Original article

Late-season fertilization of *Picea mariana* seedlings: intensive loading and outplanting response on greenhouse bioassays

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Abstract – Traditional greenhouse culture involves a late-season hardening period that withholds irrigation and fertilization from black spruce seedlings to promote frost-hardiness. Since nutrient uptake is limited without supplemental fertilization, growth during hardening may lead to internal nutrient dilution, a condition detrimental to field performance of seedlings. We examine whether late-season fertilization, applied as intensive loading, will counter dilution and build up nutrient reserves in seedlings reared conventionally or nutrient loaded before hardening. A mixed NPK fertilizer delivering 0, 12, 24, or, 48 mg N·seedling^{-1} for 9 weeks after bud set was tested. Root and shoot dry mass increased as much as 104 and 42% during hardening. Seedling biomass, however, was unchanged by late-season fertilization, but N uptake was increased 44–167% signifying induced luxury consumption. Extra K supplementation of treatments averted K dilution in plant tissues often occurring with high N addition. A 13-week outplanting trial on intact soil bioassays retrieved from a boreal site showed that growth and nutrient allocation were significantly enhanced by larger N reserves built up after intensive nutrient loading. About 72–80% of N required for new shoot growth was met from internal cycling, demonstrating the capacity of loading to enhance retranslocation. Intermediate loading (24 mg N) was most effective in promoting N accumulation and outplanting growth of both seedling types. Survival was reduced (30%) only at the highest dose. Study results demonstrate the potential advantage of these practices to improve growth of newly planted seedlings on northern forest sites.

black spruce / hardening period / nitrogen / nutrient loading / retranslocation / super-loading

Résumé – Fertilisation tardive de semis de *Picea mariana* : apport intensif et réponse après plantation en serre d'essais biologiques. Les cultures traditionnelles en serre comportent une période tardive d'endurcissement qui retarde l'irrigation et la fertilisation des semis de *Picea mariana* pour promouvoir l'endurcissement au froid. Puisque le prélèvement de nutriments est limité sans supplémentation de fertilisation, la croissance pendant la période d'endurcissement peut conduire à une dilution interne des nutriments, une situation préjudiciable pour les performances au champ des plants. Nous avons examiné si la fertilisation tardive, appliquée par des apports massifs peut contrebalancer la dilution et accroître les réserves de nutriments dans les semis élevés conventionnellement ou les nutriments apportés avant l'endurcissement. On a testé un mélange de fertilisant NPK libérant 0,12, 24 ou 48 mg d'azote par semis pendant 9 semaines après le débourrement. Le poids sec des racines et des pousses s'est accru de 104 et 42 % pendant l'endurcissement. Cependant la biomasse des semis a été inchangée par une fertilisation tardive, mais le prélèvement d'azote s'est accru de 44 à 167 %, indiquant une consommation de luxe. Une supplémentation en K évite une dilution en K des tissus, ce qui arrive souvent avec un apport important d'azote. Un essai à 13 semaines sur un sol pour essais biologiques, extrait d'une station boréale, a montré que la croissance et l'allocation des nutriments étaient significativement augmentées par de grandes réserves d'azote accumulé après des apports intensifs de nutriments. Environ 72 à 80 % de N requis pour la croissance des nouvelles pousses provenaient du cycle interne, démontrant l'importance des apports pour augmenter les retranslocations. Un apport intermédiaire (24 mg N) était plus efficace pour promouvoir l'accumulation de N et la croissance des deux types de semis. La survie a été réduite (30 %) seulement pour la plus forte dose. Les résultats de cette étude ont démontré l'avantage potentiel de ces pratiques pour l'amélioration de la croissance des semis dans les stations forestières du Nord.

Picea mariana **/ période d'endurcissement / azote / apport de nutriment / retranslocation / super apport**

1. INTRODUCTION

Intensive forestry in Canada involves large-scale reforestation programs that require high quality planting stock, conditioned for improved survival, nutrition and vigorous growth after outplanting. However, studies have shown that survival and growth of newly planted conifer seedlings may be reduced by low soil fertility or by intense plant competition [6, 20, 23].

Hence, nursery treatments that improve early plantation establishment may contribute significantly to reforestation success. Examples of current nursery techniques that have promise for improving field performance of seedlings are exponential fertilization [16, 18, 42, 45] and nutrient loading [37, 47]. These techniques nutritionally prepare and condition trees for the field environment by matching nutrient supply with crop demand to maximize nutrient reserves in seedlings prior to planting [36,

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48]. Superior growth of exponentially nutrient loaded seedlings was attributed to retranslocation of stored nutrients to active growth sinks soon after planting [24, 32, 36].

Traditional greenhouse cultural practices do not promote the build up of nutrient reserves in seedlings because of late-season cutback of fertilizer for hardening off purposes [4, 10, 22]. The hardening period is defined as the time interval following apical bud initiation when roots and shoots acquire frost hardiness [10]. Apical bud initiation is induced artificially within two-weeks of exposure to short-day photoperiod (8 h) once seed-lings attain a target height [1, 5, 9]. Subsequent fertilization and irrigation are usually reduced during hardening to induce mois-ture and nutrient stress in cultured plants. This practice may promote seedling drought and frost tolerance for winter storage and subsequent outplanting [12], but may also lower plant nutrient concentration due to reduced nutrient supply and dilu-tion effects associated with growth response despite stress induction [5]. For example, root dry mass increased as much as 200% [5] and 794% [33] in black spruce (*Picea mariana* [Mill.] B.S.P.) seedlings during hardening. Late-season fertili-zation is controversial, however, since some studies suggest it may negatively affect cold tolerance of conifer seedlings [2], others found no effects on cold tolerance of conifer seedlings [3], while still others report increased frost hardiness after application [35]. There is general consensus that fall N fertili-zation benefits subsequent field performance of newly planted trees because of increased N concentration and contents in the trees [19, 25, 39].

Biomass growth without sufficient fertilization during hard-ening can severely dilute plant nutrient reserves, thus compro-mising steady-state nutrition and nutrient loading efforts [2, 5, 28]. Steady-state nutrition is characterized by stable internal tissue nutrient concentration over time free from nutrient stress [17]. This state can be achieved by applying nutrients at expo-nentially increasing addition rates that correspond more closely with exponential growth and nutrient demand of crops during the exponential growth phase of plants [18]. Compared to con-stant feed fertilization used conventionally [44], this condition conforms more closely to the natural outplanting environment in terms of supply, flux and acquisition of soil nutrients for newly planted seedlings [30].

We have previously demonstrated that late-season fertiliza-tion as nutrient loading during hardening may counter nutrient dilution and further build nutrient reserves in seedlings for improved field performance [5]. At high rates, or extended nutrient loading, this practice effectively induced luxury uptake and raised seedling N concentration and other nutrient reserves in plants [5]. However, growth dilution still occurred when applications ended, reducing internal nutrient reserves to pre-hardening levels since uptake could not keep up with rapid growth. The results suggested more intensive loading at even higher rates for longer time periods (or "super loading") might prolong nutrient uptake, avert growth dilution and promote nutrient accumulation in seedlings. We also noted that plant K uptake was reduced at higher N addition, which indicated that intensified nutrient loading would need extra K to maintain nutrient balance in seedlings [5, 49]. The study focused on growth and nutritional development under nursery culture, but did not cover subsequent outplanting performance of test seed-lings.

This paper reports on a follow up of the previous study [5]. We test here whether application of super-loading regimes, supplying higher dose rates with longer delivery schedules, can enhance nutrient accumulation during the hardening period. A second objective was to assess whether N induced K dilution [5] could be avoided by enriching the applied fertilizer with more K (30%). The final objective was to test effects of inten-sified nutrient loading on outplanting performance of seedlings using pot bioassays. The general hypothesis addressed was that high-dose, late-season fertilization promotes seedling growth and nutrition under greenhouse culture and outplanting envi-ronments, such as on bioassays retrieved from the boreal forest.

2. MATERIALS AND METHODS

2.1. Plant material and pre-hardening fertility regimes

Black spruce seedlings were grown and over-wintered at a com-mercial greenhouse (North Gro Development Ltd.) located near Kirk-land Lake, Ontario (48° 10' N, 88° 01' W). Seeds were spring-sown (April, 1999) into 40 cm^3 cavities in Styroblock trays (format 470 con-taining 330 cavities·tray^{-1}) filled with a peat:vermiculite:perlite mix-ture (3:1:1, v/v/v). Germinated seedlings were reared under natural day-length with internal greenhouse temperatures averaging 16: 28 °C (daily night:day min.:max.). The crops were grown under two fertility regimes before hardening (Tab. I). The first treatment referred to as the conventional (C) fertilization regime (representing industry stand-ard practice), supplied a seasonal total of 19 mg N·seedling^{-1} based on a constant feed model described in detail by [44]. The second treat-ment, referred to as nutrient loading (NL) regime, delivered a seasonal total of 74 mg N·seedling^{-1} at exponentially increasing addition rates Timmer et al. [44]. A commercial water-soluble fertilizer (Plant Prod-ucts 20N-20P$_2$O$_5$-20K$_2$O, plus microelements) was applied to both regimes. The nutrient loaded treatment, however, was enriched with more K (30%) to avoid possible K dilution associated with high N addition [49]. The extra elemental K was supplied as KCl. Seedlings were sprayed with nutrients as pre-mixed fertilizer solutions using traveling booms with fixed nozzles and were subsequently rinsed with water to avoid fertilizer burn. This commenced one week after fer-nation and was carried out for 14 wk (Tab. I). Irrigation (including fer-tilization and final rinse) was to container capacity [46, 50], which assured that crops were returned to the same level of moisture avail-ability among treatments.

When seedlings reached target height (16 cm, 12 weeks after germi-nation), a two-week short-day treatment (8 h light with blackout cur-tains) was imposed to induce terminal bud-set and stop height growth [1]. The hardening period commenced after terminal bud set with a return to natural day-length and a gradual lowering of greenhouse tem-peratures (7:18 °C daily night:day min.:max.). Seedlings were hard-ened for 18 weeks (Tab. I) and then transferred to a cold storage facility (–2 °C) for over-wintering for about 5 months [11].

2.2. Late-season fertilization treatments and the experimental design

Immediately following bud set, four late-season fertilization treat-ments were tested within the conventionally (C) fertilized or nutrient loaded (NL) regimes. The four treatments were: (i) an unfertilized or control (0) simulating standard industry hardening practices, (ii) an extended loading treatment (1×) that supplied a cumulative total of 12 mg N·seedling^{-1} to avoid nutrient dilution [5], (iii) an intermediate super-loading treatment (2×) at 24 mg N·seedling^{-1}, and (iv) a high super-loading treatment (4×) at 48 mg N·seedling^{-1}. The two latter

Table I. Treatment schedule for containerized black spruce seedlings during greenhouse culture and after outplanting. The two main fertilization regimes: conventional (C) and nutrient loaded (NL) supplied cumulative totals of 19 and 74 mg N·seedling^{-1} before budset. The four late-season fertilization treatments within each of the C or NL regimes: control (0), extended loading (1×), intermediate super-loading (2×) and high super-loading (4×) supplied cumulative totals of 0, 12, 24 and 48 mg N·seedling^{-1} after bud set.

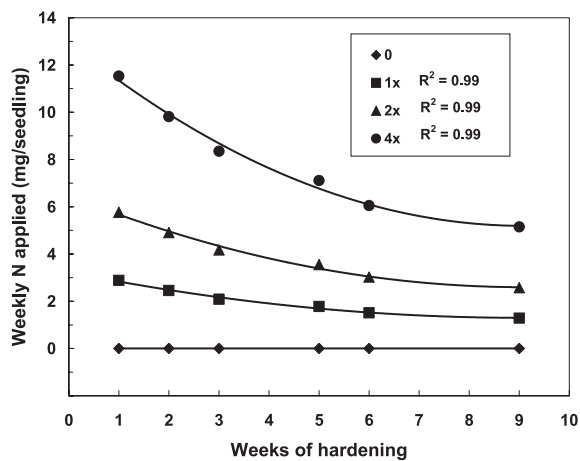| Year	Week	Cultural phase	Treatment
Year 1			
	0–1	Germination	Water only
	1–14	Exponential growth	Main fertilization (C and NL)
	14–16	Bud set	Short day
	18–27	Hardening	Late-season fertilization (0, 1×, 2× or 4×)
	27–36	Hardening	Water only
	36+	Cold-storage	
Year 2			
	0–13	Outplanting	Water only

Figure 1. Late-season fertilization regimes: unfertilized (0), extended loading (1×), intermediate super-loading (2×), and high super-loading (4×) treatments supplied cumulative totals of 0, 12, 24 and 48 mg N·seedling^{-1}, respectively, as a pre-mixed N, P, and K fertilizer solution. Delivery was scheduled for 6 wk, but was delayed for one and two weeks after wk 3 and 6, respectively, to allow adequate dry down and avoid leaching after fertilization.

treatments were expected to substantially build up nutrient reserves in seedlings during hardening (Fig. 1). All four treatments (0, 1×, 2× and 4×) were arranged within each of the two pre-hardening (C or NL) regimes as a completely randomized design with three replications.

The fertilizer delivery schedules were based on a reverse exponential function that proportionately matched nutrient addition rate with exponentially declining growth and nutrient uptake rates [18] during hardening off [1, 5, 11, 47]. In theory, the delivery schedule would avoid toxic accumulation of nutrients in the growing medium since nutrient addition rate is synchronized with plant growth and nutrient uptake rates [5, 18]. Calculation of the nutrient addition rate (r) was based on the function:

$$N_T = \sum_{i=1}^{t} N_f e^{-rt} \tag{1}$$

where N_T is the total amount of nutrients applied for t applications during hardening. The starting dose (N_f) is equal to the final dose applied

before hardening to avert potential nutrient stress during blackout [5]. Knowing r, the amount of fertilizer supplied for a specific application (N_t) was computed as:

$$N_t = N_f e^{-rt}. \tag{2}$$

The same commercial fertilizer mix (enriched with extra K as KCl) used before hardening was applied during hardening. Six ($t = 6$) weekly applications were scheduled, although the penultimate and final applications were delayed by one and two weeks, respectively, to allow adequate crop dry-down (Fig. 1). The required fertilizer amount (adjusted for over spray) was added to a watering can and sprayed on individual trays. Subsequently, trays were brought up to container capacity [50] with water only. At the end of the hardening period (week 36) seedlings were cold stored prior to outplanting.

2.3. Bioassay outplanting trial

The outplanting trial was comprised of 8 treatments: four within each of the conventional (C) and nutrient-loaded (NL) groups as unfertilized (0), extended loading (1×), intermediate super-loading (2×) and high super-loading (4×). The test seedlings were planted on blocks of intact soil substrates retrieved from a mature black spruce stand (FEC site type 4; see [27]) near Cochrane, Ontario (49º 04' N, 81º 01' W). The soil was a moderately drained, coarse sandy Orthic Humo-Ferric Podzol topped by a shallow organic layer. The ground cover was dominated by *Pleurozium* mosses. Thirty-two rectangular blocks of substrate (36 × 30 cm) were cut to a depth of 15 cm without disturbing surface vegetation and placed into plastic containers with pre-drilled drainage holes to allow free drainage and aeration. The containers were transported to the University of Toronto and placed on elevated benches in heated and ventilated greenhouse. Growth conditions were: temperature 18–25 ºC, humidity 65–85%, and an extended 18-h photoperiod supplemented with sodium vapor lamps at a light intensity of 250 µmol·s^{-1}m^{-2} at crop level. Seedlings were presorted to similar size at planting to minimize confounding effects due to initial differences in pre-plant size. Each bioassay pot was a replicate of one treatment. The pot was planted with nine seedlings at about 10 × 10 cm spacing. The pots were then arranged as a completely randomized design with four blocks as replicates. The pots were irrigated weekly to container capacity [50], and randomly re-arranged on the bench to reduce placement effects.

Table II. Effects of nutrient loading on seedling dry mass, shoot:root ratio, and N, P and K concentration (% dw) before hardening. The pre-hardening regimes: conventional and nutrient loaded treatments supplied cumulative totals of 19 and 74 mg N·seedling^{-1}.

Pre-hardening fertilization regimes[†]	Plant dry mass (mg)	Shoot/root ratio	Nutrient concentration (% dw)		
			N	P	K
Conventional (C)	560 (33)	2.73 (0.14)	1.33 (0.02)	0.26 (0.01)	0.67 (0.01)
Nutrient loaded (NL)	550 (18)	4.33 (0.21)	2.90 (0.07)	0.37 (0.01)	0.81 (0.03)

[†] Means (and standard error in parentheses) were estimated from random samples of 3 groups of 5 seedlings per tray within each of the C or NL regime treatments at the end of pre-hardening phase.

2.4. Sampling and statistical analyses

Seedlings were sampled before (week 18) and after (week 36) the hardening phase, and before and after transplanting for 13 weeks in the bioassay pots (Tab. I). After harvest, growing media was rinsed from roots and shoot lengths were recorded. Thereafter, seedlings were separated into roots and shoots, oven dried at 70 °C for 48 h, weighed, milled and composited by replicate or subsample for nutritional analysis according to Timmer and Armstrong [45]. Prehardening growth and nutrient responses were assessed on three subsamples of five trees each drawn from randomly selected trays reared under the conventional (C) or nutrient loaded (NL) regimes. Each regime was imposed in a separate part of the greenhouse without replication; hence results were presented as means and standard errors. Late-season fertilization responses were evaluated on a sample of five random seedlings per tray (replication) using a one-way ANOVA testing four treatments within each prehardening regime replicated three times. A similar one-way ANOVA was conducted on results from the outplanting trial based on the destructive sample of five trees per bioassay before and after transplanting. In this case there were eight treatments within the conventional (C) and nutrient loading (NL) regimes replicated three times. Significant treatment means were ranked according to Tukey's HSD test at $p < 0.05$ [38].

3. RESULTS AND DISCUSSION

3.1. Pre-hardening growth and nutrition

Before late-season fertilization, pre-hardening nutrient loading increased seedling N, P and K concentration without changing total dry mass (Tab. II), reflecting typical luxury uptake from extra fertilization since nutrient uptake was enhanced without biomass increase [5, 7, 36]. This finding concurs with results of Salifu and Timmer [37] where induced luxury consumption was associated with increased N content (150%) in black spruce seedlings when compared to conventionally cultured plants. Pre-hardening N content for respective C and NL seedlings were 7.50 and 16.00 mg N·plant^{-1}. Thus, nutrient loading raised N content by 113%, and concentration level as high as 2.9% dw, which is close to the target considered optimum for nutrient loaded black spruce crops at the hardening stage [5, 37]. Shoot:root biomass ratio was also increased by nutrient loading (2.7–4.3) indicating proportionally more carbon partitioning to shoots rather than roots presumably because of greater nutrient availability in the growing medium [37]. Similarly, high soil N availability lowered root:shoot biomass ratios in longleaf pine (*Pinus palustris* Mill.) seedlings grown under water stress [21].

3.2. Growth allocation and nutrient uptake during hardening

After hardening off (week 36), late-season fertilization increased biomass production by 40–59% ($p < 0.3513$, Tab. III) in conventional (C) and 47–70% ($p < 0.1611$) in nutrient loaded (NL) seedlings demonstrating that considerable growth occurred during this period [5, 32], although differences were not significant. As expected, most growth occurred in roots (104%) as compared with shoots (42%), lowering shoot:root ratios from 4.3 to 2.5 within the nutrient loaded regime ($p < 0.0838$) and from 2.7 to 1.9 within the conventional regime ($p < 0.4954$) (Tab. III and Fig. 2). This plastic response was presumably triggered by short-day treatments that induced bud-set and shifted carbon allocation to roots rather than shoots [5, 29]. Increased proliferation and development of the root systems is considered beneficial for newly planted seedlings. The carbon shift promotes nutrient and moisture acquisition in comparatively less fertile field soils, especially soon after planting when root extension is slow [6, 8].

Although total dry matter production was unaffected by late-season fertilizer additions (Tab. III), N content was significantly increased by 44–167% ($p < 0.0001$) in the NL-treated seedlings, and by 100–312% ($p < 0.0001$) in the C-treated seedlings, demonstrating significant luxury uptake of N in both regimes (Fig. 3). The accumulation was stimulated by higher N rates illustrating the efficacy of supplemental fertilization at this stage in the greenhouse rotation. Plant N concentration ranged from 1.33–2.90 % dw before hardening and 0.93–4.36% dw after hardening (Tabs. II and III). Evidently, growth dilution (reduced N concentration) occurred in unfertilized (0) seedlings and N built up in fertilized (1×, 2×, 4×) seedlings in both C and NL regimes. For instance, late-season fertilization raised N concentration by 265% ($p < 0.0001$) and 61% ($p < 0.0001$) within C and NL regimes, respectively, when compared to controls (Tab. III). Overall concentrations were higher than those reported (maximum 2.88% dw) in our previous study [5], apparently because of longer (6 vs. 9 wks) and (or) higher (12 vs. 48 mg N·seedling^{-1}) rates of late-season fertilization. These trends although high, are in general agreement with results of other studies [19, 39] where fall N fertilization benefited seedling nutrition by increasing N concentration and contents in plant tissues that subsequently stimulated growth during outplanting.

Phosphorus uptake was also improved by 12–20% ($p < 0.0235$) within NL-regime seedlings during hardening (Fig. 3), but not as markedly as with N. The difference probably reflects that N is more readily incorporated into organic molecules than P,
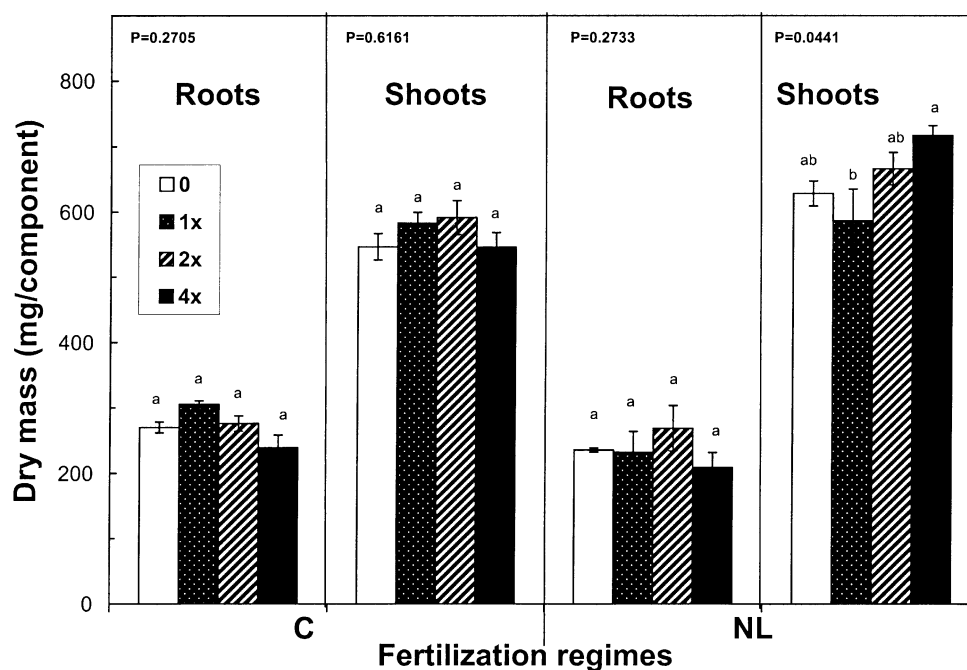
Figure 2. Root and shoot dry mass after late-season fertilization. Treatments as indicated in Figure 1. Bars represent one standard error of the mean. For each plant component, bars sharing the same letter within regime are not statistically different according to Tukey's HSD test ($\alpha = 0.05$).

Table III. Effects of late-season fertilization on seedling dry mass, shoot:root ratio, and N, P and K concentration (% dw) and associated ANOVA. The two pre-hardening regimes: conventional (C) and nutrient loaded (NL) supplied cumulative totals of 19 and 74 mg N·seedling^{-1}. The four late-season fertilization treatments conducted within each regime were: control (0), extended loading (1×), intermediate super loading (2×) and high super loading (4×) supplying cumulative totals of 0 12, 24 and 48 mg N·seedling^{-1}.

Prehardening fertilization	Late-season fertilization[†]	Plant dry mass (mg)	Shoot/root ratio	Nutrient concentration (% dw)		
				N	P	K
C-regime						
	0	817a	2.06a	0.93d	0.21b	0.61a
	1×	888a	1.90a	1.78c	0.31a	0.77a
	2×	867a	2.17a	2.79b	0.32a	0.69a
	4×	785a	2.32a	3.39a	0.28a	0.68a
NL-regime						
	0	863a	2.75a	2.71c	0.33a	0.68b
	1×	818a	2.54a	3.52b	0.33a	0.69b
	2×	935a	2.50a	3.86b	0.34a	0.80b
	4×	926a	3.43a	4.36a	0.36a	1.40a
ANOVA $p > F$						
C-regime		0.3513	0.4954	0.0001	0.0001	0.1639
NL-regime		0.1611	0.0838	0.0001	0.1757	0.0001

[†] Late-season fertilization means (0, 1×, 2× and 4×) sharing similar letters within each regime (C or NL) are not significantly different according to Tukey's HSD test, $p < 0.05$.

and (or) that more N (20%) than P (9%) was supplied in the fertilizer formulation [29]. Super-loading (2×, 4× rates) raised P concentrations of conventional (C) seedlings (range 0.26– 0.32% dw) but lowered (range 0.37–0.33% dw) levels of the nutrient loaded (NL) seedlings that were initially high (Tab. III). The relatively low (12–20%) boost in P uptake suggests that
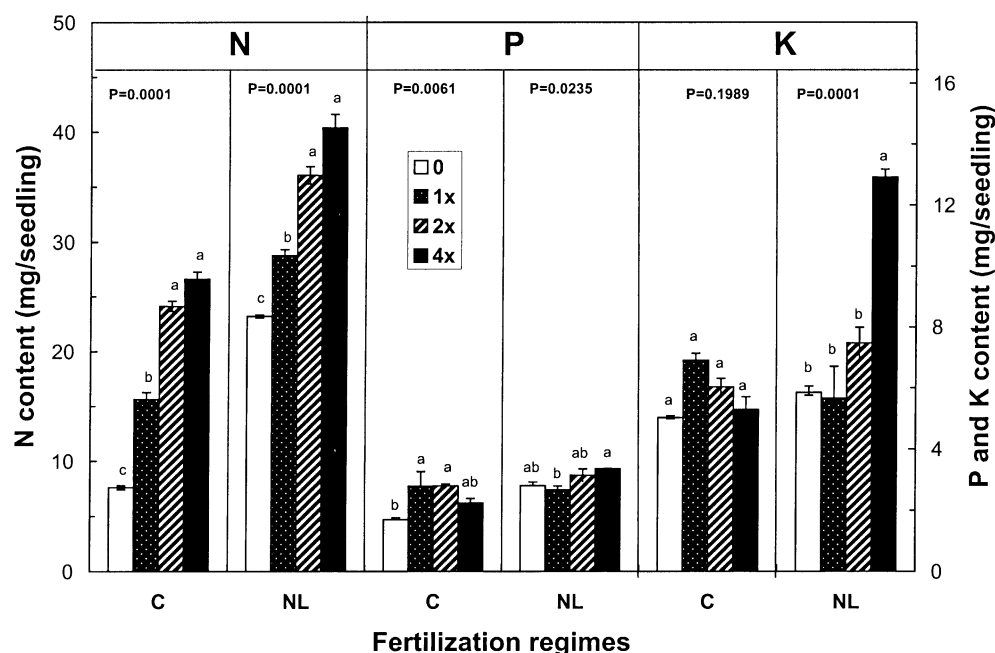
Figure 3. Seedling N, P, and K content after late-season fertilization. Treatments as indicated in Figure 1. Bars represent one standard error of the mean. For each nutrient, bars sharing similar letters within regime are not statistically different according to Tukey's HSD test ($\alpha = 0.05$).

higher P additions may be necessary for late-season fertilization to maintain steady-state levels.

Ammonium (NH_4^+) and potassium (K^+) ion uptake appear to be similar and competitive [14]. Consequently, K uptake may decline with high N supply [16, 51] because NH_4^+ acts as an uptake antagonist to K [15]. Increased K addition may counter this effect for conifer nursery stock [49]. This inhibitory effect was noted in our previous study with late-season fertilized black spruce [5], but was not evident here presumably because of the higher K (30%) supplied (Fig. 3). Seedling K uptake was unchanged by late-season fertilization in the C-regime ($p < 0.1989$), but was increased ($p < 0.0001$) by 28% and 121% in the 2×- and 4×-treated seedlings, respectively, of the NL-regime (Fig. 3). As expected, K concentration was reduced (diluted) in unfertilized (0) seedlings, but levels remained at steady-state (undiluted) after super-loading signifying that the extra high K application was adequate for late season applications (Tabs. II and III). The exception was elevated K (1.40% dw) in the high super-loaded (4×) seedlings of the nutrient loaded (NL) regime, which may reflect excess accumulation (Tab. III).

3.3. Outplanting response

When outplanted on bioassays for a 13-week growing period, late-season fertilization (1×, 2×, and 4× rates) increased biomass production as much as 78% for conventional (C) and 115% for nutrient loaded (NL) ($p < 0.0001$) seedlings when compared to the C-regime controls (Fig. 4a). There was no mortality, except for some (30%) of the nutrient loaded 4×-treated trees that were associated with the highest shoot/root

ratio (4.33) and highest N and K concentration (4.36 and 1.40% dw) before transplanting (Tab. III). Growth response was also sub-maximum for the surviving trees (Fig. 4a), suggesting possible moisture stress and (or) excessive nutrient accumulation. Although pre-plant biomass of all treatments was similar ($p < 0.1163$) (Fig. 4a), N content differed ($p < 0.0001$) widely, depending on loading intensity (Fig. 4b). Presumably the higher reserves functioned as a crucial N source that was rapidly remobilized for sinks of new growth when outplanted [33, 34, 36].

Growth increases in new shoots were closely associated with amounts of N accumulated in these shoots (Fig. 4). In contrast, N content in old shoots declined to a common threshold range (8–10 mg N) presumably reflecting mostly structurally bound N [7]. The decline signified substantial N depletion, particularly for loaded seedlings. Most N in new growth originates from two main sources: internally from plant tissues and externally from the soil [33, 36]. Assuming that the old and new shoots act as respective major source and sink for nutrients [23, 36], N content differences in old shoots before and after planting provide estimates of net N retranslocated to new growth. The remaining N in new shoots then represent estimates of soil derived N [24, 26, 32]. Nitrogen distribution among seedling components in Figure 4b shows that net N retranslocation (or net N depletion from old shoots to new growth) was associated with higher pre-plant N reserves built up by pre-hardening nutrient loading and late-season fertilization [25, 28, 43, 49, 51]. The depletion was most severe for nutrient loaded (NL) rather than conventional (C) regime seedlings (max. 10 vs. 18 mg N, see Fig. 4b), exemplifying typical "opportunistic"
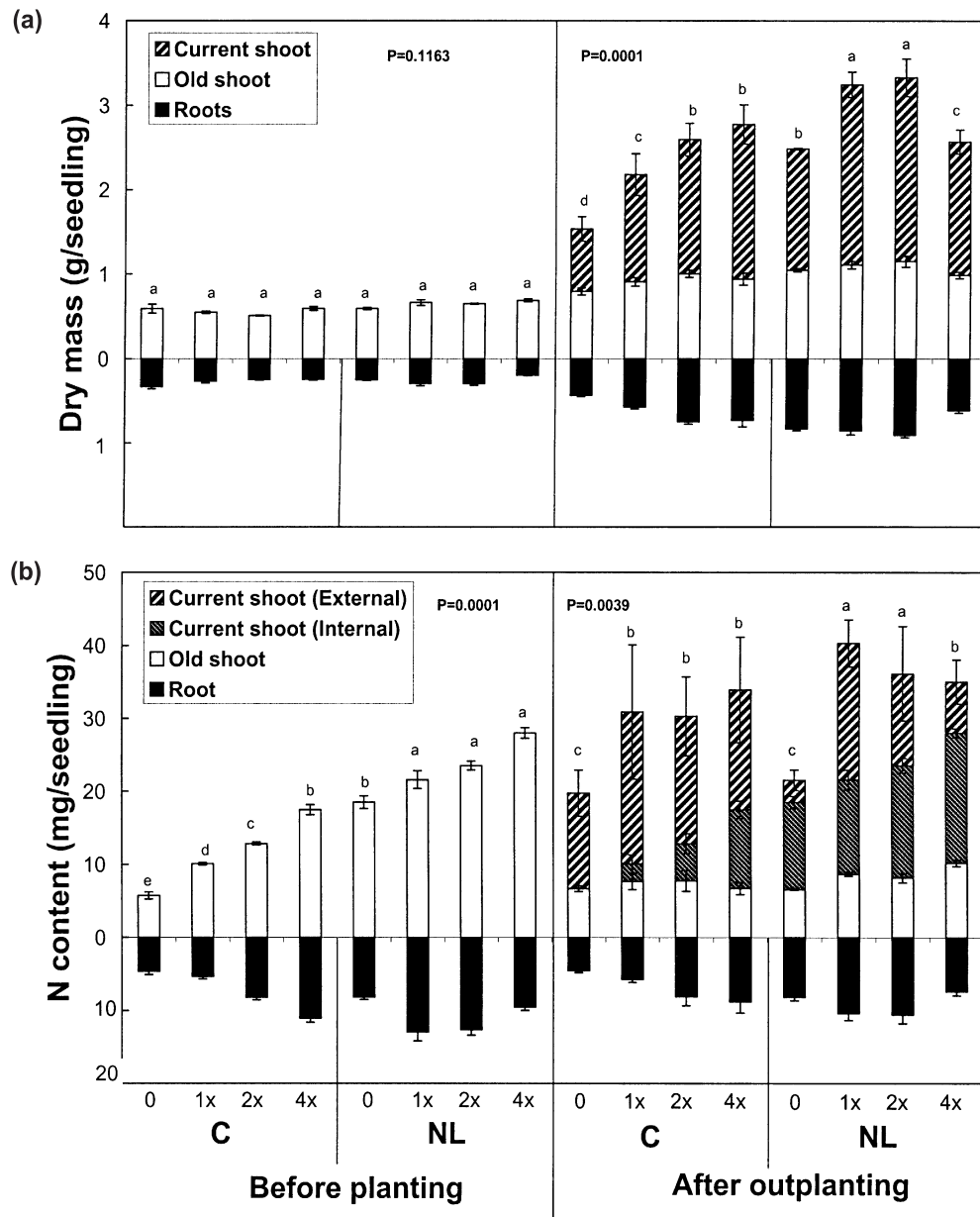
Figure 4. Component dry mass (a) and N content (b) of conventional (C) and nutrient loaded (NL) seedlings before and after transplanting on intact bioassays of soil substrates retrieved from a Boreal forest site. Vertical error bars are standard errors of the mean. Late-season fertilization means (0, 1×, 2× and 4× as in Fig. 1) sharing the same letter before or after outplanting are not statistically different according to Tukey's HSD test ($\alpha = 0.05$).

and "conservative" nutrient use strategies for respective loaded and non-loaded seedlings [24]. Thus, retranslocation met about 11–40% of the N required for new growth in C-regime seedlings and up to 72–80% in NL-regime plants, demonstrating the efficacy of loading to meeting sink demand for N through internal cycling [24, 36]. Isotope studies have shown black spruce seedlings can rely entirely on N retranslocation (100%) to meet early growth demands in [36], a capacity enhanced by nutrient loading. Conventionally reared seedlings were more dependent on soil derived N (as high as 77%), suggesting these plants may

be more prone to nutrient stress when planted on competitive or poor fertility sites [24].

Although root biomass increased substantially after planting (31–231%), N uptake (content) in roots changed relatively little (1–28%) (Fig. 4). These responses contrast with higher N accumulations in shoots, which seems to support the contention that the expanding shoot system is the primary sink for retranslocated nutrients in newly planted seedlings [7]. This allocation pattern promotes light capture and photosynthesis (6, 13, 24, 26). It must be kept in mind, however, that the 13-week outplanting

period represented the summer growing season only, hence root system N reserves may build up later in the autumn as found by others [3, 25, 28].

3.4. Implications

The improved growth and nutritional status of the intensively fertilized seedlings after planting demonstrate the potential benefit of nutrient loading and late-season fertilization practices on early plantation performance. Accelerated outplant growth and enhanced nutrition is consistent with our hypothesis and concurs with results of other studies [19, 28, 39, 40]. The increased biomass of seedlings may be partly attributed to higher photosynthetic rates associated with increased N status [13, 41] that promote soluble protein accumulation for carboxylation during photosynthesis. There is of course a limit to the intensity of nutrient loading of seedlings. Our results show that the high super-loading rate (48 mg N·seedling^{-1}) is toxic for black spruce culture, particularly when raised in relatively small volume containers. Based on short term responses so far, intermediate super-loading (24 mg N·seedling^{-1}) was most effective in stimulating nutrient accumulation in the nursery and promoting outplanting response with both conventional and nutrient loaded seedlings.

The bioassay approach adopted in this study is considered an effective tool for evaluating initial outplanting response of seedlings. Other studies have noted close correspondence between first year growth responses of nutrient loaded black spruce seedlings under field conditions with growth on intact soil bioassays [23, 31]. Consequently, our results may have relevance to expected stock performance at plantation establishment. A field trial in northern Ontario has shown that prehardening nutrient loading increased tree biomass by 39% when compared with non-loaded plants after two growing seasons [23]. A similar trial noted 49% more biomass after 6 years [42]; indicating some persistence in the loading response probably because of initial growth and nutritional advantages at plantation establishment. These findings suggest that nutrient loading at both the pre-hardening and hardening stage in the nursery may contribute to shortening the time required to attain crown closure, thus reducing competition with neighboring vegetation [19].

4. CONCLUSIONS

Super-loading regimes significantly increased N uptake, which prevented N dilution, and consistently stimulated N accumulation in seedlings during hardening. These results suggest that nutrient loading practices can effectively be prolonged until the end of greenhouse culture to build up greater nutrient reserves for outplanting. The intermediate super loading (2×) treatment was most effective in promoting nutrient storage in plants during hardening. This treatment was also associated with improved outplanting response and is recommended for late-season nutrient loading of black spruce seedlings. Potassium dilution was averted with high K enrichment of the fertilizer mix, but extra P may be needed to maintain steady-state uptake and avoid P imbalance under intensive super-loading. When outplanted for a 13-week period, biomass production increased by 115% in intermediate super loaded compared with conventionally reared seedlings. However, higher loading intensities may induce mortality. The improved growth response was associated with increased retranslocation (72–80%) of built-up nutrient reserves to sites of new growth. Higher tissue nutrient concentration and uptake rates by loaded seedlings may have promoted photosynthesis that improved growth in these plants. Results show that nutrient loading during the entire greenhouse rotation enhanced outplanting performance on pot bioassays, and may contribute to the success of reforestation efforts in Ontario.

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