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Title: Effects of nutrient loading and fertilisation at planting on growth and nutrient status of Lutz spruce (*Picea x lutzii*) seedlings during the first growing season in Iceland

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Abstract

The low availability of nitrogen is believed to be one of major limiting factors of forest regeneration in the nitrogen-poor volcanic soils of Iceland. To investigate the effects of nutrient loading seedlings in nursery combined with fertilizing at planting, Lutz spruce (*Picea x lutzii Littl*.) seedlings were nutrient loaded using four fertilization regimes (0.0, 0.9, 2.7 or 3.9 g N/m^2 per week) in the end of nursery rotation and planted next spring 2009. Planting was done at two places in northern Iceland and seedlings were either single dose fertilized or not. The highest loading level in the nursery without field fertilization increased new needle mass by 122% and 152%, for the poor and more fertile site, respectively. The highest loading level with field fertilization increased new needle mass by 188% and 189% compared to no loading and field fertilization for the poor and more fertile site, respectively. Retranslocation of N increased with more loading in the nursery. The results illustrate the significance of the retranslocation of stored nutrients to support new growth early in the season when root growth and nutrient uptake are still low. It was however clear that nutrient loading could not replace field fertilization, as the seedlings generally showed an additive response to field fertilization and nutrient loading in the present study. This also meant that field fertilization did not either replace the effect of nutrient loading; doing both always gave the best results in seedling performance the first field season.

Key words: Field fertilization, Lutz spruce seedlings, nutrient loading, seedling establishment

Introduction

Sitka spruce (*Picea sitchensis* (Bong.) Carr.) and Lutz spruce (*Picea x lutzii* Littl.) are the second most planted coniferous species in Iceland, with 24,2% of the total annual planting (Gunnarsson, 2011). Lutz spruce is a hybrid of Sitka spruce and white spruce (*Picea glauca* (Monech) Voss). Lutz spruce has proven more resistant to autumn frosts than Sitka spruce in Iceland (Skúlason *et al*. 2001), but has a similar growth rate (Blöndal 2004). This makes it more suitable for plantations in northern Iceland, where summers are shorter and drier than those in southern Iceland. In the southern parts Sitka spruce is more suitable because of the area's milder climate and greater precipitation in the form of rain (Skúlason *et al*. 2001).

The first growing season is critical because then seedling roots have minimal contact with the soil causing high risks for water shortage and nutrient stress (Burdett et al. 1984; van den Driessche 1991). Furthermore, newly planted seedlings depend on the internal mobilization of nutrients, a natural phenomenon called retranslocation (Burdett et. al 1984). Retranslocation is depleted elements from older plant components that are made available for new growth (Lim & Cousens 1986). Accumulation of nitrogen (N) in plants can occur in nature when availability of N is abundant or when supply exceeds the capacity of plants to utilize N for growth (Millard 1988). The accumulated N can be stored and used later to support new growth during times of N limitation (Chapin III 1980).

Nutrient status of conifer seedlings when planted is considered to be one of the key factors in their survival because of their initial slow root development that limits uptake from the soil

(Burdett et al. 1984). They depend therefore heavily on the retranslocation of internal nutrient reserves to sinks of new growth soon after transplanting (Chapin III 1980).

Nutrient status of seedlings is especially critical when forest plantations are established in treeless areas with low nutrient availability. The low availability of nitrogen is believed to be one of the major limiting factors in plantations establishment in the nitrogen-poor volcanic soils of Iceland (Óskarsson & Sigurgeirsson 2001; Ritter 2007).

The mineral nutrient shortage can be ameliorated either by ensuring a good nutrient status of seedlings when they leave the nursery (Grossnickle 2000; Rytter et al. 2003) or by an addition of fertilizer at planting. The latter has been a common practice in afforestation in Iceland since the 1990s, where a low-dose fertilizer application of ca. 2-3 g N per seedling has been applied (Óskarssson & Sigurgeirsson 2001).

Óskarsson (1997) examined the effects of various fertilizer applications at the time of planting on the survival and growth of three tree species: downy birch (*Betula pubecsens* Ehrh.), Siberian larch (*Larix sibirica* Ledeb.) and Sitka spruce in southern Iceland. After two growing seasons, results showed that application of N and P improved both survival and growth. Survival was improved with fertilization at planting by 30-40%, which was related to reduced frost heaving of seedlings during the first winter after planting. Furthermore, seedlings in unfertilized treatments all suffered from N and P deficiency (Óskarsson 1997).

A fertilization practice called nutrient loading utilizes the ability of plants, when they have stopped shoot elongation, to consume and store excessive nutrients towards the end of nursery cultivation (Grossnickle 2000). The term nutrient loading has been defined by Timmer

(1996) as fertilization in excess of the demand for current growth during nursery cultivation to induce luxury uptake of nutrients characterized by increased internal concentration in plants without significantly changing the plant's total dry mass.

In a field experiment with downy birch and Sitka spruce in western Iceland Óskarsson and Brynleifsdóttir (2009) found that nutrient loading can increase growth and survival. Other studies elsewhere have also showed that the fertilization practices in the nursery can significantly improve subsequent field performance (van den Driessche 1991; Timmer 1996; Salifu & Timmer 2003a).

The approach of this study was to (1) load seedlings of Lutz spruce (*Picea x lutzii*) with different amounts of nutrients in nursery in the autumn, without significantly changing their total biomass, and (2) plant them in the spring with or without a single low-level dose of fertiliser at two places in N-Iceland with contrasting soil fertility. This way we wanted to create a large range in seedling nutrient status and nutrient availability at planting, but of otherwise comparable seedlings.

To our knowledge very few studies have compared the effects of nutrient loading and field fertilization on different soil fertility sites. Therefore the aims of this study were to investigate: a) the effects of nutrient loading on biomass allocation and nutrient status after the nursery rotation. b) how important initial seedling nutrient status and translocation is for growth during the first growing season. c) if nutrient loading in the nursery would give the similar result as field fertilisation at planting or if their effects were additive. d) if the response of nutrient loading and/or field fertilisation was dependent on the site quality.

Material and Methods

Pretreatments

The provenance of Lutz spruce (*Picea x lutzii*) used in the present study was 'Seward' (60,06°N, 149,26°W). The nursery treatments were applied in *Sólskóga*r, a nursery in Akureyri in northern Iceland (65,66°N, 18,10°W). Lutz spruce was sown on the 11th of April 2008 one seed per cavity in plastic conical multipots (BCC, HIKO- 93, Sweden). Each pot has a volume of 93 cm^3, with 526 cells per square meter (40 cavities per tray). The growing media was Finnish peat (M6, Kekkilä Oy, Tuusula, Finland). Fertilization began on the 28th of April by mixing the fertilizer (Kekkilä Stock Superex, NPK 19-4-20, Kekkilä, Co., Tuusula, Finland) with the irrigation water to an electrical conductivity (EC) rate of 1.0 mS/cm. Seedlings were cultivated in a heated greenhouse until the 3rd of July and then moved outdoors for further growth. On the 6th of August the seedlings were divided into different nutrient loading treatments and the seedlings were moved to unheated plastic greenhouse. Trays with seedlings of similar size were chosen for the experiment, in total 128 trays (5120 seedlings). The trays were randomly divided into four fertilizer treatments and fertilized with either 0.0, 0.9, 2.7 or 3.9 g N/m^2 per week (Table 1).

[TABLE 1 HERE]

Each treatment had four replicates in the greenhouse, each containing eight trays. The fertilizer was applied in solution twice per week between 6th of August until 27th of September. The fertilizer was mixed into 1.2 l of water. A watering can with a mini boom was use to spread the fertilizer. The seedlings were rinsed with two liters of clean water after each fertilization. If the weight of the trays was reduced to 65-70% of the container capacity between fertilizer applications, supplemental irrigation with no fertilizer was applied. All seedlings were treated with long nights (8 hours/day) from the 12th to the 26th of August. On the 18th of November all seedlings were packed in cardboard boxes and stored over the winter in a freezer at -3 °C.

Biomass measurements and nutrient analysis

Eight seedlings were randomly sampled from each replication on the 18th of November 2008. The seedlings sampled were kept at -18°C until biomass- and nutrient analyses were conducted. The shoots were cut from the root systems at the root collars. All the soil was washed from the root systems. Root systems and shoots were oven dried for 24 hours at 85°C. Then all needles were removed from shoots and branches. Before weighing the different parts of the seedlings they were once more oven dried once more for 24 hours at 85°C.

Needle total nitrogen (N) was analysed at Efnagreiningar Keldnaholti (ICETEC, Reykjavik, Iceland) using Kjeldahl's wet combustion on a Tecator Kjeltec Auto 1030 Analyzer. Other mineral elements were measured with Spectorflame D Sequential Instrument.

Assessment of freezing tolerance

Freezing tolerance was measured with the Shoot Electrolyte Leakage technique (SEL) (see Lindström & Håkansson, 1995) on two occasions. On the 20th of October samples were frozen to – 25 °C and on the 14th of November samples were frozen to -35°C. For these tests 30 seedlings from each replication were randomly sampled. The uppermost 4 cm. of the shoots were cut off and rinsed in deionised water. Three shoots were put in a screw-capped plastic bottle, in total 10 bottles per replicate. Half of the samples were slowly frozen to -25° C (or -35°C). The other half was kept at 2°C. The temperature was lowered from room temperature to 2°C over a period of two hours. The freezing rate was then set to 2-3°C/hour. When -25°C (or -35°C) was reached, these temperatures were maintained for two hours to ensure complete freezing. Samples were slowly thawed by raising the temperature 2°C/hour until a temperature of 2°C was reached. After thawing, all bottles were filled with 40 ml. of deionised water and put in a shaker for 24 hours. Conductivity from the water was measured from all bottles, frozen and unfrozen, with a conductivity meter (Jenway, 4070). All the tissue samples were then killed by autoclaving (at 121°C for 15 minutes at 2,1 bars pressure) to release any remaining electrolytes. When the samples had cooled to room temperature the total conductivity from each sample was measured. The freezing damage was then estimated as relative conductivity, the ratio of conductivity after freezing to the total conductivity after killing the tissues. If the difference between relative conductivity of the control sample and the frozen sample was less than 5, the seedlings are considered to have reached enough freezing tolerance to be safely stored in a freezer (Lindström & Håkansson 1995).

Field planting

The effect of the nutrient loading on seedling performance was tested at two locations with different soil fertility levels in northern Iceland. The first, site A in Figure 1, at Stóru Hámundarstaðir (65°57´ N, 18°27’ W) is located approximately 44 km. north of Akureyri. This site is dominated by heath vegetation, including *Vaccinium uliginosum* L., *Empetrum nigrum* L., and *Betula nana* L. This site-type is considered poor for spruce (Skúlason et al. 2006). The site was scarified at 26th of May 2009, using shallow scarification with a homemade plough to remove the vegetation cover. Site B at Reykir (65°29´ N, 19°22’ W) is located approximately 120 km W of Akureyri (Figure 1). The site is dominated by a grassland vegetation, including *Festuga rubra* L. and *Poa pratensis* L. This site-type is considered to be more fertile and suitable for spruce (Skúlason et al. 2006). At this site the vegetations surface was scarified with a TTS-10 disc trencher (TTS Forest Oy, Finland) and planting was done in 2nd of June.

[FIGURE 1 HERE]

Field experiment design

The experimental design consisted of four randomized blocks on both sites. The seedlings deriving from the four loading treatments were randomly placed in two rows of 40 plants, in each block. Of these one row was fertilized immediately after planting. This increased the number of treatments to eight (Table 2). Ten g of solid mineral fertilizer ‘Sprettur’ (Carrs Fertilisers, Scotland) were applied by hand in a 15 cm circumference around each seedling selected for fertilization. The fertilizer is a blend of NP (23-5.2-0) and also contains S (2.4%), Ca (2.65%) and Mg (1.5%).

[TABLE 2 HERE]

Field performance

The seedlings were measured after the first growing season in September 2009. Height and annual increment of the leading shoot was measured for each seedling. If the leading shoot was missing because of damage, height growth was measured on the topmost side shoot.

The height of all seedlings per treatment was dived into five classes and the aboveground parts of three seedlings per treatment in each block were harvested from the most frequent height-class (in total 96 seedlings from each site). The seedlings were divided into current (C+0) and old (C+1) branches, needles and stems, dried at 70 °C for 48 hours and weighed. Needles were analyzed for nutrient content using the same methods described earlier.

Calculation of nutrient dynamics

The contribution of external N (reserves of N in roots or other plant parts other than old needles, and/or soil) to the total N content in current needles was estimated by the difference in total N content in current needles and the amount of N retranslocated from old needles (Figure 7) as described by Malik and Timmer (1998) and Imo and Timmer (2001).

Statistical analysis

First the normality of each treatment was checked by inspecting normal probability plots and stem and leaf diagrams in SAS 9.1 software (SAS Institute, Inc., Cary, NC, USA). Only annual increment required transformation. The four nursery nutrient loading means were compared using a One-Way ANOVA and the results from the eight field treatments were analysed with Two-Way ANOVA, followed by a pairwise Fisher's Least Significant Difference Tests when main factors proved significant.

Results

Nutrient loading

The nutrient loading in nursery in August-September did not affect the seedlings total biomass when put into winter storage in November (Figure 2). Dry mass ratios (needle mass ratio, branch mass ratio, stem mass ratio and root mass ratio) were not affected either, except that root mass ratio was slightly lower for seedlings receiving higher doses of fertilizer (see table 1), treatments II and III (both $P < 0.05$ from treatments 0 and I; data not shown).

[FIGURE 2 HERE]

Although total dry mass of non-loaded and loaded seedlings was similar, their nutrient concentrations differed significantly, especially in N concentration (Table 3). Only the unloaded treatment had N concentrations below the given optimal N concentration for spruce. The increase in N caused by the nutrient loading was 29%, 41% and 48% for treatments I, II and III, respectively. There were no significant differences in the P:N ratios of the treatments, but the K:N ratio decreased significantly with increased loading. Treatment III, receiving the highest nutrient loading (31.4 g N m^{-2}), had 42% lower K:N ratio than the unloaded treatment, but still above the given optimal ratio. The Mg:N ratio also declined significantly with increased loading, and was below the optimal Mg:N ratio for all loaded treatments, but treatments II and III both had significantly lower Mg:N ratio than the two other treatments (Table 3).

[Table 3 Here]

Shoot freezing tolerance was not significantly affected by the loading treatments (data not shown). In October the seedlings tolerated -25 °C and in November -35 °C.

Field performance

Survival was overall good after the first growing season in the field. It was not affected by loading treatment, but was on average 91% and 97% for unfertilized seedlings in the field and seedlings receiving field fertilization, respectively (ANOVA, P<0.001; data not shown).

Both nutrient loading and field fertilization at planting improved seedling shoot growth during the first growing season (Figure 3). The seedlings receiving the highest loading treatment without field fertilization increased annual shoot elongation by 51-54% for sites A and B, respectively. The loading effect on shoot elongation was highly significant (P<0.001). Field fertilization also increased annual shoot elongation on average by 48-65% at sites A and B, respectively. At the poor site A there was no interaction between nutrient loading and field fertilization, both treatments tended to increase height growth, even if the difference was not significant between loading treatments II-0 + II-F and III-0 + III-F, respectively (statistics not shown). There was a significant interaction between nutrient loading and field fertilization at the more fertile Site B (Load. x Fert. P = 0.04). This was because increased loading constantly increased height growth, while field fertilization did not significantly differ between loading treatments I, II and III (Figure 3; statistics not shown).

[FIGURE 3 HERE]

Shoot mass was divided into mass of old wood, old needles (C+1), current branches and current needles (Table 4). All biomass fractions were significantly increased both by nutrient loading and field fertilization at both sites, except old needles (Table 4). No significant interaction was detected between loading and field fertilization. Unfertilized loaded seedlings increased their old wood mass, on average 17% and 36% on sites A and B respectively. The increase of old wood mass was on average 51% and 70% for the fertilized seedlings in sites A and B, compared to the 0-0 treatment. The III-0 treatment increased new needle mass by 122% and 152%, for sites A and B, respectively (Table 4). Similarly the III-F treatment increased new needle mass by 188% and 189% compared to the 0-0 treatment for sites A and B, respectively.

[TABLE 4 HERE]

Nitrogen status

At the end of the first growing season the nitrogen status current needles of all treatments was considerably lower than optimum (Figure 4, the dotted line). Nutrient loading in the nursery only led to significantly higher N status at site A (Figure 4; Loading effect), where the three nutrient loading treatments had on average 10% higher N concentrations in the autumn than the unloaded treatments. It was, however, still below the critical N-stress limit in the autumn, indicated by the dashed line (Figure 4). The field fertilization had a positive significant effect on seedling N status but the N-levels were still below the optimum levels in the autumn (Figure 4, the dotted line).

[FIGURE 4 HERE]

Nitrogen contents

Total nutrient content for N, was calculated by multiplying nutrient concentration (mg g^{-1}) by total needle mass. Nutrient loading and field fertilization both significantly stimulated total N content at both sites (Figure 5). On average, loaded seedlings without field fertilization increased N content in current needles by 104% and 109% for sites A and B, respectively, compared to the 0-0 treatment. Corresponding values for field fertilized seedlings showed an increased in N content of 612% respectively 559%.

[FIGURE 5 HERE]

There was also a significant loading x fertilization interaction at the more infertile site A (Figure 4). This was caused by a relatively smaller seedlings in the 0-F treatment, compared to I-F, II-F and III-F treatments there. This pattern was also seen at the more fertile site B, but the differences were not enough to cause a significant interaction (Figure 4). The N content in old needles was not affected by the loading treatments on either site, but field fertilization had highly significant effect on N content in old needles (data not shown).

Retranslocation of nitrogen

Since the N content of the needles in 2008 was known and the N content of both old and current needles in 2009, the retranslocation of N from older needles to current needles could be calculated. The contribution of internal N to current needles was estimated as the difference in the amounts of N in old needles before and after planting. Both nutrient loading and field fertilization significantly increased retranslocation between old and new needles at both sites, while no significant interaction was observed (Figure 6).

[FIGURE 6 HERE]

The most loaded seedlings without field fertilization (treatments II-0 and III-0) showed more retranslocation than 0-0 and I-0 (Figure 6). The difference was on average 42% and 32% on sites A and B, respectively. This was also the case for seedlings receiving a combination of nursery loading and fertilizer at planting (II-F and III-F), where retranslocation was on average 84% higher at both sites.

Seedlings that were only fertilized at planting showed a reduced N retranslocation at both sites (Figure 6). Unfertilized seedlings had on average 62% and 40% more retranslocation than fertilized at sites A and B, respectively.

[FIGURE 7 HERE]

Unfertilized seedlings in sites A and B showed negative values when their external N contribution was calculated (Figure 7). Negative values indicate retranslocation of N to plant parts other than current needles. The loaded seedlings at site A received significantly more N (P=0.03) from external sources than the unloaded seedlings. Conversely, there was no significant difference between loading treatments at site B. Fertilization at planting increased external N contribution dramatically at both sites (Figure 7).

Discussion

Effects of nutrient loading in nursery

This study showed that by nutrient loading Lutz spruce seedlings toward the end of nursery cultivation it was possible to improve nutrient status without changing their total biomass. This is an important finding for the nurseries, as loading can be done without exceeding the seedling size standards for delivery. Our results show that nutrient loading induced luxury nutrient uptake without altering the total biomass of the seedlings is consistent with previous findings (Malik & Timmer 1998; Idris et al. 2004).

The loading treatments (see table 1) I and II had needle N concentrations just within optimum values for spruce (Ingestad 1991). The III treatment, which received the highest nutrient loading (31.4 g N/m^2), had N concentrations that were slightly above the optimum values for spruce (26 mg N g^{-1} DM). This did not have much negative effects during nursery phase, except that root mass ratio was slightly lower for seedlings receiving higher doses of fertilizer (treatments II and III). This effect is well-known from previous studies (Troeng & Ackzell 1988).

Moreover, there was no significant difference in N concentration between the two highest loading treatments (II and III), indicating that the loading efficiency was reduced at highest loading levels. This is consistent with the existence of an upper threshold for effective loading of seedlings (Malik & Timmer 1998).

All target nitrogen ratios between different nutrients and N were considered optimal for seedlings in all treatments (cf. Linder 1995), except for the Mg:N ratio in the two highest loading treatments. So far, we have not seen any negative effects of this suboptimal ratio on seedling performance in the field. This could be important in nursery fertilization in Iceland, but most nurseries use the nutrient solution used in this project.

Seedling growth after one growing season in field

In the field trials nutrient loading in the nursery stimulated shoot elongation and development of above ground biomass as have been reported earlier in similar studies (Timmer & Munson 1991; Malik & Timmer 1995, 1998; Timmer 1996; McAlister & Timmer 1998; Salifu & Timmer 2001, 2003b; Imo & Timmer 2001; Kaakinen et al. 2004; Óskarsson & Brynleifsdottir 2009; Heiskanen et al. 2009).

It was however clear that nutrient loading could not replace field fertilization, as the seedlings generally showed an additive response to field fertilization. Both treatments together always resulted in better field performance than the selection of one treatment.

The effects of nutrient loading and field fertilization were affecting all above ground parts of the seedling except for the old needles (C+1). Their role was probably mainly to translocate nutrients to new growth. The old wooden parts of the seedlings showed a positive growth response as well as a strong treatment effect on growth of current needles. This is essential since current needles has the potential of increasing the seedling photosynthesis and simultaneously create a large N-sink from where it can be retranslocated for future growth (van den Driessche 1991).

N concentration

The positive loading effect from fertilizing the seedlings in the nursery on the N concentration of the seedlings had totally disappeared at the end of the first growing season. Seedlings not fertilized at planting became critically low in N-status at both sites (<9 mg N g^{-1}; Ingestad 1962), whereas field fertilization resulted in higher N concentrations but still somewhat below optimum N-status. Probably, most of the available N in the seedlings that was not fertilized at planting was used up during growth. Conifer seedlings are known to have a slow initial root development (Burdett et al. 1984), which may limit their possibilities to replenish their N status with an uptake from the soil during the first year. Similar depletion of nutrients was also observed by Munson and Bernier (1993). In their study the benefit of increased nutrient status in black spruce (*Picea mariana* (Mill.) Britton, Sterns & Poggenburg) was short lived and the nutrient reserves declined after planting, due to dilution in tissue nutrient concentrations especially if external nutrient sources could not meet the demands of new growth.

Only at the poor site A, nutrient loading led to a significantly higher N concentration at the end of the season. We also found, besides N concentration, that growth was significantly lower at the poor site A compared to the more fertile site B without any nutrient loading in the nursery before planting (0-F). Even though we did not find a study that has looked at effects of preloading seedlings, field fertilization and site effects together there are studies indicating better relative growth responses on the more nutrient deficient sites. (Timmer 1996; Timmer & Munson 1991; Idris et al. 2004).

Nitrogen content

It must be recognized that a change in nutrient concentration alone is not an unequivocal indication of change in nutrient status, since dry weight changes, due to growth or respiration, will result in changes of nutrient concentration (van den Driessche 1991). Nursery loaded and field fertilized seedlings (I-F, II-F and III-F) partitioned significantly more N to current needles than unloaded field fertilized seedlings (0-F). The high N content indicated that current needles were a major sink for absorbed N in the first growing season. Imo and Timmer (2001) also report current shoots to be a major sink for absorbed N in their study with black spruce seedlings. Furthermore, Malik and Timmer (1998) reported that nutrient loaded black spruce seedlings depleted their nutrient reserves when planted, but also took up more nutrients from soil than unloaded seedlings. This was attributed to improved root growth in the nutrient loaded seedlings (Malik & Timmer 1998; Salifu & Timmer 2001).

Retranslocation of N.

Using N content in calculating retranslocation is considered more robust than using N concentration data alone that may over or under estimate retranslocation because of confounding by dilution due to high biomass accumulation (Malik & Timmer 1998). The calculations are therefore based on the content of N in the needles as that method has been shown to be more reliable than using concentration alone in estimating N flux between plant parts (Nambiar & Fife 1987; Munson *et al.* 1995; Imo & Timmer 2001). When calculating retranslocation, it must be kept in mind that nutrients are distributed in various plant parts. Van den Driessche (1991) found that two year old white spruce seedlings contained 12 mg N in their needles, 5 mg N in the stem and 5 mg N in roots. Only the N content in old and current needles was used to calculate retranslocation in the present study; same as was done by Nambiar and Fife (1987) and Munson et al. (1995).

Retranslocation from old to current needles increased with more loading in the nursery for both fertilized and unfertilized seedlings. This has also been observed in other studies (McAlister & Timmer 1998; Malik & Timmer 1998; Salifu & Timmer 2001, 2003a; Imo & Timmer 2001). The reason for higher retranslocation in nursery loaded seedlings, may be that N reserves in these seedlings were less structurally bound, hence readily available for depletion to active metabolic sinks. However, in this study, field fertilization at planting seemed to reduce N retranslocation, which is consistent with the findings of others (Imo & Timmer 2001; Salifu & Timmer 2001).

Fertilized seedlings had partitioned more N to current needles than unfertilised seedlings in the end of the growing season and therefore their estimated retranslocation was lower than for unfertilized seedlings. This has to be kept in mind when those results are interpreted. The only way to calculate retranslocation with certainty is to use labelled isotopes. Without labelled isotopes the N uptake from the soil cannot be separated from that remobilized internally by the plants (Salifu & Timmer 2003a).

Nutrient loading late in the nursery cultivation was successful in changing seedling nutrient status without altering the size of the seedling or affecting the freezing tolerance.

Both nutrient loading and fertilization at planting stimulated growth during the first growing season in field, and their effects were additive. Based on these results, nutrient loading in nurseries could improve field performance of newly planted seedlings, especially when planted at unfertile and treeless sites which are common in Iceland.

Nutrient translocation from older needles to younger was an important response where nutrient status was getting close to critical values. It also seems to be the dominant supply for N for new growth on poor soils in spruce seedlings during the establishment phase following planting. This finding has many implications, besides explaining why good nutrient status is so important during the first growing season. It also indicates that if young spruce trees lose their older needles at a young age, e.g. because of autumn insect damage or frost episodes, it is very important to fertilize the plantation to replace the lost retranslocation potential from the older needles.

The differences between the two sites were not large and the loading and field fertilization were much more important for seedling N-status at the end of the first growing season than site quality. Since conifer seedlings are known to have slow initial root development, site-type effects are most likely to develop in subsequent growing seasons when root uptake has increased. Therefore it is important to follow what happens in these field experiments during the forthcoming years.

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TABLES

Table 1. The nutrient loading treatments applied to Lutz spruce seedlings in the nursery. Electric conductivity in the irrigation water (EC) and amounts of N applied per area or seedling. The different fertilizing regimes started in August 6 and ended September 27. Before that seedlings were equally fertilized.

Nursery treatm.	EC mS/cm	Total N (g m^{-2})	Weekly N (g m^{-2})	Seedling N (g $seedl.^{-1}$)
0	0	0	0	0
I	1.1	7.8	0.9	0.015
II	3.2	22.2	2.7	0.042
III	4.2	31.4	3.9	0.059

Table 2. Treatments tested in the field trials on Luzt spruce and number of seedlings used in each field treatment. 0 – 0 = no nursery loading or field fertilization, I = low nursery loading, II = medium nursery loading, III = high nursery loading, F = field fertilization (see also Table 1).

Treatm.	Nursery (g N $seedl^{-1}$)	Field (g N $seedl^{-1}$)	No. of seedlings
0-0	0	0	160
0-F	0	2.4	160
I-0	0.015	0	160
I-F	0.015	2.4	160
II-0	0.042	0	160
II-F	0.042	2.4	160
III-0	0.059	0	160
III-F	0.059	2.4	160

Table 3. Nutrient concentrations (mg g^{-1} DM) and N-ratios in needles of Lutz spruce seedlings exposed to different nutrient loading treatments, indicated by the total amount of N supplied from 6. August – 27. Sept 2008 (see Table 1). Samples were taken in early November, n=4.

Nutrients	Treatments				Optimum
	0	I	II	III	values[1]
Concentrations					
N	17.55 ± 0.43 a	22.71 ± 0.62 b	24.81 ± 036 c	25.97 ±0.82 c	20-25
P	2.15 ± 0.03 a	2.88 ± 0.11 b	3.05 ± 0.09 b	3.18 ± 0.14 b	
K	12.43 ± 0.20 a	13.73 ± 0.44 a	13.58 ± 0.39 a	12.95 ± 0.45 a	
S	1.15 ± 0.03 a	1.48 ± 0.05 b	1.63 ± 0.03 c	1.70 ± 0.04 c	
Ca	3.05 ± 0.13 a	2.90 ± 0.16 a	2.80 ± 0.11 a	2.78 ± 0.12 a	
Mg	0.90 ± 0.04 a	0.98 ± 0.05 a	0.93 ± 0.03 a	0.88 ± 0.03 a	
Ash	30.45 ± 0.29 a	33.03 ± 0.97 a	32.35 ± 0.76 a	33.90 ± 2.77 a	
N-ratios					
P:N	12.21±1.8 a	12.62±0.19 a	12.38 ±0.19 a	12.29±0.14 a	>10
K:N	70.82 ± 19.6 a	60.47±1.90 b	54.72 ±0.94 c	49.80 ±0.61 d	>35
S:N	6.46 ±1.5 a	6.52 ±0.09 a	6.57 ±0.05 a	6.55 ±0.05 a	>2.5
Ca:N	17.38 ±5.6 a	12.79 ±0.43 b	11.35 ±0.38 c	10.62 ±0.09 c	>4
Mg:N	5.07 ± 1.6 a	4.28 ± 0.15 b	3.72 ±0.09 c	3.40 ±0.09 c	>5

[1] According to Ingestad (1962), Linder (1995) and Sigurdsson (2001).

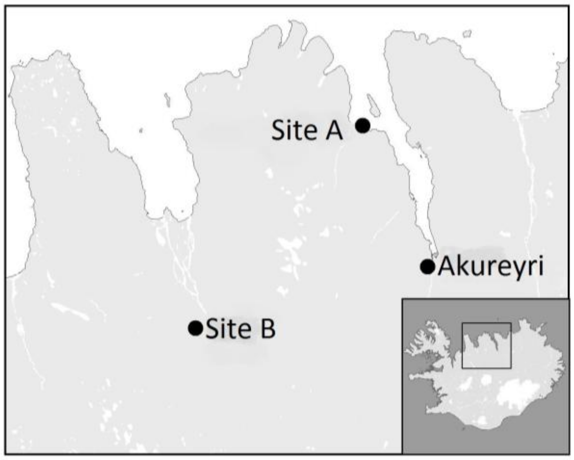
Table 4. Dry mass of old wood, old needles, current branches and current needles (g/plant) of unloaded and loaded (see Table 1) Lutz spruce seedlings in nursery, planted with or without fertilization (see Table 2). Samples were taken in late November 2009. Column values in the same site (A or B) followed by the same letter are not statistically different at the P< 0.05 level. Results were found using a Two-Way ANOVA and post-ANOVA Fisher's Least Significant Difference test, number of sampled seedlings 12 per treatment, n = 4.

Treatments	Site	Old wood	Old needles	Current branches	Current needles
0/0	A	1.22 ± 0.07 a	1.09 ± 0.10	0.12 ± 0.01 a	0.78 ± 0.05 a
I/0		1.32 ± 0.09 ab	1.10 ± 0.09	0.18 ± 0.02 b	1.16 ± 0.08 b
II/0		1.43 ± 0.17 b	0.92 ± 0.10	0.29 ± 0.05 c	1.46 ± 0.22 bc
III/0		1.54 ± 0.11 b	1.13 ± 0.12	0.35 ± 0.04 c	1.73 ± 0.14 c
0/F		1.37 ± 0.14 a	1.06 ± 0.08	0.22 ± 0.05 a	1.00 ± 0.20 a
I/F		1.68 ± 0.16 ab	1.02 ± 0.11	0.49 ± 0.06 b	1.90 ± 0.19 b
II/F		1.94 ± 0.15 b	0.96 ± 0.08	0.61 ± 0.07 c	2.34 ± 0.16 bc
III/F		1.91± 0.22 b	0.97 ± 0.09	0.57 ± 0.08 c	2.25 ± 0.30 c
0/0	B	1.05 ± 0.10 a	0.99 ± 0.11	0.13 ± 0.01 a	0.79 ± 0.07 a
I/0		1.27 ± 0.14 ab	1.04 ± 0.16	0.25 ± 0.02 b	1.40 ± 0.16 b
II/0		1.46 ± 0.17 b	1.09 ± 0.16	0.31 ± 0.03 c	1.61 ± 0.19 bc
III/0		1.55 ± 0.11 b	1.07 ± 0.11	0.44 ± 0.04 c	1.99 ± 0.14 c
0/F		1.41 ± 0.10 a	1.15 ± 0.09	0.30 ± 0.06 a	1.25 ± 0.14 a
I/F		1.58 ± 0.12 ab	0.95 ± 0.10	0.57 ± 0.07 b	1.93 ± 0.19 b
II/F		1.91 ± 0.12 b	1.24 ± 0.09	0.63 ± 0.07 c	2.06 ± 0.19 bc
III/F		1.88 ± 0.22 b	1.07 ± 0.13	0.63 ± 0.08 c	2.28 ± 0.27 c
Site	Sources of variation:				
A	Loading	0.023	ns	<0.0001	<0.0001
B	Loading	0.007	ns	<0.0001	<0.0001
A	Fertilization	0.002	ns	<0.0001	<0.0001
B	Fertilization	0.0007	ns	<0.0001	0.001
A	Load x Fert	ns	ns	ns	ns
B	Load x Fert	ns	ns	ns	ns

ns = ANOVA model not significant (P>0.05).

FIGURES

Fig 1. Location of the two planting sites, site A (poor) and site B (more fertile).



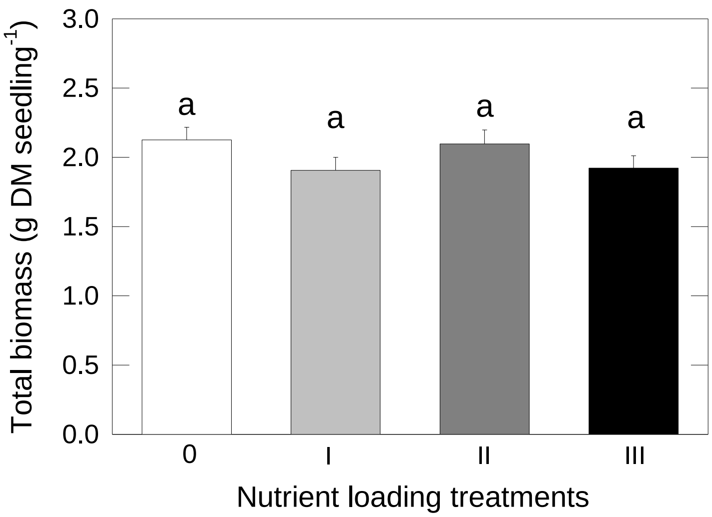


Fig. 2. Average (±SE) total biomass of Lutz spruce seedlings exposed to different nutrient loading treatments (see Table 1). Samples were taken in early November 2008. Different letters above the bars indicate significant differences between total biomass means, found by One-Way ANOVA and post-ANOVA Fisher's Least Significant Difference test, total number of seedlings in a treatment 32, n=4.

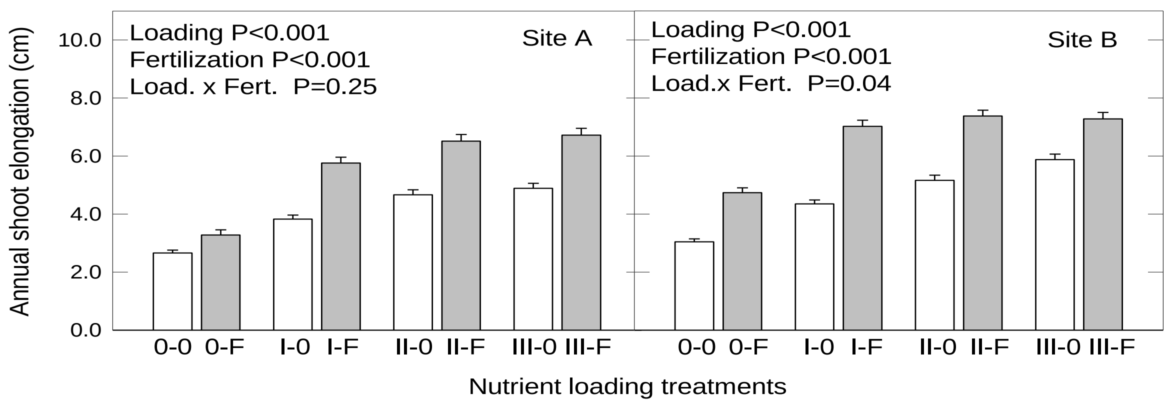


Fig. 3. Average (±SE) annual shoot elongation (cm) of Lutz spruce seedlings exposed to different nutrient loading treatments (see Table 1) and planted in the spring 2009 with (filled bars) or without (empty bars) field fertilization (see Table 2) at sites A and B. Seedlings measured in autumn 2009. Statistical results were found by using a Two-Way ANOVA, n=4 .

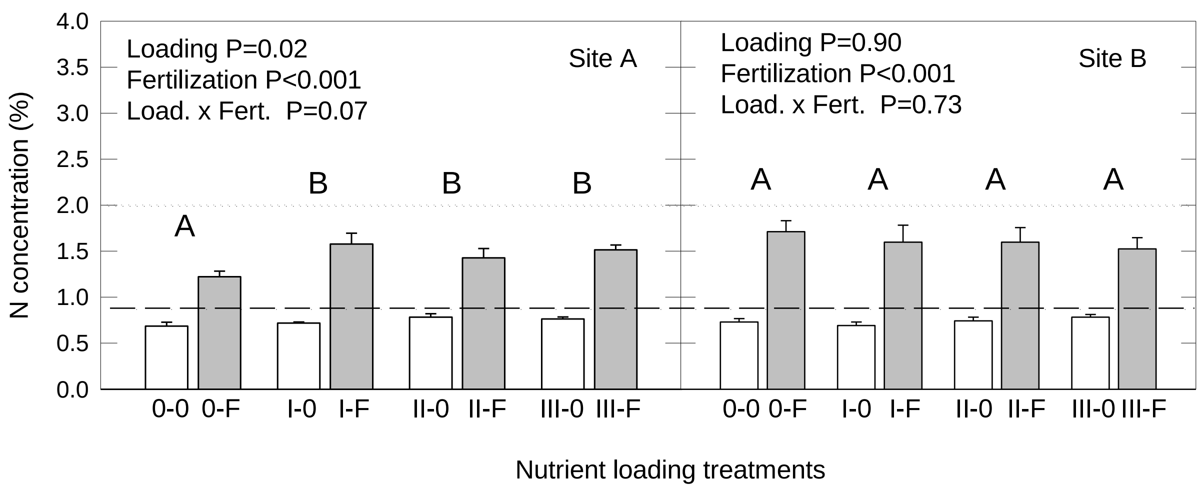


Fig. 4. Average (±SE) nitrogen concentration in current needles of Lutz spruce seedlings that had been exposed to different nutrient loading treatments (see Table 1) and planted with (filled bars) or without (empty bars) field fertilization (See table 2) at sites A and B. The dotted line indicates optimum N status according to Ingestad, (1962) and Roberntz, (1998). Dashed line

indicates a N deficiency (<9 mg N/g) according to Ingestad, (1962). Different letters above the bars indicate significant differences between loading treatments at each site. Statistics were obtained by using a Two-Way ANOVA and post-ANOVA Fisher's Least Significant Difference test.Tvelwe seedlings sampled per treatment, n=4.

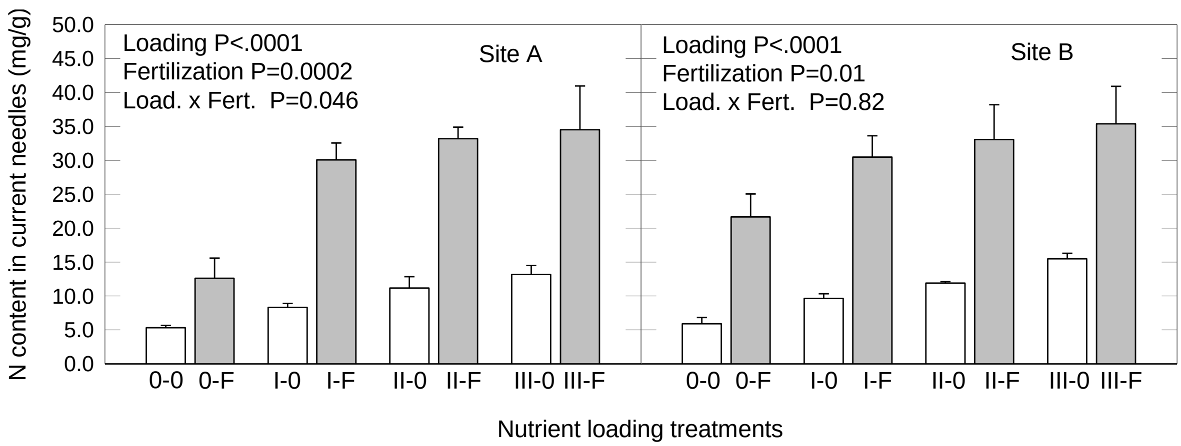


Fig. 5. Average (±SE) total N content in current needles of Lutz spruce seedlings exposed to different nutrient loading treatments (see Table 1) and planted with (filled bars) or without (empty bars) field fertilization (see table 2) at sites A and B. Statistical results were obtained by using a Two-Way ANOVA. Tvelwe seedlings sampled per treatment, n=4.

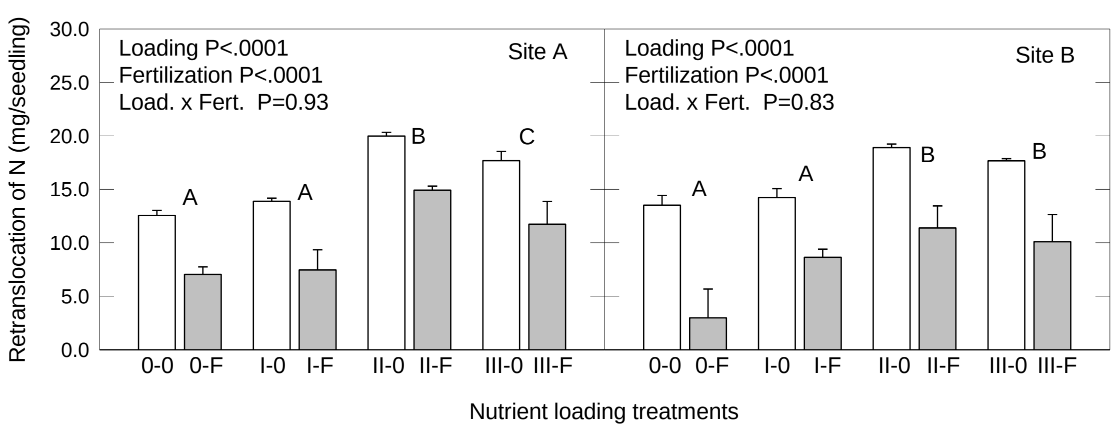


Fig. 6. Average (±SE) retranslocation of N from old needles (2008) to current needles (2009) of Lutz spruce seedlings exposed to different nutrient loading treatments (see table 1) and planted with (filled bars) or without (empty bars) field fertilization (see Table 2) at sites A and B. Different letters above the bars indicate significant differences between loading treatments at each site. Statistics were obtained by using a Two-Way ANOVA and post-ANOVA Fisher's Least Significant Difference test. Tvelwe seedlings sampled per treatment, n=4.

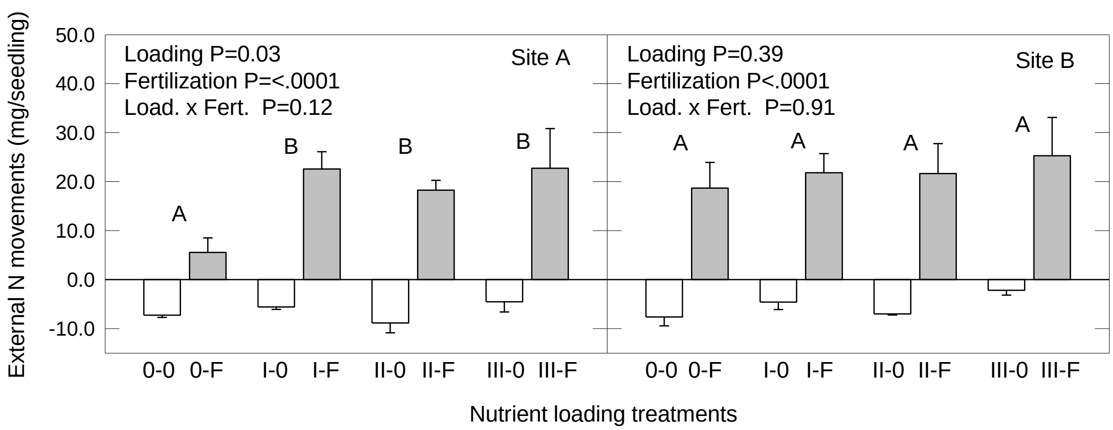


Fig. 7. Average (±SE) external uptake of N in current needles of Lutz spruce seedlings exposed to different nutrient loading treatments (see Table 1) and planted with (filled bars) or without (empty bars) field fertilization (see Table 2) at sites A and B. The horizontal line represents no external N uptake. Negative values show retranslocation of N to plant parts other than current needles. Different letters above the bars indicate significant differences between loading treatments at each site, found by Two-Way ANOVA and post-ANOVA Fisher's Least Significant Difference test. Tvelwe seedlings sampled per treatment, n=4.