Nutrient Loading of Norway Spruce Seedlings Hastens Bud Burst and Enhances Root Growth after Outplanting

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We studied the effects of late season nutrient loading (NLOAD) on the timing of bud burst, growth and changes in nitrogen (N) concentrations in the first growing season after seedlings were outplanted. Two-year-old Norway spruce (Picea abies (L.) Karst.) seedlings with three foliar nitrogen concentration levels (NLOAD levels 11.3, 22.5 and 27.5 g N kg⁻¹ for L-, M- and H-seedlings, respectively) were examined in the following three experiments: root growth capacity test (RGC), rooting experiment in the field and soil fertility experiment (‘rich’ or ‘poor’ soil) in the field. Bud burst in RGC was monitored daily and foliar N concentration (field experiments), height and root growth (rooting experiment) at monthly intervals. With respect to the RGC test, no differences in root growth were observed among the three NLOAD levels, but buds of H-seedlings burst 2–6 days earlier than others. In the rooting experiment, nutrient loading increased height and root growth but did not affect the timing of height growth. In the soil fertility experiment, foliar N of H- and M-seedlings decreased rapidly, but the decline was slower in rich soil. Current-year needles had more N in seedlings growing in rich soil and the N concentration declined until height growth ceased whereafter it increased until autumn. Improved growth from nutrient loading seems to last only for the first season after planting and the greatest benefits are enjoyed by seedlings planted in poor soils.

Keywords late-season fertilization, nitrogen concentration, root growth capacity, Picea abies

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1 Introduction

In spring, root growth and nutrient uptake of newly-planted seedlings are low (e.g., Vapaavuori et al. 1992, Amponsah et al. 2004). As such, seedlings depend on stored rather than acquired nutrients for the first few weeks of growth (van den Driessche 1991, Salifu and Timmer 2001). The amount of stored nutrients can be increased by nutrient loading after height growth has ceased in the previous autumn.


Nutrient loading has been shown to enhance root egress and first-year shoot growth of Norway spruce (Rikala et al. 2004) and black spruce (Salifu and Timmer 2001, Idris et al. 2004) seedlings. The effects of nutrient loading may, however, be temporary and variable among species. For example, nutrient loading enhanced the growth of Norway spruce seedlings only for the first season after planting (Heiskanen et al. 2009) but black spruce grew better for 2–6 years (Malik and Timmer 1996, Way et al. 2007).

Nutrient loading can improve field performance of outplanted seedlings due to enhanced nutrient uptake and increased root growth (Malik and Timmer 1996), and may also affect growth rhythm of the shoot. Higher springtime foliar nitrogen levels have either hastened bud burst (Benzian et al. 1974, Fløistad and Kohmann 2004) or had no effect (Bigras et al. 1996, Fløistad 2002, Timmer and Munson 1991). In boreal forests, timing of bud burst is critical because early flushing may expose seedlings to springtime frosts that could nullify any advantage it would otherwise confer. Thus, early bud burst may enhance growth by lengthening the growing period but the nutrient-loaded seedling runs a higher risk of suffering frost damage due to early exposure of delicate tissues.

First year root growth and nitrogen status of outplanted seedlings, especially Norway spruce, are poorly understood but Rikala et al. (2004) and Heiskanen et al. (2009) found nutrient loading in later years to have no effect on growth. With these results in mind and to investigate the transitory effect of nutrient loading in Norway spruce seedlings (Picea abies (L.) Karst.), we organized our study according to the following hypothesis: differences in foliar nutrient concentration caused by loading disappear in few weeks and untreated seedlings grow as well as those that have been nutrient loaded when planted in fertile soil.

Earlier studies (Luoaranen et al. 2008, Heiskanen et al. 2009) examined the response of seedlings to frost hardiness and soil fertility with the same seedling material. In this study, we monitored the effects of nutrient loading on timing of bud burst, height and root growth, and the effects of soil fertility on needle nitrogen concentration.

2 Material and Methods

2.1 Seedling Material

Norway spruce seedlings were sown on 12 June 2000 and grown for their first year in the nursery of Fintaimi Inc. at Tuusjärvi in Finland (62°53´N, 28°19´E) in hard-walled plastic containers with air slits (Plantek, PL-64F, volume 110 cm³, growing density 432 cells m⁻², Lännen Corp., Iso-Vimma, Finland). For their second growing season, seedlings were transferred to the Finnish Forest Research Institute nursery at Suonenjoki (62°40´N, 27°03´E) on 27 June 2001 and placed under a blackout frame where they were short day (SD) treated for three weeks between 04 and 25 July.

Seedling trays were distributed in three blocks during the nutrient loading (NLOAD) experiment. Growth conditions and seedling attributes were detailed in Luoranen et al. (2008). After the SD treatment, seedlings were nutrient loaded to three levels: low (L), medium (M) and high (H) by supplying the 0.8 L fertilizer solution per tray (Taimi Superex, Kekkilä Oy, Tuusula, Finland; L: 0–0.1% solution, M: 0.1–0.15% solution and H: 0.15–0.30% solution) with a watering can five times at weekly intervals (02–30 August). The quantity of fertilizer supplied for each treatment..
was maintained within recommended values by monitoring the foliar nitrogen concentration and the electrical conductivity (EC) of the press water in the peat (see details in Luoranen et al. 2008). The amounts of N provided during the loading period were 8, 22 and 37 mg per plant, respectively, for the L, M, and H treatments. Nitrogen concentrations in previous year needles after frozen storage were 11.3, 22.5 and 27.5 g N kg⁻¹, respectively for the L, M and H treatments.

NLOAD did not affect either seedling height (mean of 16 cm) or diameter (2.8 mm) (Luoranen et al. 2008). Seedlings were packed in cardboard boxes on 10 October 2001 and stored at –3 °C until 02 April 2002.

2.2 Root Growth Capacity (RGC) Test

On 02 April 2002, 90 seedlings were transferred from frozen storage for thawing at 7–10 °C. Six days later, seedlings were planted into 0.75 l sand-filled plastic pots, arranged in a randomized block design with three blocks and 10 seedlings per block and treatment. Seedlings were grown in a greenhouse for three weeks: day/night temperature 20/15 °C, 16 h supplementary light from 400 W high-pressure sodium lamps, irrigated with tap water when required. At the time of planting and at the end of the experiment, seedling height was measured to the nearest 1 mm. Seedling buds were monitored daily and scored as burst when needle tips were visible. Differences between treatments were determined as the date when the first bud burst, when 50% of buds were burst, and the time between these two dates. On 30 April 2002, root growth capacity (RGC) was determined as the dry mass (48 hours at 60 °C) of all new roots emerged from each of the root plug.

2.3 Rooting Experiment

On 10 May 2002, seedlings were removed from frozen storage and thawed as in RGC. After five days boxes were opened and placed outdoors under a shelter for one day. On 16 May, 15 seedlings were planted into a former nursery field composed of sandy soil and peat (organic content 5.1%, pH 5.1, 0.12% tot.-N, soluble N 2.2 mg/100 g, exchangeable P 1.5 mg and K 2.8 mg/100 g) in five blocks, 75 seedlings per NLOAD treatment, according to a randomized block design. Soil characteristics were based on four samples taken from the test field at 0–10 cm depth. The soluble soil N concentrations were determined spectrophotometrically from the extract of 1 M KCl with a FIA analyzer (Tecator 5012, Tecator Ab, Sweden). Total soil N was determined using a CHN analyzer (LECO-1000). Extractable P and K concentrations were analyzed from an extract of acidic (pH 4.65) 1 M ammonium acetate with inductively coupled plasma atomic emission spectrophotometry (ICP/AES, ARL 3800, Applied Research Laboratories, Ecublens, Switzerland).

At planting, seedling height (to the nearest 0.5 cm) and diameter (0.01 mm) were measured. Fifteen seedlings per treatment (three seedlings from each NLOAD treatment and block) were harvested five times at monthly intervals. Height of harvested seedlings was measured and the roots emerging from the root plug were cut, washed and dried at 60 °C for 48 h. Height of the current year shoot was calculated as the difference between initial height and height at harvesting. The dry mass of new roots was weighed to the nearest 1 mg. In each harvesting, previous year (C+1) needles were dried and ground for N analysis. Needle N concentration was determined with a CHN analyzer (LECO-900, LECO Corp., St Joseph, MI, USA).

2.4 Soil Fertility

This experiment was established at the same time as and adjacent to the rooting experiment with the same soil type and employed a split-plot layout in which two soil fertility levels were considered plots and NLOAD treatments as subplots divided into five blocks. Rich-soil plots were fertilized with a slow-release NPK fertilizer (1300 kg ha⁻¹) (Taimiston kestolannos 1, Kemira Oy, Finland; current producer Yara Suomi Oy, Finland) for one growing season, which contains 9% N (methyl-ene-urea), 3.5% P (apatite), and 5% K (biotite) and micronutrients yielding 11.7, 4.6 and 6.5 g m⁻² N, P and K, respectively. The poor-soil plots were left unfertilized. Heiskanen et al. (2009) detailed the experimental design and results of
seedling heights at the end of each growing season for years 1–3 after outplanting. In order to monitor N levels in C+1 and C needles, one needle from the middle part of the southern side of the previous and current years’ shoots were taken every 10 days from two random seedlings in each block and treatment (different seedlings in each sampling) and pooled. For the first sampling on 17 May, needles from previous year shoots were taken from each NLOAD treatment from a separate seedling lot designated for the analysis of N concentration. Needles were pooled for a total of three samples per treatment. N concentration was analysed as described above.

2.5 Weather

Weather data were obtained from the Suomenjoki Research Unit of the Finnish Forest Research Institute. Monthly mean air temperatures were higher during the 2002 growing season and the temperature sum (1462 d.d.) was higher than the long-term (1974–2004) average (1219 d.d.). Monthly mean temperatures (°C) were May: 10.8 (long-term average 9.0), June: 15.7 (14.1), July: 18.3 (16.7), August: 17.1 (14.2) and September: 8.7 (9.2). Between 18 and 26 May, minimum air temperature 15 cm above the soil surface varied between −5.5 and 0 °C. Total precipitation in May (20 mm), July (73 mm), August (48 mm) and September (36 mm) were lower than the long-term averages (38, 82, 82 and 58 mm, respectively), but it was higher in June (104 mm compared with 69 mm). Precipitation from the planting on 16 May to the end of May or June was 10 mm and 74 mm, respectively. On 16 May, when seedlings were planted, daily mean soil temperature was 14 °C at a depth of 5 cm.

2.6 Statistical Analysis

Differences in seedling height and root growth in RGC were analyzed between NLOAD treatments by one-way ANOVA (SPSS 17.0 for Windows). Differences in N concentration, seedling height or diameter at planting, height and root growth between NLOAD treatments in the rooting experiment were analyzed by two-way ANOVA. The differences between means of NLOAD and harvesting dates were evaluated with Tukey’s test. To homogenize variance, shoot growth in RGC and root growth in rooting experiments were square-root transformed, and shoot growth log-transformed prior to exposure to ANOVA. In the soil fertility experiment, a linear mixed model (MIXED procedure in SPSS 17 for Windows) was used to analyze differences in C and C+1 needles. C needles were log-transformed prior to MIXED analysis. The differences between means of NLOAD and harvesting dates were evaluated with a Bonferroni test. Differences between NLOAD treatments on dates when 50% of buds had burst and diameter of seedlings planted on the second planting date were analyzed via a non-parametric Kruskall-Wallis test. The probability of multiple leaders was analyzed with the generalized linear model in SPSS for binary data and included block effects and NLOAD treatment.

3 Results

3.1 Bud Burst and Root Growth Capacity (RGC)

In the RGC test, the initial mean height of seedlings was 14 cm and uniform among NLOAD treatments (p = 0.749). The first buds burst three days earlier in H- and M-seedlings than in L-seedlings (p = 0.049). Half of the buds burst between 2 and 6 days earlier in H-seedlings than in L- or M-seedlings (p = 0.023), and in L-seedlings four days later than in M-seedlings (Fig. 1). No difference was found in the duration of bud burst (p = 0.304). During three weeks after planting, L-seedlings grew on average only 6 mm whereas M- and H-seedlings grew 18–27 mm (p < 0.001; Fig. 2). Mean dry mass of new roots was 33–38 mg/seedling but no differences (p = 0.238) among treatments were observed.

3.2 Morphology and Growth Rhythm

The mean initial seedling height in all NLOAD treatments was 17 cm (p = 0.921; Fig. 3) and the
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initial root collar diameter was 2.4 mm (p = 0.238). At the final harvest in September, L-seedlings were shorter (20 cm) than M- (24 cm) and H-seedlings (25 cm; p = 0.028). While harvesting seedlings in the rooting experiment for root and shoot growth monitoring, multiple-leaders were noticed of 7±4%, 8±4% and 12±4% (not significant) for L-, M- and H-seedlings, respectively. Seedlings with multiple leaders were excluded from the statistical analysis and figures displaying root and shoot growth.

There were differences in height and root growth both among NLOAD treatments (p < 0.001) and harvest dates (T) (for height growth p = 0.004, for root growth p < 0.001), and no interaction was detected. L-seedlings grew less than M- and H-seedlings (Fig. 3a). Root growth of H-seedlings was faster than that of L-seedlings (Fig. 3b).

### 3.3 Changes in Foliar Nitrogen Concentrations

In the rooting experiment, N concentration in C+1 needles decreased in M- and H-seedlings and initial differences among NLOAD treatments had become negligible by August (Fig. 4; NLOAD × T, p < 0.001). In all treatments, N concentration seemed to slightly increase from July onwards.

In the soil fertility experiment, foliar N concentration of C+1 needles in H- and M-seedlings decreased from initial concentrations of 2.7%
Fig. 3. A) Height (cm) and B) root growth (g) from planting to harvesting date of nutrient-loaded seedlings during the first growing season after planting in 16 May 2002. Vertical bars indicate the standard errors of means in each harvesting date and NLOAD treatment (N=5 blocks).

Fig. 4. Changes in N concentration of C+1 needles in nutrient-loaded seedlings loaded during the previous year and planted on 16 May 2002. Letters describe the statistically significant differences between NLOAD treatments within the harvesting date. Vertical bars indicate standard errors of block means (N=5 blocks).
and 2.2% to 1.4% and 1.1% in 11 weeks (T, p < 0.001)(Fig. 5a). The steepest decline occurred in H-seedlings and then in M-seedlings while foliar N concentration of L-seedlings remained stable until the beginning of August (NLOAD × T, p < 0.001), after which foliar N concentrations in all NLOAD treatments started to gradually increase. Differences in foliar N concentrations between seedlings planted in rich and poor soil appeared within two weeks and increased to a maximum 4–9 weeks after planting and then decreased (T × S, p < 0.001). However, in rich soil N concentration of C needles declined less than in poor soil (T × S, p < 0.001, but NLOAD × T × S, p = 0.842). In poor soil, N concentration of C needles was lowest irrespective of NLOAD treatment (NLOAD × S p = 0.032, S p < 0.001).

**Fig. 5.** Changes in N concentration of a) C+1 and b) C needles in seedlings loaded with nutrients during the previous year and planted into the poor or rich soil on 16 May 2002. Vertical bars indicate standard errors of block means (N = 5 blocks, except for first sampling in C+1 needles where N = 3).

4 Discussion

4.1 Earlier Bud Burst

Initial bud burst was hastened by a maximum of 3 days and 50% of buds burst at most 6 days earlier in nutrient loaded seedlings. Previously, Benzian et al. (1974) observed that late-season
nitrogen fertilization advanced the bud burst of several conifer seedlings growing under field conditions in Great Britain. In contrast, Bigras et al. (1996) observed in black spruce (*P. mariana* [Mill.] B.S.P.), and Fløistad (2002) and Fløistad and Kohmann (2004) in Norway spruce no or only one to three days difference in the onset of bud burst in the spring following nutrient loading. These conflicting results may be explained by different N concentrations in seedlings. In the study of Bigras et al. (1996), N concentration was low (0.6–1.28%) and only slightly higher (0.9–1.8%) in Fløistad and Kohmann (2004). This explanation is supported by an earlier field study (Rikala, unpublished) in which seedlings with N concentrations of 1.7% burst their buds two days earlier than seedlings with 1.2%. Growing conditions may also affect bud burst. We monitored bud burst in the optimal light and temperature conditions of the RGC experiment and this may have amplified the difference among NLOAD treatments.

The importance of a few days difference in the timing of bud burst depends on the weather conditions. The week following planting in May, air temperatures in several successive nights dropped below 0 °C at ground level, and the lowest temperature was −5.6 °C. In our earlier study with the same seedling material, we did not find differences in frost hardiness (LT50 −14…−17 °C) of buds among NLOAD treatments at the time of planting (Luoranen et al. 2008). However, in another experiment with Norway spruce seedlings, frost hardiness (LT50) of swollen and burst buds was between −9 and −5 °C (Luoranen et al. 2011). Thus, earlier bud burst may increase the risk of injury caused by spring frosts especially if several frosty nights occur successively, as shown by Holopainen (1988) in Scots pine (*Pinus sylvestris* L.).

### 4.2 Enhanced Growth

We observed that nutrient loading increased the height attained by Norway spruce seedlings as in previous studies (Rikala et al. 2004, Heiskanen et al. 2009). Idris et al. (2004) and Heiskanen et al. (2009) found that nutrient loading stimulates growth, especially in low fertility sites where the relative difference between loaded and unloaded seedlings was greater compared with seedlings grown in richer soil. Our results concerning nutrient loading and soil fertility are in line with their findings.

Nutrient loading did not affect root growth of seedlings growing under the favorable conditions of the RGC experiment (Fig. 2). However, the higher initial nutrient concentration enhanced the root growth of outplanted seedlings later in summer (Fig. 3b). This is consistent with the new root growth observed at the end of the first growing season in a parallel soil fertility study (Heiskanen et al. 2009). An earlier bud burst of a few days and faster shoot growth in loaded seedlings probably produced more current photosynthates that may also explain their enhanced root growth later in summer.

The discrepancy in the results of root growth between the RGC and rooting experiment may be due to the ease of water uptake of seedlings grown in the former. This is supported by a previous study with Norway spruce seedlings where differences in root growth among seedlings with different autumn N fertilization levels were negligible if seedlings were watered (Rikala et al. 2004). Notably, in the rooting experiment the weather after planting was warm but precipitation was low, which may have induced drought stress that in turn may have stimulated seedling root growth.

### 4.3 Nitrogen Concentrations

Nitrogen concentration in C+1 and C needles decreased after planting under both soil fertility levels. The decrease in C+1 needles but not in C needles was greater in seedlings with higher initial N concentration. The decrease of N concentration in needles of rapidly growing seedlings is likely caused by nitrogen dilution due to a sudden increase in biomass (Timmer 1991). Increase of carbohydrates in July may also have decreased N concentration although total N content would not have changed (Linder 1995). Foliar N concentration in C needles of all NLOAD treatments declined less in rich than in poor soil, which means that nitrogen retranslocation from old to new tissues could not compensate nitrogen uptake even in H-seedlings but site fertility already has
an observable effect on seedling nutrient status the first summer after planting.

The slower decline of N in C+1 needles of M- seedlings and the increase seen in L-seedlings growing in rich soil suggests that seedlings could begin to absorb nitrogen within two weeks. Probably due to the high initial N concentration in H-seedlings, its uptake was delayed compared with the two other treatments. The delay in N uptake of H-seedlings may also indicate an inability of the roots to absorb nitrogen due to heavy autumn fertilization, which may have hampered nutrient uptake the following spring by increasing the rhizosphere electrical conductivity beyond safe levels and ultimately impair root uptake of water or nutrients (Jacobs and Timmer 2005). This, however, appears unlikely because electrical conductivity of the peat after the nutrient loading period did not exceed 1 mS cm$^{-1}$ (Luoranen et al 2008). Or from another perspective, because L-seedlings had the least (if any) surplus of nitrogen that could be used for growth, they were more dependent on its uptake. The availability of N in rich soil may have compensated for a lower initial N in seedlings and help to explain the lack of a difference in seedling growth among NLOAD treatments planted in rich soil (Heiskanen et al. 2009).

At the end of the planting season, nutrient-loaded seedlings had larger root systems than seedlings with lower initial N concentrations. This should translate to enhanced growth in subsequent seasons due to the improved uptake of water and nutrients. However, Heiskanen et al. (2009) did not observe any such growth effects of nutrient-loaded Norway spruce seedlings in the second or third season in poor or rich soil of either a field or forested site. This result conflicts with the other studies concerning black spruce that have observed benefits at two (Malik and Timmer 1996) or even six years (Way et al. 2007). However, it remains unclear why the effect of nutrient loading lasts only for the first summer after planting although both photosynthetic leaf area and mass of new roots were increased by higher initial nitrogen concentration.

Compared with the concentration of available N measured in mounds with a double humus layer in which Norway spruce are typically planted in Finland (Smolander and Heiskanen 2007), N concentrations in the soil fertility experiment were fairly low, especially in autumn. Heiskanen and Rikala (2006) have shown that N concentration in needles can increase during the first growing season when grown in a mound system. Thus, our results may overemphasize the effects of nutrient loading when seedlings are planted in mounds. This explanation is consistent with previous observations in that when these seedlings were planted in mounds, no differences were observed in shoot growth among nutrient load levels 1–3 years after planting (Heiskanen et al. 2009).

4.4 Conclusions

The buds of loaded seedlings burst earlier than buds in unloaded seedlings and initial differences in nitrogen concentrations disappeared during the planting season. However, we found that nutrient loading increased the shoot and root growth of seedlings and did not explain why loading effects lasted only the planting season in Norway spruce seedlings. As a conclusion of results reported here and from our earlier studies (Heiskanen et al. 2009, Luoranen et al. 2008), nutrient loading confers several advantages in the form of improved hardiness to autumn frosts in the application year and enhanced root growth after outplanting, especially in low fertility soils. However, earlier bud burst caused by nutrient loading may increase the risk of seedling exposure to frost damage after outplanting in spring. The growth effect of nutrient loading was shorter than expected and found in previous studies (Malik and Timmer 1996, Way et al. 2007).

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