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Original article

Late-season fertilization of *Picea mariana* seedlings under greenhouse culture: biomass and nutrient dynamics

Joseph R. Boivin, Brad D. Miller and Vic R. Timmer*

Faculty of Forestry, University of Toronto, 33 Willcocks Street, Toronto, Ontario, Canada

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Abstract – Conventional nursery culture of containerized black spruce (*Picea mariana* Mill. B.S.P.) seedlings usually involves a late-season interval, commonly called the "hardening period", when fertilization and water are withheld to promote frost-hardiness. Considerable growth may occur during this time which may lead to internal nutrient dilution, a condition often detrimental to subsequent field performance. Continued late season fertilization (at 6 or 12 mg N seedling^{-1}) of seedlings during the hardening period was tested as a technique to prevent late season nutrient dilution and possibly to increase nutrient reserves. Root growth was increased much more than shoot growth during this period. Late-season fertilization raised N, P and K uptake as much as 164, 70 and 32% respectively, compared to conventionally fertilized seedlings with no late-season fertilization. Depending on dose rate and pre-hardening nutrient loading, this technique demonstrates the potential to build internal nutrient reserves in seedlings. Nutrient dilution was temporarily averted by late-season fertilization suggesting that intensive and prolonged nutrient supplementation during the hardening period may further delay or eliminate nutrient dilution in seedlings.

black spruce / hardening period / nitrogen / nutrient dilution / nutrient loading

Résumé – Fertilisation en fin de saison des plants de *Picea mariana* cultivés en serre : dynamique de la biomasse et des éléments nutritifs. Dans les pépinières, l'élevage en container de plants de *Picea mariana* (Mill. B.S.P.) comporte normalement en fin de saison une phase appelée « période d'endurcissement » pendant laquelle fertilisation et arrosage sont supprimés pour améliorer la résistance au froid. La croissance, au cours de cette période, peut être importante d'où une dilution interne des éléments nutritifs affectant souvent les performances ultérieures sur le terrain. On a testé une technique consistant à prolonger la fertilisation pendant la période d'endurcissement (6 à 12 mg N par plant) pour éviter, en fin d'élevage, une dilution des éléments nutritifs, voire en augmenter la teneur. Pendant cette période, le gain de croissance du système racinaire a été plus élevé que celui des parties aériennes. Cette fertilisation en fin de saison se traduit par un prélèvement en N, P et K accru de respectivement 164, 70 et 32 % par rapport à celui observé avec le régime de fertilisation classique. Dépendant du régime de fertilité antérieur avant endurcissement et de la dose d'éléments nutritifs adoptée, cette technique démontre qu'il est possible d'agir sur la quantité de réserves en éléments des plants. Une fertilisation en fin de saison interrompt temporairement le processus de dilution des éléments. Ceci permet de penser que l'apport intensif et prolongé d'éléments pendant la période d'endurcissement peut retarder ou éviter la dilution en éléments des plants.

***Picea mariana* / période d'endurcissement / azote / dilution des éléments nutritifs / changements nutritifs**

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* Correspondence and reprints
Tel. + 416 978 6774; Fax. +416 978 3834; e-mail: vic.timmer@utoronto.ca

1. INTRODUCTION

Commercial greenhouse production of containerized conifer seedlings usually involves a late-season hardening period imposed to improve drought and frost tolerance during winter storage and subsequent outplanting [1, 11]. Once seedlings reach a target height during greenhouse culture, apical bud initiation is induced artificially by shortening daily photoperiod [9, 12] resulting in budset in about two weeks for black spruce (*Picea mariana* Mill. B.S.P.) [8]. The hardening period is defined as the time interval following apical bud initiation (bud-set) when roots and shoots acquire frost hardiness [9]. Irrigation and fertilization are generally reduced to induce nutritional and environmental stress thus promoting frost hardiness development in seedlings [5, 28]. However, substantial growth, particularly in the roots, occurs during hardening despite stress induction [33, 36]. Black spruce seedlings may gain as much as 142% in shoot dry mass and 794% in root dry mass during hardening [33]. Since nutrient uptake is limited without continued fertilization, growth of this magnitude can severely dilute plant nutrient reserves, compromising nutrient loading efforts [2, 33].

Nutrient loading, or extra-high fertilization that builds up internal nutrient reserves during nursery culture, has been shown to improve outplanting performance of overwintered seedlings both in the field [30, 32, 42] and in pot trials [34, 46] as the stored nutrients are retranslocated to growing apices during the initial flush of shoot expansion after transplanting when new root growth is restricted [41]. Nutrient loading before hardening may counter late-season dilution [39], although some growers are reluctant to adopt this practice because of concerns that high N fertilization may jeopardize frost-hardiness development in seedlings prior to winter storage [2, 17, 43]. More recent studies, however, have shown that high N supply does not affect cold tolerance of conifers [4] and may actually increase frost-hardiness [7, 14, 16, 27, 35]. Presumably autumnal accumulation of free amino acids and proteins may lessen cellular freezing damage by reducing the symplastic water volume [3, 26, 38]. Beside increasing plant nutrient reserves, nutrient loading may also build up nutrients in the growing medium that seedlings can draw on during hardening hence reducing later nutrient dilution. Although plant nutrient status was increased during hardening and nutrient dilution was delayed, this carry-over effect from loading was temporary because of subsequent leaching and inadequate nutrient release from the peat rooting medium [33].

Compared to pre-hardening nutrient loading, late-season fertilization may be more effective in overcoming late-season nutrient dilution in seedlings because nutrient supplementation is extended or prolonged into the hardening period [4, 35]. Ideally fertilizer additions during this period should continue to match growth and nutrient demand rates of seedlings to maintain stable tissue nutrient concentrations, thus preserving desirable steady-state nutrition [22, 23]. Steady-state nutrition is usually achieved by exponentially increasing fertilization during the exponential growth phase of seedlings [19, 39]. Following bud-set, however, black spruce seedlings usually exhibit a gradual decline in growth rate and physiological activity until dormancy requirements are met [1, 10, 29]. Consequently, nutrient supplementation during hardening should match this decline pattern [25] by following a reverse exponential function that synchronizes nutrient supply rate with growth rate. The objective of this study was to test late-season fertilization regimes on a commercial crop of black spruce seedlings utilizing declining delivery rates. The expectation was that, depending on application level, late-season fertilization would build up nutrient reserves in the seedlings to counter and delay nutrient dilution.

2. MATERIALS AND METHODS

2.1. Plant material and main fertilization regimes

Black spruce seedlings were grown in a greenhouse at a commercial forest tree nursery (North Gro Development Ltd.) near Kirkland Lake, Ontario (48° 10’ N, 88° 01’ W), as detailed in Miller and Timmer [33]. The treatment and cultural schedule is outlined in *table I*. Each seed was planted in late April into a peat-filled cavity (40 cm^3) of Styroblock trays containing 330 cavities $tray^{-1}$. Seedlings grew under natural daylength with day: night temperatures averaging 22:15 °C, respectively. Weekly application of fertilizer solutions commenced one week following germination and was carried out for 15 weeks. The application frequency was predominantly controlled by exterior temperature and humidity (which impacted watering frequency) and was occasionally delayed to permit adequate crop dry-down, thus avoiding fertilizer loss due to leaching. Four main fertility regimes, providing cumulative totals of 14.7, 41.2, 38.7, and 57.6 mg N $seedling^{-1}$, were applied during the first 16 weeks of growth (*table I*). These regimes are hereafter respectively referred to as conventional (C),

Table I. Treatment schedule of containerized black spruce seedlings during greenhouse culture. The four main fertilization regimes: conventional (C), conventional loading (CL), exponential loading (EL), and high exponential loading (HEL) supplied cumulative totals of 14.7, 41.2, 38.7, and 57.6 mg N seedling^{-1}. Late-season fertilization supplied 0 (U), 6 (XF) and 12 (XL) mg N seedling^{-1} as in *figure 1*.

Week	Cultural phase	Treatment
0 – 1	Germination	Water only
1 – 16	Exponential growth	Main fertilization (C, CL, EL, or HEL)
15 – 17	Bud set	Short day
18 – 23	Hardening	Late-season fertilization (U, XF, or XL)
23 – 32	Hardening	Water only
32 +	Cold-storage	

conventional loading (CL), exponential loading (EL), and high-dose exponential loading (HEL). The conventional regime (C) simulated standard industry practice for pre-hardening nutrient delivery in northern Ontario [33]. The loading (CL and EL) regimes and high loading (HEL) regime represented two moderate and a high nutrient loading level, respectively. Conventional loading and exponential loading (CL and EL) delivered about the same cumulative total of nutrients, at either constant or exponentially increasing rates. The high exponential loading (HEL) treatment delivered the most nutrients and was designed to build nutrient reserves for the initial 16 weeks as described in [40]. A commercial water soluble fertilizer (Plant Products 20-20-20, containing 20% N, 9% P, and 17% K plus micronutrients) was sprayed on to the seedlings as a pre-mixed solution using traveling booms with fixed nozzles. Seedlings were rinsed after each application to dilute the fertilizer and avoid fertilizer burn.

A two-week shortday treatment commenced 14 weeks after germination (*table I*) by reducing photoperiod from natural day-length to 8 h using blackout curtains. Seedlings were hardened for 15 weeks after shortday treatment by return to natural day-length and gradual reduction of greenhouse temperatures (18–10:12–4 ºC day:night) before transfer to a cold storage facility (–2 ºC).

2.2. Late-season fertilization, experimental design and statistical analysis

After shortday exposure, late-season fertilization was evaluated on a subsample of nine randomly selected seedling trays from each unreplicated main fertilization regime (C, CL, EL, and HEL). Each set of nine trays (holding 330 trees per tray) was arranged in a completely randomized design with three replicates testing three late-season treatments: an unfertilized control (U), an extended fertilization (XF) treatment that provided a cumulative total of 6 mg N seedling^{-1}, and an extended loading (XL) treatment that provided a cumulative total of 12 mg N seedling^{-1} (*figure 1*). The control represented standard practice of periodic irrigation without fertilization during hardening. Extended fertilization (XF) was expected to maintain steady-state nutrition, the dose rate reflecting N content differences (4–6 mg N) usually found between conventional and nutrient loaded seedlings [40]. The 12 mg N seedling^{-1} extended loading (XL) treatment was intended to increase N concentration, thus building nutrient reserves during hardening. Weekly additions of premixed fertilizer solutions declined exponentially with time (*figure 1*), starting one week after termination of shortday treatment (week 18, *table I*) and continuing for six weeks using the same application procedure as before. Final harvest treatment responses within each main fertilization regime were tested by one-way analysis of variance for a completely randomized design of three treatments and three replications using SAS Institute Inc.

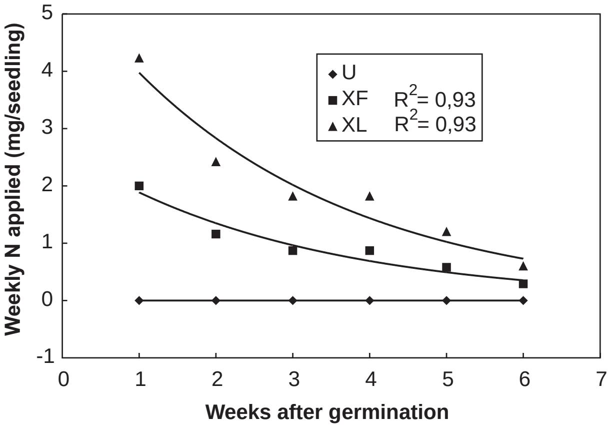

Figure 1. Late-season fertilization regimes applied during the first six weeks of the hardening period (week 18–32). Unfertilized control (U), extended fertilization (XF), and extended loading (XL) supplied cumulative totals of 0, 6, and 12 mg N seedling^{-1}, respectively, at exponentially declining rates.

[37] procedures. Means separation was by Tukey's HSD test ($p < 0.05$).

2.3. Sampling and vector diagnosis

Ten seedlings per treatment-replicate were randomly sampled at week 18, 20, 22, 24, 26, 28, and 32 during hardening. Growing media was washed from roots and shoot lengths were recorded. Seedlings were rinsed in distilled water, separated at the root collar, composited by treatment-replicate, dried in an oven at 70 ºC for 48 hours, and weighed. Chemical analysis was then conducted according to methods described in Timmer and Armstrong [41]. Vector diagnosis [20] was used to examine temporal changes in growth and nutrient status during the hardening period as demonstrated with N using sequential sampling data. Treatment responses were portrayed as vectors that reflect changes in seedling dry mass, N content, and N concentration, progressively with time relative to the initial sampling event (week 18, before late-season fertilization). Three diagnostic trends were apparent: nutrient dilution, steady-state nutrition, or a deficiency associated nutrient accumulation, depicted respectively as Shift *A*, *B*, or *C* (*figure 1*, in [20]). Nutrient dilution (Shift *A*) depicted as a downward sloping vector was characterized by increased dry mass and nutrient uptake but decreased nutrient concentration. A right pointing vector with no slope signified steady-state nutrition (Shift *B*) reflecting increased dry mass and nutrient uptake with no change in nutrient concentration. An accumulation of nutrient reserves over time defined by an upward sloping vector (Shift *C*) represented increased dry mass, nutrient uptake, and nutrient concentration [20].

3. RESULTS AND DISCUSSION

3.1. Growth and biomass partitioning

At final harvest (*tables II* and *III*), late-season fertilization significantly influenced total biomass production

Table II. Means of seedling dry mass (mg), shoot root ratio, and seedling N, P, and K concentration (% d.w.), and N/K ratio before (week 18) and after (week 32) late season fertilization. The four main fertilization regimes: conventional (C), conventional loading (CL), exponential loading (EL),and high exponential loading (HEL) supplied cumulative totals of 14.7, 41.2, 38.7, and 57.6 mg N seedling^{-1}. Late-season fertilization treatment abbreviations as in *figure 1*.

Main fertilization regime	Before or after late-season fertilization	Total dry mass[1]	Shoot/root ratio	Seedling nutrient concentration			K/N ratio
				N	P	K	
C	Before	220.73	2.78	2.04	0.37	0.98	0.48
	After (U)	654.40a	0.97c	1.36c	0.27b	0.68a	0.51a
	After (XF)	601.43a	1.09b	1.74b	0.37a	0.68a	0.39a
	After (XL)	599.70a	1.21a	2.62a	0.38a	0.58b	0.22b
CL	Before	286.87	5.20	2.54	0.37	1.18	0.46
	After (U)	904.77a	1.42b	1.35c	0.26c	0.70a	0.52a
	After (XF)	805.57a	1.36b	1.72ba	0.34b	0.74a	0.43b
	After (XL)	789.97a	1.77a	2.10a	0.37a	0.66a	0.31c
EL	Before	318.30	5.37	2.62	0.35	1.10	0.42
	After (U)	957.73a	1.69a	1.49c	0.24b	0.68a	0.45a
	After (XF)	847.63b	1.42b	2.05b	0.32a	0.65a	0.32b
	After (XL)	794.80b	1.82a	2.47a	0.35a	0.64a	0.26c
HEL	Before	286.03	6.82	2.91	0.36	1.13	0.39
	After (U)	967.53a	1.66a	2.39b	0.30a	0.60a	0.25a
	After (XF)	807.40b	1.30b	2.27b	0.34a	0.56a	0.25a
	After (XL)	809.73b	1.55ab	2.88a	0.37a	0.61a	0.21a

[1] Within each regime, late-season fertilization means (U, XF and XL) sharing a common letter are not significantly different according to Tukey's HSD test, $p < 0.05$.

Table III. Analysis of variance associated with *table II* and *figures 2* and *3* testing dry mass, shoot/root ratio and plant nutrient concentration and content, and K/N ratio of seedlings after late season fertilization treatments. Conventional (C), conventional loading (CL), exponential loading (EL), and high exponential loading (HEL) regimes supplied cumulative totals of 14.7, 41.2, 38.7, and 57.6 mg N seedling^{-1} respectively, before hardening.

	$p > F$								
Source of variation within	Dry mass	Shoot/ root ratio	Nutrient concentration			Nutrient content			K/N ratio
			N	P	K	N	P	K	
C regime	0.4755	0.0014	0.0001	0.0001	0.0096	0.0010	0.0423	0.0770	0.0017
CL regime	0.3352	0.0001	0.0001	0.0001	0.2435	0.0360	0.0949	0.0861	0.0003
EL regime	0.0099	0.0003	0.0001	0.0001	0.5288	0.0003	0.0031	0.0003	0.0001
HEL regime	0.0292	0.0144	0.0297	0.2188	0.3102	0.0547	0.4635	0.0456	0.1280

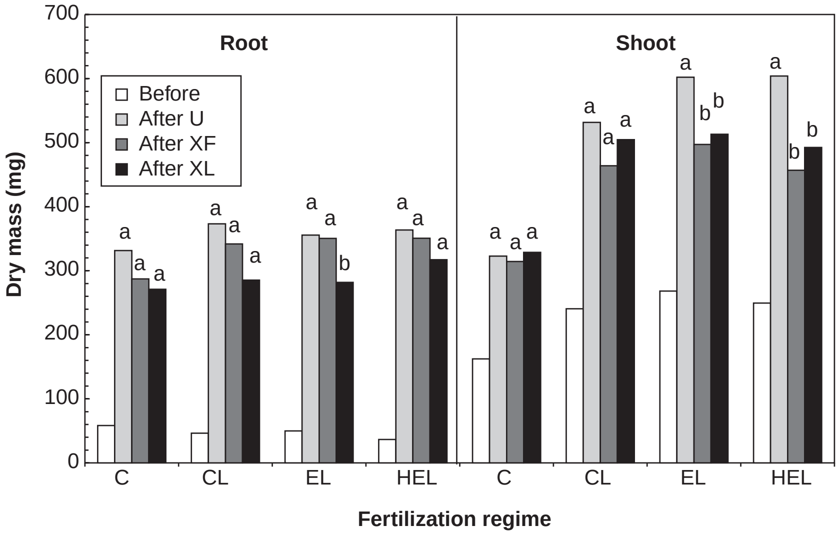

Figure 2. Root and shoot dry mass before and after hardening. Pre-hardening regimes abbreviations (C, CL, EL, and HEL) as in *table II*. Late-season fertilization treatment abbreviations (U, XF, and XL) as in *figure 1*. Within each regime, late-season fertilization means sharing a common letter are not significantly different according to Tukey's HSD test, $p < 0.05$.

of exponentially loaded (EL and HEL) seedlings ($p = 0.0099$–0.0292) but not the conventionally (C and CL) treated seedlings ($p = 0.3352$–0.4755). Dry matter production increased 170–200% for all treatments after budset, exemplifying the large growth increase that can occur during the 15 week hardening phase (*tables II* and *III*). The pre-hardening nutrient loading regimes (CL, EL and HEL) had little effect on subsequent root growth, but shoot growth was stimulated (44–87%) during hardening (*figure 2*). On the other hand, extended fertilization (XF) and extended loading (XL) induced a relatively small negative effect (13–27%) on total biomass compared to unfertilized (U) seedlings (*tables II* and *III*), which may be related to induced K/N imbalance in the plants as will be discussed later.

As expected, proportionately more growth was partitioned to the roots than to the shoots (*figure 2*) during hardening, significantly ($p = 0.0003$–0.0144) lowering shoot: root biomass ratios from an average of 5.0 to 1.4 (*tables II* and *III*). The shift in carbon allocation presumably occurred because terminal bud-set induced by shortday treatments restricted further height growth [10, 13, 33]. This practice is often used operationally to control height growth of crops once a target height has been achieved [1]. Although height growth was restricted after budset [33], shoot dry mass increased by 89 to 122% (*figure 2*) attributed mainly to thickening of the stem and cell walls, and lignification of secondary xylem [7, 8]. The late-season reallocation of biomass to roots may also contribute to enhanced outplanting performance because increased root size at planting is often beneficial for subsequent water and nutrient uptake [6, 24, 30].

3.2. Nutrient uptake

Nutrient content in the seedlings increased substantially during hardening, and uptake was promoted further by pre-hardening loading regimes and late-season fertilization practices (*figure 3*). Compared with the conventional unfertilized (C-U) seedlings, final N, P, and K content was increased as much as 164, 70 and 32% (for HEL-XL, EL-XL, and CL-XF trees, respectively) reflecting the high potential for building up nutrient reserves in tree crops by combining both types of fertilization practices in nursery culture. Late-season fertilization stimulated N and P uptake for all treatments ($p = 0.001–0.423$) except for the high exponential loading (HEL) treated trees (*table III, figure 3*) associated with high residual nutrient pools in the growing media before hardening [33] that sustained N and P uptake without dilution (*table II).*

Comparisons between initial (week 18) and final (week 32) N and P concentrations for all treatments indicate that extended loading (XL) was generally more effective than extended fertilization (XF) in reducing nutrient dilution, demonstrating the advantage of adopting higher application rates (more insight into the dynamic nature of the dilution process is given in the next section). As anticipated, late-season fertilization (XF and XL treatments) proved more effective in increasing seedling N and P status when compared to pre-hardening low-dose nutrient loading (CL and EL) alone (*figure 3*) even though less total fertilizer was involved (*figure 1*). Thus late-season nutrient supplementation shows promise as an efficient technique to boost final nutrient status of seedling crops.

Plant K content was consistently raised during the hardening period, but the increase was reduced by late-season fertilization especially at high dose rates (*table II, figure 3*). Since K uptake did not keep up with N uptake, it may well be that higher levels of ammonium (NH_4^+) ions in late-season fertilizers induced an inhibitory effect on K uptake, because NH_4^+ acts as a strong uptake antagonist to other nutrient cations [18]. Internal K/N ratios declined markedly (as low as 0.21) after late-season treatment (*table II*) probably inhibiting biomass production somewhat (*figure 2*). Ingestad [21] considered K/N concentration ratios between 0.45–0.55 as optimum for pine and spruce seedlings, which was achieved by most unfertilized (U) trees during hardening (*table II*). The drop noted with the highly-loaded (HEL-U) trees reflect the carry-over effect of high prehardening fertilization in the rooting medium [33]. Induced K deficiency was reported with other conifer seedlings exposed to high N supplementation [15, 45] and has been countered by increased K supplementation [44]. A similar approach to avoid internal K/N imbalance may be needed for intensive late-season fertilization with black spruce seedlings.

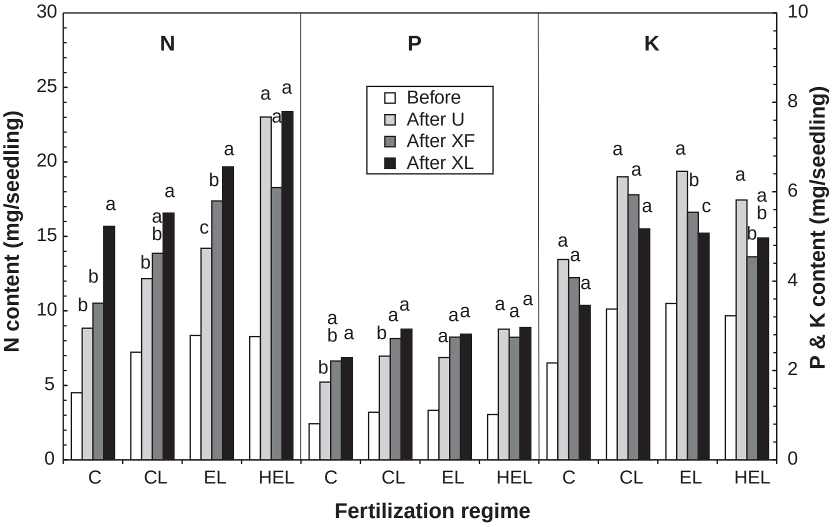

Figure 3. Seedling N, P, and K content before and after hardening. Pre-hardening treatment abbreviations (C, CL, EL, and HEL) as in *table II.* Late-season fertilization treatment abbreviations (U, XF, and XL) as in *figure 1*. Within each regime, late-season fertilization means sharing a common letter are not significantly different according to Tukey's HSD test, $p < 0.05$.

3.3. Nutrient dynamics

Vector analysis of sequential sampling data was conducted to monitor temporal changes in biomass and N status of black spruce seedlings during hardening [20, 33]. Initial status (week 18) of each late-season fertilization treatment (U, XF, and XL) was normalized to 100, and sequential changes in dry mass, N concentration and N content were plotted as positive, negative or unchanged responses relative to initial status (*figures 4* and *5*). Progressions in time were depicted as vectors reflecting the magnitude and direction of each response shift. Three major response trends were evident during the hardening period: nutrient dilution, steady-state nutrition, and nutrient deficiency reflecting respectively Shift *A*, Shift *B* and Shift *C* as described previously, and also in [20]. These responses were strongly influenced by both pre-hardening nutrient status and late-season fertilization rates. Thus, conventional (C) seedlings exhibited increased growth and N concentration and content initially (Shift *C*) for all treatments at week 18–20 (*figure 4a*). This may reflect a recovery from chlorosis after shortday treatment, observed as a darkening in needle colour [33]. Subsequently, N dilution (Shift *A*) characterized by increased biomass and N uptake but reduced N concentration was rapid for unfertilized (U) seedlings, but was delayed about 2 weeks by extended fertilization (XF), and for 6 weeks by extended loading (XL). Near steady-state nutrition (Shift *B*) was achieved during the delay, as plant growth and N uptake increased without appreciable concentration change indicating that N uptake matched growth (*figure 4a*).

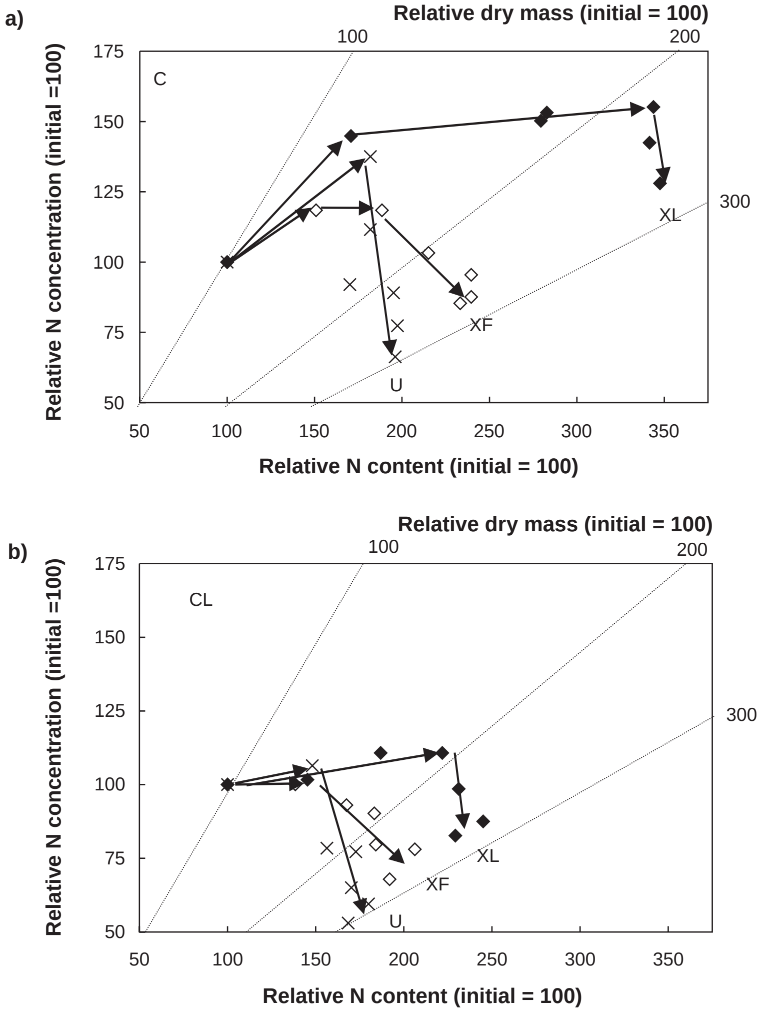

Figure 4. Progressions of relative N concentration, N content and dry mass of seedlings sampled during the hardening period. Initial seedling status (week 18) was normalized to 100. Pre-hardening treatment abbreviations (C, CL) as in *table II*. Vectors reflect sequential growth and nutrient dynamics of seedlings at week 18, 20, 22, 24, 26, 28 and 32. Late-season fertilization occurred week 18 to 24, treatment abbreviations (U, XF, XL) as in *figure 1*.

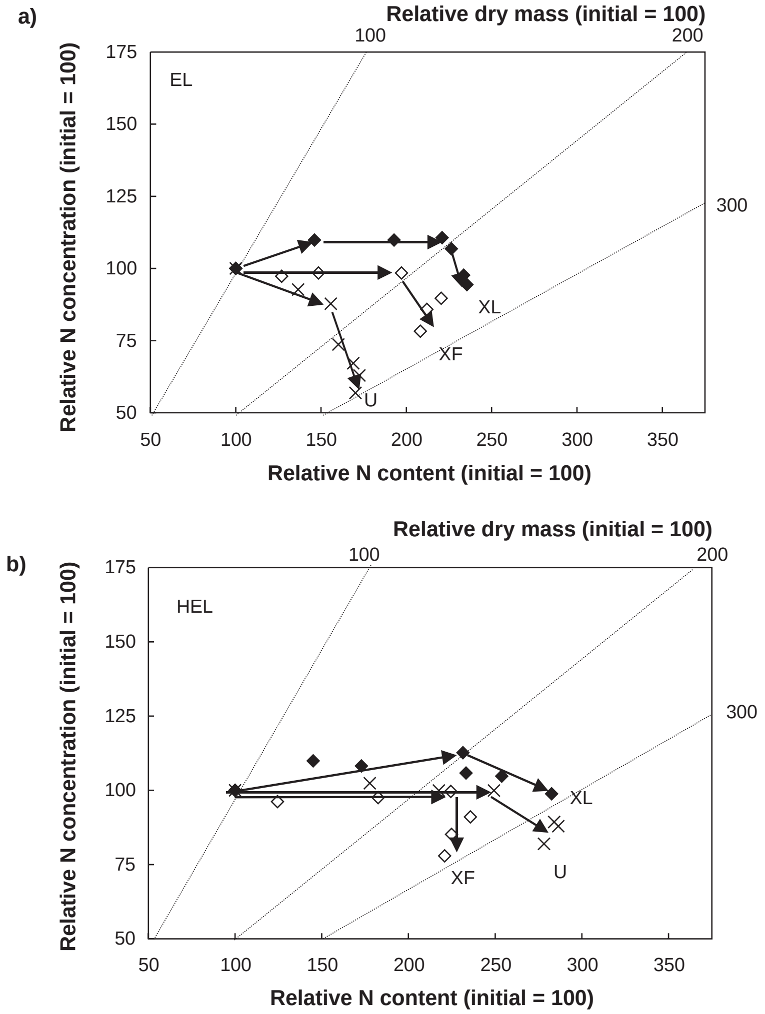

Figure 5. Progressions of relative N concentration, content, and dry mass of seedlings sampled during the hardening period. Initial seedling status (week 18) was normalized to 100. Pre-hardening treatment abbreviations (EL, HEL) as in *table II.* Vectors reflect sequential growth and nutrient dynamics of seedlings at week 18, 20, 22, 24, 26, 28 and 32. Late-season fertilization occurred week 18 to 24, treatment abbreviations (U, XF, XL) as in *figure 1.*

Unlike the conventional seedlings (C), the loaded seedlings (CL, EL, and HEL) did not exhibit a strong initial deficiency response (Shift *C* in *figures 4b* and *5*). This was likely due to the higher nutrient status of these trees at budset (*table II*). However, a similar pattern of delayed dilution from extended fertilization (XF) and extended loading (XL) was apparent that was also prolonged by the higher dose rate. In general, the onset of dilution (Shift *A*) occurred one week after late-season fertilization ended reflecting the sensitivity of the seedlings to nutrient supplementation during this period. These response patterns also illustrate the feasibility of continuing and prolonging late-season fertilization applications, both to minimize dilution during the hardening period and to build up nutrient reserves.

Under extended fertilization (XF), steady-state nutrition (Shift *B*) was more consistently attained with the exponentially loaded trees (EL and HEL) than with conventional (C) and conventionally loaded (CL) trees, presumably due to their higher initial nutrient status (*figures 4* and *5*). The build up of nutrient reserves (Shift *C*) was evident in the extended loading treatment (XL), most notably in the conventional (C) trees, exemplifying that extended loading can effectively increase reserves. There was no toxic accumulation of N (increased concentration and content accompanied with decreased growth, Shift *E* in [20]) in response to high dose fertilization, suggesting that even higher late-season rates than applied in this study may be used to load seedlings even more successfully. We intend to pursue these practices in further studies.

4. CONCLUSIONS

The results show that fertilizer supplementation during fall hardening promoted nutrient uptake and minimized dilution of nutrients associated with traditional hardening practices employed in containerized black spruce seedling production. Late-season fertilization was usually more effective in increasing plant nutrient reserves than low-level nutrient loading applied before hardening. Vector analysis confirmed increased uptake or steady-state accumulation of nutrients in seedlings for the 6-week application interval. Nevertheless, N dilution occurred soon after late-season nutrient additions stopped, demonstrating the nutritional sensitivity of these seedlings during the hardening period. Plant K uptake was reduced to some extent when combined with high N addition, indicating that intensified nutrient loading regimes may require higher proportional K than present treatments to maintain nutrient balance in seedlings. Implications from these findings are that late-season nutrient supplementation may prevent nutrient dilution in seedlings during the hardening-off stage, and that even higher rates of balanced fertilizer may promote nutrient uptake to augment internal nutrient reserves for improved outplanting performance.

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