emphasis on complexation and adsorption processes—A review of literature. Water Air Soil Pollut. 56:667–680.

Sorensen, J.A., G.E. Glass, K.W. Schmidt, J.K. Huber, and G.R. Rapp, Jr. 1990. Airborne mercury deposition and watershed characteristics in relation to mercury concentrations in water, sediments, plankton, and fish of eighty northern Minnesota lakes. Environ. Sci. Technol. 24:1716–1727.

Steinnes, E., and E.M. Andersson. 1991. Atmospheric deposition of mercury in Norway: Temporal and spatial trends. Water Air Soil Pollut. 56:391–404.

Swain, E.B., D.R. Engstrom, M.E. Brigham, T.A. Henning, and P.L. Brezonik. 1992. Increasing rates of atmospheric mercury deposition in midcontinental North America. Science (Washington, DC) 257:784–787.

Urban, N.R., S.E. Bayley, and S.J. Eisenreich. 1989. Export of dissolved organic carbon and acidity from peatlands. Water Resour. Res. 25:1619–1628.

Verry, E.S., K.N. Brooks, and P.K. Barten. 1988. Streamflow response from an ombrotrophic mire. p. 25–29. *In* Proc. Sym. Hydrology of Wetlands in Temperate and Cold Climates. Vol. 1. Joensuu, Finland. 8 June 1988. Academy of Finland, Helsinki, Finland.

Verry, E.S., and D.R. Timmons. 1982. Waterborne nutrient flow through an upland–peatland watershed in Minnesota. Ecology 63:1456–1467.

Nutrient Retranslocation Response of *Picea mariana* Seedlings to Nitrogen Supply

K. F. Salifu and V. R. Timmer*

ABSTRACT

The hypotheses that retranslocation is controlled by soil nutrient availability or plant nutrient reserves were tested under field conditions for one growing season using nutrient-loaded and non-loaded (conventional) black spruce [*Picea mariana* (Mill.) BSP] seedlings planted on a poor, medium, and rich fertility soil created by equivalent applications of 0, 200, and 400 kg N ha^{-1}, respectively. Growth and nutrient uptake increased with N supply, and was consistently higher in loaded than conventional seedlings, demonstrating the advantage of nutrient loading practices to accelerate seedling growth across the range of soil N tested. Compared to the poor soil, new shoot biomass of loaded seedlings increased by 34 and 134% on the medium and rich soils, suggesting loaded seedlings may be more efficiently transplanted on more fertile sites. Net retranslocation of N, P, and K increased by 569, 185, and 102% by nutrient loading in the nursery, supporting the hypothesis of translocation driven by the magnitude of plant nutrient reserves. However, net N retranslocation diminished with time due to root system expansion that promoted uptake and reduced the need for N redistribution. Net retranslocation of N (the most limiting nutrient) declined with soil N supply, but that of P and K were relatively independent of soil fertility. Increased N availability in the soil enhanced N accumulation in the plants but lowered N retranslocation. We conclude that higher net retranslocation of N on poor soils is a phenotypic adjustment by *P. mariana* seedlings to maximize N use at low availability.

Newly planted seedlings depend highly on retranslocation of internal nutrient reserves to sinks of new growth soon after transplanting because initial slow root development limits uptake from the soil (Burdett et al., 1984; van den Driessche, 1985; Burdett, 1990). Retranslocation can be defined as the amount of an element depleted from older plant components and made available for new growth (Lim and Cousens, 1986), and has demonstrated prime significance in the nutrient economy of plants (Kim et al., 1987).

New cultural techniques, such as nutrient loading, have been designed specifically to build higher plant nutrient reserves in seedlings during nursery culture (Timmer and Aidelbaum, 1996). These reserves are depleted later to support current growth in newly transplanted seedlings. For example, nutrient-loaded seedlings depleted 67% N from old shoots to support new growth in black spruce (Malik and Timmer, 1998), and 51 to 60% in Chinese fir [*Cunninghamia lanceolata* (Lamb) Hook] (Xu and Timmer, 1999). Nutrient-loaded seedlings also grew faster, took up more nutrients, and suppressed weeds more effectively in the field (Malik and Timmer, 1998). Since nutrients often limit growth of seedlings in boreal forests under interspecific competition (Nilsson et al., 1986; Orlander et al., 1986), the higher internal reserves acquired by nutrient-loaded trees may ameliorate nutrient shortages when transplanted on such sites (Timmer, 1997; Timmer and Aidelbaum, 1996).

Malik and Timmer (1998) contend that net retranslocation is a key mechanism driving the improved transplanting growth response and competitiveness of nutrient-loaded seedlings on weed prone sites. However, the retranslocation response seems to decline with soil N availability (Timmer and Munson, 1991; Xu and Timmer, 1999), raising the question whether nutrient-loaded seedlings should be more efficiently transplanted on poor rather than rich soils? Furthermore, variability of retranslocation with soil nutrient availability is still controversial (Hawkins et al., 1999). Current work suggests that retranslocation may be regulated by soil nutrient supply, nutrient uptake rates, the size of plant nutrient reserves, and age of trees (Munson et al., 1995; Hawkins et al., 1998, 1999; Malik and Timmer, 1998), although the mechanisms involved are not well understood (Hawkins et al., 1999). Furthermore, the relative contributions of nutrients from retranslocation and from external uptake to sinks of new growth are unknown.

In this study, we used a conceptual model (Lim and Cousens, 1986) to quantify relative contributions of nutrients from net retranslocation and external uptake to new growth under field conditions and to explain the mechanisms driving retranslocation on a N supply gradient. In addition, we examined site specific planting for loaded trees and determined whether net retranslocation is controlled by plant nutrient reserves. We focused mainly on N, because this element is usually most limiting for growth of black spruce seedlings on Boreal

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mixedwood sites (Malik and Timmer, 1996). Our research addressed four major questions:

1. Should nutrient-loaded trees be more efficiently transplanted on poor rather than rich soils for enhanced net retranslocation for growth of newly established seedlings?
2. Does N retranslocation in spruce decrease in response to N supply? Such a decrease has been demonstrated with Chinese fir under greenhouse conditions (Xu and Timmer, 1999) and in other studies (Miller et al., 1976; Lim and Cousens, 1986), but does it also occur with spruce under field conditions?
3. Is greater retranslocation and growth response in transplanted nutrient-loaded seedlings controlled by higher plant N reserves acquired during nursery culture?
4. Will increased root growth over time following transplanting enhance nutrient uptake from external soil sources and reduce the need for internal redistribution of nutrients as Millard and Neilsen (1989) suggested?

MATERIALS AND METHODS

Plant Material and Prior Nursery Culture

Black spruce seedlings were reared in commercial greenhouses at North Gro Development located at Kirkland Lake in northeastern Ontario. Seedlings were spring sown (April, 1998) in styro-block trays containing 40-mL cavities (165 cavities per tray), filled with peat/vermiculite (3:1, v/v) growing media, and reared under similar environmental conditions (moisture, light, and temperature) except for N fertilizer treatments. A commercial water soluble fertilizer (20-20-20, Plant Products Co. Ltd., Brampton, ON) was supplied in solution form with the irrigation. A seasonal total of 10 mg N seedling^{-1} was delivered to one group of seedlings at a constant weekly rate, starting 1 wk after germination to represent industry's normal practice for containerized black spruce seedlings production in northern Ontario (called the conventional treatment) (Timmer and Aidelbaum, 1996; Timmer, 1997). The other treatment (referred to as the loaded treatment hereafter) received a seasonal total of 64 mg N seedling^{-1}. Weekly loading rates for this latter treatment were calculated according to exponential functions described in detail by Timmer (1997) and Timmer and Aidelbaum (1996) for steady-state nutrient culture (Ingestad and Lund, 1986; Ingestad and Agren, 1995). Fertilization started 1 wk after germination and was carried out for 18 wk using traveling boom-sprayers. After each spray, the seedlings were washed with water to avoid possible burning. Seedlings were reared until budset in summer, hardened, and cold stored (−2°C) for overwintering.

Site Description and Transplanting Procedure

Experimental plots were established on a clear-cut area that had lain fallow for 3 yr in Kirkland Lake, ON, Canada (48°10'N, 88°01'W). The site type is classified as ST3b, characterized by mixedwood stands on fresh to moderately moist, sandy to coarse loamy soils (McCarthy et al., 1994). The soil belongs to the Orthic Humo-Ferric Podzol subgroup, equivalent to the Cryorthods (Soil Classification Working Group, 1998). Mechanical analysis (Day, 1965) of composite mineral soil samples collected from the 0- to 10-cm depth at 20 random points within the study site showed particle-size distribution averaged 72, 26, and 2% sand, silt, and clay, respectively. Total N and soil organic matter (Ball, 1964) averaged 0.4 and 19%, respectively. Mean values for available P (Bray and Kurtz, 1945) and pH$_{(\mathrm{water})}$ were 63 mg kg^{-1} soil and 4.0. Previous overstory vegetation included trembling aspen (*Populus tremuloides* Michx.), jack pine (*Pinus banksiana* Lamb.), black spruce, and white spruce [*Picea glauca* (Moench) Voss]. Shrubs included bush honeysuckle (*Diervilla lonicera* Mill.) and velvetleaf blueberry (*Vaccinium myrtilloides* Michx.), while mosses were schreber's moss [*Pleurozium schreberi* (Brid.) Mitt] and plume moss [*Ptilium crista-castrensis* (Hedw.) De Not.].

Prior to plot establishment, a reconnaissance survey was conducted to locate a uniform area devoid of vegetation to avoid possible confounding effects of competing vegetation on treatment responses. Twelve 6 by 12 m^2 blocks (main plots) separated by 2-m buffers were established within this area. Each main plot was then split into two subplots (6 by 6 m^2). One subplot was planted with 25 conventional seedlings and the other with 25 nutrient-loaded seedlings at 1 by 1 m spacing. A nutrient gradient was created by incorporating 0, 30, and 60 mg N tree^{-1} (20-20-20 water soluble fertilizer) around each seedling within a 0.5-m radius, equivalent to 0, 200, and 400 kg N ha^{-1}, respectively, on the main plots at planting. These treatments are referred to as the poor, medium, and rich soils, respectively, hereafter. After fertilization, water was sprayed on the seedlings to avoid possible burning. Planting was conducted on 28 May 1999 with visually presorted seedlings of similar size to minimize possible confounding due to initial size differences.

Experimental Design, Plant Sampling and Nutrient Analysis

The experiment tested three levels of N supply (0, 30, and 60 mg N seedling^{-1}), two levels of nursery nutrition (loaded and conventional), at two time periods (60 and 120 d) after transplanting (Table 1). The experimental design was a split plot design, with a 3 by 2 by 2 factorial treatment structure replicated four times, for a total of 48 experimental units. At planting, five batches of four seedlings (a total of 20 seedlings) per loading regime were sampled, roots were washed free of soil, and the plants were separated into stems and roots. A random sample of three seedlings within the 6 by 6 m^2 plot, for a total of 12 seedlings per treatment, were harvested 60 and 120 d after establishment. Seedlings were washed free of soil and separated into new shoots, old shoots, and roots. Samples were composited by replicate for chemical analysis, but measured individually and averaged for growth analysis. Plant material was oven-dried for 72 h at 68°C, ground, and wet-digested in a block digester using an H_2SO_4-H_2O_2 solution (Lowther, 1980). Total tissue N was determined using a Technicon II Autoanalyzer System (Technicon Industrial Systems, Tarrytown, NY) (Eastin, 1978), P by the molybdate blue method (Allen, 1974), and K by atomic absorption (Model 3100, Perkin-Elmer, Norwalk, CT).

Net Retranslocation Estimates

Net retranslocation determined in two successive years was quantified as the total amount of an element in year n, minus leaching in intervening months and the total amounts retained in older tissues in the year $n + 1$ (Switzer and Nelson, 1972; Lim and Cousens, 1986). Since net retranslocation in our study is determined in the same year, it is quantified as the total amount of an element at Day 0 (preplant status) minus the total amount in old tissues at Day x (60 or 120 d after transplanting). Thus, net retranslocation from old shoots was quantified by

Table 1. Summary of analysis of variance, testing effects of single and factorial treatments [soil N supply (S); nursery nutrition (F); and time since transplanting (T)] on net N, P, and K retranslocation and uptake in old shoots of black spruce seedlings under field conditions.

		N		P		K	
Source†	**df**	**MS‡**	**$P > F$**	**MS**	**$P > F$**	**MS**	**$P > F$**
			Net retranslocation				
Main plot comparisons							
S	**2**	**28.84**	**0.0046**	**0.00**	**0.2733**	**0.668**	**0.0866**
Main plot error (e)	**9**	**2.77**		**0.00**		**0.206**	
Subplot comparisons							
F	**1**	**164.69**	**0.0001**	**0.002**	**0.0001**	**4.008**	**0.0195**
S × F	**2**	**11.34**	**0.0122**	**0.000**	**0.7655**	**0.158**	**0.7368**
e × F	**9**	**1.52**		**0.000**		**0.499**	
Sub-subplot comparisons							
T	**1**	**94.73**	**0.0001**	**0.002**	**0.0001**	**35.381**	**0.0001**
S × T	**2**	**29.88**	**0.0004**	**0.000**	**0.8392**	**0.668**	**0.0866**
e × T	**9**	**1.39**		**0.000**		**0.206**	
F × T	**1**	**32.46**	**0.0021**	**0.001**	**0.0020**	**4.008**	**0.0195**
S × F × T	**2**	**20.00**	**0.0036**	**0.000**	**0.5951**	**0.158**	**0.7368**
e × F × T	**9**	**1.79**		**0.000**		**0.499**	
Cor. total	**47**						
Mean	**1**						
Raw total	**48**						
			Uptake				
Main plot comparisons							
S	**2**	**4 604.70**	**0.0013**	**0.014**	**0.0028**	**331.373**	**0.0059**
e	**9**	**302.42**		**0.001**		**34.51**	
Subplot comparisons							
F	**1**	**3 847.43**	**0.0021**	**0.010**	**0.0012**	**405.364**	**0.0001**
S × F	**2**	**1 473.26**	**0.0146**	**0.004**	**0.0141**	**98.430**	**0.0044**
e × F	**9**	**210.37**		**0.001**		**9.358**	
Sub-subplot comparisons							
T	**1**	**21 712.42**	**0.0001**	**0.102**	**0.0001**	**1346.519**	**0.0001**
S × T	**2**	**2 651.72**	**0.0011**	**0.007**	**0.0052**	**60.293**	**0.1485**
e × T	**9**	**167.66**		**0.001**		**25.386**	
F × T	**1**	**3 760.19**	**0.0005**	**0.017**	**0.0001**	**265.974**	**0.0005**
S × F × T	**2**	**2 390.06**	**0.0008**	**0.006**	**0.0002**	**107.682**	**0.0033**
e × F × T	**9**	**134.72**		**0.001**		**9.330**	
Cor. total	**47**						
Mean	**1**						
Raw total	**48**						

† S (soil) = poor, medium, and rich; F (fertilization) = conventional and loaded; T (time) = 60 and 120 d.
‡ MS = mean square.

$$\mathrm{Re} = A - B - (D + \mathrm{Le}) \qquad [1]$$

where Re is the amount of nutrient retranslocated, A is the nutrient content in shoot at Day 0 (preplant status), B is the nutrient content in old shoots at Day 60 or 120 after transplanting, D is the nutrient content of dead tissues between Day 0 and Days 60 or 120, and Le is the amount of nutrient leached. Since D + Le is assumed negligible as noted by others (Miller et al., 1976; Chapin and Kedrowski, 1983; Lim and Cousens, 1986; Helmisaari, 1992), and as for short-term trials like ours, percentage net retranslocation was calculated as

$$\%\ \mathrm{Re} = \left(\frac{A - B}{A}\right)100 \qquad [2]$$

Net uptake from external source (U) was quantified by

$$U = \mathrm{Req} - \mathrm{Re} \qquad [3]$$

where Req was nutrient content in new tissues at Day 60 or 120 after transplanting.

Statistical Analysis

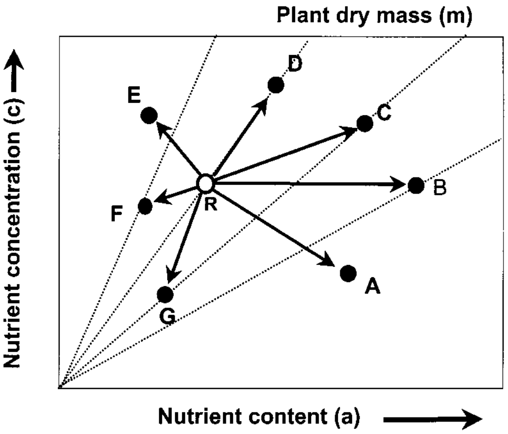
Analysis of variance (ANOVA) was conducted on growth and nutrient variables using SAS and where appropriate means were ranked according to Waller-Duncan's multiple range tests (SAS Institute, 1989). The Anderson and McLean's (1974) linear model for the ANOVA is given as:

$$\begin{aligned} \mathrm{Y}_{ijklm} = {} & \mu + S_i + \omega_{(i)j} + \delta_{(ij)} + F_k + SF_{ik} + \omega F_{(i)jk} \\ & + \delta'_{(ijk)} + T_l + ST_{il} + \omega T_{(i)jl} + FT_{kl} + SFT_{ikl} \\ & + \omega FT_{(i)jkl} + \varepsilon_{(ijkl)m} \end{aligned} \qquad [4]$$

where Y_{ijklm} is seedling biomass, nutrient content or concentration of the mth replicate (m = 1), estimated at lth time (l = 1, 2), for the kth nursery nutrition (k = 1, 2), within the ith soil N supply (i = 1, 2, 3) from the jth block (j = 1, 2, 3, 4); μ is overall mean; S_i is fixed effect of the ith soil N supply; $\omega_{(i)j}$ is whole plot error; $\delta_{(ij)}$ is first restriction error; F_k is fixed effect of the kth nursery nutrition; $\delta'_{(ijk)}$ is second restriction error; T_l is fixed effect of the lth time; followed by the interactions among the main and block effects; and ε is error associated with measured seedling biomass, nutrient content, or concentration from bulk replicates.

Vector Diagnosis

Vector diagnosis involves comparing nutrient concentration, nutrient content, and biomass of plants or plant components in a graphic format known as a vector nomogram (Timmer, 1991, Fig. 1). Plant tissues sampled at time intervals are usually compared to a control or reference (R) usually on a relative basis, or on an absolute basis as in our case. The technique has undergone periodic refinement to enhance diag-



Vector Direction with Time (T)	Change in			Interpretation	d(c)/dT	Possible Diagnostic
	m	c	a			
A	+	-	+	Dilution	< 0	Growth dilution
B	+	0	+	Sufficiency	0	Steady-state
C	+	+	+	Deficiency	<1,>0	Limiting
D	0	+	+	Luxury consumption	1	Accumulation
E	-	++	±	Excess	>1	Toxic accum.
F	-	-	-	Excess	< 0	Antagonistic
G	0, +	-	-	Depletion	< 0	Retranslocation

Fig. 1. Nutritional interpretations of directional changes with time in dry mass, nutrient content, and nutrient concentration during plant development. The reference point (R) represents an initial plant sample taken at time T_0 (usually normalized to 100). Vector orientation and magnitude characterize parameter relationships [increase (+), decrease (−), or unchanging (0)] relative to seedlings sampled at subsequent time intervals. Arrow direction also depicts time progression from time T_0 to T_1, T_2... A new *Shift G*, characterizing net depletion often associated with retranslocation, is included in the box (Modified from Imo and Timmer, 1997; Malik, 1998).

nostic precision, capacity, and flexibility. New concepts, such as steady-state nutrition characterized by stable internal tissue nutrient concentration with time **(Shift B)**, were introduced by incorporating temporal parameters in the system (Imo and Timmer, 1997). Recently a new vector **(Shift G)** was added to expand the diagnostic power of vector analysis to examine retranslocation processes (Malik, 1998). **Shift G** depicts depletion of nutrients (see box under Fig. 1) since nutrient concentration and contents decline in plant components with or without change in biomass often associated with net nutrient retranslocation from older plant parts to sinks of new growth (Malik and Timmer, 1998). In the nomogram (Fig. 1), slanting lines or diagonals represent lines of equal biomass; thus, **Shifts C** and **G** may contrast markedly in nutrition at similar biomass. Shifts to the left and right of the reference (R) treatment depict respective net depletion and gain of nutrients. For comprehensive reviews of vector diagnosis, see Timmer (1991), Haase and Rose (1995), and Imo and Timmer (1997). The technique is employed here to elucidate the net retranslocation response and examine temporal biomass and nutrient allocation dynamics during seedling development.

RESULTS AND DISCUSSION

Seedling Growth and Allocation Dynamics

Growth in structural plant components increased with time (Fig. 2), and was consistently greater ($P < 0.0001$) in nutrient-loaded than conventional seedlings, demonstrating the value of nutrient loading over conventional fertilization methods in promoting rapid seedling growth. For example, compared with Day 0, root biomass increased ($P < 0.0001$) by 431% in nutrient-loaded, compared with only 77% in conventional seedlings on the rich soil 120 d after transplanting. The greater root growth in nutrient-loaded seedlings probably enhanced

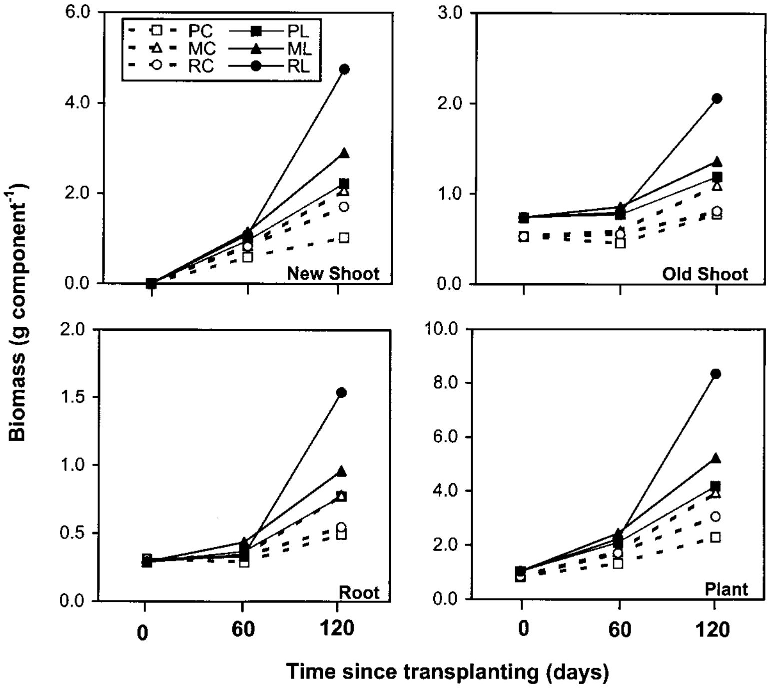


Fig. 2. Component dry mass production of nutrient-loaded (L) and conventional (C) black spruce seedlings 0, 60, and 120 d after transplanting on poor (P), medium (M), and rich (R) soils.

rapid exploitation of available pools of soil N that increased growth by 173% in loaded vs. conventional plants on the rich soil at final harvest (Day 120, Fig. 2). Irrespective of N supply level, significantly higher growth trends occurred in all plant structural components in nutrient-loaded vs. conventional seedlings, indicative of the robustness of loaded seedlings to acclimate to the full range of simulated N supply. For example, plant growth increased 711% in loaded, compared with only 264% in conventional seedlings at final harvest with respect to initial status. Furthermore, compared with the poor soil at final sampling, growth increased by 34 and 134% in new shoots ($P < 0.0023$), and 25 and 100% ($P < 0.0067$) in nutrient-loaded plants on the medium and rich soils, respectively. In contrast, growth increased by 103 and 68% in new shoots, and 72 and 33% in conventional plants on the respective medium and rich soils with respect to the poor soil. The consistently higher growth of conventionally fertilized seedlings on the medium compared with the rich soil suggested that the 400 kg N ha^{-1} dose rate induced nutrient toxicity inhibiting growth (Timmer, 1997).

All three-way and two-way interaction effects significantly ($P < 0.0022$–0.0001) affected growth in all structural plant components except for roots ($P = 0.1433$). For example, significant ($P < 0.0001$) loading × N supply × time interaction effects promoted growth in plants and increased biomass production by 100% at final harvest in loaded seedlings compared with their status at Day 0 (Fig. 2). The lack of early growth (Days 0–60) in old shoots may reflect nutrient depletion to support sinks of new growth as elucidated by vector diagnosis below.

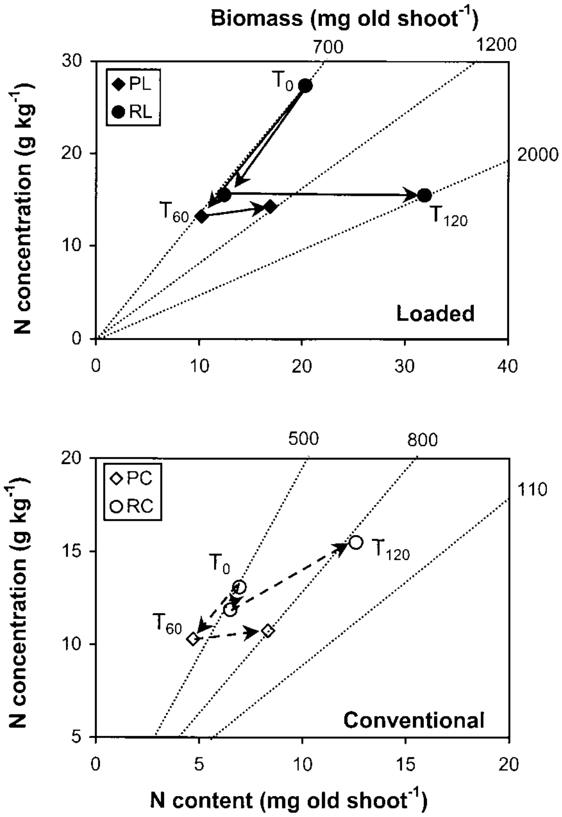


Fig. 3. Vector nomograms of absolute change in dry mass, N uptake, and concentration in old shoots of nutrient-loaded (L, top) and conventional (C, bottom) spruce seedlings after transplanting on poor (P) and rich (R) soils. Vectors reflect progressions in time (d) from T_0 to T_{60}, and T_{60} to T_{120}. Note scale difference between nomograms.

Vector Diagnosis of Biomass and Nitrogen Allocation Dynamics

Temporal changes in biomass and N status in old shoots of *P. mariana* seedlings are shown in Fig. 3. Rapid early growth of new shoots (Fig. 2) was at the expense of old shoots that exhibited N depletion, apparently severe for loaded (Fig. 3, top), and mild for conventional trees (Fig. 3, bottom), as reflected by differential vector length (**Shift G**, Fig. 1 and 3) during the initial 60 d only. These shifts demonstrated typical net retranslocation of N from source organs in old shoots to sinks of new growth (Malik, 1998; Malik and Timmer, 1998; McAlister and Timmer, 1998). The loaded seedlings exhibited an exploitive nutrient use strategy (Malik and Timmer, 1996, 1998) by depleting higher N reserves for investment in active metabolic sinks in new shoots and roots that promoted growth in these components (Fig. 2). In contrast, conventional seedlings showed a conservative strategy characterized by less N depletion from lower reserves (Fig. 3, bottom). For example, biomass and N uptake in new shoots increased by 336 and 366%, respectively, in loaded, compared with only 100 and 82% in conventional seedlings at final sampling (Fig. 2). Moreover, biomass increased by 1300 mg in old shoots of loaded, compared with 300 mg in conventional seedlings planted on the same soil at final harvest with respect to Day 0 (Fig. 3). Steady-state nutrition (Imo and Timmer, 1992; Quoreshi and Timmer, 2000) characterized by stable internal tissue N concentration with time was achieved in old shoots of loaded seedlings on the rich soil after Day 60 (**Shift B**, Fig. 1 and 3, top) suggesting growth rate matched N uptake rate. In contrasts, conventional seedlings on the rich soil were N deficient as shown by increased dry mass, N uptake and concentration after Day 60 (**Shift C**, Fig. 1 and 3, bottom) presumably because of the lower N reserves and uptake in these seedlings (Imo and Timmer, 1997).

Net Retranslocation and Nutrient Uptake Kinetics as Influenced by Loading

The proportional contribution of N, P, and K from net retranslocation and external uptake to new growth were quantified according to Eq. [1] and [3], respectively, and presented for main effects (Fig. 4 and 5). The ANOVA table associated with Eq. 4 is given in Table 1. Figure 4 (left) shows that the amount of N remobilized from old shoots to new growth is affected ($P < 0.0001$, Table 1) by nursery nutrition. Loaded seedlings retranslocated 50% net N that met 46% of requirements for new growth, compared with 32% net N that met 18% of the requirement in conventional seedlings 60 d after transplanting (Fig. 4, left).

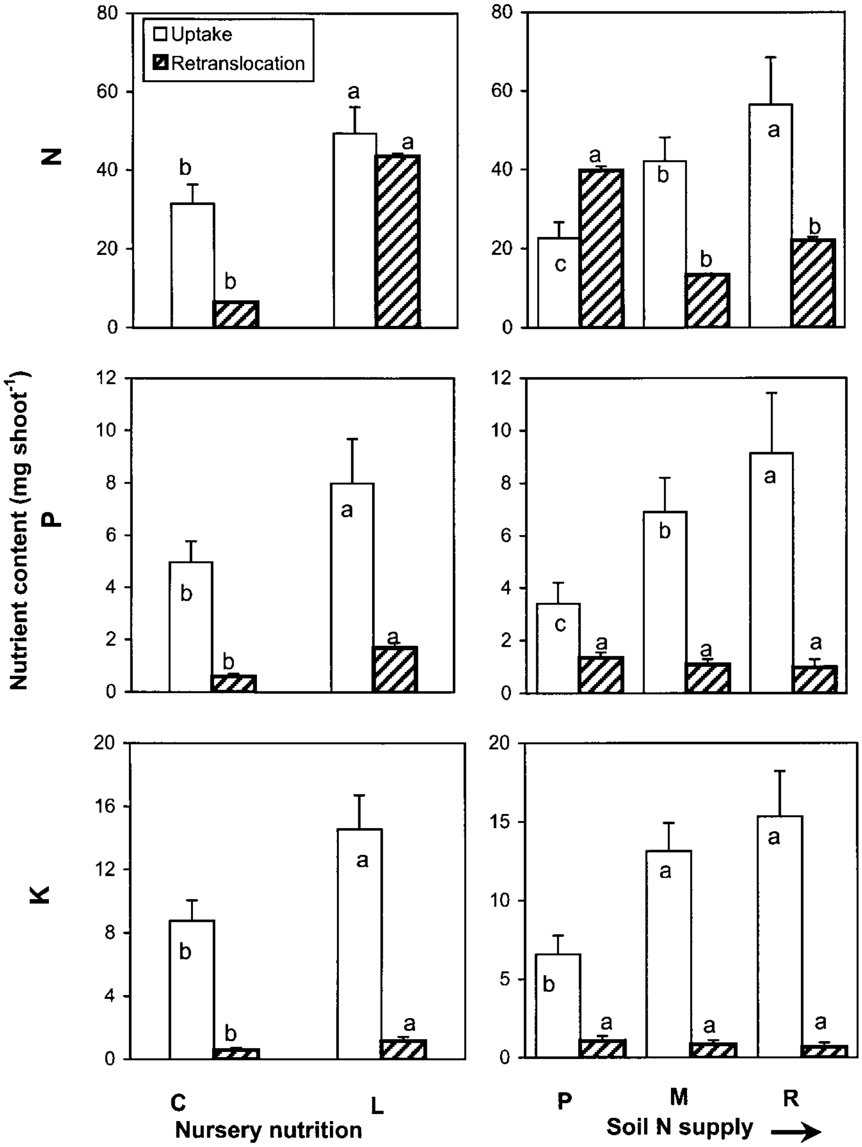


Fig. 4. Estimated amounts of N, P, and K mobilized from old shoots (net retranslocation/10) and from the soil (uptake) for new growth in nutrient-loaded (L) and conventional (C) black spruce seedlings [left]; transplanted on poor (P), medium (M) and rich (R) soils [right]. Vertical bars are standard errors of mean. Similar bars marked with different letters are significantly different according to DMRT, $P < 0.05$.

The greater N depletion associated with higher preplant reserves in nutrient-loaded (20.28 mg N, Fig. 3, top) than in conventional (6.94 mg N, Fig. 3, bottom) seedlings found in this study supports the hypothesis that net retranslocation is driven by the amount of plant N reserves acquired during nursery culture (Malik, 1998; Malik and Timmer, 1998; Nambiar and Fife, 1991). Millard and Proe (1993) also noted that the amount of N stored is closely related to the amount of N remobilized in the following year for new growth in Sitka Spruce [*P. sitchensis* (Bong.) Carrière]. Furthermore, N content in the foliage of Tasmanian blue gum (*Eucalyptus globulus* Labill.) in Southern Australia was positively and significantly correlated ($r^2 = 0.95$) with the amount retranslocated to new growth the following season (Saur et al., 2000).

Higher net retranslocation in loaded seedlings suggests N reserves in these seedlings were probably less structurally bound, hence readily available for depletion to active metabolic sinks (Chapin, 1990; Miller et al., 1979; Fagerstrom and Lohm, 1977). Nitrogen in plant tissues is distributed in either the mobile or structurally bound forms (Fagerstrom and Lohm, 1977). The mobile pool is thought to consist of nonfunctional amino acids and proteins that are readily depleted from source organs to support new growth (Chapin, 1990). Amino acid analysis showed that arginine, glutamic acid, and proline were the major N forms in the mobile pool in black spruce seedlings (Kim et al., 1987). We speculate that in addition to building N reserves, nutrient loading may also increase the mobile N pool in plant tissues that are then rapidly remobilized to sinks of new growth soon after transplanting as demonstrated in this study and others (Malik and Timmer, 1998; Xu and Timmer, 1999). The degree of N retranslocation (50%) by loaded seedlings 60 d after transplanting in this study is lower than

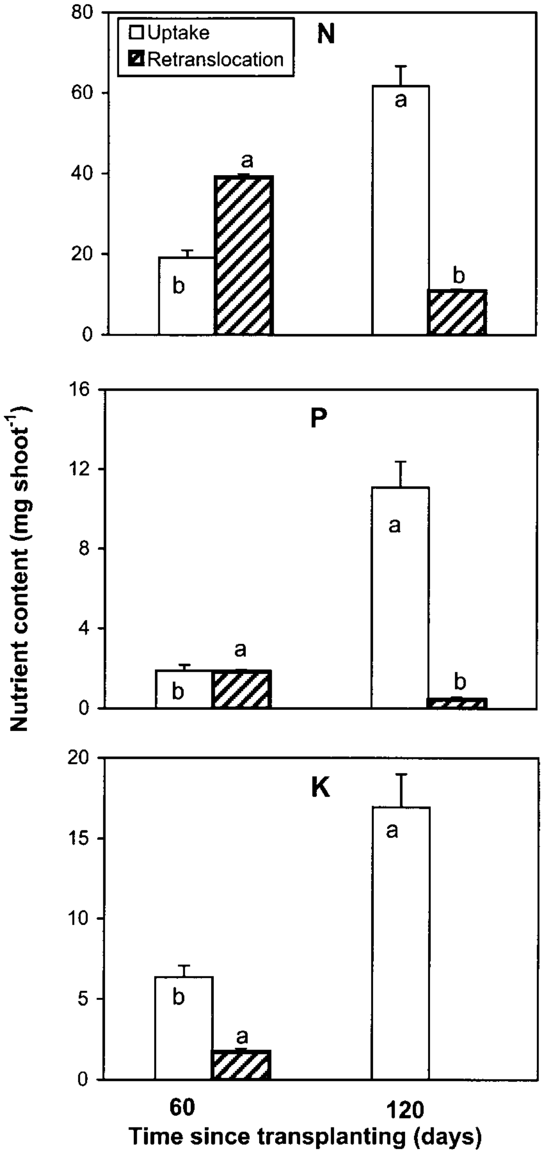


Fig. 5. Estimated amounts of N, P, and K mobilized from old shoots (net retranslocation/10) and from the soil (uptake) for new growth in black spruce seedlings 60 and 120 d after transplanting. Vertical bars are standard errors of mean. Similar bars marked with different letters are significantly different according to DMRT, $P < 0.05$.

55% reported for Chinese fir seedlings (Xu and Timmer, 1999) and 55% for radiata pine (*Pinus radiata* D. Don) (Nambiar and Fife, 1987) and white pine (*Pinus strobus* L.) (Munson et al., 1995), suggesting that N retranslocation capacity may differ between species (Ralhan and Singh, 1987; Schlesinger et al., 1989; Munson et al., 1995; Keenan et al., 1995).

Loaded seedlings also exhibited higher net retranslocation of P ($P < 0.0001$) and K ($P < 0.0195$) compared with conventional seedlings, but response magnitude was lower than occurred with N (Fig. 4, left and Table 1). Compared with conventional seedlings, N, P, and K net retranslocation increased by 569, 185, and 102% in loaded seedlings (Fig. 4, left), exemplifying the ability of nutrient-loaded seedlings to redistribute nutrients for sinks of new growth soon after transplanting.

Net Retranslocation as Influenced by Soil Nutrient Supply

Studies suggest nutrient retranslocation may be enhanced on poor sites (Miller et al., 1976; Johnson et al., 1982) or may be independent of nutrient gradients (Millard and Proe, 1992; Nelson et al., 1995), or may increase with nutrient availability (Proe and Millard, 1994; Munson et al., 1995). We found that N net retranslocation declined ($P < 0.0001$) with N supply (Fig. 4, right), supporting a hypothesis of reduced retranslocation on rich soils (Miller et al., 1976; Turner, 1977; Johnson et al., 1982; Shaver and Milillo, 1984; Lim and Cousens, 1986; van den Driessche, 1991). Increased N uptake at higher availability, 86 and 149%, respectively, on the medium and rich soils compared with the poor soil (Fig. 4, right), seemed to lower net retranslocation as noted by Turner (1977). In contrast, P and K net retranslocation were independent of soil nutrient supply, suggesting that for less-limiting nutrients retranslocation is independent of nutrient gradients (Fife and Nambiar, 1982, 1984; Chapin and Kedrowski, 1983; Birk and Vitousek, 1986; Nambiar and Fife, 1991; Chapin and Moilanen, 1991; Del Arco et al., 1991; Millard and Proe, 1992; Nelson et al., 1995).

Net Retranslocation as Influenced by Time since Transplanting

Net retranslocation of N, P, and K significantly diminished ($P < 0.0001$) with time (Fig. 5, Table 1); supporting the hypothesis that retranslocation is influenced by seedling development (Clark, 1983; Potter et al., 1987; Hawkins et al., 1998; Xu and Timmer, 1999). Apparently, N, P, and K uptake from external sources significantly increased ($P < 0.0001$) with time (Fig. 5, Table 1), presumably because of greater root establishment and growth (Fig. 2). This supports the contention of Millard and Neilsen (1989) that an established root system would allow greater exploitation of soil N to occur and lower the need for N retranslocation. For example, greater root growth (Fig. 2) resulted in increased N, P, and K uptake from the soil by 223, 493, and 166% ($P < 0.0001$, Table 1) at final harvest with respect to Day 60 (Fig. 5). Interaction effects were also significant (data not presented). Compared with the rich soil at Day 60, for example, N and P uptake increased by 336 and 433% at Day 120 on the same soil, exemplifying significant ($P < 0.001$– 0.005, Table 1) soil N supply × time interaction effects. Similarly, greater N acquisition by loaded (313%) compared with conventional (131%) seedlings at final sampling with respect to Day 60 status exemplifies significant ($P < 0.001$, Table 1) nursery nutrition × time interaction effects.

Retranslocation from old foliage provided about 66% of the nutrients required for new growth in closed conifer stands (Miller, 1995). Also, 43% of N required for new growth in Italian stone pine (*Pinus pinea* L.) (Rapp et al., 1979), and between 50 and 60% in Austrian pine (*Pinus nigra* Arnold) (Miller, 1984) were met by retranslocation. Furthermore, retranslocation met about 39% N, 60% P, and 22% K demand for new growth of a 20-yr-old Loblolly pine (*Pinus taeda* L.) plantation (Switzer and Nelson, 1972). The estimates of respective N and K net retranslocation in newly planted black spruce seedlings were 50 and 40% in this study, and retranslocation was most critical for growth soon after transplanting when nutrient stress was most severe (Xu and Timmer, 1999). Similarly, about 32 to 40% of N used for leaf growth in young conifers was derived from retranslocation (Millard, 1996). We propose that net retranslocation is critical at seedling establishment, but declines with root system development because of increased nutrient uptake from external sources. It may become significant at crown closure where two-thirds of nutrients required for growth could be met by retranslocation from tight nutrient budgets (Miller, 1995).

CONCLUSIONS

Results show that nutrient loading practices promoted depletion of higher plant N, P, and K reserves to sinks that stimulated rapid new growth and nutrient uptake in transplanted seedlings. In contrast, conventional fertilization practices induced nutrient conservation in seedlings and reduced new growth and nutrient uptake. Consistently greater growth and nutrient uptake on the simulated N supply gradient suggest loaded seedlings may be transplanted on the full range of soil N supply tested, with enhanced growth response on rich soils. Net retranslocation of N and P increased by 569 and 185% in nutrient-loaded vs. conventional seedlings, and higher preplant nutrient reserves accounted for the greater retranslocation in loaded seedlings, supporting a hypothesis of retranslocation driven by the amount of plant nutrient reserves. Root growth in loaded seedlings increased N, P, and K uptake from external sources by 223, 493, and 166%, respectively, and lowered the need for internal redistribution of nutrients over time. Net N retranslocation declined with increased soil N availability due to enhanced plant uptake that lowered the need for internal redistribution of N. Nutrient loading has been shown to offset early ambient deficiencies, promote vigorous growth, and may accelerate early dominance (McAlister and Timmer, 1998), which is essential for the development of an even-aged stand (Miller, 1995). We speculate that the greater physiological plasticity exhibited by nutrient-loaded seedlings in net retranslocation, nutrient uptake, and biomass allocation kinetics soon after transplanting will contribute to enhanced future survival, growth, and nutrition when compared with conventional seedlings.

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REFERENCES

Allen, S.E. (ed.) 1974. Chemical analysis of ecological materials. John Wiley and Sons, New York.

Anderson, V.L., and R.A. McLean. 1974. Design of experiments: A realistic approach. Marcel Dekker, New York.

Ball, D.F. 1964. Loss on-ignition as an estimator of organic matter and organic carbon in non-calcareous soils. J. Soil Sci. 15:84–92.

Birk, M., and P.M. Vitousek. 1986. Nitrogen availability and nitrogen use efficiency in loblolly pine stands. Ecology 67:69–79.

Bray, R.H., and L.T. Kurtz. 1945. Determination of total organic, available forms of phosphorus in soils. Soil Sci. 59:39–45.

Burdett, A.N. 1990. Physiological process in plantation establishment and development of specifications for forest planting stock. Can. J. For. Res. 20:415–427.

Burdett, A.N., I.J. Herring, and C.F. Thompson. 1984. Early growth of planted spruce. Can. J. For. Res. 14:644–651.

Chapin, F.S. III. 1990. The ecology and economics of storage in plants. Ann. Rev. Ecol. Syst. 21:423–447.

Chapin, F.S., and R.A. Kedrowski. 1983. Seasonal changes in fractions and autumn retranslocation in evergreen and deciduous taiga trees. Ecology 64:376–391.

Chapin, F.S. III, and L. Moilanen. 1991. Nutritional controls over nitrogen and phosphorus resorption from Alaskan birch leaves. Ecology 72:701–708.

Clark, R.B. 1983. Plant genotype differences in the uptake, translocation, accumulation, and use of mineral elements required for plant growth. Plant Soil 77:175–196.

Day, P.R. 1965. Particle fractionation and particle-size analysis. p. 545–566. *In* C.A. Black (ed.) Methods of soil analysis. Part 1. Agron. Monogr. 9. ASA, Madison, WI.

Del Arco, J., A. Escudero, and M.V. Garrido. 1991. Effects of site characteristics on nitrogen retranslocation from senescing leaves. Ecology 72:709–715.

Eastin, E.F. 1978. Use of an AutoAnalyzer for total nitrogen determination in plants. Comm. Soil Sci. Plant. Anal. 9:107–113.

Fagerstrom, T., and U. Lohm. 1977. Growth in Scot pine (*Pinus sylvestris*): Mechanisms of response to nitrogen. Oecologia (Berlin) 26:305–315.

Fife, D.N., and E.K.S. Nambiar. 1982. Accumulation and retranslocation of mineral nutrients in developing needles in relation to seasonal growth of young radiata pine trees. Ann. Bot. 50:817–829.

Fife, D.N., and E.K.S. Nambiar. 1984. Movement of nutrients in radiata pine in relation to growth of shoots. Ann. Bot. 52:303–314.

Haase, D.L., and R. Rose. 1995. Vector analysis and its use for interpreting plant nutrient shifts in response to silvicultural treatments. For. Sci. 41:54–66.

Hawkins, B.J., G. Henry, and S.B.R. Kiiskila. 1998. Biomass and nutrient allocation in Douglas-fir and Amabilis fir seedlings: Influence of growth rate and nutrition. Tree Physiol. 18:803–810.

Hawkins, B.J., S.B.R. Kiiskila, and G. Henry. 1999. Biomass and nutrient allocation in Douglas-fir and Amabilis fir seedlings: influence of growth rate and temperature. Tree Physiol. 19:59–63.

Helmisaari, H. 1992. Nutrient retranslocation within the foliage of *Pinus sylvestris*. Tree Physiol. 10:45–58.

Imo, M., and V.R. Timmer. 1992. Nitrogen uptake of mesquite seedlings at conventional and exponential fertilization schedules. Soil Sci. Soc. Am. J. 56:927–943.

Imo, M., and V.R. Timmer. 1997. Vector diagnosis of nutrient dynamics in mesquite seedlings. For. Sci. 43(2):268–273.

Ingestad, T., and G.I. Agren. 1995. Plant nutrition and growth: Basic principles. Plant Soil 168/169:15–20.

Ingestad, T., and A. Lund. 1986. Theory and techniques for steady-state mineral nutrition and growth of plants. Scand. J. For. Res. 1:439–453.

Johnson, D.W., D.W. Cole, C.S. Bledsoe, K. Cromack, R.L. Edmonds, S.P. Gessel, C.C. Grier, B.N. Richards, and K.A. Vogt. 1982. Nutrient cycling in the forest of the Pacific Northwest. p. 186–232. *In* R.L. Edmonds (ed.) Analysis of coniferous forest ecosystems in the western United States. US/IBP Synthesis series 14. Hutchinson Ross Publishing Company, Stroudsburg, PA.

Keenan, R.J., C.E. Prescott, and J.P. Kimmins. 1995. Litter production and nutrient resorption in western red cedar and western hemlock forest on northern Vancouver Island. British Columbia. Can. J. For. Res. 25:1850–1857.

Kim, Y.T., C. Glerum, J. Stoddart, and S.J. Colombo. 1987. Effect of fertilization on free amino acid concentration in black spruce and jack pine containerized seedlings. Can. J. For. Res. 17:27–30.

Lim, M.T., and J.E. Cousens. 1986. The internal transfer of nutrients in Scot pine stand. I. Biomass components, current growth and their nutrient contents. Forestry 59:1–16.

Lowther, J.R. 1980. Use of a single sulphuric-hydrogen peroxide digest for the analysis of *Pinus radiata* needles. Commun. Soil Sci. Plant Anal. 11:175–188.

Malik, V. 1998. Growth and nutrient dynamics of nutrient-loaded black spruce seedlings in relation to neighbouring vegetation on boreal mixedwood sites. Ph.D. thesis. Univ. of Toronto, Canada.

Malik, V., and V.R. Timmer. 1996. Growth, nutrient dynamics, and interspecific competition of nutrient-loaded black spruce seedlings on a boreal mixedwood site. Can. J. For. Res. 26:1651–1659.

Malik, V., and V.R. Timmer. 1998. Biomass partitioning and nitrogen retranslocation in black spruce seedlings on competitive mixedwood sites: a bioassay study. Can. J. For. Res. 28:206–215.

McAlister, J.A., and V.R. Timmer. 1998. Nutrient enrichment of white spruce seedlings during nursery culture and initial plantation establishment. Tree Physiol. 18:195–202.

McCarthy, T.G., R.W. Arnup, J. Nieppola, B.G. Merchant, K.C. Taylor, and W.J. Parton. 1994. Field Guide for Forest Ecosystems of Northeastern Ontario. NEST Field Guide FG-001. Queen's Printer for Ontario, Timmins, ON, Canada.

Millard, P. 1996. Ecophysiology of the internal cycling of nitrogen for tree growth. Z. Pflanzenernaehr. Bodenkd. 159:1–10.

Millard, P., and G.H. Neilsen. 1989. The influence of nitrogen supply on the uptake and remobilization of stored N for the seasonal growth of apple trees. Ann. Bot. 63:301–309.

Millard, P., and M.F. Proe. 1992. Storage and internal cycling of nitrogen in relation to seasonal growth of Sitka spruce. Tree Physiol. 10:33–43.

Millard, P., and M.F. Proe. 1993. Nitrogen uptake, partitioning and internal cycling in *Picea sitchensis* (Bong.) Carr. as influenced by nitrogen supply. New Phytol. 125:113–119.

Miller, H.G. 1984. Dynamics of nutrient cycling in plantation ecosystems. p. 137–146. *In* J.R. Aldous (ed.) Wood for energy: The implications for harvesting, utilization, and marketing. Inst. of Chartered Foresters. Edinburgh, England.

Miller, H.G. 1995. The influence of stand development on nutrient demand, growth, and allocation. Plant Soil 168/169:225–232.

Miller, H.G., J.M. Cooper, and J.D. Miller. 1976. Effect of nitrogen supply on nutrient in litter fall and crown leaching in a stand of Corsican pine. J. Appl. Ecol. 13:233–256.

Miller, H.G., J.M. Cooper, J.D. Miller, and O.J. Pauline. 1979. Nutrient cycles in pines and their adaptation to poor soils. Can. J. For. Res. 9:19–26.

Munson, A.D., H.A. Margolis, and D.G. Brand. 1995. Seasonal nutrient dynamics in white pine and white spruce in response to environmental manipulation. Tree Physiol. 15:141–149.

Nambiar, E.K.S., and D.N. Fife. 1987. Growth and nutrient retranslocation in needles of radiata pine in relation to nitrogen supply. Ann. Bot. 60:147–156.

Nambiar, E.K.S., and D.N. Fife. 1991. Nutrient retranslocation in temperate conifers. Tree Physiol. 9:185–207.

Nelson, L.E., M.G. Shelton, and G.L. Switzer. 1995. The influence of nitrogen application on resorption of foliar nutrients in sweetgum. Can. J. For. Res. 25:298–306.

Nilsson, U., P. Gemmel, and J.E. Hallgren. 1986. Competing vegetation effects on initial growth of planted *Picea abies* N.Z. J. For. Sci. 26:84–98.

Orlander, G., U. Nilsson, and J.E. Hallgren. 1986. Competition for water and nutrients between grown vegetation and planted *Picea abies*. N.Z. J. For. Sci. 26:99–117.

Potter, C.S., H.L. Ragsdale, and C.W. Berish. 1987. Resorption of foliar nutrients in a regenerating southern Appalachian forest. Oecologia (Berlin) 73:268–271.

Proe, M.F., and P. Millard. 1994. Relationship between nutrient supply, nitrogen partitioning, and growth in young Sitka spruce (*Picea sitchensis*). Tree Physiol. 14:75–88.

Quoreshi, M., and V.R. Timmer. 2000. Growth, nutrient dynamics, and ectomycorrhizal development of container-grown *Picea mariana* seedlings in response to exponential nutrient loading. Can. J. For. Res. 30:191–201.

Ralhan, P.K., and S.P. Singh. 1987. Dynamics of nutrients and leaf mass in central Himalayan forest trees and shrubs. Ecology 68:1974–1983.

Rapp, M., M.C.L. Lecterc, and P. Lossaint. 1979. The nitrogen economy in *Pinus pinea* L. stand. For. Ecol. Manage. 2:221–231.

SAS Institute. 1989. SAS/START User's guide. SAS Inst. Cary, NC.

Saur, E., E.K.S. Nambiar, and D.N. Fife. 2000. Foliar nutrient retranslocation in *Eucalyptus globulus*. Tree Physiol. 20:1105–1112.

Schlesinger, W.H., E.H. De Lucia, and W.D. Billings. 1989. Nutrient efficiency of woody plants on contrasting soils of the western Great Basin, Nevada. Ecology 70:105–113.

Shaver, G.R., and J.M. Melillo. 1984. Nutrient budgets of Marsh plants: efficiency concepts and relation to availability. Ecology 65:1491–1510.

Soil Classification Working Group. 1998. The Canadian system of Soil Classification. Publ. 1646 (Revised) Agric. and Agric. Food Can. NRC Research Press, Ottawa, ON.

Switzer, G.L., and L.E. Nelson. 1972. Nutrient accumulation and cycling in loblolly pine (*Pinus taeda* L) plantation ecosystems: the first 20 years. Soil Sci. Soc. Am. Proc. 36:143–147.

Timmer, V.R. 1991. Interpretation of seedling analysis and visual symptoms. p. 113–114. *In* R. van den Driessche (ed.) Mineral nutrition of conifer seedlings. CRC Press, Boca Raton, FL.

Timmer, V.R. 1997. Exponential nutrient loading: a new fertilization technique to improve seedling performance on competitive sites. New For. 13:279–299.

Timmer, V.R., and A. Aidelbaum. 1996. Manual for exponential nutrient loading of seedlings to improve transplanting performance on competitive forest sites. NODA/NFP Tech. Rep. TR25. Nat. Res. Canada. Canadian Forest Services, Sault Ste Marie, ON, Canada.

Timmer, V.R., and A.D. Munson. 1991. Site-specific growth and nutrition of planted *Picea mariana* in the Ontario Clay Belt. IV Nitrogen loading response. Can. J. For. Res. 21:1058–1065.

Turner, J. 1977. Effects of nitrogen availability on nitrogen cycling in a Douglas-fir stand. For. Sci. 23:307–316.

van den Driessche, R. 1985. Late season fertilization, mineral nutrient reserves, and retranslocation in planted Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco) seedlings. For. Sci. 31:485–496.

van den Driessche, R. 1991. Effects of nutrients on stock performance in the forest. p. 229–260. *In* Mineral nutrition of conifer seedlings. CRC Press. Boca Raton, FL.

Xu, X.J., and V.R. Timmer. 1999. Growth and nitrogen nutrition of Chinese fir seedlings exposed to nutrient loading and fertilization. Plant Soil 216:83–91.