

## Response of Douglas-fir seedlings to a brief pulse of $^{15}\text{N}$ -labeled nutrients

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Received December 20, 2002; accepted June 7, 2003; published online November 3, 2003

**Summary** The temporal distribution of soil nutrients is heterogeneous, and thus the uptake, storage and later remobilization of brief nutrient pulses may be critical for growth in nutrient-limited habitats. We investigated the response of 2-year-old Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) seedlings receiving a low nutrient supply to a 15-day nutrient pulse (containing 250 ppm nitrogen (N) as 10 atom %  $^{15}\text{NH}_4^{15}\text{NO}_3$ ). The nutrient pulse was imposed in late July, toward the end of the seedlings' third growing season, and subsequent changes in dry mass and N content over the following 3 months were determined from destructive harvests. We tested three hypotheses: (1) N from the nutrient pulse is rapidly assimilated and accumulated primarily in needles and roots; (2) this accumulated N is later remobilized to support new growth; and (3) the nutrient pulse leads to a larger second flush of shoot growth. Seedlings increased their N content by 175 mg (67%) in response to the nutrient pulse. Nitrogen was taken up preferentially into younger tissues, especially the secondary flush and current-year roots. Immediately after the nutrient pulse, tissue N concentrations were high and supported subsequent increases in dry mass. Over 3 months, seedlings receiving the nutrient pulse added twice as much dry mass as control seedlings, and even after 3 months of growth, N concentrations remained greater than in controls. Current-year and older needles were the only components whose dry mass did not increase over this period. The nutrient pulse increased the size of the second flush, but it was still a minor component of increments in dry mass (~10% of the total dry mass increment) and N content (23%). The relatively modest increases in N content during autumn could be accounted for by soil uptake and there was no evidence that N was remobilized to support growth of new tissues. Short-term (15 days) elevated N uptake led to sustained growth in the long term (>3 months), and thus growth rate was to a large extent decoupled from current nutrient supply.

**Keywords:**  $^{15}\text{N}$ , allocation, dry mass, growth, height, nitrogen, nutrient flush, nutrient pulse, partitioning, *Pseudotsuga menziesii*, remobilization.

### Introduction

In forest soils, such as those of the Pacific Northwest of the USA, nitrogen (N) is the element most commonly limiting growth (Gessel et al. 1973). It was commonly assumed that organic N must be converted into inorganic N (nitrate and ammonium) to be available for plant uptake, but recent studies indicate that many species can utilize organic N and thus partially overcome this bottleneck (Näsholm and Persson 2001). Nevertheless, because the quantitative importance of organic N uptake in field situations is poorly characterized (Näsholm and Persson 2001), we restrict our discussion to inorganic N. The supply of inorganic N is determined partly by rates of mineralization and nitrification, which vary spatially and temporally (Turner et al. 1993, Laverman et al. 2000). Consequently, inorganic N may be present only during brief periods when conditions for mineralization or nitrification are favorable (e.g., optimal soil water content, temperature). The window of opportunity for uptake by roots is reduced further because inorganic N can be rapidly immobilized by soil microbes (Vitousek et al. 1982, Lodge et al. 1994). Hence, inorganic N may be available for plant uptake only as brief pulses. These pulses of inorganic N may constitute a potentially large N source for plants in infertile environments (Chapin 1980, Grime 1988).

Application of nitrogenous fertilizer leads to similar large and transient pulses in the availability of inorganic N (Miller 1981). In *Pseudotsuga menziesii* (Mirb.) Franco, for example, most fertilizer N is taken up within 24 weeks of application (Heilman et al. 1982). Nevertheless, many conifers show large and sustained growth responses to N fertilizer (e.g., Mitchell et al. 1996). Long-term growth responses are not due to additional uptake of fertilizer N, but are a result of internal N cycling (Proe et al. 1992). Therefore, the ability to take up, store and later remobilize brief N pulses may be critical for survival in N-limited habitats and a determinant of responses to N fertilizer, yet the importance of brief pulses of inorganic N has received little attention in coniferous species.

Storage and internal cycling of N allows plants to uncouple growth from current nutrient supply. Plants take up N when it

is available and grow when conditions are conducive for growth (Nambiar and Fife 1987). Internal cycling may contribute a large proportion of the N demand of trees (Nambiar and Fife 1991, Millard 1996). In closed-canopy conifer stands, up to 66% of required nutrients may be obtained from older foliage (Miller 1995). In seedlings of *Picea sitchensis* Bong. (Carr.) and *P. menziesii*, N demand during bud break in spring is met almost entirely by remobilization of N taken up and stored during the previous autumn (van den Driessche 1985, Millard and Proe 1992).

Studies on N remobilization in conifers have primarily examined removal of N from foliage prior to senescence (e.g., Vitousek 1982), or remobilization from old to expanding foliage during spring (Krueger 1967, Millard and Proe 1992, Hawkins and Henry 1999). Few studies have examined N remobilization within seasons, in response to a brief but large pulse of N. In the present study, we examined the response of Douglas-fir seedlings grown with a low nutrient supply to a 15-day nutrient pulse imposed at the end of the main growing season. The nutrient pulse contained 250 ppm N (as 10 atom %  $^{15}\text{NH}_4^{15}\text{NO}_3$ ) to allow us to distinguish between N uptake from the nutrient pulse and N uptake from the soil. We tested three hypotheses: (1) N from the nutrient pulse is rapidly assimilated and stored primarily in needles and roots; (2) this stored N is later remobilized to support new growth; and (3) the nutrient pulse leads to a larger second flush of growth, as has been shown previously for Douglas-fir (Carter and Klinka 1986).

## Materials and methods

### *Plant material*

Cold-stored clonal 1 + 1 transplanted, nursery-grown seedlings (Line #615A, CellFor, Victoria, Canada) of coastal Douglas-fir were obtained in mid-May 2001. Seedlings were defrosted and planted into 3-dm<sup>3</sup> pots filled with quartz sand. The root plug (about 0.300 dm<sup>3</sup> in volume) of peat and perlite was left intact and served as the low background nutrient supply. Seedlings were grown outdoors at the University of Victoria (Victoria, Canada) in a shade-house transmitting 70% of incident light. Seedlings were watered to field capacity every second day and received no external nutrients for 2 months. For Douglas-fir seedlings growing in Victoria, the primary period of shoot extension is from May until mid-July (Hawkins and Henry 1999). After this date, seedlings may undergo a second flush and continue height growth. Treatments were imposed after primary extension was complete, but before the second flush of growth commenced.

### *Experimental design and treatments*

Fifty-four seedlings were studied. The experiment comprised two nutrient treatments (control, nutrient pulse) and three harvest dates with nine replicate seedlings per treatment at each harvest. Seedlings were arranged in three classes based on their height on July 23, and each treatment and harvest date was randomly allotted three seedlings from each height class. The stratified random design ensured that there was no signifi-

cant difference in seedling height (on July 23) between treatments or among harvest dates (*t*-test,  $P > 0.05$ ). Once seedlings had been assigned to treatments and harvest dates, they were randomly placed in the shade-house and rearranged every 2 weeks throughout the experiment.

From July 24 to August 8 (15 days), seedlings in the nutrient pulse treatment were irrigated every day with 0.1 dm<sup>3</sup> of a balanced nutrient solution containing 250 ppm N (as 10 atom %  $^{15}\text{NH}_4^{15}\text{NO}_3$ ). Only the soil was irrigated to avoid  $^{15}\text{N}$  contamination of shoots. The nutrient solution, modified from Hawkins et al. (1998), contained in addition to N: 60 ppm P, 100 ppm K, 50 ppm Mg, 80 ppm Ca and a commercial micro-nutrient mixture (Plant Products Micromix, Grace-Sierra Horticultural Products, Milipitas, CA) delivering 1.05 ppm Fe, 0.3 ppm Mn, 0.06 ppm Zn, 0.015 ppm Cu, 0.19 ppm B, and 0.009 ppm Mo. The total amount of N added to each pot was 375 mg. At the same time, seedlings in the control treatment received the same volume of water. On August 9, seedlings in both treatments were irrigated with about 5 dm<sup>3</sup> of water—equivalent to 10 to 15 times the soil water solution—to flush the nutrient solution from the pots. From August 9, seedlings in both treatments received only water.

### *Destructive harvests*

Destructive harvests of nine seedlings per treatment were made on August 8, September 26 and November 11. Seedling height was measured and tissues separated into current-year roots (RC), old roots (RO), current-year stem (SC), old stem (SO), current-year needles (NC) and old needles (NO). Primary growth was complete when treatments were implemented, and thus we could easily identify shoot and needle material produced after the treatments because it was the second flush of growth (Sec). Current-year roots were defined as those that extended beyond the peat plug into the sand, whereas roots inside the plug were classified as old roots. The sand from each pot was sieved and any remaining roots were recovered and added to the current-roots category. Roots were washed three times with deionized water and subsamples of all components were placed in muslin bags and stored in liquid N. The remainder was dried at 70 °C to constant mass and weighed.

### *Total N and $^{15}\text{N}$ enrichment*

Samples stored in liquid N were freeze-dried, and weighed before analysis. Subsamples for determination of total N and  $^{15}\text{N}$  were ground in a ball mill (Wig-L-Bug C32003A, Rinn Dentsply, IL). Total N and  $^{15}\text{N}$  enrichment were determined on all samples from the N-pulse treatment and a subsample from the control treatment with a continuous-flow isotope ratio mass spectrometer (Integra, Europa Scientific, Crewe, U.K.) at the Stable Isotope Facility of UC Davis. Total N of remaining samples from the control treatment was determined with an elemental analyzer (Flash EA 1112 Series, ThermoQuest, Rodano, Italy).

To characterize isotopic enrichment and partitioning patterns, calculations were adapted from Deléens et al. (1994).

We calculated  $^{15}\text{N}$  atom % (A%) as:

$$A\% = 100 \frac{^{15}\text{N}}{^{14}\text{N} + ^{15}\text{N}}$$

Relative specific allocation (RSA), defined as the proportion of newly acquired N relative to total N of that component, was calculated as:

$$\text{RSA} = \frac{A\%_{\text{pulse}} - A\%_{\text{control}}}{A\%_{\text{labeled solution}} - A\%_{\text{unlabeled solution}}}$$

We assumed  $A\%_{\text{unlabeled solution}}$  was 0.366%, and used a mean value of 0.37% determined on a subsample of control samples for  $A\%_{\text{control}}$ . Given that  $A\%_{\text{pulse}}$  varied between 2.9 and 6.7%, the use of mean  $A\%_{\text{control}}$  would have only a minor effect on calculations. The mass of N taken up from the labeled nutrient solution (Pulse N) was calculated as:

$$\text{Pulse N} = \text{RSA}_{\text{component}} \times \text{DM}_{\text{component}} \times N_{\text{mass}}$$

where  $\text{DM}_{\text{component}}$  and  $N_{\text{mass}}$  are dry mass and N concentration of that component, respectively. The size of the unlabeled background pool of N (Background N) was determined similarly:

$$\text{Background N} = (1 - \text{RSA}_{\text{component}}) \times \text{DM}_{\text{component}} \times N_{\text{mass}}$$

The partitioning coefficient (%P) describes the partitioning of N among the different components as a percentage of total N, and was calculated separately for the Pulse N and Background N:

$$\%P_{\text{Pulse N}} = 100 \frac{\text{Pulse N}_{\text{component}}}{\text{Pulse N}_{\text{seedling}}}$$

$$\%P_{\text{Background N}} = 100 \frac{\text{Background N}_{\text{component}}}{\text{Background N}_{\text{seedling}}}$$

Uptake of labeled N during the chase period (see Figure 1) meant that we could not distinguish between uptake of  $^{15}\text{N}$  from the soil and remobilization of  $^{15}\text{N}$  within seedlings. Therefore, we present only RSA and %P data for the first harvest.

## Results

### Uptake and partitioning of N

Seedlings contained between 250 and 300 mg of N not derived from the nutrient pulse (Background N), and this amount did not vary between treatments or among harvest dates ( $P > 0.05$ ) (Figure 1). Seedlings took up, on average, 175 mg of N from the nutrient pulse between July 24 and August 8 (Table 1, Figure 1). After August 8, seedlings continued to take up N from the nutrient pulse, and thus pulse-derived N was significantly

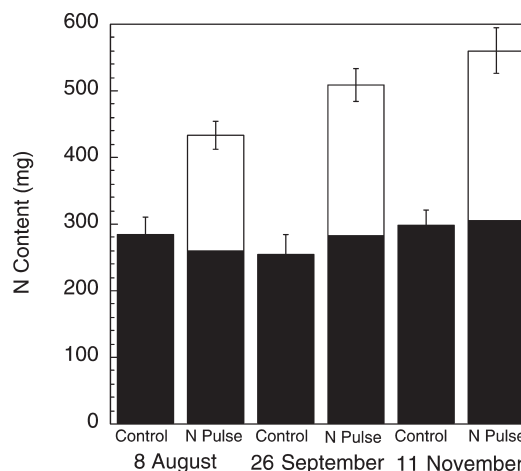


Figure 1. Nitrogen (N) content of Douglas-fir seedlings harvested on August 8, September 26, and November 11. Half of the seedlings received a nutrient pulse (N pulse) from July 24 until August 8. Control seedlings did not receive the nutrient pulse. Nitrogen derived from the N pulse and background N from the peat:perlite root plug are indicated by the unshaded and shaded bars, respectively. Data are means of nine replicates, the error bars are one standard error.

greater on September 26 (227 mg) and November 11 (255 mg) compared with August 8 (175 mg).

We determined RSA to evaluate the capacity of different components to accumulate Pulse N, independently of their size. On August 8, RSA was 0.40 for whole seedlings (Table 1) and varied significantly among different components. The RSA was greater in current-year tissues than in older tissues, and was especially high in the secondary flush (0.66) and current-year roots (0.64). The RSA of current-year needles (0.40) was the same as for the whole seedling.

The percentage of whole-seedling N in any given component (%P, partitioning) was determined separately for Background N and Pulse N. Partitioning of Background N and Pulse N was similar for current-year needles, which contained between 33 and 34% of whole-seedling N. For all other components, partitioning of Pulse N and Background N differed significantly, with more Pulse N being partitioned to younger components and less to older components. For example, compared with Background N, more Pulse N was partitioned to the secondary flush (2.5 versus 0.9%), current-year roots (18.7 versus 7.1%), and current-year stems (7.5 versus 5.4%). Conversely, less Pulse N than Background N was partitioned to old needles (11 versus 20%), old roots (16 versus 19%) and old stems (12 versus 14%).

### Temporal changes in height and dry mass

There was no significant difference in seedling height between August 8 and November 11 in either treatment, or between treatments (Figure 2).

On August 8, there was no difference between treatments in dry mass (DM) of whole seedlings or seedling components, whereas on September 26 and November 11, DM was significantly greater in N-pulsed seedlings than in control seedlings

Table 1. Uptake of nitrogen (N) into different organs of Douglas-fir seedlings harvested on August 8, following the supply of 250 ppm N from July 24 until August 8. Seedlings were divided into current-year roots (RC), old roots (RO), current-year stem (SC), old stem (SO), current-year needles (NC), and old needles (NO). Shoots and needles produced after treatments commenced were placed in their own category (Sec). Background N is unlabeled N not derived from the nutrient pulse. Pulse N is the amount of N derived from the nutrient pulse. Relative specific allocation (RSA) is the proportion of newly incorporated N relative to the total N content of that component. Partitioning of Background N and Pulse N (%P) is the amount of Background N (or Pulse N) in that component as a percentage of whole-seedling Background N (or Pulse N). Data are means of nine replicates, one standard error is given in parenthesis. Differences in the partitioning of Background N and Pulse N were compared by paired *t*-test, and significance (*P*) is indicated: ns = not significant; \* = *P* < 0.05; \*\* = *P* < 0.01; and \*\*\* = *P* < 0.001.

Component	Background N (mg)	Pulse N (mg)	RSA	%P <sub>Background N</sub>	%P <sub>Pulse N</sub>
Sec	2.3 (0.2)	4.3 (0.4)	0.66 (0.02)	0.88 (0.07)	2.5 (0.2) ***
NC	88 (6)	57 (4)	0.40 (0.01)	33.8 (0.9)	33 (1) ns
NO	51 (5)	19 (3)	0.27 (0.02)	20 (1)	11 (1) ***
RC	18 (2)	33 (3)	0.64 (0.01)	7.1 (0.5)	18.7 (0.7) ***
RO	48 (5)	27 (3)	0.36 (0.01)	19 (2)	16 (2) **
SC	14 (1)	13 (1)	0.48 (0.02)	5.4 (0.2)	7.5 (0.3) ***
SO	38 (4)	21 (2)	0.36 (0.02)	14.4 (0.7)	11.9 (0.4) ***
Total	259 (14)	175 (9)	0.40 (0.01)		

(Figure 3, Table 2). The increase in dry mass ( $\Delta$ DM) between August 8 and November 11 was twice as great in N-pulsed seedlings (32 g) as in control seedlings (14 g). Relative changes in dry mass of the different components were generally similar between treatments. In seedlings in both treatments, current-year needles and old needles were the only components whose dry mass did not increase between August 8 and November 11. In both control and N-pulsed seedlings, about one-third of the increase in dry mass occurred in older stems and a further 27–28% in current-year roots. The increase in dry mass of old roots accounted for 13% of the increase in dry mass of N-pulsed seedlings and 24% in control seedlings. The secondary flush contributed only 3.2% to the increase in dry mass of N-pulsed seedlings and 1.5% in control seedlings. The ratio of root dry mass to shoot dry mass in-

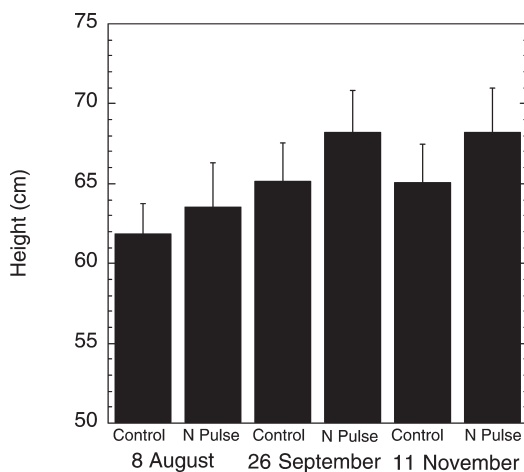


Figure 2. Heights of Douglas-fir seedlings harvested on August 8, September 26 and November 11. Half of the seedlings received a nutrient pulse (N pulse) from July 24 until August 8. Control seedlings did not receive the nutrient pulse. Data are means of nine replicates, the error bars are one standard error.

creased with time in both treatments, and was significantly less in N-pulsed seedlings than in control seedlings (Figure 3).

#### Temporal changes in nitrogen content and concentration

In control seedlings, neither total N content nor the N content of any component varied between August 8 and November 11 (Table 3). In contrast, the N content of N-pulsed seedlings increased by 127 mg between August 8 and November 11 (Figure 1, Table 3). Increases in N content occurred in current-year roots (+51 mg), old stems (+41 mg), the secondary flush (+29 mg) and current-year stems (+16 mg). The N content of current-year needles, old needles and old roots did not change during the same period.

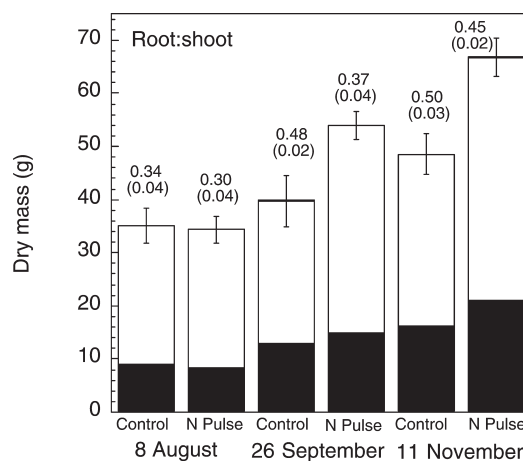


Figure 3. Dry mass of Douglas-fir seedlings harvested on August 8, September 26 and November 11. Half of the seedlings received a nutrient pulse (N pulse) from July 24 until August 8. Control seedlings did not receive the nutrient pulse. Shown separately are shoot dry mass (unshaded bars) and root dry mass (shaded bars). Numbers above bars are ratios of root dry mass to shoot dry mass, with standard error in parentheses. Data are means of nine replicates, the error bars are one standard error.

Table 2. Dry mass (DM; g) of different components of Douglas-fir seedlings harvested on August 8, September 26 and November 11. Seedlings were divided into current-year roots (RC), old roots (RO), current-year stem (SC), old stem (SO), current-year needles (NC) and old needles (NO). Shoots and needles produced after treatments commenced were placed in their own category (Sec). Also indicated are differences in dry mass between August 8 and November 11 ( $\Delta$ DM). Data are means of nine replicates, one standard error is given in parentheses. Unpaired *t*-tests were used to assess the significance of (a) differences in dry mass between nutrient pulse and control seedlings (within dates), and (b) between August 8 and November 11 ( $\Delta$ DM) (within treatments). Significance (*P*) is indicated: ns = not significant; \* = *P* < 0.05; \*\* = *P* < 0.01; and \*\*\* = *P* < 0.001.

Component	N Pulse				Control			
	August 8	September 26	November 11	$\Delta$ DM	August 8	September 26	November 11	$\Delta$ DM
Sec	0.22 (0.02) ns	2.8 (0.5) ***	3.4 (0.7) ***	+3.2 ***	0.15 (0.03)	0.5 (0.1)	0.39 (0.08)	+0.2 *
NC	8.2 (0.7) ns	9.4 (0.7) ns	9.1 (0.7) ns	+0.8 ns	8.2 (0.9)	8 (1)	8.9 (0.8)	+0.8 ns
NO	5.1 (0.5) ns	5.0 (0.4) ns	5.1 (0.4) ns	-0.01 ns	5.7 (0.5)	4.5 (0.6)	5.3 (0.6)	-0.4 ns
RC	2.2 (0.2) ns	8.0 (0.5) ***	11 (1) **	+8.7 ***	2.6 (0.5)	3.7 (0.6)	6.4 (0.8)	+3.8 ***
RO	5.8 (0.6) ns	7 (1) ns	9.9 (0.9) ns	+4.1 **	6.7 (0.6)	9.0 (0.9)	9.9 (0.9)	+3.3 *
SC	2.0 (0.2) ns	3.7 (0.2) ***	5.6 (0.6) ***	+3.6 ***	1.5 (0.2)	2.0 (0.3)	2.8 (0.3)	+1.4***
SO	10.8 (0.9) ns	18 (1) ***	23 (1) ***	+11.8 ***	10.4 (0.9)	12 (1)	15 (1)	+4.4 *
Total	34 (2) ns	54 (3) *	67 (4) **	+32.3 ***	35 (3)	40 (5)	49 (4)	+13.5 *

In both N-pulsed and control seedlings, N concentration ( $N_{\text{mass}}$ ) of all components except old needles decreased significantly between August 8 and November 11 (Table 4). In all cases, the decrease was greater in N-pulsed seedlings than in control seedlings. Nevertheless, on November 11,  $N_{\text{mass}}$  in all components was still significantly greater in N-pulsed seedlings than in control seedlings. The temporal decrease in  $N_{\text{mass}}$  was particularly large in the secondary flush (N-pulsed,  $-20.8 \text{ mg g}^{-1}$ ; control,  $-5.7 \text{ mg g}^{-1}$ ) and current-year roots (N-pulsed,  $-14.6 \text{ mg g}^{-1}$ ; control,  $-5.0 \text{ mg g}^{-1}$ ).

## Discussion

Douglas-fir seedlings increased their N content by 67% in response to a 15-day pulse of high nutrient availability (Table 1) and in the three subsequent months, added twice as much dry mass as control seedlings (Figure 3). Immediately after the nutrient pulse, tissue N concentrations were high (Table 4, cf. Hawkins and Henry 1999), and supported subsequent in-

creases in dry mass (Table 2). Three months after the nutrient pulse, N concentrations remained greater in the N-pulsed seedlings than in control seedlings, and if the experiment had not been terminated, this probably would have led to continued differences in growth rates between treatments during the next growing season (e.g., Timmer 1997). Douglas-fir typically inhabits N-deficient soils (Gessel et al. 1973), and as soil fertility declines, nutrient supply normally becomes restricted to brief unpredictable flushes (Chapin 1980). Therefore, the large responses of growth and N uptake to a brief nutrient pulse are probably a major determinant of Douglas-fir's fitness in N-limited habitats (Crick and Grime 1987). The large response of Douglas-fir to N fertilizer (e.g., Mitchell et al. 1996) also is probably due to the capacity of this species for elevated nutrient uptake in the short term, leading to sustained growth in the long term.

There are few comparative data for coniferous species despite the significance of temporal variation in nutrient supply. In contrast to the large growth response that we observed,

Table 3. Nitrogen content (N; mg) of different components of Douglas-fir seedlings harvested on August 8, September 26 and November 11. Seedlings were divided into current-year roots (RC), old roots (RO), current-year stem (SC), old stem (SO), current-year needles (NC) and old needles (NO). Shoots and needles produced after treatments commenced were placed in their own category (Sec). Also indicated are differences in N content between August 8 and November 11 ( $\Delta$ N). Data are means of nine replicates, one standard error is given in parentheses. Unpaired *t*-tests were used to assess the significance of (a) differences in N content between nutrient pulse and control seedlings (within dates), and (b) between August 8 and November 11 ( $\Delta$ N) (within treatments). Significance (*P*) is indicated: ns = not significant; \* = *P* < 0.05; \*\* = *P* < 0.01; and \*\*\* = *P* < 0.001.

Component	N Pulse				Control			
	August 8	September 26	November 11	$\Delta$ N	August 8	September 26	November 11	$\Delta$ N
Sec	6.6 (0.5) ***	26 (4) ***	35 (8) ***	+29 **	1.8 (0.3)	3.1 (0.7)	2.4 (0.5)	+0.7 ns
NC	144 (9) ***	142 (9) ***	138 (9) ***	-6 ns	75 (8)	67 (10)	74 (6)	-2 ns
NO	70 (6) ns	68 (5) ***	68 (5) **	-2 ns	56 (5)	39 (5)	48 (5)	-8 ns
RC	51 (4) *	96 (6) ***	103 (10) ***	+51 ***	31 (6)	26 (4)	44 (6)	+13 ns
RO	74 (8) ns	62 (9) ns	73 (7) ns	-1 ns	64 (5)	68 (7)	66 (6)	+2 ns
SC	27 (2) ***	30 (2) ***	43 (4) ***	+16 **	10 (2)	10 (1)	15 (1)	+4 ns
SO	58 (5) ns	83 (5) ***	99 (8) ***	+41 ***	46 (5)	40 (5)	50 (3)	+3 ns
Total	434 (27) ***	509 (25) ***	560 (35) ***	+127 **	285 (25)	253 (30)	299 (22)	+14 ns

Table 4. Nitrogen concentration ( $N_{\text{mass}}$ ;  $\text{mg g}_{\text{DM}}^{-1}$ ) of different components of Douglas-fir seedlings harvested on August 8, September 26 and November 11. Seedlings were divided into current-year roots (RC), old roots (RO), current-year stem (SC), old stem (SO), current-year needles (NC) and old needles (NO). Shoots and needles produced after treatments commenced were placed in their own category (Sec). Also indicated are differences in N concentration between August 8 and November 11 ( $\Delta N_{\text{mass}}$ ). Data are means of nine replicates, one standard error is given in parentheses. Unpaired *t*-tests were used to assess the significance of (a) differences in N concentration between nutrient pulse and control seedlings (within dates) and (b) between August 8 and November 11 ( $\Delta N_{\text{mass}}$ ) (within treatments). Significance (*P*) is indicated: ns = not significant; \* =  $P < 0.05$ ; \*\* =  $P < 0.01$ ; and \*\*\* =  $P < 0.001$ .

Component	N Pulse				Control			
	August 8	September 26	November 11	$\Delta N_{\text{mass}}$	August 8	September 26	November 11	$\Delta N_{\text{mass}}$
Sec	31 (2) ***	9.8 (0.3) ***	10.1 (0.3) ***	-20.8 ***	11.8 (0.4)	6.3 (0.4)	6.1 (0.2)	-5.7 ***
NC	17.9 (0.7) ***	15.2 (0.5) ***	15.5 (0.6) ***	-2.4 *	9.2 (0.2)	8.2 (0.3)	8.2 (0.2)	-1.0 **
NO	14.0 (0.5) ***	13.7 (0.4) ***	13.7 (0.4) ***	-0.3 ns	9.8 (0.3)	8.9 (0.3)	9.1 (0.2)	-0.7 ns
RC	24 (2) ***	12.1 (0.3) ***	9.4 (0.2) ***	-14.6 ***	11.8 (0.2)	7.1 (0.3)	6.8 (0.1)	-5.0 ***
RO	12.8 (0.5) ***	8.6 (0.2) ***	7.4 (0.2) *	-5.4 ***	9.7 (0.5)	7.6 (0.2)	6.6 (0.2)	-3.1 ***
SC	14.1 (0.9) ***	8.3 (0.5) ***	7.7 (0.4) **	-6.4 ***	7.9 (0.3)	5.1 (0.2)	5.4 (0.6)	-2.5 **
SO	5.5 (0.2) ***	4.7 (0.1) ***	4.4 (0.3) *	-1.1*	4.4 (0.1)	3.4 (0.1)	3.4 (0.2)	-1.0 ***

*Picea sitchensis* (Bong.) Carrière seedlings preconditioned to low N showed no growth response to 22-day high-N pulses despite a 20–40% increase in N content (Proe and Millard 1995). The absence of a growth response and the smaller increase in N content in *P. sitchensis* may indicate that the background N supply, which was higher than in our study, was sufficient to support growth of *P. sitchensis*. In our study, there was no measurable uptake of unlabeled N (i.e., N from the peat/perlite root plug, Figure 1) and thus the background N supply was effectively nonexistent, which probably explains the large response of growth and N uptake to the nutrient pulse (cf. Proe and Millard 1995).

Our nutrient pulse treatment mimicked the situation in natural soils insofar as a fraction of N from the nutrient pulse was retained in the soil, and thus plants were to some extent buffered against extreme changes in N availability (Lodge et al. 1994). The most likely explanation for soil retention of N and its subsequent uptake after treatments stopped (the chase period) (Figure 1) is that N was taken up and immobilized in microbial biomass during the 15-day pulse treatment. The N in microbial biomass, together with that in recalcitrant, and physically protected N pools would not have been removed when pots were flushed with excess water. Hence, seedlings were buffered against an extreme drop in N availability that the termination of the nutrient pulse might otherwise have produced. Studies examining plant responses to temporally heterogeneous nutrient supply rarely consider this buffering effect of soil. Without the use of a  $^{15}\text{N}$ -labeled nutrient pulse, this continued uptake would have gone unnoticed, and we might have incorrectly assumed that the increases in N content in N-pulsed seedlings were associated with N uptake from the peat plug. Unfortunately, because of uptake of labeled N during the chase period, we could not distinguish between uptake of  $^{15}\text{N}$  from the soil and remobilization of  $^{15}\text{N}$  within seedlings, and thus our RSA and %P data were valid only for the first harvest.

Nitrogen from the nutrient pulse was taken up preferentially into younger components, with the secondary flush and current-year roots having particularly high relative uptake (RSA)

(Table 1). Current-year needles are considered to be major sites of N storage in Douglas-fir (e.g., Krueger 1967, van den Driessche 1985), and the largest absolute uptake was in current-year needles (57 mg, 33% of Pulse N). However, relative uptake (RSA = 0.40) of current-year needles was similar to that of the whole seedling, indicating that N was not preferentially allocated to current-year needles. In any case, trends in relative uptake of N may well be determined by growth rates of different organs, rather than any sort of preordained allocation to storage organs. Although it is difficult separating cause from effect (i.e., N uptake from growth rate), the data are consistent with N being preferentially allocated to those organs with high growth rates. Our conclusions must be tempered in light of the phenology of growth, and the observation that growth and N uptake of different organs vary seasonally (Hawkins et al. 1998).

There was little evidence that N was remobilized to support new growth, inasmuch as no component decreased its N content significantly (Table 3). Instead, growth was largely supported by N present in the tissues in early August, and the modest increases in N content could be accounted for by soil uptake. The nutrient pulse increased the size of the secondary flush, but there was no evidence that remobilized N supported this growth. This is consistent with previous studies in *P. sitchensis* (Millard and Proe 1992) and *Pinus radiata* D. Don (Nambiar and Fife 1987) where there was no evidence that internal cycling provided N for the second flush of growth. The absence of measurable remobilization may be accounted for by one or more of the following explanations. (1) Significant yet small remobilization may have been missed because of the relatively poor precision of our methodology. (2) The secondary flush may not constitute a large enough N sink for remobilization to be necessary. (3) Treatments may have been administered too late in the growing season. Generally, remobilization of N in conifers is important for spring growth, but becomes insignificant as the season progresses and soil uptake supports continued growth (Millard and Proe 1992, Proe et al. 2000). (4) The absence of remobilization may also be a

function of the young age of our seedlings. In young Douglas-fir, soil uptake is the main source of nutrients for growth (Hawkins and Henry 1999), with no remobilization evident until the third growing season (Hawkins et al. 1998).

The large response of Douglas-fir to temporal variation in nutrient supply contrasts with its inconsistent response to a spatially heterogeneous distribution of nutrients (Friend et al. 1990, George et al. 1997). Local N addition to a peat:vermiculite mixture increased root growth of Douglas-fir, particularly when seedlings were N-deficient (Friend et al. 1990), but no root response was seen in similar experiments in a forest mineral soil (George et al. 1997). The artificial substrate of Friend et al. (1990) may have led to higher root growth rates than in natural soil, and the lack of response noted by George et al. (1997) with a forest soil is likely a better representation of behavior in the field. Possession of a root system that is relatively unresponsive in terms of growth may be advantageous to Douglas-fir on sites where nutrients become temporarily available (Crick and Grime 1987, Campbell and Grime 1989). These findings are consistent with there being a trade-off or negative correlation between success in spatially variable habitats and success in temporally variable habitats.

We conclude that Douglas-fir seedlings have a large capacity for capturing N from brief nutrient pulses. Luxury uptake of N during brief and unpredictable pulses led to sustained increases in growth in the long-term. This was made possible by the storage and subsequent dilution of N in all tissues except old needles. These factors effectively decoupled growth rate from current nutrient supply and provide one means by which Douglas-fir copes with a temporally heterogeneous N supply.

#### Acknowledgments

This research was supported by grants from the Natural Sciences and Engineering Research Council of Canada. Samantha Robbins and Lindsay White are warmly thanked for expert technical help. CellFor Inc. is thanked for supplying seedlings of Douglas-fir and Brad Binges is acknowledged for his expert assistance growing seedlings. We thank Barbara Hawkins for many useful comments on a previous version of this paper.

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