



Effects of nutrient loading in Lutz spruce seedlings (*Picea x lutzii* Littl.) during nursery rotation and on subsequent growth in field



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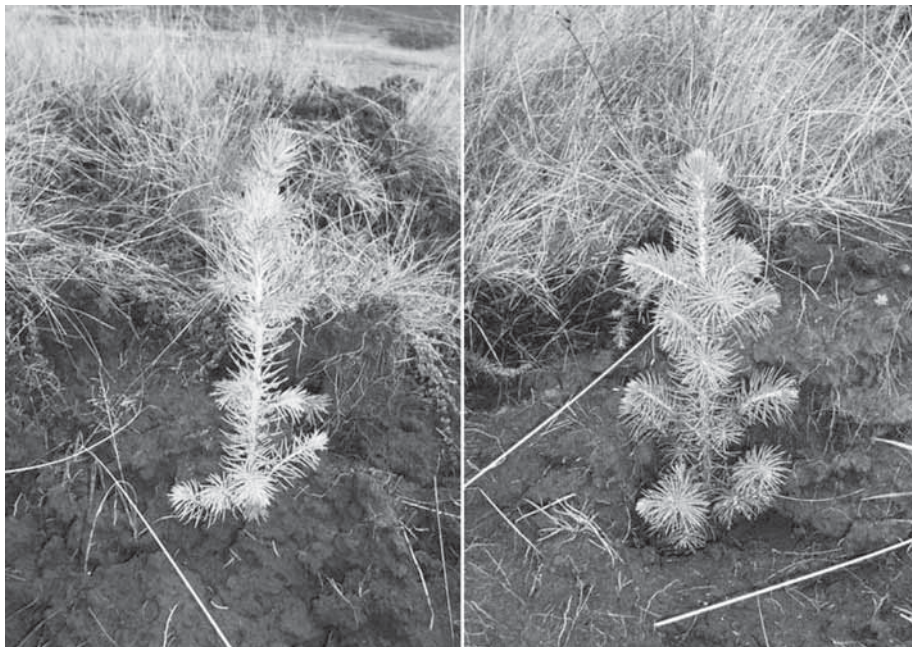
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Abstract

Lutz spruce (*Picea x lutzii* Littl.) seedlings were nutrient loaded using four fertilization regimes, receiving in total 0, 7.8, 22.2 and 31.4 g N/m² over a period of eight weeks (6th august – 27th September) to investigate the effects on biomass allocation, frost hardiness, root growth capacity and nutrient status after the nursery rotation. The total biomass of loaded seedlings was similar to unloaded seedlings after the nursery culture, but the increase in N from the nutrient loading was 29%, 41% and 48% for loaded treatments 7.8 – 31.4 mg N, respectively. A delay in accumulation of frost hardiness during the autumn was not detected, and the root growth capacity was not affected by the nutrient loading when measured the following spring. To investigate if the internal N status of the seedlings affected growth, survival and N content after one growing season in field, the seedlings were planted at two field trials (sites A and B), with or without field fertilizer. The shoots of loaded treatments without fertilizer application in the field had on average 31% and 52% more dry mass than unloaded treatments without fertilizer at sites A and B, respectively, after one growing season in field. Field fertilization also increased total shoot mass on average by 35% and 52% at sites A and B, respectively. The loading treatments without field fertilization increased N content in current needles by 104% and 109% for sites A and B, respectively. Field fertilization also increased N content on average in loaded treatments by 33% and 33% at site A and B, respectively. Nitrogen retranslocation from old to new needles was detected. The results illustrate the significance of retranslocation of stored nutrients to support new growth early in the season when root growth and nutrient uptake are still low. Survival was not affected by the nutrient loading after the first growing season, but fertilizing significantly decreased the damage caused by *Otiorhynchus* larvae in heath land. It was concluded that loading might provide an additional input for faster plantation establishment during the first crucial growing season after planting.

Key word: Lutz spruce seedlings, nutrient loading, frost hardiness, root growth capacity, biomass allocation, N content, N concentration, growth, survival, retranslocation.

Ágrip

Áhrif næringarefnahleðslu sitkabastarðs í gróðrarstöð á vöxt og lifun í foldu

Orsakir fyrir afföllum plantna í nýgróðursetningum á Íslandi eru m.a. taldar vera vegna köfnunarefnisskorts í jarðvegi og/eða hægrar umsetningu þess. Niðurstöður íslenskra tilrauna undanfarin ár hafa aukið skilning á mikilvægi áburðargjafar við gróðursetningu. Í þessu verkefni var kannað hvort ávinningur hlytist af því að nesta plönturnar næringarefnum fyrir gróðursetningu.

Hvítgreniplöntur (*Picea x lutzii* Littl.) voru vökvaðar með mismunandi miklum styrk næringarefna í gróðrarstöð. Á átta vikna tímabili (6. ágúst- 27. sept.) fengu plöntur í fjórum meðferðum í heild 0, 7,8, 22,2 og 31,4 g N/m² með vökvunarvatni. Markmiðið var að kanna áhrif næringarefnahleðslu á lífmassa, frostþol, rótarvöxt og næringarefnainnihald plantna eftir vaxtarlotu í gróðrarstöð. Heildarlífmassi plantnanna í hleðslumeðferðunum (7,8, 22,2 og 31,4 g N/m²) var svipaður og óhlöðnu plantnanna (0 g N/m²) eftir vaxtarlotuna í gróðrarstöðinni um haustið. Köfnunarefnisinnihald hleðslumeðferðanna þriggja var samt sem áður marktækt meira en í óhlöðnu meðferðinni, eða sem nam 29%, 41% og 48%, í hverri meðferð um sig í áðurnefndri röð. Frostþolsmyndun seinkaði ekki vegna næringarefnahleðslunnar og hún olli ekki auknum rótarvexti að vori. Til þess að kanna hvort mismunandi styrkur köfnunarefnis í plöntum hefði áhrif á vöxt, lifun og köfnunarefnisinnihald plantna eftir eitt vaxtartímabil í foldu voru meðferðirnar gróðursettar í tvær tilraunir (A og B) með og án áburðargjafar við gróðursetningu. Eftir eitt vaxtartímabil í foldu var yfirvöxtur næringarefnahlaðinna plantna, sem fengu ekki áburð við gróðursetningu, að meðaltali 31% meiri í tilraun A og 52% meiri í tilraun B en í óhlöðnum, óabornum plöntum. Áburðargjöf við gróðursetningu jók vöxt næringarefnahlaðinna plantna að meðaltali um 31% í tilraun A og 52% í tilraun B. Köfnunarefnisinnihald hleðslumeðferða sem fengu enga áburðargjöf við gróðursetningu, jókst að meðaltali um 104% í A og 109% í B. Áburðargjöf á hleðslumeðferðirnar jók köfnunarefnisinnihald þeirra um 33% að meðaltali í báðum tilraunum. Tilfærsla köfnunarefnis frá eldri nálum til nýrra nála var merkjanleg. Niðurstöðurnar sýna fram á mikilvægi þessarar tilfærslu til að auka vöxt snemma á vorin þegar rótarvöxtur og upptaka næringarefna er lítil. Eftir eitt vaxtartímabil í foldu, hafði næringarefnahleðslan ein og sér ekki haft áhrif á lifun. Áburðargjöf við gróðursetningu dró hinsvegar marktækt úr afföllum af völdum ranabjöllulirfa í mólendinu í tilraun A. Niðurstöðurnar benda engu að síður til þess að næringarefnahleðslan stuðli að því að plöntur nái fyrir rótfestu og vaxi meira en óhlaðnar plöntur á fyrsta vaxtartímabili eftir gróðursetningu.

Lykilorð: Hvítgreni, næringarefnahleðsla, frostþol, rótarvöxtur, lífmassi, köfnunarefnisinnihald, köfnunarefnisstyrkur, vöxtur, lifun, tilfærsla næringarefna.

Table of contents

Abstract.....	3
Ágrip.....	4
1. Introduction	7
1.1 Plant establishment and retranslocation.....	9
1.2 Nutrient loading in the nursery.....	10
1.3 Effects of nutrients on morphology of seedlings.....	12
1.4 Frost hardiness and fertilization	13
1.5 The effects of nutrient loading in nursery on performance in field	13
1.6 The effects of nutrient loading in nursery on survival of seedlings.....	14
1.7 Objectives.....	15
2. Material and methods	16
2.1 Study location and plant material.....	16
2.2 Biomass measurements	17
2.3 Nutrient analysis	18
2.4 Assessment of freezing tolerance	18
2.5 Assessment of seedling vitality	18
2.6 Field planting.....	19
2.7 Field experiment	20
2.8 Measurements	21
2.9 Biomass measurements after one season in field	21
2.10 Statistical analysis.....	21
3. Results.....	23
3.1 Growth responses during nursery rotation	23
3.2 Dry-mass allocation responses.....	24
3.3 Nutrient responses.....	26
3.4 Frost tolerance	27
3.5 Plant quality after winter storage	27
3.6 Seedling growth after the first growing season.....	29

3.7 Dry mass allocation after the first growing season.....	30
3.8 Nutrient dynamics after first growing season.....	33
3.8.1 Nitrogen status.....	33
3.8.2 Nitrogen contents	33
3.8.3. Retranslocation of Nitrogen.....	36
3.9 Survival	38
4. Discussion.....	40
4.1 Growth responses during the nursery rotation	40
4.2 Nutrient responses after nursery rotation.....	40
4.3 Frost tolerance	41
4.4 Root growth capacity	41
4.5 Seedling growth after one season in field.....	41
4.6 Nutrient dynamics after first growing season.....	42
4.6.1 Nitrogen concentration.....	42
4.6.2 Nitrogen content.....	43
4.6.3 Interaction effects of nutrition	44
4.6.4 Retranslocation of N	44
4.7 Survival after one growing season in field	46
5. Conclusions	48
6. Acknowledgements.....	49
7. References	50

1. Introduction

Large scale afforestation in Iceland only began at the end of the 20th century (Eggertsson *et al.* 2008). Former president, Vigdís Finnbogadóttir made afforestation a priority and a popular concern and during her term in office, from 1980-1990, the Icelandic state budget for forestry increased mainly because of support provided for farmer's afforestation programmes. Farmers could thereby acquire some income for planting on their own land. In the following years annual planting increased dramatically (Fig. 1) counting more than 5 million seedlings per year after 2003. Today, five Regional Afforestation Projects have been established and were responsible for roughly 80% of planting in Iceland in 2007 (Eysteinnsson, 2009). Their function is to manage the state grants scheme for afforestation on farms, each in its own region of the country.

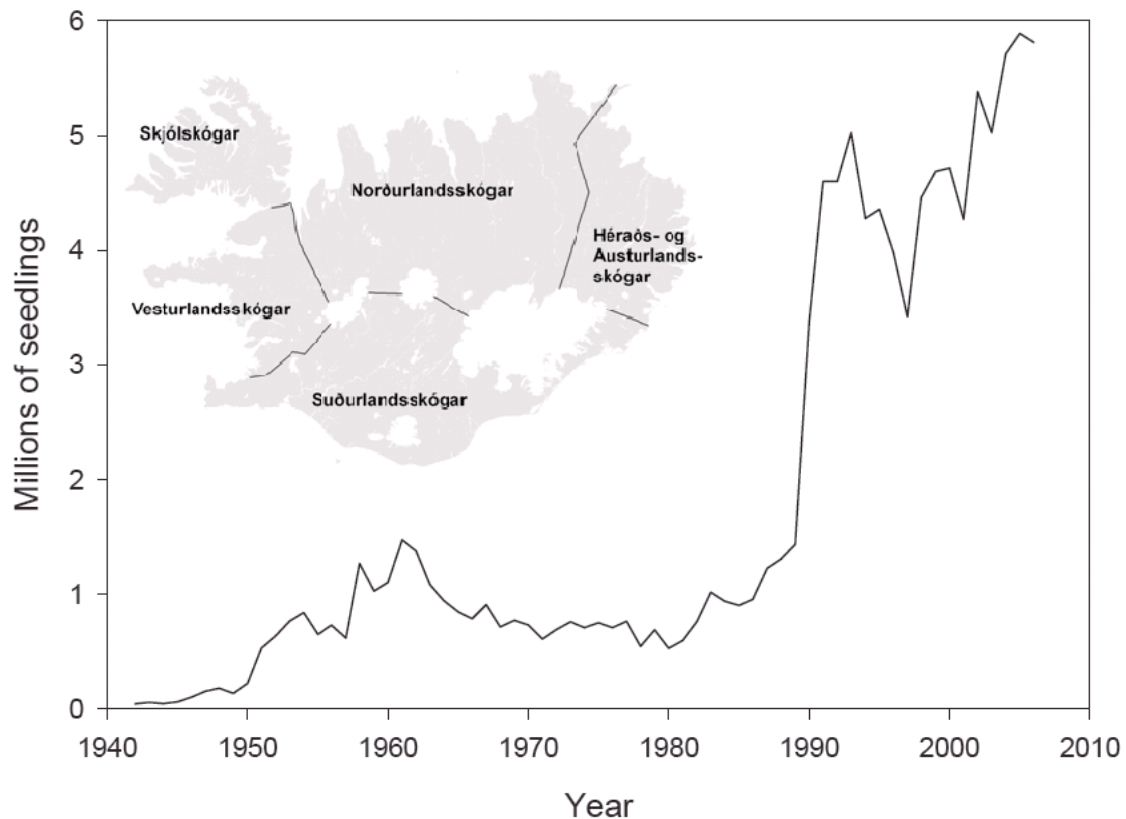


Fig. 1. Annual planting in Iceland during 1942-2006 (adopted from Eggertsson et al. 2008) and the regions of the five Regional Afforestation Projects.

With increased afforestation the surveillance of new plantings has increased. Many Icelandic studies and reports have revealed high mortality of newly planted seedlings. Snorrason (2007) reported average survival rate in new plantations for a period of 16 years, from 1990 – 2006, of

only of 45–52%. These measurements were made on permanent plots, both in plantations and native birch woodlands and collected as a part of the Icelandic Forest Inventory. Thorsson (2008) found that average survival rate of seedlings planted in the period 2000-2006 in the region of *Norðurlandsskógar* (Fig. 1) was 70%. Reynisson (2007) reported a 73% survival rate in the region of *Héraðs-* and *Austurlandsskógar*. For the region of *Suðurlandsskógar* an average survival rate of 40 -71% was reported (Eggertsson, 2005) with considerable variability in survival between sites and vegetation types. Assessments of survival for the Land Reclamation Forest project showed an average survival rate for 4–6 year old seedlings was 70% for birch (*Betula pubescens*), 63% in pine (*Pinus sp.*) and 44% for larch (*Larix sp.*)(Aradottir & Arnalds, 2001). The highest mortality in this study was found in plantations on gravel sites with sparse vegetation.

Several limiting factors have been identified as the cause for the high mortality in young plantations. Harsh windy climate, with cold, wet soils and low growing-season temperatures can be built up into a combination of climate factors that can damage young seedlings on very exposed sites. These problems are aggravated by the fact that most planted seedlings are the first generation of an introduced tree species, which may to greater or lesser extent be poorly adapted to the Icelandic climate (Óskarsson & Sigurgeirsson, 2001).

The Icelandic soil is also a limiting factor (Óskarsson & Sigurgeirsson, 2001). It is characterized by volcanic activity and has the structure of the volcanic parent materials. Most of the soil is classified as Andosol (Arnalds *et al.* 1995). Andosol has fine, sandy texture with a high water retention capacity that enhances cryogenic processes in the surface of the soils (Arnalds, 1998), leading to severe frost heaving of young seedlings and making afforestation difficult (Óskarsson & Sigurgeirsson, 2001). Frost heaving is caused by the formation of ice crystals in the deeper soil layers or at the surface. When air temperatures are just below freezing, temperatures in the upper soil layer fluctuate around 0°C, resulting in the formation of ice crystals. The crystals grow from below and push upwards generating a vertical uplift of tree seedlings. This causes root breakage and desiccation that can result in mortality, deteriorated growth and instability. Newly planted seedlings are especially prone to frost heaving due to lack of adequate root system development needed to anchor the seedlings to the soil (Goulet, 1995). The Icelandic climate fluctuations around 0° C and limited snow cover contributes to a great risk of frost heaving (Pétursson & Sigurgeirsson, 2004). Aradottir & Gretarsdottir (1995) reported frost heaving as a major seedling mortality factor, especially on sparsely or unvegetated sites. Other factors reported as the causes for mortality are: herbivory of *Otiorhynchus spp.* larvae, seedling size and competition (Halldorsson *et al.* 1999).

Another characteristic of the Icelandic soil is the high P-retention and low availability of N in the soils, believed to be another major limiting factor of plantation establishment in Iceland (Óskarsson & Sigurgeirsson 2001; Ritter, 2007). Low atmospheric deposition (less than 1 kg/ha

per year) of nitrogen and slow decomposition and mineralization rates, caused by the cold climate, are also growth limiting factors (Óskarsson & 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 pubescens*), Siberian larch (*Larix sibirica*) and Sitka spruce (*Picea sitchensis*) 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%. Unfertilized treatments all suffered from N and P deficiency and furthermore, application with a NPK fertilizer reduced frost heaving of seedlings the first winter after planting.

Results from other Icelandic studies examining the effects of nutrient loading on performance in the field revealed that nutrient loading prior to planting can increase growth and survival and in the same study fertilization also reduced frost heaving of newly planted seedlings (Óskarsson & Brynleifsdóttir, 2009). These results are of particular interest for those who want to increase survival in young plantations. Other studies done outside Iceland showed that the fertilization practices in the nursery can significantly improve subsequent field performance (van den Driessche, 1991; Timmer, 1996; Salifu & Timmer, 2003 a) because seedlings with a high internal nutrient status have more reserves to utilize after planting to support the nutrient demand of new growth (Grossnickle, 2000; Rytter *et al.*, 2003). In the following chapter the necessity for nutrients in plant establishment is discussed.

1.1 Plant establishment and retranslocation

Newly planted seedlings have minimal root contact with the soil and can be short of water and mineral nutrients (Burdett *et al.*, 1984; van den Driessche, 1991). The mineral nutrient shortage can be ameliorated by the addition of fertilizer at planting. This can improve root growth and unfavourable soil nutrient conditions at plantation sites. Furthermore, newly planted seedlings depend on the internal mobilization of nutrients, a natural phenomenon called retranslocation (Burdett *et al.*, 1984). Retranslocation is the amount of an element depleted from older plant components and made available for new growth (Lim & Cousens, 1986). Nutrient status of conifer seedlings when planted is considered one of key factors in their survival because they depend on the retranslocation of internal nutrient reserves to sinks of new growth soon after transplanting. This is caused by the initial slow root development that limits uptake from the soil (Burdett *et al.*, 1984). The storage and retranslocation of mineral nutrients seems to be particularly important in conifers because N is stored during the winter in needles and remobilized in the spring during the growth of new foliage (Millard & Proe, 1993; van den Driessche, 1991). The amount of stored nutrient available for growth after planting depends on the concentration in the tissues, and is affected by seedling size and seedling age. Seedling age is important because nutrients are normally retranslocated from older tissues to those actively growing. Thus, a three-year-old seedling has a larger amount of old needle tissue from which to

withdraw nutrients to supply new growth, compared to a one-year-old seedling. The size and the form of seedlings can influence the amount of stored nutrients if they affect the proportions of the important nutrient storage sites, such as foliage, stems, or roots (van den Driessche, 1991).

Retranslocation in plants has been studied in numerous experiments with and without nutrient loading in nursery. Salifu and Timmer (2003b) reported that nutrient loading improved retranslocation by 218% in black spruce (*Picea mariana*), increasing new biomass production by 156%. Their research also showed the total reliance of unfertilized plants on internal N reserves for growth on poor soil demonstrating the importance of retranslocation in meeting the plant's N demands. Imo and Timmer (2001) concluded in their study of nutrient loading and differing vegetation management for black spruce, that factors affecting retranslocation were the type and level of internal N reserves, biomass- and N-accumulation and partitioning. Other external factors including N supply and other stresses that limit plant growth such as competition were important for retranslocation. Nitrogen in plant tissues is distributed in either the mobile or structurally bound forms. The mobile pool is thought to consist of non-functional amino acids and proteins that are readily depleted from source tissues to support new growth (Chapin, 1990). It has been speculated that nutrient loading may increase the mobile N pool in plant tissues that are then rapidly remobilized to sinks of new growth soon after transplanting (Malik and Timmer, 1998; Salifu and Timmer, 2001).

Studies of the effect of nutrient availability in the soil on retranslocation show somewhat contradictory results. Some studies suggest nutrient retranslocation may be enhanced on poor sites (Salifu and Timmer, 2001), others have found increased retranslocation with nutrient availability (Millard & Proe, 1993) and some conclude that retranslocation may be independent of nutrient gradients (Millard & Proe, 1992). However, the key rationale for nutrient loading in nurseries is the ability of conifers to retranslocate nutrients to support new growth (Grossnickle, 2000; McAlister & Timmer, 1998).

1.2 Nutrient loading in the nursery

Luxury consumption and 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, 1980). A fertilization practice called nutrient loading utilizes this ability of plants by inducing excessive consumption of nutrients towards the end of nursery cultivation when seedlings have stopped shoot elongation (Grossnickle, 2000) (Fig. 1). 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.

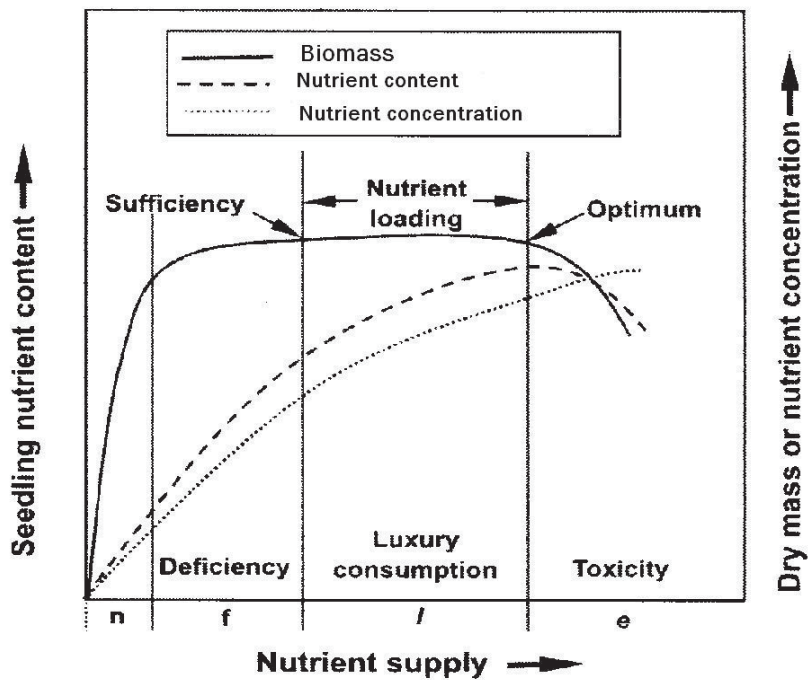


Fig 2. Relationships among nutrient supply with plant growth, tissue nutrient content, and concentration. Conventionally, fertilizer (f) is added to supplement native supply (n) to prevent nutrient deficiency and maximize growth to the sufficiency level. Optimum nutrient loading is achieved by adding fertilizer (l) that induces luxury consumption to build up plant nutrient reserves for out planting. Excess fertilization (e) inhibits growth because of toxicity (adopted from Salifu & Timmer, 2003 b).

A model of nutrient loading (Fig. 2) was proposed by Timmer (1996) to rationalize fertilization regimes in nursery cultivation in relation to plant nutrition, plant growth and nutrient supply (Salifu & Timmer, 2003 b). Fertilization is divided into phases to distinguish nutrient deficiency, sufficiency, luxury consumption and toxicity in plants. The model shows how growth, N content (uptake) and N concentration is increased by increasing the supply of N in the deficiency range until the luxury consumption level is reached. In the phase of luxury consumption N uptake and concentration increase further without significantly changing total plant dry mass. Nutrient sufficiency is reached when growth is maximized and toxicity occurs when growth declines. The major sources of N for uptake by containerized seedlings are from native supplies (n) in the growing media and from fertilizer added (f) extra high fertilization, or nutrient loading (l) or excess fertilization (e) (Salifu & Timmer, 2003 b). Two studies of the relationship between growth, nutrient content and concentration are consistent with this conceptual model. Salifu & Timmer (2003 b) used containerized black spruce and Salifu *et al.* (2005) used red oak (*Quercus rubra L*) in their experiment. The nutrient loading technique demands careful fertilizer application to prevent toxicity and the disruption of nutrient balance.

Thus a familiarity with loading thresholds and fertilization limits, and frequent nutrient monitoring of plants soils, and fertilizer solutions is an important part of the procedure (Timmer, 1996) as optimum dose rates for nutrient loading have not been determined for specific planting stock (Salifu & Timmer, 2003 b).

In the United States some nurseries apply late-season fertilizer after shoot elongation has ceased to prevent nutrient dilution (Haase & Landis, 2010). Dilution occurs because late in the growing season, a significant amount of root and stem growth is possible. This increase in biomass can lead to dilution unless more nutrients are supplied through fertilization. If nutrient concentrations drop below the adequate range, there are inadequate reserves for vigorous growth following planting. Nutrient loading is a relatively recent cultivation practice and many nursery growers are concerned that fertilizing too late in the season will cause budbreak, stimulate additional shoot growth, or delay or reduce cold hardiness. These issues are addressed in chapters 1.3 and 1.4.

1.3 Effects of nutrients on morphology of seedlings

According to Mattson (1996) seedling morphological and physiological parameters are used for assessing seedling quality. The most common morphological parameters are seedling height, stem diameter, bud diameter or bud height and sturdiness. Sturdiness is measured as the root: shoot ratio. The importance of balance in root: shoot ratio is explained by Grossnickle (2000) who states that seedlings with a balanced shoot to root system can avoid water stress because root absorption meets transpiration demands. To support that theory Burdett *et al.* (1984) found that in cases of water stress and on a site with restricted supply of nutrients, large seedlings with a low root: shoot ratio show lower survival rates and restricted growth after planting. Plant nutrition has a drastic effect on the partitioning of dry matter to the different parts of plant. It has been observed for a long time that general response to nutrient limitation induces an increased root: shoot ratio. (Ingestad & Ågren, 1991; Troeng & Ackzell, 1988) while high nitrogen supply improves shoot growth rate (Troeng & Ackzell, 1988). Miller and Timmer (1997) found that shoot growth was favoured much more than root growth in treatments with high N uptake during the hardening phase in the nursery. Limitation of N will produce plants with relatively large roots (Troeng & Ackzell, 1988), but it will also lead to planting stock with a low N status and plants with low N reserves (Ingestad, 1979). Rytter *et al.* (2003) and Miller and Timmer (1997) point out that if nutrient loading is done close to the end of the growing period, after the plants have perceived the critical night length for initiating dormancy, there is too little time for the plants to change their root: shoot ratios, but the N status is still improved without delaying frost hardiness development.

1.4 Frost hardiness and fertilization

Nitrogen fertilization in late summer may predispose seedlings to autumn frost damage by encouraging seedlings to continue growing late in the season. Recently formed buds may flush prematurely in the year they are formed, predisposing shoots to late season frost damage (Colombo *et al.* 2001). It has been shown that nutritional factors affect frost hardiness of plants, but the results have been somewhat contradictory. Some results show that fertilization, especially nitrogen application, applied during the growing season may improve frost hardiness or extend the growth period and delay hardening (Rikala and Repo, 1997; Landis, *et al.* 1989). Others show no effect of fertilization on the hardening phase of seedlings and others show low nitrogen application impairs frost hardiness (Rikala and Repo, 1997; Bigras *et al.* 1996) or delays the development of tolerance to freezing, and limits acclimation during the initial stages of cold hardening in the fall (Grossnickle, 2000). Fløistad (2002) however found no evidence that excessive N concentrations in needles of Norway spruce (*Picea abies*) lead to increased autumn frost damage.

Fertilization can also result in early flushing the following spring, predisposing the plants to frost damage (Colombo *et al.* 2001). In studies of N or NPK fertilizers and conifer cold hardiness the results are variable. Fløistad (2004) examined the influence of nutrient supply on spring frost hardiness and time of bud break in Norway spruce (*Picea abies*) seedlings. Her results showed the lower the N concentration in needles, the more frost damage occurred in seedlings and those seedlings that received the most N in the nursery broke bud earliest. The reason for contradictory results in nutritional studies of cold hardening likely reflects the variability among species, differences in tissue nutrient concentrations, growth phase during nutrient application or the season in which cold hardiness was assessed (Colombo *et al.* 2001).

1.5 The effects of nutrient loading in nursery on performance in field

Many research studies on effects of nutrient loading on performance in field have been conducted and results have been variable. One explanation for this variation is that studies have been done on different crops, with different fertilizers, at different rates and applied at different times (Haase and Landis, 2010). In most studies about nutrient loading of conifer seedlings, improved field performance was reported (Grossnickle, 2000; Haase & Landis, 2010). This improvement is seen in increased shoot (Óskarsson & Brynleifsdottir, 2009; Heiskanen *et al.* 2009; Troeng & Ackzell, 1998; Timmer & Munson, 1991; Malik & Timmer, 1995 and 1998; Salifu & Timmer, 2001 and 2003 a; McAlister & Timmer, 1998; Imo & Timmer, 2001) and root production (Heiskanen *et al.* 2009; Way *et al.* 2007; McAlister & Timmer, 1998) in recently planted seedlings and is attributed to greater internal nutrient reserves in nutrient loaded seedlings. Timmer & Munson, (1991) reported nitrogen loaded black spruce seedlings exhibit consistently greater growth and increased nutrient uptake after planting when compared to conventionally fertilized seedlings of similar pre-planting size. Nitrogen loading significantly

increased height growth and dry matter production and relative response was higher on the more nutrient deficient sites. The greater root biomass enhances rapid exploitation of available pools of soil N that increases growth in loaded seedlings in comparison to conventional grown seedlings of black spruce (Salifu & Timmer 2003 a; Way *et al.*, 2007).

The effect of nutrient loading can also appear as increased nutrient uptake (Heiskanen *et al.* 2009; Timmer & Munson, 1991; Malik & Timmer, 1995 and 1998; Salifu & Timmer, 2001 and 2003 a; McAlister & Timmer, 1998; Imo & Timmer, 2001) and increased retranslocation (Malik & Timmer, 1998; Salifu & Timmer, 2001 and 2003 a; McAlister & Timmer, 1998; Imo & Timmer, 2001). Malik and Timmer (1998) suggested that improved growth of nutrient loaded black spruce seedling is attributable to the higher pre-planting nutrient status. Nutrient loaded seedlings were presumably less stressed by belowground nutrient levels and hence favoured shoot growth. The retranslocation of N to metabolically active tissues such as current needles and roots also increased metabolic efficiency by allocation of relatively more N than carbon. Higher levels of available N promoted carbon fixation by increasing needle size and numbers, chlorophyll and carotenoid concentrations and could also increase nutrient uptake by accelerating fine root growth (Ingestad and Ågren, 1991).

Any potential benefit of increased fertility in the nursery in terms of improved seedling performance in the field is short lived (Grossnickle, 2000; Troeng & Ackzell, 1998). Nutrient reserves in spruce seedlings decline after planting, due to dilution in tissue concentrations if external nutrient sources do not meet the demands of new growth.

1.6 The effects of nutrient loading in nursery on survival of seedlings

Results from an Icelandic study about effects of nutrient loading on performance in the field revealed that nutrient loading prior to planting increased growth and survival and reduced frost heaving of newly planted seedlings (Óskarsson & Brynleifsdóttir, 2009). This was also observed in white spruce (*Picea glauca*) after one year in field (van den Driessche, 1992) and in Sitka spruce after three years in field (van den Driessche, 1984). Heiskanen *et al.* (2009) reported that nutrient loading had a transitory effect on plants after planting since after one growing season, nutrient loading did not affect seedling performance of Norway spruce. However, they suggested although nutrient loading does not compensate for the unavailability of nutrients to the seedlings from the soil, it might provide an additional input for fast plantation establishment on poorer sites during the first crucial growing season after planting.

1.7 Objectives

The goals of this study were to (1) load seedlings of Lutz spruce with nutrients in nursery without significantly changing their total biomass, (2) examine whether different nutrient concentrations in seedlings resulted in different root growth capacity and (3) delayed frost hardiness and (4) examine growth, survival, retranslocation and nutrient status of these treatments after one growing season in the field. Nitrogen was the focus of the study because it is one of the most limiting nutrients for plant growth in Icelandic soils (Óskarsson & Sigurgeirsson 2001; Ritter, 2007).

2. Material and methods

2.1 Study location and plant material

The plant material used in this study was Lutz spruce (*Picea x lutzii* Littl.) of the provenance 'Seward'. Lutz spruce is a hybrid of Sitka spruce (*Picea sitchensis* (Bong.) Carr.) and white spruce (*Picea glauca* (Monech) Voss). The species has proven more resistant to autumn frosts than Sitka spruce in Iceland (Skúlason *et al.* 2004), 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 the south where Sitka spruce is more suitable because of the area's milder climate and greater precipitation in the form of rain (Skúlason *et al.* 2004).

The study was performed in *Sólskógar*, a nursery in Akureyri (65,66°N, 18,10°W) in northern Iceland (Fig.3). Lutz spruce was sown on the 11th of April, one seed per hole in multipots. Plastic conical multipots (BCC, HIKO- 93, Sweden) were used in the study. Each pot had a volume of 93 cm³, with 526 cells per square meter (40 cavities per tray). The growing media was Finnish peat (M6, Kekkilä Oy, Tuusula, Finland).

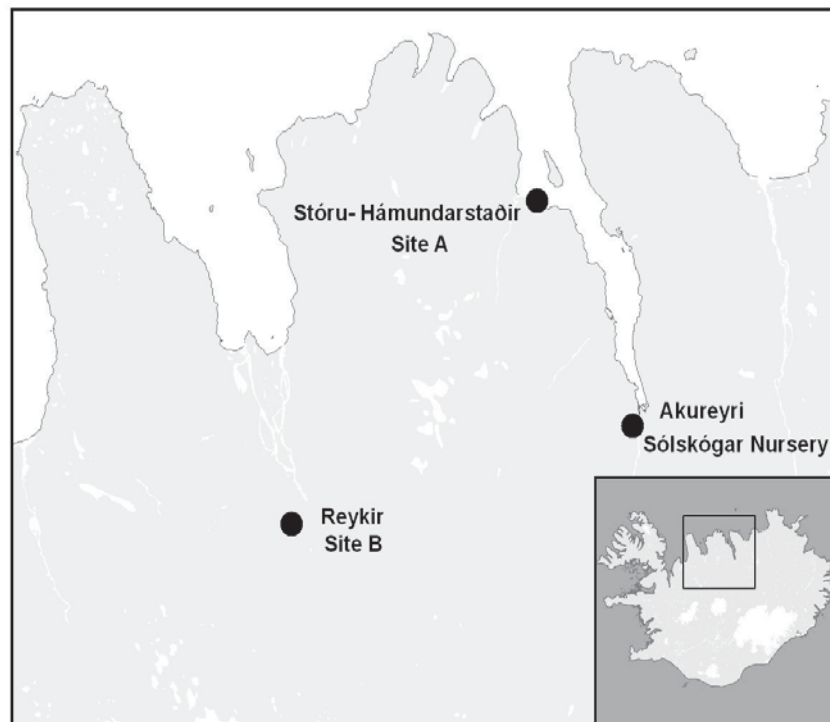


Fig 3. Location of *Sólskógar* nursery in Akureyri and the two planting sites, *Stóru Hámundarstaðir* (site A) and *Reykir* (site B).

Fertilization began on the 28th of April (2008) using electrical conductivity (EC) at a rate of 1.0 mS/cm. The mineral nutrient solution (Kekkilä Stock Superex, NPK 19-4-20, Kekkilä, Co.,

Tuusula, Finland) was dissolved in the irrigation water. Seedlings were cultivated in a heated greenhouse until the third of July, and then moved outdoors for further growth. On the 6th of August the seedlings were divided into different nutrient loading treatments and seedlings were moved to unheated plastic greenhouse. Trays with seedling of similar size were chosen for the experiment, in total 128 trays with 5,120 seedlings. The trays were randomly divided into four fertilizer treatments and fertilized with either 0, 0,9, 2,7 or 3,9 g N/m² per week (Table 1).

Table 1. *The treatments applied to Lutz spruce seedlings in the nursery trial. The fertilizer was mixed with irrigation water and applied twice per week for eight weeks, from the 6th of August until the 27th of September. The numbers in the treatment names indicate the total amount of N applied during that period.*

Treatments				
Total g N/m ²	g N/m ² /week	EC mS/cm	Total mg N/seedling	Nr. of seedlings
0	0	0	0	1280
1.1	0.9	1.1	14.7	1280
3.2	2,7	3.2	41.5	1280
4.2	3.9	4.2	58.7	1280

Each treatment had four replicates in the greenhouse, each replication containing eight trays. The fertilizer was applied in solution form twice per week until late September. The fertilizer was mixed into 1,2 liters of water. A watering can with a mini boom was use to spread the fertilizer. After each irrigation, the seedlings were rinsed with 2 liters of clean water. If the weight of the trays had been reduced to 65 - 70% of the container capacity, supplemental irrigation with no fertilizer was applied. The last irrigation with fertilizer was conducted on the 27th of September. All seedlings were long night treated (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 minus 3°C. Before packing, the height and diameter of the seedlings were measured.

2.2 Biomass measurements

Eight seedlings were randomly sampled from each replication on the 18th of November 2008. The seedlings sampled were kept at -18°C until growth analyses was conducted. The shoots were cut from the root systems at the root collars. All the soil was washed from the root systems. The branches were also separated from the shoots. Branches, root systems and shoots were oven dried for 24 hours at 85°C. Then all needles were removed from the branches and the shoot parts. All plant parts were oven dried for 24 hours at 85°C again. After drying the dry mass of all plant parts were weighed.

2.3 Nutrient analysis

When the needles were dried, as described in previous chapter, it was used in a nutrient analyses performed at the Centre for Chemical Analyses (*Efnagreiningar Keldnaholti*), ICETEC, Reykjavik, Iceland, where their total nitrogen (N) was measured using Kjeldahl's wet combustion on a Tecator Kjelttec Auto 1030 Analyzer. Other minerals were measured with Spectorflame D Sequential instrument, Spectra.

2.4 Assessment of freezing tolerance

Frost tolerance was measured with the Shoot Electrolyte Leakage technique (SEL) 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 frost 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 have reached enough frost tolerance to be safely stored in a freezer (Lindström & Håkansson, 1995).

2.5 Assessment of seedling vitality

Root Growth Capacity test (RGC) was used to estimate the vitality of the root systems after winter storage as described in Mattson (1986). Five seedlings from each replicate, in total 20 seedlings from each treatment, were planted in the RGC-table on the 24th of April 2009 and grown for three weeks. An assessment of vitality was done on the 15th of May. The growing medium used when conducting the RCC-test was 50% peat and 50% sand. The seedlings were planted in trays and put in the RGC-table filled with water (Fig. 4). The air temperature and the temperature of the growing medium were maintained at 20 °C. The daily period of light was 18 hours. The seedlings were watered twice a week. An hour after watering, excess water was

drained from the trays. After cultivation for three weeks, growing medium and seedlings were lifted from the trays. The growing medium was removed carefully from the root system. All new, white roots that had grown outside the original root system were counted. The number of roots was used as a measure of the quality of the seedlings, using a scale (table 2) developed by Lindell (1986).



Fig 4. Seedlings of lodge pole pine (Pinus contorta) in a RGC table.

Table 2. *The scale used to estimate quality of seedlings (adapted from Lindell, 1986).*

Index	New roots
0	none
1	some, none > 1 cm
2	1-3 > 1 cm
3	4-10 > 1 cm
4	11-30 > 1 cm
5	31-100 > 1 cm
6	101 -300 > 1 cm
7	Over 300 > 1 cm

2.6 Field planting

The seedlings were taken out of winter storage on the 19th of May 2009. The seedlings were thawed in the packing boxes for two days and then put in Hiko trays in a plastic greenhouse and irrigated without fertilizer until the planting date. 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 (fig. 5) at *Stóru Hámundarstaðir*, (65°57' N, 18°27' W) is located approximately 44 km. north of Akureyri (fig.2). This site is dominated by heath, *Vaccinium uliginosum* L., crowberry, *Empetrum nigrum* L., and dwarf birch, *Betula nana* L. The site was scarified prior to planting using shallow scarification with a homemade plough to remove the vegetation cover. Site A is considered poor for spruce (Brynjar Skúlason *et.al*, 2006). The seedlings were planted on site A on the 26th of May.



Fig 5. Site A (left) and B (right).

Site B at *Reykir* (65°29' N, 19°22' W) is located approximately 120 km W of Akureyri (fig. 2 and 5). The site is characterized by grass species (*Kobresia sp.*, *Festuga sp.* and *Poa sp.*). At this site the vegetations surface was scarified prior to planting with TTS-10 disc trencher (TTS Forest Oy, Finland). The planting of the seedlings in site B was conducted second of June.

2.7 Field experiment

The experimental design consisted of four randomized blocks on both sites. The four loading treatments were randomly planted in each block, 80 seedlings from each treatment and 40 seedlings from each treatment were fertilized immediately after planting. This increased the number of treatments to eight (table 3). Ten grams of fertilizer was scattered by hand in a 15 cm. circumference around each fertilized seedling. The fertilizer, *Sprettur*, (Carrs Fertilisers, Scotland) is a blend of NP (23-5.2-0). It also contains Sulfur 2.4%, Calcium 2,65% and Magnesium 1,5%.

Table 3. *Treatments tested in the field trials on Lutz spruce.*

Treatments in nursery	Fertilizer in field	Nr. of seedlings for each site
No fertilizer	Without	160
No fertilizer	Fertilizer	160
7.8 g N/m ²	Without	160
7.8 g N/m ²	Fertilizer	160
22.2 g N/m ²	Without	160
22.2 g N/m ²	Fertilizer	160
31.4 g N/m ²	Without	160
31.4 g N/m ²	Fertilizer	160

2.8 Measurements

The seedlings were measured in September 2009 on both sites. The stem diameter was measured directly below the lowest branch. Two measurements of height were done for each seedling. First, from the soil surface to the beginning of the current growth. And the second from the soil surface to the apex of the shoot's terminal bud. The first height measurement was subtracted from the second to find the annual increment of the seedlings. If the leading shoot was missing because of damage, height growth was measured on side shoots. Vitality of the seedlings was registered by giving the seedlings grades. The following scale was used: 0=dead seedling, 1=affected seedling, 2=vital seedling. The cause of any damage to the seedlings was recorded when known, otherwise the cause was recorded as unknown.

2.9 Biomass measurements after one season in field

To ensure that the size of samples was not skewed for this diagnosis, the frequency distribution of height within treatments was examined. The height was divided into five classes and seedlings were randomly chosen from the classes with the highest frequency. Samples were taken between the 7th and 13th of December. Three seedlings per treatment in each block (in total 96 seedlings from each site) were cut at the soil surface. The shoots were stored in the dark at a temperature of -5°C. The seedlings were then taken apart and the plant materials were divided into new (current=C) and old (C+1) branches, needles and stems. These materials were dried at 70°C for 48 hours and their dry masses weighed. Needles were analyzed for nutrient content using the same method described earlier.

2.10 Statistical analysis

The four nursery nutrient loading treatments were measured after one growing season in the nursery, in autumn 2008, before the plants were stored in freezer. Treatment means were compared using a One-Way ANOVA, and when significant ($P < 0.05$), additional pair-wise

comparisons were done using Fisher's Least Significant Difference Test (LSD) in the general linear model procedure (GLM) of the SAS 9.1 software (SAS Institute, Inc., Cary, NC, USA).

Prior to ANOVA analysis, the normality of each treatment was checked by inspecting normal probability plots and stem and leaf diagrams. When there were clear violations of the normality assumption, all the treatments for that parameter were transformed either by logarithmic or square root transformation before the treatment means were compared. Only annual increment and some morphological weights (old needles, old wood) required transformation, however most parameters, including total biomass, diameter, apical bud dry matter content, shoot electrolyte leakage and nutrient analyzes could be compared directly.

The transformations were, however, not successful in correcting the skewed distribution in plant height. Its overall treatment differences were therefore compared by a Kruskal-Wallis test in the NPAR1WAY procedure of the SAS, followed by a Mann-Witney U test to compare treatment pairs.

Field trials at two places were set up as randomized block experiments, with four blocks, where one fixed factor was added, i.e. fertilization at planting. Treatment effects were investigated separately for each field experiment. All variables measured at continuous scale were first checked for normal distribution for each treatment and then analyzed with Two-Way ANOVA for each place, where nutrient loading and field fertilization were the main factors. When the nutrient loading was significant as a main factor, but the interaction between the two main factors was not significant, the nutrient loading pairs across both fertilization levels (6 comparisons) were investigated with pair-wise LSD tests. When, however, the interaction term was significant, all possible pairs of nutrient loading and fertilization (12 comparisons) had to be checked by LSD tests.

Variables that were not continuous, such as grading of needle colour, survival or damage were first tested by Kruskal-Wallis' test across all treatments for each site. If significant, the treatment "main effects" were tested by testing for fertilization effects across all nutrient loading treatments, and testing for effects of nutrient loading across unfertilized and fertilized treatments separately. If either of the nutrient loading "main effects" tested proved significant, Mann-Witney's U test was used to compare nutrient loading treatment pairs.

3. Results

3.1 Growth responses during nursery rotation

There was a significant difference in height between treatments after cultivation in the nursery ($P=0.0013$) (Fig. 6). The treatment receiving no fertilizer and the one receiving 22.2 g N/m² were significantly higher than the others. Diameter of the 22.2 g N/m² treatment was also significantly higher than the other treatments ($P=0.0046$).

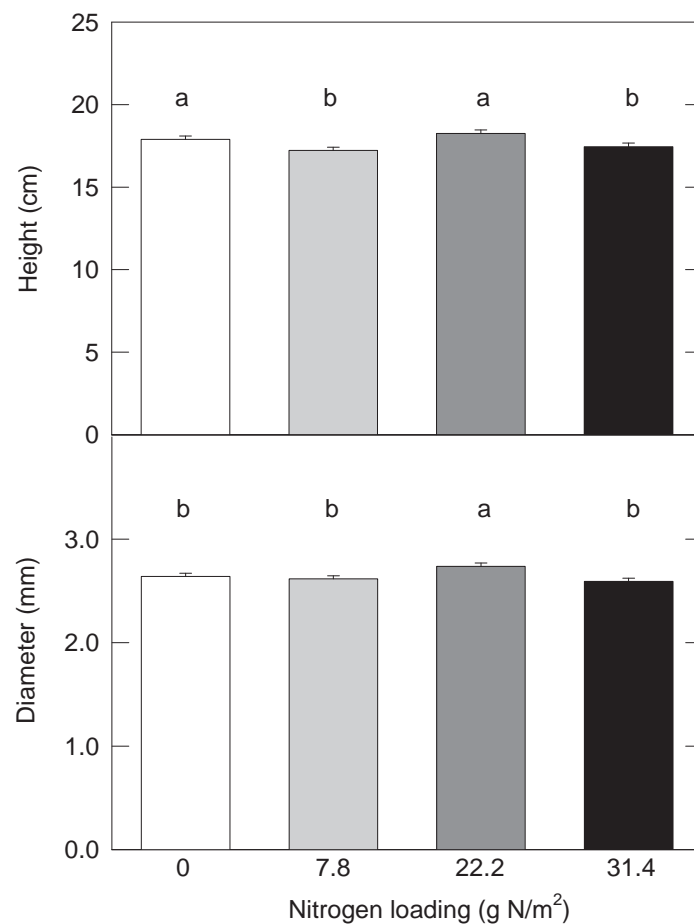


Fig. 6. Average (\pm SE) height (at the top) and diameter (at the bottom) of Lutz spruce seedlings exposed to different nutrient loading treatments. Samples were taken in early November 2008. Different letters above the bars indicate significant differences between loading treatments, found by One-Way ANOVA and post-ANOVA Fisher's Least Significant Difference test, ($n=4$).

3.2 Dry-mass allocation responses

The BMR (Branch mass ratio), SMR (Shoot mass ratio) and LMR (Leaf mass ratio) were similar for all treatments (Fig. 7). Only RMR (Root mass ratio) of treatments receiving the most fertilizer 22.2 and 31.4 g N/m² was significantly lower. Treatments receiving the most fertilizer had 11% lower root: shoot ratio than the non-loaded treatment (Fig. 8).

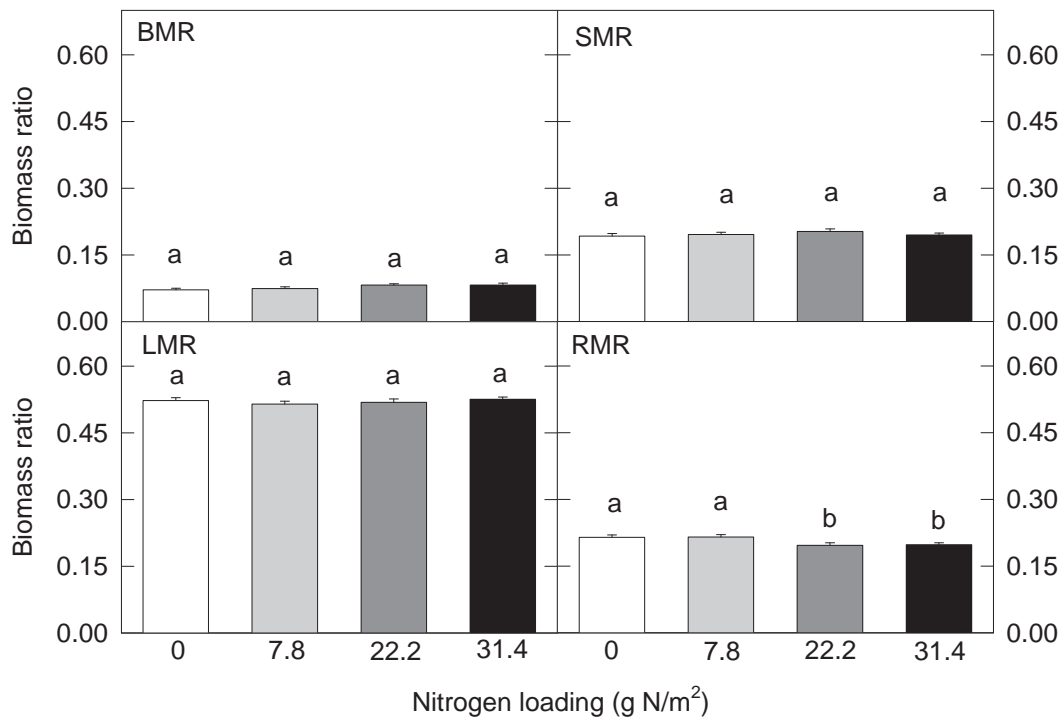


Fig. 7. Average (\pm SE) of Branch mass ratio (BMR), Shoot mass ratio (SMR), Leaf mass ratio (LMR) and Root mass ratio (RMR) of Lutz spruce seedlings exposed to different nutrient loading treatments. Samples were taken in early November 2008. Different letters above the bars indicate significant differences between loading treatments, found by One-Way ANOVA and post-ANOVA Fisher's Least Significant Difference test, ($n=4$).

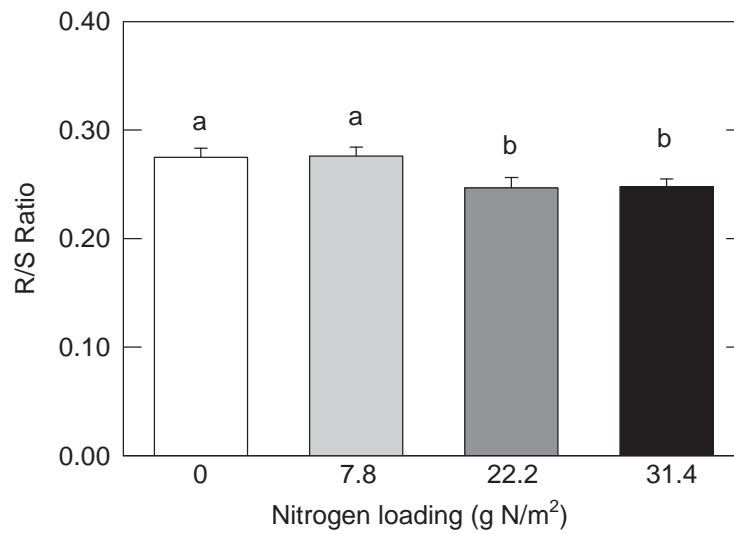


Fig. 8. Average (\pm SE) Root: shoot ratio of Lutz spruce seedlings exposed to different nutrient loading treatments. Samples were taken in early November 2008. Different letters above the bars indicate significant differences between loading treatments, found by One-Way ANOVA and post-ANOVA Fisher's Least Significant Difference test, ($n=4$).

Though the seedlings were of different heights and RMR, this did not affect their total biomass weight (Fig. 9). There was no significant difference between total biomass between the treatments.

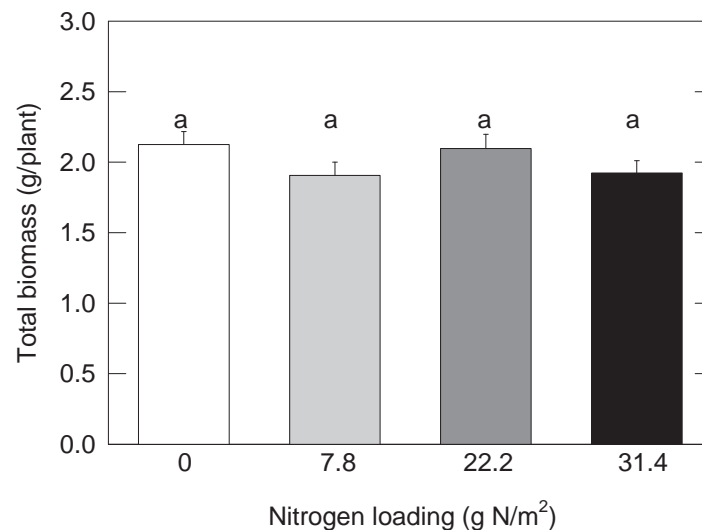


Fig. 9. Average (\pm SE) of total biomass of Lutz spruce seedlings exposed to different nutrient loading treatments. Samples were taken in early November 2008. Different letters above the bars indicate significant differences between loading treatments, found by One-Way ANOVA and post-ANOVA Fisher's Least Significant Difference test, ($n=4$).

3.3 Nutrient responses

Although total dry mass of non-loaded and loaded seedlings was similar, the nutrient concentrations differed significantly between treatments especially in N concentration (Table 4). The increase in N caused by nutrient loading was 29%, 41% and 48% for loaded treatments 7.8 – 31.4 mg. N, respectively. There was no significant difference in P: N ratio of the treatments, but the effects of the nursery loading significantly decreased the K: N ratio with increased loading. Therefore the treatment receiving the most nutrient loading (31.4 g N/m²) had 42% lower K: N ratio than the unloaded treatment. The Mg: N ratio declined with increased loading. The most loaded treatments 22.2g N/m² and 31.4g N/m² had significantly lower Mg: N ratio than the other treatments.

Table 4. Nutrient concentrations (mg/g 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. Samples were taken in early November. Different letters for the same element indicate significant differences between treatments found using an One-Way ANOVA and AdHoc Fisher's Least Significant Difference test, (n=4).

Nutrients	Treatments				Optimum values ¹
	0 g N/m ²	7.8 g N/m ²	22.2 g N/m ²	31.4 g N/m ²	
<i>Concentrations</i>					
N	17.55 ± 0.43 a	22.71 ± 0.62 b	24.81 ± 0.36 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	
<i>N-ratios</i>					
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).

3.4 Frost tolerance

The first frost hardiness measurement, using shoot electrolyte leakage, was conducted on the 20th of October and by then, all treatments were tolerant to a temperature of -25 °C (Fig. 10). In an attempt to distinguish between treatments, all treatments were frozen to -35 °C the 14th of November. The results showed no significant differences in frost hardiness between treatments at that time (Fig. 10, lower panel).

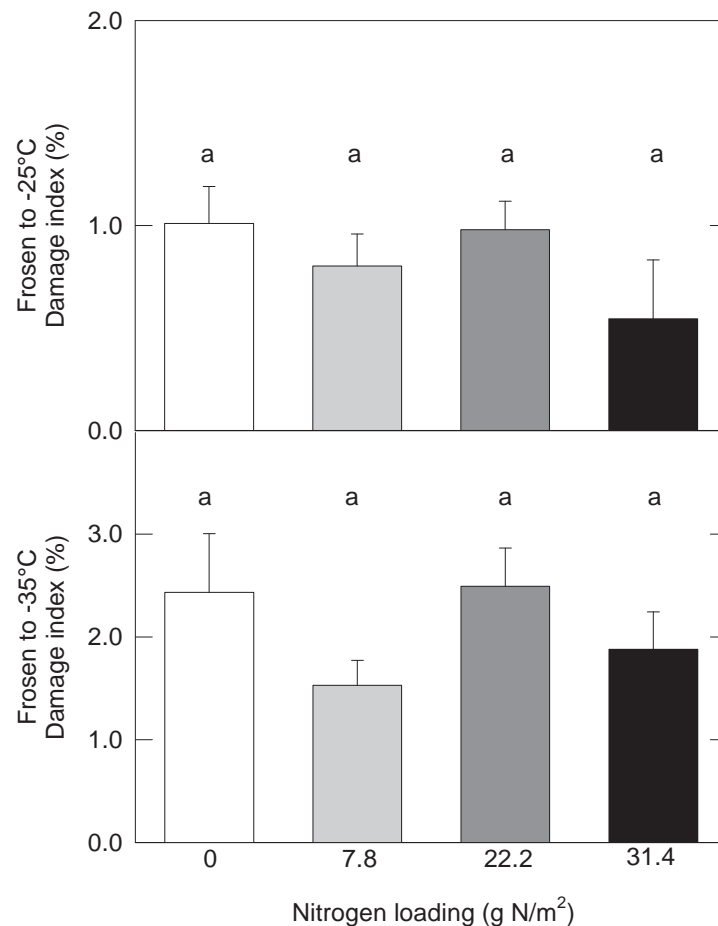


Fig. 10. Average (\pm SE) shoot electrolyte leakage in Lutz spruce seedlings exposed to different nutrient loading treatments. Samples were tested on Oct. 20th and frozen to -25°C (top panel) and on the 14th of Nov. they were frozen to -35°C. Different letters above the bars indicate significant differences between loading treatments, found by One-Way ANOVA and post-ANOVA Fisher's Least Significant Difference test, ($n=3$).

3.5 Plant quality after winter storage

To examine if loading treatments in the nursery affected root growth capacity of the seedlings after winter storage, RGC measurements were conducted. An index (Table 2) was used to estimate root growth after three weeks of growth in a RGC-table. Root growth capacity showed

a small negative response to loading treatments (-9% between non-loaded and the highest loading treatment)(Fig. 11). This effect was, however, not significant.

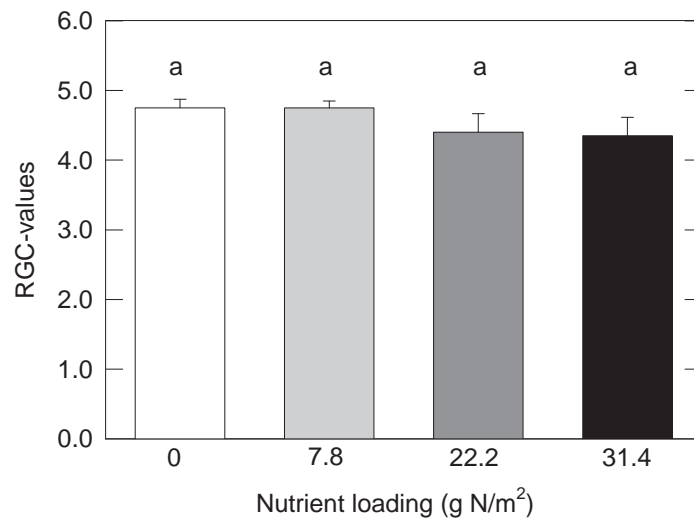


Fig. 11. Average (\pm SE) root growth in Lutz spruce seedlings exposed to different nutrient loading treatments. Samples were tested in May 2009 after winter storage in a freezer. Different letters above the bars indicate significant differences between loading treatments, found by using an One-Way ANOVA and post-ANOVA Fisher's Least Significant Difference test. Each bar represents a mean of 20 seedlings ($n=4$).

3.6 Seedling growth after the first growing season

Nutrient loading and field fertilization stimulated height growth similarly on both sites (Fig. 12). The highest loading treatment without field fertilization increased annual shoot elongation by 51-54% for sites A and B, respectively. This effect was highly significant ($P < 0.001$). Field fertilization also increased annual shoot elongation on average by 48-65% at sites A and B, respectively. There was a significant interaction between nutrient loading and field fertilization at Site B (Load. x Fert. $P = 0.04$). This was caused by the relative increase in shoot elongation diminishing as the loading increased. The increase attributable to field fertilization was 55%, 61%, 42% and 24% for 0 – 31.4 mg N loading, respectively.

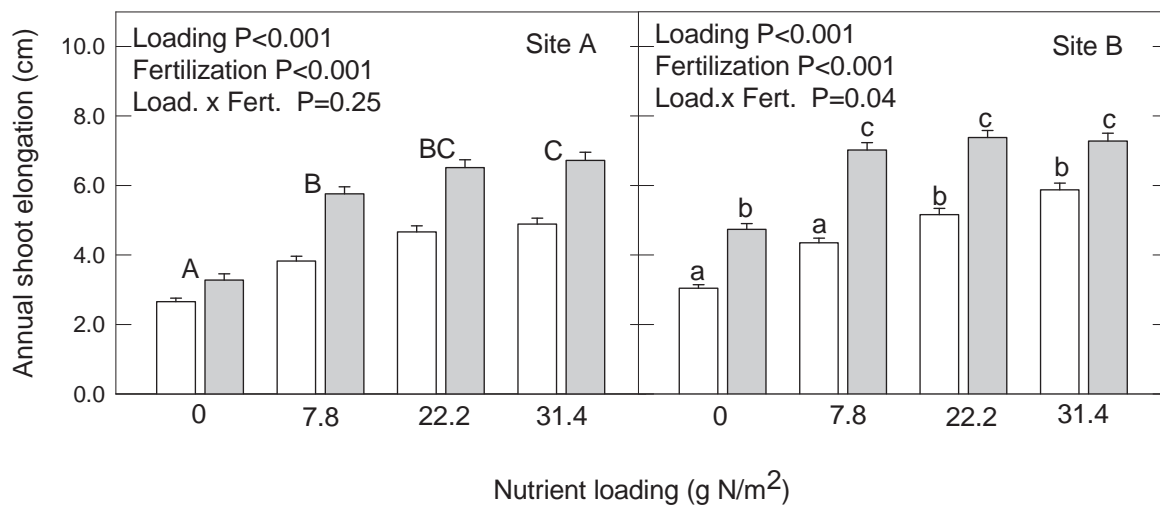


Fig. 12. Average (\pm SE) annual shoot elongation (cm) of Lutz spruce seedlings exposed to different nutrient loading treatments and planted in the spring with (filled bars) or without (empty bars) field fertilization at sites A and B. Different letters above the bars indicate either significant differences between both fertilization and loading treatments (small letters) or only between loading treatments (capital letters) depending on whether the interaction was significant or not. These results were found by using a Two-Way ANOVA and post-ANOVA Fisher's Least Significant Difference tests ($n=4$).

3.7 Dry mass allocation after the first growing season

Both nursery loading and fertilizer at the time of planting significantly increased total the shoot mass of seedlings (Fig. 13). Shoots from loaded treatments without fertilizer in the field had on average 31% and 52% more dry mass than the unloaded treatments without fertilizer in sites A and B, respectively ($P=0.002$). Field fertilization also increased total shoot mass on average by 35% and 52% at Site A and B, respectively.

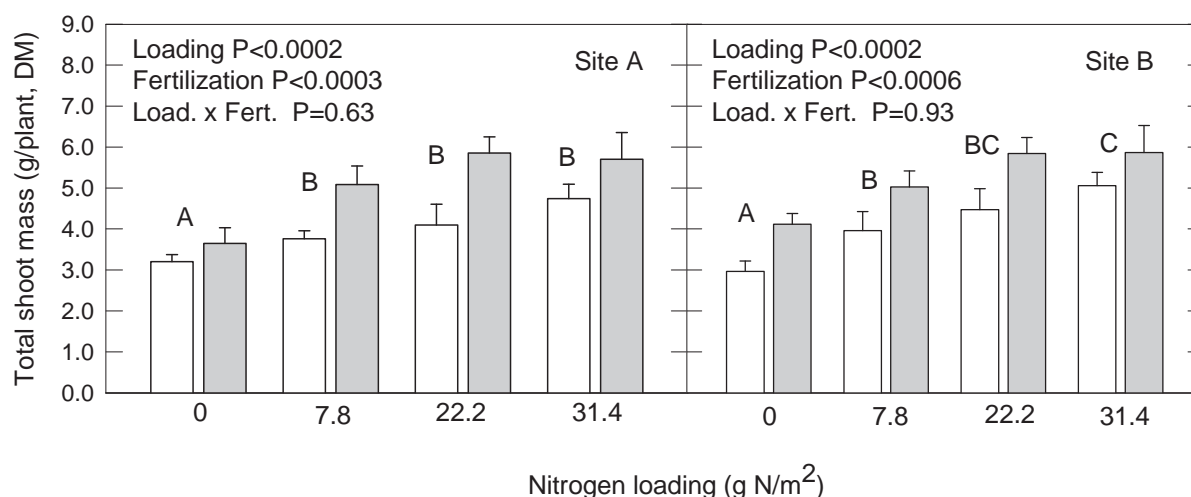


Fig. 13. Average (\pm SE) weight (dry weight) of biomass above ground for Lutz spruce seedlings exposed to different nutrient loading treatments and planted in the spring with (filled bars) or without (empty bars) field fertilization at sites A and B. Different letters above the bars indicate significant differences between loading treatments at each site, found by using a Two-Way ANOVA and post-ANOVA Fisher's Least Significant Difference test ($n=3$).

Shoot mass was divided into mass of old wood, old needles, current branches and current needles and the proportion of each was examined (Fig. 14). Nursery loading and fertilizer at the time of planting significantly increased old wood (shoot and branches from previous year) on both sites. Unfertilized loaded seedlings increased their old wood mass on average 17% and 35% on sites A and B, respectively (Table 5). These increases for fertilized loaded seedlings were on average 34% and 26% in sites A and B. All treatments showed no significant difference in mass of old needles at both sites. The highest loading treatment (31.4 g N/m²) without field fertilization increased new needles mass by 119% and 152%, for sites A and B, respectively. This was also the case for highest loading treatment with field fertilization, were new needle mass increased by 124% and 84 % for sites A and B, respectively.

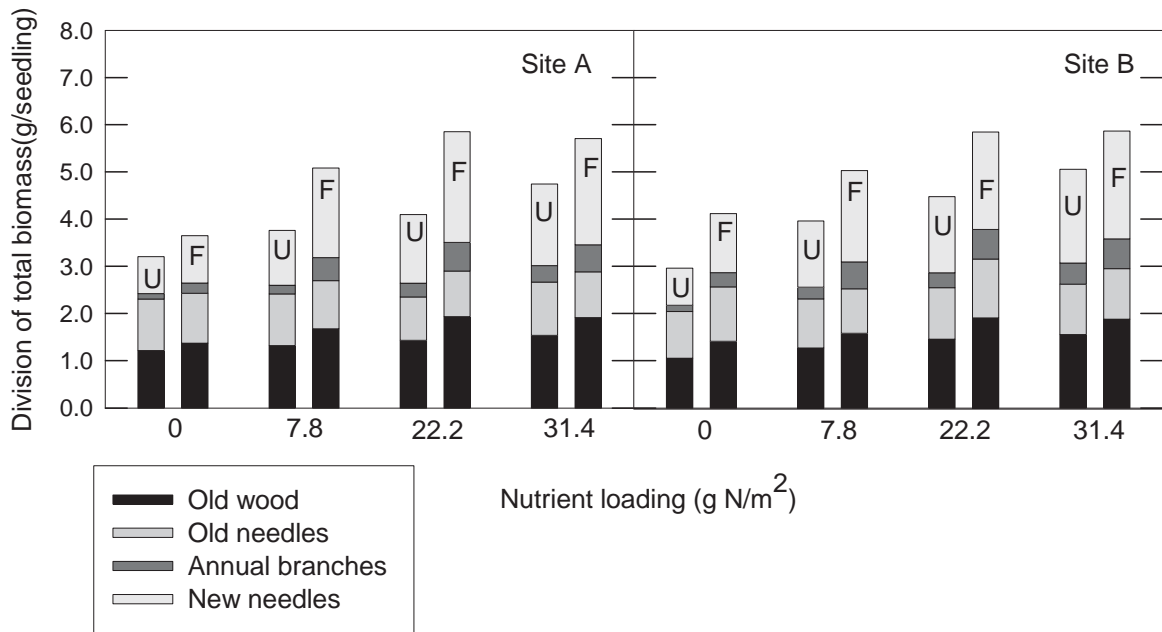


Fig. 14. The division of average total biomass into old wood, old needles, current branches and current needles of Lutz spruce seedlings exposed to different nutrient loading treatments and planted in the spring at sites A and B. Bars marked with the letter U represent unfertilized treatments after planting. Bars marked with F represent fertilized treatments after planting. Statistical information can be found in Table 5.

Table 5. Effects of unloaded (no fertilizer) and loaded (7.8, 22.2, 31.4 g N/m²) Lutz spruce seedlings in nursery, planted with or without fertilizer in the spring, on dry mass of old wood, old needles, current branches and current needles (g/plant). 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 ($n=3$).

Site	Treatments	Fertilizer in field	Old wood	Old needles	Current branches	Current needles
A	No fertilizer	Without	1.22 ± 0.07 a	1.09 ± 0.10	0.12 ± 0.01 a	0.78 ± 0.05 a
		Without	1.32 ± 0.09 ab	1.10 ± 0.09	0.18 ± 0.02 b	1.16 ± 0.08 b
		Without	1.43 ± 0.17 b	0.92 ± 0.10	0.29 ± 0.05 c	1.46 ± 0.22 bc
		Without	1.54 ± 0.11 b	1.13 ± 0.12	0.35 ± 0.04 c	1.73 ± 0.14 c
	7.8 g N/m ²	Fertilizer	1.37 ± 0.14 a	1.06 ± 0.08	0.22 ± 0.05 a	1.00 ± 0.20 a
		Fertilizer	1.68 ± 0.16 ab	1.02 ± 0.11	0.49 ± 0.06 b	1.90 ± 0.19 b
		Fertilizer	1.94 ± 0.15 b	0.96 ± 0.08	0.61 ± 0.07 c	2.34 ± 0.16 bc
		Fertilizer	1.91 ± 0.22 b	0.97 ± 0.09	0.57 ± 0.08 c	2.25 ± 0.30 c
B	No fertilizer	Without	1.05 ± 0.10 a	0.99 ± 0.11	0.13 ± 0.01 a	0.79 ± 0.07 a
		Without	1.27 ± 0.14 ab	1.04 ± 0.16	0.25 ± 0.02 b	1.40 ± 0.16 b
		Without	1.46 ± 0.17 b	1.09 ± 0.16	0.31 ± 0.03 c	1.61 ± 0.19 bc
		Without	1.55 ± 0.11 b	1.07 ± 0.11	0.44 ± 0.04 c	1.99 ± 0.14 c
	7.8 g N/m ²	Fertilizer	1.41 ± 0.10 a	1.15 ± 0.09	0.30 ± 0.06 a	1.25 ± 0.14 a
		Fertilizer	1.58 ± 0.12 ab	0.95 ± 0.10	0.57 ± 0.07 b	1.93 ± 0.19 b
		Fertilizer	1.91 ± 0.12 b	1.24 ± 0.09	0.63 ± 0.07 c	2.06 ± 0.19 bc
		Fertilizer	1.88 ± 0.22 b	1.07 ± 0.13	0.63 ± 0.08 c	2.28 ± 0.27 c
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	0.55	ns	0.18	0.42
B		Load x Fert	0.74	ns	0.33	0.92

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

3.8 Nutrient dynamics after first growing season

3.8.1 Nitrogen status

To ascertain the plant's nitrogen status at the end of the first growing season, current needles were analysed for total N. The nitrogen status of all treatments was considerably lower than optimum (Fig. 15, the dotted line). Nutrient loading in the nursery only led to significantly higher N status at site A (Fig. 15; Loading effect), where the three nutrient loading treatments had 10% higher N concentrations in the autumn than the unloaded treatments. It should be noted that even if the N status was elevated by the spring fertilization, it did not reach optimum levels (Fig. 15, the dotted line).

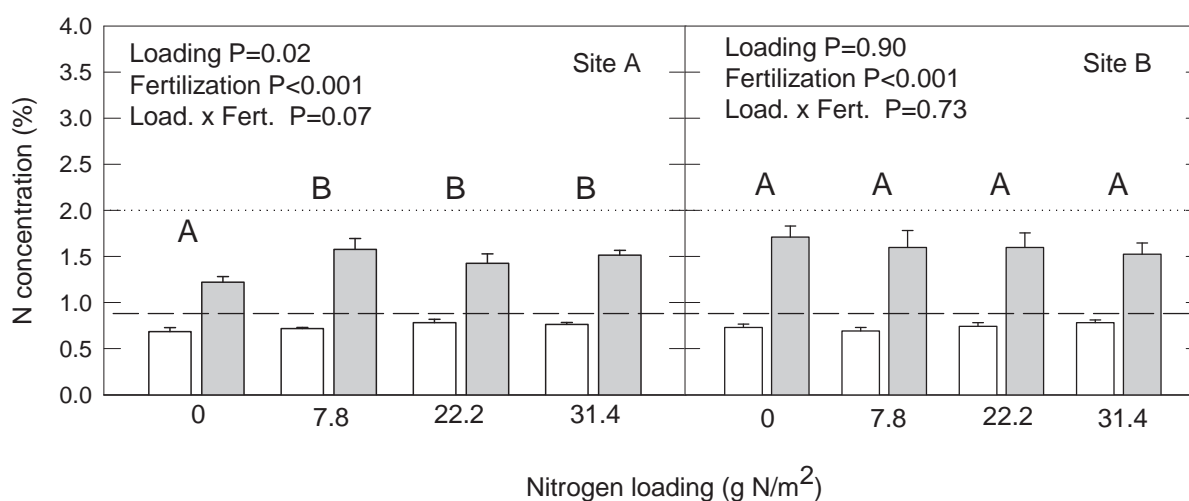


Fig. 15. Average (\pm SE) nitrogen concentration in current needles of Lutz spruce seedlings that had been exposed to different nutrient loading treatments and planted with (filled bars) or without (empty bars) field fertilization at sites A and B. The dotted line indicates optimum N status according 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, found by using a Two-Way ANOVA and post-ANOVA Fisher's Least Significant Difference test.

3.8.2 Nitrogen contents

Total nutrient content for N, was calculated by multiplying nutrient concentration (mg/g) by total needle mass. Nutrient loading and field fertilization stimulated total N nutrient content in site A (Table 6), were loaded treatments with field fertilization increased N content significantly ($P=0.0007$) by 83%. Loading treatments without field fertilisation increased N content by 51% at site A. This effect was highly significant ($P<0.001$). At site B fertilized treatments were significantly different ($P<0.0001$) from unfertilised treatments. The loading effect was not significant at site B ($P=0.0531$).

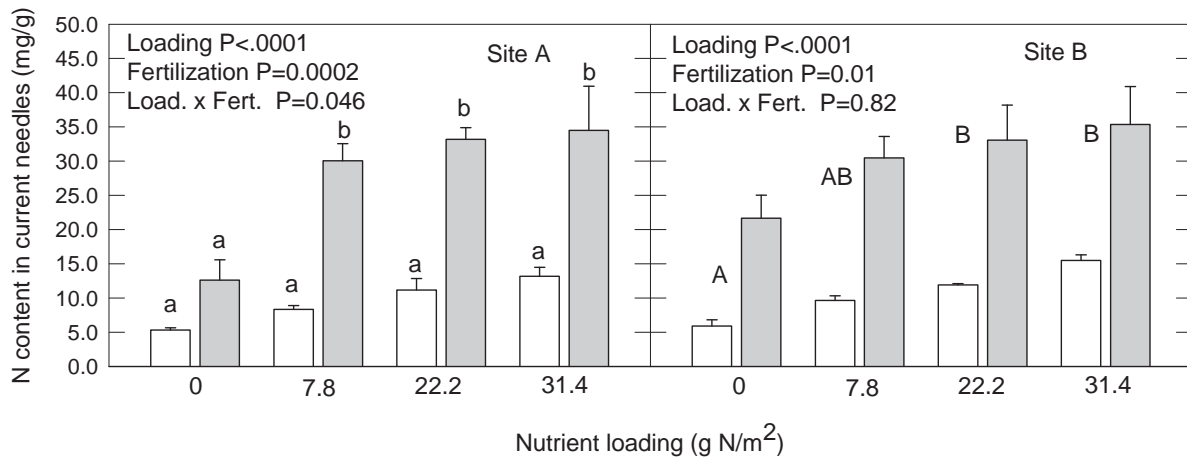


Fig. 16. Average (\pm SE) total N content in current needles of Lutz spruce seedlings exposed to different nutrient loading treatments and planted with (filled bars) or without (empty bars) field fertilization at sites A and B. Different letters above the bars indicate either significant differences between both fertilization and loading treatments (small letters) or only between loading treatments (capital letters) depending on whether or not the interaction was significant. These results were obtained by using a Two-Way ANOVA and post-ANOVA Fisher's Least Significant Difference tests.

The loading treatments without field fertilization increased N content in current needles (Fig. 16, Table 6) by 104% and 109% for sites A and B, respectively. This effect was highly significant ($P < 0.001$). Field fertilization also increased N content on average in loaded treatments by 33% and 33% at sites A and B, respectively. There was a significant interaction between nutrient loading and field fertilization at site A (Load. x Fert. $P = 0.046$). This was caused by the relative increase in N content varying as the loading increased. The increase caused by the field fertilization was 199%, 262%, 197% and 262% for 0 – 31.4 mg N loading, respectively. The N content in old needles (Table 6) was not affected by the loading treatments on either site. On the other hand, fertilization had highly significant effect on N content in old needles.

Table 6. Effects of unloaded (no fertilizer) and loaded (7.8, 22.2, 31.4 g N/m²) Lutz spruce seedlings in the nursery, planted with or without fertilizer in the spring, on N content in old needles, current needles and total N content(g/plant). 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 $P < 0.05$ level. The results were found by using a Two-Way ANOVA and post-ANOVA Fisher's Least Significant Difference test ($n=3$).

Site	Treatments	Fertilizer in field	N content		
			Old needles	Current needles	Total
A	No fertilizer	Without	7.05 ± 0.48	5.32 ± 0.32 a	12.38 ± 0.49 a
	7.8 g N/m ²	Without	7.90 ± 0.29	8.31 ± 0.60 a	16.21 ± 0.50 b
	22.2 g N/m ²	Without	7.18 ± 0.35	11.16 ± 1.69 a	18.33 ± 2.02 b
	31.4 g N/m ²	Without	8.67 ± 0.87	13.18 ± 1.31 a	21.84 ± 9.09 b
	No fertilizer	Fertilizer	12.58 ± 0.71	12.60 ± 2.99 a	25.18 ± 2.97 a
	7.8 g N/m ²	Fertilizer	14.33 ± 1.89	30.06 ± 2.48 b	44.38 ± 3.52 b
	22.2 g N/m ²	Fertilizer	12.24 ± 0.39	33.17 ± 1.72 b	45.41 ± 2.04 b
	31.4 g N/m ²	Fertilizer	14.61 ± 2.13	34.49 ± 6.46 b	49.10 ± 8.10 b
B	No fertilizer	Without	6.10 ± 0.92	5.90 ± 0.92 a	12.00 ± 1.82
	7.8 g N/m ²	Without	7.55 ± 0.82	9.64 ± 0.68 ab	17.19 ± 1.50
	22.2 g N/m ²	Without	8.27 ± 0.33	11.89 ± 0.22 b	20.16 ± 0.21
	31.4 g N/m ²	Without	8.68 ± 0.20	15.49 ± 0.81 b	24.17 ± 1.00
	No fertilizer	Fertilizer	16.64 ± 2.70	21.65 ± 3.37 a	38.29 ± 5.27
	7.8 g N/m ²	Fertilizer	13.13 ± 0.76	30.47 ± 3.16 ab	43.59 ± 3.90
	22.2 g N/m ²	Fertilizer	15.78 ± 2.07	33.06 ± 5.13 b	48.84 ± 6.08
	31.4 g N/m ²	Fertilizer	16.26 ± 2.55	35.37 ± 5.54 b	51.63 ± 7.84
Sources of variation:					
A		Loading	0.24	0.0002	0.0007
B		Loading	0.61	0.012	0.0531
A		Fertilization	<0.0001	<0.0001	<0.0001
B		Fertilization	<0.0001	<0.0001	<0.0001
A		Load x Fert	0.93	0.046	0.12
B		Load x Fert	0.52	0.82	0.99

3.8.3. Retranslocation of Nitrogen

Since N content of needles 2008 was known, as was the N content of both old and current needles 2009, the retranslocation of N from older needles to current needles, could be calculated. The calculations are based on the content of N in the needles as that method has been shown to be more successful and more robust than using concentration alone in estimating N flux between plant parts (Nambiar and Fife, 1987; Munson *et al.*, 1995; Imo and Timmer, 2001). 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. The most loaded treatments 22.2 and 31.4 g N/m² without field fertilizing had more retranslocation than less loaded treatments, 0 and 7.8 g N/m² without field fertilizing (Fig. 17). The difference was on average 42% and 32% on sites A and B, respectively. This was also the case in spring fertilized treatments with the most loading, were the most loaded treatments 22.2 and 31.4 g N/m² had on average 84% more retranslocation in both sites. Fertilization after planting reduced N retranslocation at both sites. Unfertilized treatments had on average 62% and 40% more retranslocation than fertilized in site A and B, respectively.

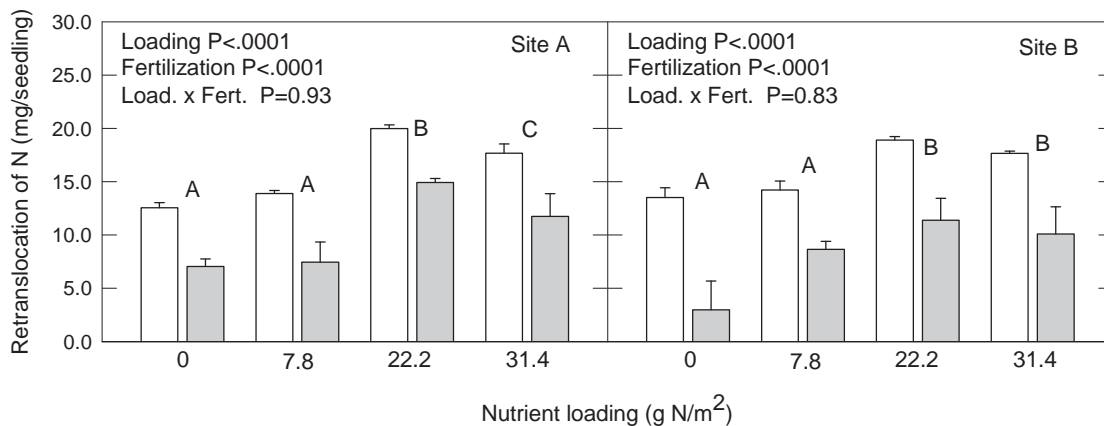


Fig. 17. Average (\pm SE) retranslocation of N from old needles (2008) to current needles (2009) of Lutz spruce seedlings exposed to different nutrient loading treatments and planted with (filled bars) or without (empty bars) field fertilization at sites A and B. Different letters above the bars indicate significant differences between loading treatments at each site, results were found using a Two-Way ANOVA and post-ANOVA Fisher's Least Significant Difference test ($n=3$).

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 as described by Malik and Timmer(1998) and Imo and Timmer (2001). Unfertilized treatments in sites A and B showed negative values when their external N contribution was calculated (Fig. 18). Negative values show retranslocation of N to plant parts other than current needles. The loaded treatments at site A received significantly more N ($P=0.03$) from external sources than the unloaded treatments. Conversely, there was no significant difference between treatments at site B. Fertilization in the spring increased external N contribution dramatically in both sites and the effects were highly significant ($P<0.0001$).

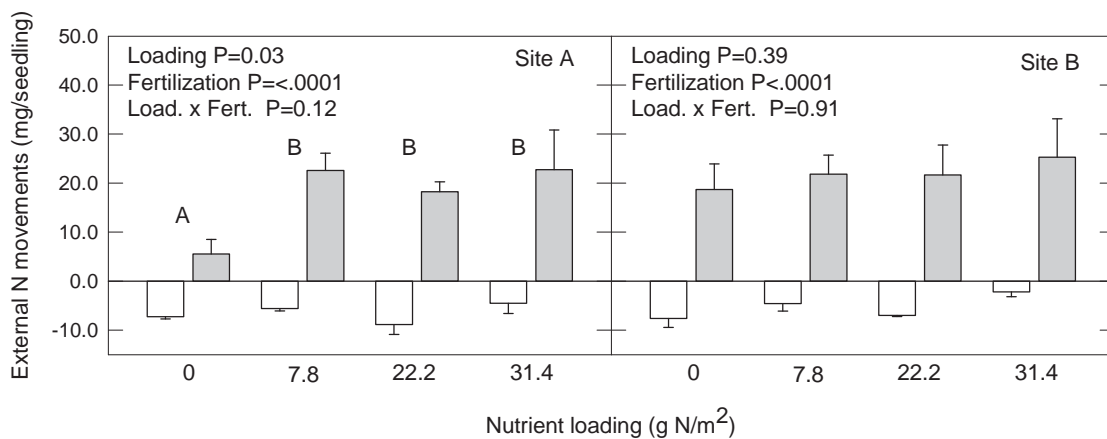


Fig. 18. Average (\pm SE) external uptake of N in current needles of Lutz spruce seedlings exposed to different nutrient loading treatments and planted with (filled bars) or without (empty bars) field fertilization at sites A and B. The horizontal line represents no external N uptake. Negative values 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 ($n=3$).

3.9 Survival

Survival was overall good after first growing season in field, but fertilized treatments had a significantly better survival rate at site A ($P < 0.0001$), independent of loading treatments (Fig. 19). Seedlings receiving spring fertilization had on average a 97% survival rate, while unfertilized had a 91% survival rate. The reason for the lower survival rate in unfertilized treatments at site A was damage caused by *Otiorhyncus* (Fig. 20). Unfertilized loaded treatments showed a significant difference in survival at site B (Fig. 19), ($P < 0.004$). The treatment loaded with 22.2 g N/m² had lower survival than the other treatments. The reason for this was unknown.

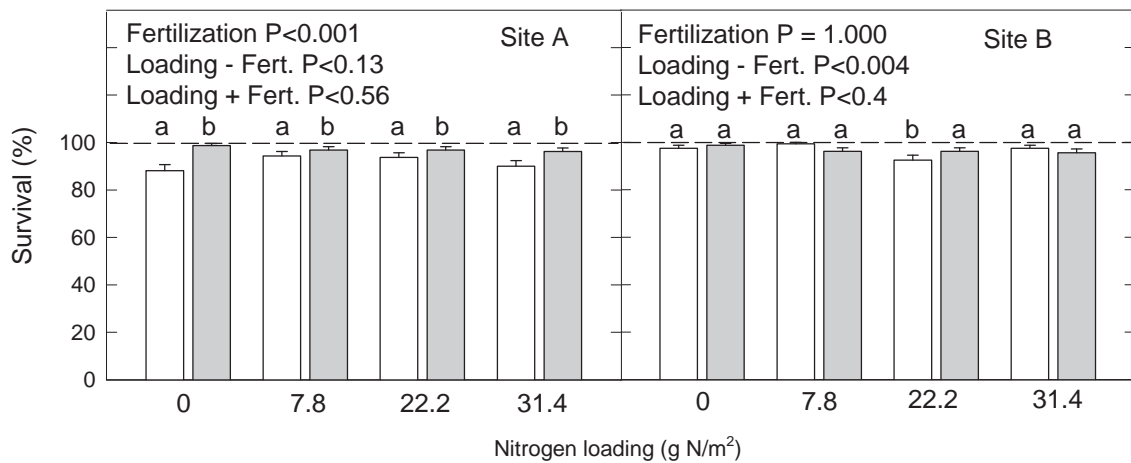


Fig. 19. Average (\pm SE) survival in Lutz spruce seedlings exposed to different nutrient loading treatments and planted with (filled bars) or without (empty bars) field fertilization at sites A and B. The dashed line indicates 100% survival. Different letters above the bars indicate significant differences between loading treatments at each site, found by Two-Way ANOVA and post-ANOVA Fisher's Least Significant Difference test ($n=4$).

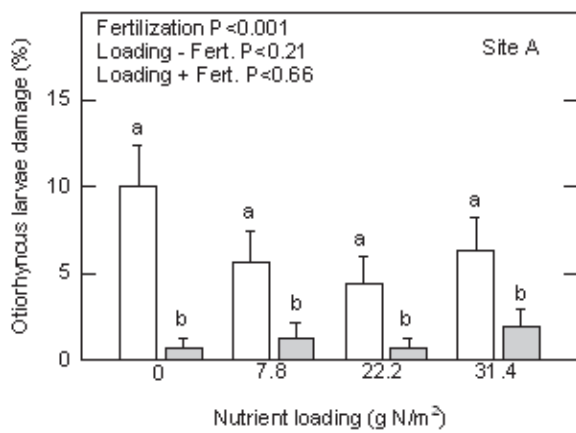


Fig. 20. Average (\pm SE) *Otiorynchus* larvae (*Otiorynchus nodosus* Fabr.) damage in Lutz spruce seedlings exposed to different nutrient loading treatments and planted with (filled bars) or without (empty bars) field fertilization at sites A and B. Different letters above the bars indicate significant differences between loading treatments at each site, found by using a Two-Way ANOVA and post-ANOVA Fisher's Least Significant Difference test ($n=4$) (left). The picture shows typical symptoms of root herbivory by the larvae (right).

4. Discussion

4.1 Growth responses during the nursery rotation

After the nursery rotation, there was significant difference in both the height and diameter of the seedlings (Fig. 6). This was probably due to difference in height before the loading treatments began. At that time, the seedlings had stopped height growth and they did not reflush. The LMR (Leaf mass ratio), SMR (Shoot mass ratio) and BMR (Branch mass ratio) were not significantly affected by the nutrient loading treatments (Fig. 7). Only the two highest loading treatments showed a significantly lower RMR than the others. Therefore the root: shoot ratio (Fig. 8) declined with increased fertilization in nursery, demonstrating a typical reaction of plants to nutrient stress. This reaction by the seedlings allocates proportionally more carbon to root production in order to better reach to belowground nutrient resources (Ingestad & Agren, 1988). The reduced root: shoot biomass ratios with increased fertilization found in this study are consistent with those of other studies. Ericsson (1995) reported that root growth in silver birch (*Betula pendula*) and Norway spruce was favoured when N was the major growth constrain. The lower root: shoot ratios of the 22.2 and 31.4 g N/m² treatments did however not lead to less total shoot mass or nutrient content of these treatments after one growing season in field (Fig. 12 and Table 6). Although the seedlings in the various treatments differed slightly in height, diameter and root growth, the total biomass of the four treatments was not affected (Fig.9) as there was no significant difference between treatments after the nursery rotation. Therefore, the goal of loading the seedlings with nutrients without changing their total biomass was reached.

4.2 Nutrient responses after nursery rotation

The nutrient loading of seedlings during the nursery phase increased nutrient concentration in all three loading treatments (Table 4). The results demonstrate that loading induced luxury nutrient consumption because uptake, probably into storage pools, was increased without altering the total biomass of the seedlings (Fig. 9). This is consistent with the findings of others (Malik & Timmer, 1998; Idris *et al.*, 2004). The loading treatments, 7.8 and 22.2 g N/m² had N concentration within optimum values (Table 4), (Ingestad, 1991). The loading treatment receiving the highest nutrient application (31.4 g N/m²) was slightly above the optimum value (25.97± 0.82 mg/g Dry matter). This did not have negative effects during nursery phase or subsequent growth in field. There was no significant difference in N concentration between the two highest loading treatments, indicating that the loading efficiency was reduced at higher loading levels and an upper threshold for effective loading of seedlings (Malik & Timmer, 1998). All target nitrogen ratios found by Linder (1995) were considered optimal in all treatments except for the Mg: N ratio in the two highest loading treatments.

4.3 Frost tolerance

When the first frost tolerance test was done on the 20th of October all treatments had gained adequate frost tolerance and there was no significant difference in leakage between treatments (Fig. 10). In an attempt to distinguish between treatments, all treatments were frozen to -35°C on 14th of November. This did not show any significant difference between treatments (Fig. 10, lower panel). Therefore it cannot be demonstrated here that one of the treatments gained frost tolerance sooner or later than the others. Comparing data from this study with data collected by Hrefna Jóhannesdóttir at the Icelandic forest service (Unpublished data, collected by Hrefna Jóhannesdóttir 2002-2010) that recorded frost tolerance by shoot electrolyte leakage since 2002 for Icelandic nurseries, conventionally grown spruce seedlings reach adequate frost tolerance at the beginning of November. The results of the frost tolerance test indicate that frost tolerance was not delayed by any treatment when compared to conventional cultivation of spruce. Fløistads (2002) found that even high needle nitrogen concentration (3.3% N) did not delay the development of frost hardiness in *Norway spruce* compared to treatments with lower needle nitrogen concentration. Her findings also show that short day treatment increased frost hardiness. Therefore, short day treatment may be used as a tool to secure frost hardiness for nutrient loaded seedlings since luxury consumption of macro elements by plants may reduce frost hardiness (Bigras *et al.*, 1996).

4.4 Root growth capacity

After three weeks of cultivation in a RGC table, root growth capacity showed a slight negative response for the two highest nutrient loading treatments (Fig. 11). The difference was on average -9% between non-loaded and the highest loading treatments. This effect was, not significant. As root growth increases with more constraints of N (Ingestad & Agren, 1988) one could speculate that the root growth would be more in less nutrient loaded seedling as the results show. However, studies have shown approximately double root growth capacity, measured at the time of planting, in white spruce (*Picea glauca*) due to high N loading (van den Driessche, 1992; van den Driessche, 1991).

4.5 Seedling growth after one season in field

Nutrient loading in the nursery and fertilization in the field both stimulated shoot elongation and dry mass allocation above ground (Fig. 12 and 13). These results are consistent with the findings of many others examining nutrient loading and subsequent field performance in field (McAlister & Timmer, 1998; Malik & Timmer, 1995 and 1998; Salifu & Timmer, 2001 and 2003 b; Timmer & Munson, 1991; Óskarsson & Brynleifsdottir, 2009; Imo & Timmer, 2001; Heiskanen *et al.* 2009, Timmer, 1996; Kaakinen *et al.* 2004). Timmer (1996) reports nutrient loaded black spruce seedlings have higher relative growth responses on the more nutrient deficient sites in his field trial. Previous studies have also shown the positive effect of nutrient loading on growth

in rich soils, but usually to a lesser degree than in poor soils (Timmer & Munson, 1991; Idris *et al.* 2004).

Both loading and field fertilization increased biomass allocation significantly (Table 5). The high effects on growth of current needles (Fig. 14 and Table 5.) was concluded to be essential as important amounts of nutrients can accumulate in and be retranslocated from needles (van den Driessche, 1991). Old wood (stem and old branches) and current branches (Table 5) also increased biomass with more loading and fertilization at planting. Although no direct measurements of the root systems were conducted, the strong response to fertilization in loaded treatments concerning shoot elongation (Fig. 12) and biomass allocation (Fig. 13 and Table 5.) indicated more root system activity in loaded seedlings than unloaded seedlings at both sites. More root growth in nutrient loaded seedlings compared to unloaded seedlings is reported in many similar studies. In black spruce, (Timmer & Munson, 1991; Idris *et al.* 2004; Imo & Timmer, 2001) Norway spruce, (Heiskanen *et al.* 2009) white spruce, (McAlister & Timmer, 1997) silver birch. (Rytter *et al.*, 2003) and Scots pine (*Pinus sylvestris*) (Iivonen *et al.*, 2001). Salifu & Timmer (2001) speculate that the greater root growth in nutrient loaded black spruce seedlings enhances rapid exploitation of available pools of soil N increasing growth markedly.

4.6 Nutrient dynamics after first growing season

4.6.1 Nitrogen concentration

After one growing season in the field, loaded and unloaded treatments without field fertilization had depleted the nutrient reserves accumulated in the nursery (Fig. 15). Only at site A nutrient loading led to a significantly higher N concentration. The unfertilized seedlings suffered from N deficiency (<9 mg N/g) (Ingstad, 1962). The depletion of nutrients was also observed by Munson and Bernier (1993). In their study the benefit of increased nutrient status in black spruce seedlings in the nursery 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. This was clearly demonstrated when fertilized seedlings in this study were observed (Fig. 15). Their nutrient status was significantly better than unfertilized seedlings although the nitrogen status of all treatments was considerably lower than optimum (Ingstad, 1962; Roberntz, 1998) indicating infertile conditions at the sites. The colour of unfertilized treatments compared with fertilized also indicated nutrient deficiency (fig. 21) (unpublished data). Malik and Timmer (1998) reported that nutrient loaded black spruce seedlings when planted depleted their nutrient reserves, but also took up more nutrients than unloaded seedlings, from external soil sources significantly stimulating new growth in shoots and roots. This was attributed to improved root growth in these plants (Malik and Timmer, 1998; Salifu and Timmer, 2001).



Fig. 21. The colour of unfertilized (left) treatments compared with fertilized (right) indicated nutrient deficiency.

4.6.2 Nitrogen content

It must be recognized that a change in nutrient concentration alone is not an unequivocal indication of change in nutrient content, since dry weight changes, due to growth or respiration, will result in changes of nutrient concentration (van den Driessche, 1991). A dilution in nutrient concentrations in seedlings was detectable in this study (Fig. 15), but at the same time dry matter increase was considerable (Fig. 13). Loaded fertilized seedlings partitioned significantly more N to current needles than nonloaded fertilized seedlings. The high N content indicated that current needles were a major sink for absorbed N. Imo and Timmer (2001) report current shoots to be a major sink for absorbed N and in their study with black spruce seedlings, loaded seedlings partitioned more N to current shoots than nonloaded seedlings as was the case in this study.

However, loading effects were not significant in total N content at site B ($P=0.0531$) but highly significant at site A (Table 6). The treatment receiving no loading at the nursery in site B but was fertilized at planting did not deplete N from old needles as much as the loaded treatments (Table 6). The N content in old needles was not significantly different between treatments so the high N content of this treatment affected the outcome of total N content in site B. Salifu

and Timmer (2001) compared nutrient loaded and non loaded (conventional) seedlings of black spruce for one growing season, planted on sites with varying fertility. They found growth and nutrient uptake increased with N supply, and was consistently higher in loaded than in conventional grown seedling. In the same experiment rapid early growth of new shoots was done at the expense of old shoots that exhibited N depletion. This depletion was severe for loaded seedling, but milder for conventional seedlings. They concluded that loaded seedlings exhibited an exploitive nutrient use strategy by depleting higher N reserves for investment in active metabolic sinks in new shoots and roots. In contrast, conventional seedlings used a conservative strategy characterized by less N depletion from lower reserves. This was also observed by Kaakinen *et al.* (2004) and is presumably the reason for high N content, in old needles, in unloaded fertilized seedlings in site B.

4.6.3 Interaction effects of nutrition

Results from this study indicated higher root growth for loaded seedlings resulting in greater N uptake (chapter 4.5). In a series of experiments conducted to examine the importance of current photosynthate for the development of new roots in planted Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco) and Sitka spruce, results showed current carbohydrates were the primary carbon source for new roots (van den Driessche, 1987). There is a strong positive correlation between leaf nitrogen concentration and photosynthetic rate (Luxmoore *et al.*, 1995) and it is possible that the loaded treatments, with a higher initial N concentration, produced more carbohydrates favoring root growth.

Malik and Timmer (1998) reported loaded seedlings to be more effective in partitioning carbon and N to metabolically active tissues as current needles and roots. They suggested that higher levels of available N in loaded seedlings promoted photosynthesis by increasing needle size and numbers, chlorophyll and carotenoid concentrations and may also increase nutrient uptake by accelerating root growth. On the other hand, it has been well documented that a typical response in plants to high N is reduced growth of the root system. (Ingstad & Agren, 1988; Ericsson, 1995). Despite that, many studies where root growth of nutrient loaded seedlings were observed over one or more growing seasons, have reported more root growth in the field for loaded seedlings compared with unloaded (Kaakinen *et al.* 2004; Malik and Timmer, 1998; McAlister & Timmer, 1997). Increased root growth results in more nutrient uptake (Ingstad & Agren, 1988), which may have positively affected the nutrient loaded seedlings in this study.

4.6.4 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 and Timmer, 1998). When calculating retranslocation it must be kept in mind that nutrients are distributed in various plant

parts. In one and two year old conifer seedlings, sampled at the end of one growing season, needle nutrient content represented more than half the total content of nutrients in the seedlings. A two year old white spruce seedling contained 12 mg/seedling of N in the needles while only 5 mg. were retained in the stem and 5 mg. in roots, showing that needles are important storage sites for nutrients in seedlings (van den Driessche, 1991). Therefore, in this study only the N content in old and current needles was used to calculate retranslocation as in Nambiar and Fife (1987) and Munson *et al.* (1995). 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.

Retranslocation increased with more loading in the nursery for both fertilized and unfertilized seedlings. This has been observed in other studies. (Malik & Timmer, 1998; Salifu & Timmer, 2001 and 2003 a; McAlister & Timmer, 1998; Imo & Timmer, 2001). Chapin (1990) associates greater N depletion with higher preplanting reserves in nutrient loaded seedlings than unloaded seedlings. The higher retranslocation in loaded seedlings, suggests that N reserves in these seedlings were probably less structurally bound, hence readily available for depletion to active metabolic sinks. However, in this study, fertilization at planting seemed to reduce N retranslocation which is consistent with the findings of others (Imo and Timmer, 2001; Salifu and Timmer, 2001). Salifu and Timmer (2001) reported decreasing net N retranslocation in black spruce seedlings with an increasing N supply, supporting a hypothesis of reduced retranslocation in rich soils. They also reported that retranslocation diminished with time. Retranslocation was greatest early in the growing season when new growth is the most intensive and then declined late in season. Conversely, external uptake increased with time presumably because of greater root establishment and growth. McAlister & Timmer (1998) showed that the N content of all plant components first reduced with time early in the growing season and then increased. As there was only one measurement of N content in the end of the growing season in this study, it was not possible to state that retranslocation was less in fertilized seedlings. Fertilized seedlings had partitioned more N to current needles than unfertilized seedlings in the end of the growing season and therefore their estimated retranslocation was lower than for unfertilized seedlings. 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 and Timmer, 2003 a).

An attempt was made to calculate if the entire retranslocated N ended up as N content in current needles. This was done by assessing the difference of the N content in current needles and the amount retranslocated from old needles as described other studies (Malik and Timmer, 1998; Imo and Timmer, 2001). The calculated retranslocation in fertilized seedlings was probably not reliable. The unfertilized treatments all showed negative values (Fig. 18) meaning

only part of the N retranslocated from old needles to current needles. This indicates that part of the retranslocated N was moved to plant parts other than current needles, probably to the roots as they have been reported to be one of major sinks of retranslocated N (van den Driessche, 1991). Malik and Timmer (1998) found in contrast to nutrient loaded seedling, unloaded seedlings with lower nutrient reserves partitioned more biomass to roots than to current needles indicating greater stress for belowground sources. They suggest that increasing root growth rather than shoot growth may improve long term survival, since the higher root: shoot ratio under nutrients stress represents a feedback mechanism favoring nutrient uptake.

The positive values of fertilized treatments (Fig. 18) led to the conclusion that more N ended up in current needles than the amount of retranslocated N. This indicated an external N source (not from old needles) from the soil or from the root system itself.

4.7 Survival after one growing season in field

At site B, only the treatment receiving 22.2 g N/m² in the nursery had lower survival rates than other treatments (Fig. 19). The reason for this was unknown. Neither the loading nor fertilization at the time of planting had any significant effects on survival. At site A the survival of seedlings was not affected by loading (Fig. 19), but fertilization at the time of planting increased survival significantly ($P < 0.0001$). The main reason for dead seedlings at site A was the damage caused by the larvae of *Otiorhynchus* that feed on the root system (Fig. 20). Generally the highest mortality caused by these larvae is found in areas vegetated by dwarf shrubs (Halldórsson *et al.*, 2000) This is the vegetation covering site A. Inoculation with forest soil, insect pathogenic fungi and ectomycorrhizal fungi have negative effects on *Otiorhynchus* larvae and are factors shown to increase the survival of seedlings (Oddsdottir, 2010). The results of this study indicate that fertilization at time of planting reduces the damage caused by *Otiorhynchus* larvae and increases survival.

The short duration this study limits its usefulness for predicting the effect of loading on survival. Óskarson and Brynleifsdottir (2009) reported increased survival in loaded and fertilized seedlings of birch and Sitka spruce after three years in the field and this has also been shown for nutrient loaded white spruce (van den Driessche, 1992). In this study the loaded treatments showed more above ground growth, and contained more N at the end of the growing season indicating more root growth than unloaded treatments. The root growth might also have occurred earlier in the loaded seedlings than the unloaded seedlings, allowing them to better exploit nutrients available in the surrounding soil earlier as seen in the results from Malik and Timmer (1998). The greater needle mass could favor these seedlings in the future, having more needle mass for photosynthesis and more N content (Malik and Timmer, 1998). On the other hand, others have reported unloaded seedlings partition more biomass to their roots than to current needles (Malik and Timmer, 1998) suggesting the potential of increased long term

survival rates. It can also be a disadvantage to have a large leaf mass when plants are subjected to drought stress (van den Driessche, 1991).

Only the futures can reveal if nutrient loading will increase survival rates of the seedlings in this study. However, loading might provide an additional input for faster plantation establishment during the first crucial growing season after planting.

5. Conclusions

- The seedlings could be nutrient loaded without changing their total biomass and without delaying frost hardiness.
- Nutrient loading did not significantly affect root growth capacity during the three weeks of cultivation in an RGC-table.
- After one growing season in the field, the height, biomass allocation and N content in loaded seedlings was significantly more than in unloaded seedlings, attributed to a higher internal N status prior to planting.
- Although no direct measurements of the root systems were conducted, after one growing season in the field the strong response to fertilization in loaded treatments in shoot elongation, biomass allocation and N content indicated more root system activity in loaded seedlings than unloaded seedlings at both sites.
- The results of the retranslocation calculations showed the need to include all plant parts and the importance of using labelled isotopes to calculate retranslocation. However, retranslocation of N from old needles to new was detectable.
- Survival was not affected by nutrient loading after the first growing season, but fertilizing significantly decreased the damage caused by *Otiorhynchus* larvae in the heath land.
- The shortness of this study limits its usefulness for predicting the effect of loading on survival in the long term. Never the less, 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. Since stored nutrients are the major source of nutrient reserves for initial seedling development, because newly planted seedlings cannot exploit the surrounding soil until they develop new roots, it is concluded that loading might provide an additional input for faster plantation establishment during the first crucial growing season after planting.

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