

Retranslocation, Plant, and Soil Recovery of Nitrogen-15 Applied to Bareroot Black Walnut Seedlings

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Abstract: Although retranslocation or nitrogen (N) derived from plants (NDFP) may account for more than 50% of the annual N demand in new growth of conifer seedlings, the proportional contribution of NDFP vs. current uptake or N derived from fertilizer (NDFE) in new growth of hardwood seedlings is relatively unknown. The current uptake was labeled with ammonium sulfate [$(^{15}\text{NH}_4)_2\text{SO}_4$] at the rate of $1.56\text{ g N plant}^{-1}$ and reared for 90 days in sand culture under greenhouse conditions, and NDFP vs. NDFE was quantified in new growth of half-sib bareroot black walnut (*Juglans nigra* L.) seedlings. Nitrogen derived from plants accounted for 68 to 83% of the total N demand in new shoot growth of black walnut seedlings vs. NDFE (17 to 32%). Recovered applied fertilizer was 43% in soil and 9% in plants. The greater proportion of NDFP in new growth demonstrates the importance of retranslocation in meeting early N demand of transplanted black walnut seedlings.

Keywords: Black walnut, fertilizer recovery, growth, nitrogen, retranslocation, stable isotope

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INTRODUCTION

Nitrogen (N) retranslocation from old tissues of evergreens is a key mechanism of N reuse to meet increased sink demand for N in newly transplanted seedlings (Nambiar and Fife 1991; Salifu and Timmer 2003). Although retranslocation synonymous with N derived from plant (NDFP) may account for between 40 and 100% of the annual N demand in new growth of transplanted conifer seedlings (Nambiar and Fife 1991; Millard and Proe 1993; Salifu and Timmer 2003), the proportional contribution of NDFP compared with current uptake, also referred to as N derived from fertilizer (NDFE), in new growth of hardwood seedlings is not well characterized. Nitrogen derived from fertilizer may vary from as low as 15 to 25% in lodgepole pine (*Pinus contorta*), western red cedar (*Thuja plicata*), western hemlock (*Tsuga heterophylla*), Sitka spruce (*Picea sitchensis*), and black spruce (*Picea mariana*) (Preston and Mead 1994; Chang et al. 1996; Salifu and Timmer 2003) or up to about 40 to 45% in lodgepole pine (*Pinus contorta*), trembling aspen (*Populus tremuloides* Michx.), and hybrid aspen (*Populus tremula* L. × *P. tremuloides*) (Amponsah et al. 2004; Choi, Chang, and Hao 2005), suggesting significant quantities (48 to 95%) of applied fertilizer may be retained in the soil pool (Amponsah et al. 2004), lost through ammonia (NH_3) N volatilization, or immobilized into organic fractions (Preston et al. 1990).

Nutrient budget studies have often quantified retranslocation as N content differences in plant tissues (e.g., old shoots) before and after planting based on net N transfer within plant components (Lim and Cousens 1986; Malik and Timmer 1998). Net N transfer methodology assumes that uptake into old shoots and leaching losses are negligible (Miller, Cooper, and Miller 1976; Lim and Cousens 1986), but such assumptions confound interpretation of retranslocation. For instance, net estimates are perplexing because N uptake from the soil pool cannot be discriminated from remobilized N within plant components without the use of labeled isotopes (Mead and Preston 1994; Proe and Millard 1994; Salifu and Timmer 2003).

The use of the stable ^{15}N isotope has proven more accurate for quantifying nutrient influx and efflux in plant tissues (Millard and Proe 1993; Proe and Millard 1994) because NDFP can be determined at a higher sensitivity in addition to discriminating this pool from NDFE (Nõmmik 1990; Mead and Preston 1994; Salifu and Timmer 2003). Therefore, we labeled current uptake with the stable ^{15}N isotope and directly quantified NDFP and NDFE within components of transplanted half-sib (i.e., where identity of one parent is known) black walnut seedlings. We also computed N retention in soils at the end of the study (90 days after transplanting). We tested the hypotheses that 1) NDFP will

account for a greater proportion of the N in new growth of transplanted black walnut seedlings compared with NDFP and 2) significant quantities of applied N will be retained in the soil pool beyond plant exploitation, volatilized, or immobilized into organic fractions. There is a paucity of such information for hardwoods in the central hardwood forest region of the USA. Black walnut is examined in this study because it is one of the most valued hardwood species in North America for its high-quality timber and multiple uses (Williams 1990). We focused on N because an inadequate N supply has generally been found to limit seedling growth.

MATERIALS AND METHODS

Plant Material and Growth Conditions

Half-sib bareroot black walnut seedlings were germinated using nuts collected from Purdue #188 mother tree, a grafted walnut tree from the Purdue University improvement collection. Seedlings were grown under operational conditions (Jacobs 2003) at Vallonia State Nursery (38° 85' N, 86° 10' W) in Indiana, USA. The 1-year-old half-sib bareroot walnut seedlings were transplanted (one per pot) in sand culture using 6.2 l Treepots™ (Stuwe and Sons, Corvallis, Ore., USA). Transplanted seedlings received 1.56 g N tree⁻¹ (simulating operational silvicultural prescription of 250 kg N ha⁻¹ under field conditions) based on mass of the furrow slice and of soil in our pots (12.50 kg pot⁻¹) as detailed in Salifu and Timmer (2003). The rate was chosen to be within the general fertilization rates (range 160–400 kg N ha⁻¹) recommended for black walnut (Villarrubia 1980; Ponder 1998).

A group of four pots were arranged in five replicate blocks (20 pots for the entire experiment) on a greenhouse bench in the Department of Horticulture and Landscape Architecture Plant Growth Facility at Purdue University, West Lafayette, Ind., USA (40° 25' N, 86° 55' W). The greenhouse was set for 24/20 °C day/night air temperatures, relative humidity of 60 to 70%, and 16-h photoperiod with photosynthetic photon flux density of 350 to 400 μmol m⁻² s⁻¹ measured at seedling top height. Each pot was fertigated to container capacity (ranging from 100 to 150 mL pot⁻¹) at each application (White and Marstalerz 1966). Additionally, plastic containers were placed under each pot, and any leached solution after supplemental irrigation was returned to the pots. Nitrogen was supplied with the irrigation as ammonium sulfate [(¹⁵NH₄)₂SO₄] enriched to 5 atoms% ¹⁵N (34-0-0, Isotec Inc., USA), at two split applications starting 2 and 4 weeks after planting to improve uptake efficiency. The single application rate (1.56 g N tree⁻¹) adapted in

this study is adequate to study retranslocation processes and has been successfully implemented in other studies (Mead and Preston 1994; Preston and Mead 1994; Amponsah et al. 2004). Chelated micronutrients (Plant Products Co Ltd., Brampton, Ont.) were applied at the rate of 0.03 g L^{-1} , and phosphorus (P) supplemented by monopotassium phosphate ($\text{KH}_2 \text{ P}_2\text{O}_5$; 0-52-34, Plant Products Co Ltd., Brampton, Ont.) was applied at the rate of 50 kg ha^{-1} to avert deficiency of other nutrients.

Plant Sampling and Nutrient Analysis

Two pots were randomly sampled from each block (a total of 10 seedlings) at 30 and 90 days after transplanting. After harvest, roots were washed free of sand, and seedlings were partitioned into new shoots, old shoots, and roots. Soils were retained from each pot for chemical analysis at day 90. All plant samples were composited by replication for nutritional analysis but measured individually and averaged for growth analysis. Plant material was oven dried for 48 h at 70°C and ground. Samples were processed for total N and ^{15}N analyses following protocols detailed in Rundel, Ehleringer, and Nagy (1989). Total N and ^{15}N were determined using a Stable Isotope Finnigan Delta C mass spectrometer (Thermo-Electron, Bremen, Germany) coupled to a Micro-Dumas elemental analyzer (NA1500 CHN; Carlo Erba Instruments, Milan, Italy) at the stable isotope laboratory located at the University of Georgia, Athens, Ga., USA.

Calculations and Statistical Analysis

It was assumed that plants acquired N from two sources: current ^{15}N uptake from the soil or NDFP and unlabelled N stored in plant tissues or NDFP. Thus, NDFP in new growth represented retranslocation (Millard and Neilsen 1989; Millard and Proe 1993; Salifu and Timmer 2003). Fertilizer N recovered from the soil or NDFP was calculated for plant tissues and the soil compartment using Eq. (1) (Millard and Neilsen 1989; Reddy and Reddy 1993; Hauck, Meisinger, and Mulvaney 1994).

$$\text{NDFP} = \text{TN} \left[\frac{(A - B)}{(C - B)} \right] \quad (1)$$

where TN is total N content (mg) of plant component or soil, estimated as concentration multiplied by plant component dry mass or soil weight; A is atom% ^{15}N in fertilized plant tissues or soil; B is atom% ^{15}N in natural standard (0.366); and C is atom% ^{15}N in fertilizer. NDFP was calculated using Eq. (2):

$$\text{NDFP} = \text{TN} - \text{NDF} \quad (2)$$

One-way analysis of variance (ANOVA) was conducted to examine growth and nutrient status over time using SAS (SAS Institute, Inc., 2001) and appeared statistically similar over time.

RESULTS AND DISCUSSIONS

Plant Growth

Figure 1A shows rapid growth response to fertilization in components of black walnut seedlings within the first 30 days. However, there were no significant growth differences at 30 and 90 days after transplanting. Relative to status at planting, root and old shoot growth increased by 29% and 43%, respectively. Increased root growth was associated with greater nutrient accumulation. Thus, roots acted as sinks for nutrients (Figure 1B), exemplified by increased total N uptake (56%) from planting to 30 days after transplanting compared with only about 8% for old shoots. This observation contrasts markedly with those found for conifers where shoots rather than roots may act as sinks for nutrients (Nambiar and Fife, 1991; Salifu and Timmer 2003).

Retranslocation vs. N Derived from Fertilizer

The exact quantification of NDFP vs. NDF in plant components improves understanding of which N pool is vital for promoting seedling growth early in the establishment phase. The benefit of such knowledge is twofold. First, if NDFP were highly important, we could build N reserves at the nursery stage to promote retranslocation and seedling growth (Salifu and Timmer 2003). Second, if plants were less dependent on NDF, target rates could be moderated at transplanting to increase uptake efficiency and minimize potential leaching losses.

In this study, NDFP, which is driven by preplant N reserves (Salifu and Timmer 2003), was more important than NDF in meeting new growth demand in black walnut seedlings (Table 1). Therefore, nursery nutrient loading to build N reserves (Timmer 1997; Salifu and Jacobs 2006) in walnut seedlings could benefit NDFP and growth following field transplant. For example, NDFP in new shoots was higher than NDF by 385% at day 30 and by 117% 90 days after transplanting (Table 1). Thus, NDFP accounted for about 68 to 83% of the N demand in new shoot growth of walnut seedlings compared with about 17 to 32% met by NDF (Table 2). The higher NDFP observed in black walnut compared

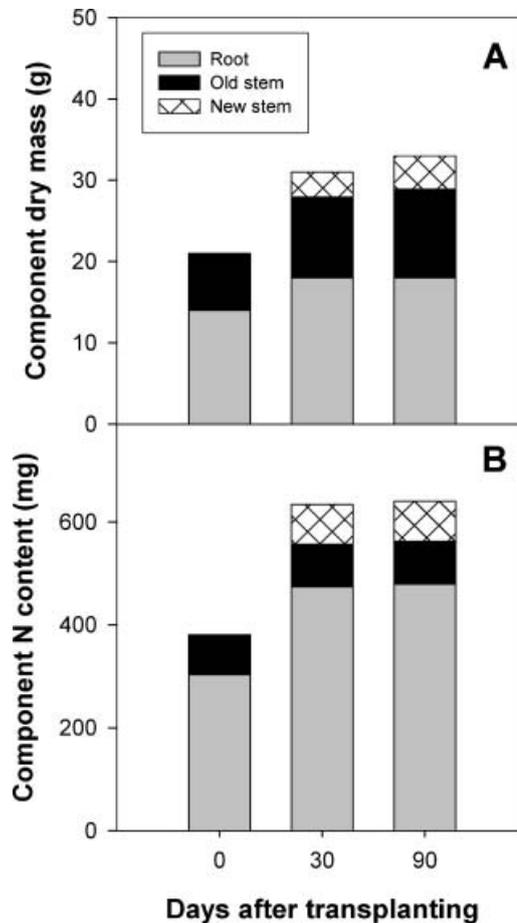


Figure 1. Component dry mass (A) and nitrogen content (B) of fertilized bareroot walnut seedlings grown in sand culture in a controlled greenhouse environment and sampled at 0, 30, and 90 days after transplanting.

with about 32% noted by Salifu et al. (2008) for northern red oak (*Quercus rubra*) suggests variation among hardwood species in retranslocation responses. The observed NDFP estimates for walnut compares favorably well with those noted for newly established black spruce seedlings (Salifu and Timmer 2003).

Fertilizer Recovery vs. Soil Retention

The proportion of mean NDFP that met new growth demand computed as a percentage of applied fertilizer for day 30 [i.e., $106/1560 \times 100$] was

Table 1. Mean (SE) of ^{15}N derived from fertilizer (NDFP) from the soil compartment or N derived from plants (NDFP) in tissues of black walnut seedlings established in sand culture for 90 days under greenhouse conditions

Sampling time (days)	Nitrogen source	N recovered in plant tissues (mg)				N recovered in soil (mg)	Total	Total (% applied)
		New stem	Old stem	Root	Plant			
30	NDFP	13 (6)	11(2)	82 (12)	106 (18)	nd ^a	nd	nd
	NDFP	63 (10)	73 (4)	392 (72)	527 (79)	nd	nd	nd
90	NDFP	24 (12)	17 (5)	104 (20)	145 (38)	675 (136)	820 (145)	53
	NDFP	52 (8)	68 (5)	376 (20)	496 (25)	nd	nd	nd

Note. Applied N = 1.56 g plant⁻¹season⁻¹.

^and = not determined.

small (7%) compared with 34% by NDFP (Table 1). The range of plant NDFP found in this study is similar to those reported (5 to 25%) elsewhere (Preston et al. 1990; Chang et al. 1996; Chang and Preston 2000; Frak et al. 2005) but lower than the higher recoveries (up to 43%) noted in other studies (Amponsah et al. 2004; Choi, Chang, and Hoi 2005). Total N recovered in the plant–soil system constituted 52% of the applied fertilizer (Table 1). Thus, the 740 mg (about 48% of applied fertilizer) unaccounted for in our study was similar to those observed in other studies (Chang et al. 1996; Amponsah et al. 2004), which could be associated with volatilization (Fenilli et al. 2007) and/or immobilization of N in organic fractions (Preston et al. 1990). Soil N recovery of applied fertilizer ranged from 48 to 95% (Amponsah et al. 2004). The inability of plants to acquire and utilize the residual soil N may be partly explained by the lack of significant plant growth response between days 30 and 90

Table 2. Mean (SE) of total N, current N uptake represented by N derived from fertilizer (NDFP), or retranslocation indicated by N derived from plants (NDFP) to meet N demand in new shoot growth of black walnut seedlings grown in sand culture and sampled at 30 and 90 days after transplanting in a greenhouse environment

Sampling time (days)	New shoot N		
	Total (mg)	NDFP (% of total)	NDFP (% of total)
30	75.62 (15)	17.19 (5)	83.31 (4)
90	76.33 (20)	31.44 (6)	68.13 (8)
ANOVA $P > F$			
Sampling time	0.7080	0.5081	0.5181

(Figure 1). Mechanisms to explain processes associated with $\text{NH}_3\text{-N}$ volatilization and/or immobilization of soil N into organic fractions needs quantification. Northern red oak, which exhibits episodic growth events with greater sink strengths, may have the capacity to exploit and utilize soil resources more effectively than walnut as observed in the current study. For example, NDFF was 68% in red oak (Salifu et al. 2008) compared with 43% noted for black walnut in this study.

CONCLUSIONS

Nitrogen derived from plants accounted for a greater proportion of the N in new shoot growth of walnut seedlings than NDFF. This demonstrates the importance of NDFF in meeting early growth demand for N in newly transplanted seedlings. Nitrogen derived from the soil pool to meet new shoot growth demand was small (7 to 9%). About half of the applied fertilizer (52%) was recovered in the plant–soil system, and the other half (48%) was perhaps lost to other processes such as volatilization or N immobilization in organic fractions. Observed trends may differ under field conditions and with different species and stock types. Building nutrient reserves in walnut seedlings at the nursery stage through nutrient loading could benefit retranslocation and outplanting performance. Further studies are needed to better understand the inability of plants to utilize residual soil N in seedling growth and to evaluate the effects of such residual N pool on plant development. Additionally, quantification of the pathways of N losses in future studies will help improve our understanding of N dynamics in plant–soil systems.

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