood occurred 30 days after treatment in trees receiving 1.42 g N or 4.27 g N, 7.7% and 20.9%, respectively. At Lisle, IL, maximum current season stem wood NDFF occurred at 14 days after treatment at both rates of application. Additionally, at Lisle, IL, the percent NDFF in current season stem wood of trees receiving 4.27 g N increased by 19.1% between 60 and 90 days after treatment.

In stem wood, maximum percent NDFF for trees receiving the 4.27 g N treatment occurred 90 days after fertilization at Arlington, WI (13.7%), and Lisle, IL (5.4%). Relative to values at 14 days, the percent NDFF in stem wood at 90 days in trees receiving 4.27 g N increased by 125% and 25% at Arlington, WI and Lisle, IL, respectively. Maximum NDFF in the stem wood of trees receiving 1.42 g N occurred at 90 days (4.5%) and 60 days (1.4%) after treatment at Arlington, WI and Lisle, IL, respectively. Between 14 and 90 days after treatment, the percent NDFF in stem wood increased by 10% at Arlington, WI. There was little change (1.2% versus 1.3%) in the percent NDFF in stem wood at Lisle, IL between these two harvest periods.

The maximum percent NDFF in root tissues of trees receiving 1.42 g N occurred 60 days after treatment at Arlington, WI and Lisle, IL, 7.3% and 2.8%, respectively (Table 3). Maximum values for trees receiving 4.27 g N occurred at 30 days in Arlington, WI (18.6%), and at 90 days in Lisle, IL (6.9%). Throughout the remaining harvest periods, root NDFF remained fairly constant at Arlington, WI. However, between 30 and 60 days after treatment, root NDFF at Lisle, IL increased by 70% and 34% in trees receiving the 1.42 g N and 4.27 g N treatments, respectively.

The combination of increases in the percent NDFF in stem wood, particularly in the 4.27 g N treatment level and declines in the percent NDFF in foliage at 90 days is consistent with theories of N conservation in higher plants growing in an N limited environment. Research found approximately 50% of foliar N is removed prior to abscission and subsequently stored as low molecular weight proteins in stem and root tissues (Chapin and Kedrowski 1983; Aerts 1996). Data from this study indicate increasing rates of application consistently enhances fertilizer N accumulation in the foliage. However, the amount of fertilizer N removed from the foliage during leaf senescence is not consistent across the treatment levels. Relative to maximum values observed during the growing season (14–60 days after treatment), the percent NDFF in the foliage of trees receiving 4.27 g N declined by 27% at 90 days after treatment in both locations. In contrast, foliar NDFF at 90 days in trees receiving the 1.42 g N rate of application was 44% lower than maximum growing season values at Arlington, WI, and 58% lower at Lisle, IL. However, at both locations and

across the treatment levels, there were consistent declines in foliar total [N]. Consequently, these data suggest trees receiving the high rate of application tended to lose more fertilizer N in the leaf litter. The fertilizer N contained within the leaf litter likely consists of structural proteins associated with new growth or photosynthetic proteins trapped in the foliage when the abscission layer was activated (Escudero and Mediavilla 2003; Hoch et al. 2003). These findings support Aerts (1996) conclusion that increasing the level of available N would either suppress or have no effect upon the amount of N removed from leaves prior to senescence.

At both locations and rates of application, 61%–78% of the fertilizer N that accumulated in aboveground tissues was partitioned to the foliage 14, 30, and 60 days after fertilization (Table 5). At 90 days after treatment, the percentage of aboveground fertilizer N accumulation in the foliage declined substantially in both treatment levels and locations. These reductions, particularly at 90 days after treatment, may be attributed to leaf loss and the onset of leaf senescence. Declines in the foliar contribution to aboveground fertilizer N accumulation coincided with increases in fertilizer N accumulation in stem wood in all treatment combinations at both locations and all rates of application. These observations are consistent with patterns of internal N cycling and storage (Brinkman and Boerner 1994; Aerts 1996).

Fertilizer Recovery (% of N Applied)

The inability to consistently separate root tissues from the rooting medium prevented the determination of fertilizer N recovery in the root system. As a result, fertilizer N recovery is presented only for the aboveground tissues. Within both locations, there were no significant differences in the percentage of fertilizer N recovered between the 1.42 g N and 4.27 g N application rates in any of the harvest periods (Table 2). Pre-senescent (before 90 days after treatment), back-transformed, recovery rates in aboveground tissues ranged from 15%–25% at Arlington, WI, and 5%–10% at Lisle, IL (Figure 4). While reported, the extent of leaf senescence and loss at 90 days after treatment, particularly at Arlington, WI, prevented a meaningful interpretation of fertilizer N recovery. At Arlington, WI, maximum recovery rates occurred at 30 days for the 1.42 g N treatment (22.3%) and at 60 days for the 4.27 g N treatment (25.1%). At Lisle, IL, maximum recovery rates for trees receiving the 1.42 g N and 4.27 g N application rates occurred 30 days after fertilization, 9.4% and 7.7%, respectively.

The range of recovery rates for both treatments at Arlington, WI were slightly higher than fertilizer N recovered in a lodgepole pine (*Pinus contorta* Dougl. ex Loud.) (Preston and Mead 1994) and black spruce [*Picea mariana* (Mill.) BSP] (Salifu and Timmer 2003), comparable to fertilizer recovery rates observed in pecan [*Carya illinoensis* (Wangenh.) K. Koch]

Table 5. Percent applied fertilizer N partitioned to aboveground common hackberry tissues at Arlington, WI, and Lisle, IL in 2002.

Tissue	Trt (g)	Arlington, WI				Lisle, IL			
		Days after Fertilization				Days after Fertilization			
		14	30	60	90	14	30	60	90
Foliage	1.42	11.4 ^z	15.7	13.2	1.3	5.8	6.2	3.4	1.5
Current Stem Wood	1.42	0.8	0.9	1.0	0.1	0.5	0.5	0.3	0.2
Stem Wood	1.42	3.4	6.0	7.6	8.4	1.3	1.2	1.4	2.2
Foliage	4.27	10.9	17.4	15.2	0.7	4.8	6.0	3.4	2.2
Current Stem Wood	4.27	0.8	0.9	1.0	1.1	0.6	0.5	0.3	0.4
Stem	4.27	3.1	6.4	8.7	9.2	1.6	1.6	1.8	2.9

^z Values are means of n = 5.

Figure 4. Percent fertilizer recovery in aboveground tissues of common hackberry at (A) Arlington, WI, and (B) Lisle, IL in 2002. Symbols represent the mean ($n = 5$) $\pm$ the adjusted standard error. Scales on the y-axis are different.

(Kraimer et al. 2001), and substantially less than 50%–60% fertilizer N recovery rates in fruit-bearing orange trees [*Citrus sinensis* (L.) Osbeck.] (Feigenbaum et al. 1987) and Kentucky bluegrass (*Poa pratensis* L.) (Horgan et al. 2002). Recovery rates at Lisle, IL for both treatment levels were lower than all cited studies, including those involving conifers.

It is important to recognize that the reported fertilizer recovery rates do not include fertilizer N accumulation in the root system. A number of studies have indicated significant accumulations of N in root tissues, particularly at high rates of applications (Warren 1993; Mattos et al. 2003). The percent NDFR in root tissues at both locations were only slightly lower than values observed in the foliage and current season stem wood, indicating fertilizer N recovery at the whole plant level is significantly higher than what is reported for aboveground tissues.

Despite the observed differences in aboveground biomass between the respective locations, the treatment-induced trends in the percent NDFR and fertilizer N recovery were remarkably consistent on both a mass and percent basis. This study demonstrated fertilizer N uptake at application rates recommended under the ANSI A-300 standards were proportional, but not equal to, the amount of fertilizer N applied. Fertilization was also demonstrated to enhance the overall aboveground N status, resulting

primarily from increases in foliar total [N]. To a degree, increases in foliar total [N] status enhance photosynthetic capacity and the ability to acquire atmospheric carbon. However, 75% or more of applied fertilizer N could not be accounted for in aboveground biomass, regardless of the rate of application. It must be stressed that whole-plant fertilizer N recovery rates, which include roots, were unquestionably greater than the reported aboveground tissue recovery rates. However, substantial increases in fertilizer N recovery based on the observed concentrations of N and the percentages of NDFR in root tissues would require dramatic, and somewhat improbable, changes in the root-to-shoot ratio (Harris 1992; Ågren and Franklin 2003). Consequently, regardless of the rate of application substantial amounts of fertilizer N appears to accumulate in nontarget sinks (e.g., soil microbial biomass), or is potentially lost from the system via leaching or gaseous emissions.

CONCLUSION

In all tissue types, there were proportional increases in the percent nitrogen derived from fertilizer. On both a mass and percentage basis, the vast majority of fertilizer N in aboveground tissues was preferentially partitioned to the foliage. However, only the highest rate of application appeared to produce a significant increase in the aboveground N status, and it was confined to a single harvest period. Support for the recommended rates of application (ANSI 2004) based on increases in fertilizer N uptake at the higher rate of application must be tempered. Substantial amounts of fertilizer N applied to each tree either accumulated in nontarget sinks (e.g., soil matrix, microbial biomass), or was lost from the planting site. It is the specific nature of what happens to the unrecovered fertilizer N that is of concern. Fertilizer N immobilized within the soil microbial biomass represents a slow-release, long-term nutrient pool. However, there is mounting evidence that chronic, high-dose additions of N adversely affects soil microbial populations and their ability to immobilize readily available N, alters soil chemistry, and negatively affects tree growth (Wallenstein et al. 2006). Consequently, the low recovery rates observed in this study suggest N fertilization in accordance with the ANSI standards may become increasingly worrisome if applications are made on an annual basis. If the majority of the unaccounted for fertilizer N is lost from the site of application through leaching and/or gaseous emissions the concern for environmental degradation is immediate. The consistency of fertilizer recovery rates suggests both rates of application should be carefully considered in landscapes with a shallow depth to groundwater, adjacent to surface bodies of water, or possess soil qualities prone to leaching (i.e. coarse texture).

The ANSI A-300 standards for the fertilization of landscape trees (2004) explicitly state the application of fertilizers is a prescription that must be based on an understanding of the targeted plants' biology, impact to the environment, and desired management objective(s). The isotopic enrichment of the fertilizer used in this study provided unique and much-needed insights into our understanding of the biology and fate of fertilizer N applied at rates consistent with tree care standards. In addition to defining the extent of recovery for a fast release fertilizer, the study provided direction for continued fertilizer research. For instance, the dynamic nature of N partitioning and storage between above and below ground tissues requires examinations be extended well into the dormant period. Similarly, it is unclear

if an equivalent amount of N applied over multiple applications using low doses of N or slow release fertilizers would yield similar results. The combination of a plethora of tree species found in urban landscapes, the myriad of soil and climatic conditions, and a wide range of management objectives have made attempts to generalize fertilizer recommendations difficult. It is essential that future studies utilize a wider array of tree species, fertilizer formulations, and treatment levels, as well as incorporate repeated applications within a growing season under a variety of site conditions to build upon the results from this study.

Acknowledgments. This research was funded by the International Society of Arboriculture's TREE Fund, the Wisconsin Arborist Association, and a University of Wisconsin System Consortium grant. Use of trade names in this publication does not imply endorsement by the University of Wisconsin-Madison of products named, nor criticism of similar ones not mentioned. Technical assistance of Drs. Brian Yandell and Jun Zhu, Mr. William Schmitt, Armand Krueger, and Peter Crump is gratefully acknowledged. Special consideration is given to The Morton Arboretum, Lisle, IL and Dr. Gary Watson for providing a research location and staff and the Root Control, Inc. for their donation of planting bags. From a dissertation submitted by L.P. Werner in partial fulfillment of the requirements for the Ph.D. degree.

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Zusammenfassung. Doppelt angereichertes Ammonium-Nitrat mit ^{15}N Isotop (1,5 atom%) wurde verwendet, um Dünger N Erholung, N Partitionierung und über Grund N-Status von containergezogenen *Celtis occidentalis*-Bäumen zu bewerten, die mit natürlich gewachsenem Boden an den Standorten Arlington, Wisconsin und Lisle in Illinois, USA verfüllt waren. Die behandlungen bestanden aus 0, 1.42 g N Baum⁻¹ und 4.27 g N Baum⁻¹, dem Flächenäquivalent von 0, 0.49 und 1.47 kg N 100m⁻². Die Bäume wurden 14, 30 60 und 90 Tage nach der Düngung geerntet. Durch den Dünger verursachte Veränderungen in dem über GUND N-Status waren nur in der Behandlung mit 4.27 g Baum⁻¹ deutlich. Die Menge des N-Düngers in oberirdischen Geweben stieg mit der Rate der Applikation. Dünger N war vorzugsweise verteilt auf Blatwerk und diesjährigem Stammgewebe. Der Prozentsatz von Dünger aus oberirdischem Gewebe differierte nicht zwischen den Applikationsraten, die in Arlington, Wisconsin bei 15%-25% und 5%-9% in Lisle in Illinois betrugen. Frostscha den an den Blättern am Standort Lisle in Illinois könnte eine Ursache für die lokalen Unterschiede in der Biomasse gewesen sein, welche die Stickstoffaufnahme und Umbau beeinflusste. Diese Daten verdeutlichen, dass Dünger N außerhalb des Zielgebiets akkumulierte und/oder dass es bei beiden Applikationen verloren ging.

Resumen. El nitrato de amonio (NH_4NO_3) doblemente enriquecido con el isótopo ^{15}N (1.5 átomo %) fue usado para evaluar la recuperación del N, la distribución del mismo, y el estado en el aire del N en el almez común de virginia (*Celtis occidentalis* L.) crecido en contenedor llenado con suelo nativo en Arlington, Wisconsin y Lisle, Illinois, U.S. Los tratamientos consistieron de 0, 1.42 g N árbol⁻¹ (0.05 onz) y 4.27 g N árbol⁻¹ (0.15 onz), el área equivalente de 0, 0.49, y 1.47 kg N 100 m⁻² (0, 1, y 3 libra N 1000 pie⁻²). Los árboles fueron cosechados 14, 30, 60 y 90 días después de la fertilización. Los cambios inducidos de fertilizante en el estado aéreo de N fueron significativos solamente en el tratamiento 4.27 g N árbol⁻¹ (0.15 onz). La cantidad de N recuperado en los tejidos incrementó con la tasa de aplicación. El N fue preferentemente distribuido al follaje y la madera del tallo de la estación de crecimiento. El porcentaje de fertilizante recuperado en los tejidos no difirió entre las tasas de aplicación, variando de 15%-25% en Arlington, WI, y 5%-9% en Lisle, IL. El daño por heladas en el follaje en Lisle, IL puede haber resultado en diferencias de localidad en biomasa lo cual afectó la toma y recuperación del fertilizante de N. Estos datos sugieren que el fertilizante N acumulado en sumideros no objetivos fueron o no perdidos del sitio de aplicación en ambas tasas de aplicación.