Effect of nitrate and ammonium on growth of transplanted Norway spruce seedlings: a greenhouse study

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Effects of nitrate and ammonium nitrogen (N) on the growth of two-year-old Norway spruce seedlings (Picea abies) were studied after transplanting in a greenhouse. In a preliminary experiment (Exp. 1), seedlings were transplanted into sandy soil in 2-liter pots and irrigated either with ammonium or nitrate solution in concentrations (5 mg l⁻¹ N) commonly found in clearcut boreal forest sites. Irrigation with pure water was used as the control treatment. In a further experiment (Exp. 2), seedlings were irrigated similarly, but irrigation with ammonium and nitrate mixture in proportion of 1:2 (total 5 mg l⁻¹ N) was used as the control treatment. In addition, N treatments were combined with contrasting soil temperatures of 11.5 and 21 °C. Irrigation with N-free pure water resulted in decreased root growth (Exp. 1). In both experiments, no clear differences were found in shoot or root growth in the different N-source treatments. Regardless of N application, the most pronounced effect was increased root growth at the higher soil temperature (Exp. 2). The results question the importance of N form on the growth of Norway spruce seedlings in boreal forests.

Key words: nitrogen, nutrient availability, Picea abies, root egress, soil temperature

Introduction

In the boreal zone, Norway spruce (Picea abies) is widely planted for reforestation (Grossnickle 2000). In Finland, for example, about 60% of a total of ca. 150 million seedlings planted each year are Norway spruce (Finnish For. Res. Inst. 2002). A couple of years after outplanting to forest sites, stagnation of seedling growth in Norway spruce and other spruce species has been recorded (Björkman 1953, Vyse 1981, Grossnickle 2000). Planting shock is often associated with post-planting water stress, but with or without water stress, this slow growth may also be related to retarded adaptation to mycorrhizal colonization and to low uptake of nitrogen (N) and other nutrients after planting (Björkman 1953, Grossnickle 2000).

In general, N is the most growth-limiting nutrient in the soil of boreal forests (e.g. Viro 1965). Limited N availability is partly due to low soil temperatures, which decrease water viscosity and mass flow in the soil as well as cell metabolic activity (Lopushinsky & Kaufman 1984, Marschner et al. 1991, Ryyppö et al. 1998). After clearcutting and site preparation, soil tempera-
ture tends to increase and thus affect nutritional and other growth prerequisites (Örlander et al. 1990, Kubin & Kemppainen 1994). In addition, N availability to seedlings is affected by soil organic material and its mineralization (Kraske & Fernandez 1990, Nordborg et al. 2003). Thus, seedling growth after planting may vary, in addition to seedling material, due to site, method of site preparation and planting spot.

Based mainly on nutrient-uptake studies, Norway spruce has been suggested to prefer ammonium (NH$_4^+$) to nitrate (NO$_3^−$) as a source of N (Marschner et al. 1991, Buchmann et al. 1995, Gessler et al. 1998, Höberg et al. 1998, George et al. 1999, Öhlund & Näsholm 2001). Furthermore, low soil temperatures may limit uptake of nitrate by Norway spruce more than uptake of ammonium (Gessler et al. 1998). Organic N can also be utilized by conifer seedlings (Öhlund & Näsholm 2001, Wallander 2002, Persson et al. 2003); but recalcitrant organic N may be barely usable (George et al. 1999).

When nitrate prevails in the soil, seedlings of late-successional spruces, like Norway spruce, have been suggested to have decreased growth potential relative to other surface vegetation, thus leading to problems in plantation establishment (Kronzucker et al. 1997). However, data about the effects of nutrient availability and other soil conditions on growth of planted Norway spruce seedlings is scarce. N uptake by trees has been studied relatively extensively in soilless cultures (e.g. Ingestad 1979, Boxman & Roelofs 1987, Peuke & Tischner 1991, Garnett & Smethurst 1999, Öhlund & Näsholm 2001), but far less in soil (Crabtree & Bazzaz 1993, Buchmann et al. 1995, George et al. 1999, Persson et al. 2003). Furthermore, only a few studies have dealt with the effects of temperature on N availability to any plant species or with the subsequent growth (e.g. Clarkson & Warner 1979, Gessler et al. 1998, Garnett & Smethurst 1999, Adam et al. 2003).

Thus, there is a need for information about the effects of N source and temperature on the growth of planted Norway spruce seedlings. The aim of the present study was to test whether different combinations of nitrate and ammonium solution and soil temperature affect the growth of transplanted Norway spruce seedlings in sandy soil.

Materials and methods

Preliminary experiment (Exp. 1)

Two-year-old Norway spruce seedlings produced for practical reforestation were used in the experiment. The seedlings were sown using seed-orchard seed of local origin and grown in Sphagnum peat in containers (container size 110 cm$^3$, type PL-64F, Lannen Oyj., Iso-Vimma, Finland) according to standard nursery procedures in a greenhouse in central Finland. The seedlings were stored over winter in container trays placed in cardboard boxes in a freezer (−3 °C). Before the experiment, the seedlings were thawed for a week at +8 °C.

A total of 75 vigorous seedlings of uniform height (mean = 28.6, S.D. = 2.5 cm) were selected for the experiment. The roots of the seedlings were gently washed free of the peat medium. Most of the seedlings roots were mycorrhizal (ca. 80%–90%), almost solely with Laccaria sp. Fifteen seedlings were measured for height, diameter at root collar, shoot and root volumes (Harrington et al. 1994) and biomass fractions (as dry mass at 65 °C). In January 2002, the rest of the seedlings were transplanted into 2-liter pots filled with sand containing 23.4% by mass of fractions 0.2–0.6 mm in diameter and 5.2% of fractions below 0.2 mm in diameter. Soil organic matter concentration was 0.6% by mass. Soil bulk density was ca. 1.50–1.55 g cm$^{-3}$.

The transplanted seedlings were divided into three groups (3 × 20 seedlings), each of which received either ammonium solution, nitrate solution or pure water. Ammonium was applied as NH$_4$Cl and nitrate as KNO$_3$. Both solutions applied had a concentration of 5 mg l$^{-1}$ N, which is similar to that found on clearcut forest sites (e.g. Nohrstedt et al. 1996, Smolander et al. 2000). The used solutions were buffered to pH 4.5 with HCl since the most favorable soil pH for Norway spruce and Scots pine (Pinus sylvestris) seedlings is 4–5 (Rikala & Jozefek 1990) and nitrification is very limited at pH 4 or lower (Persson & Wiren 1995). Application of pure deionized water, which was also buffered to pH 4.5, was used as the control treatment.

Combined samples within treatments were collected from the leachates from the pots three
times during the experiment. Leachates were analyzed for total N, NH$_4$ and (NO$_2$ + NO$_3$) with a flow-injection analyser (FIA) or ion chromatography. Organic N was estimated as total N – NH$_4$ – (NO$_2$ + NO$_3$). During the experiment, the total N concentration of the leachates was higher in the KNO$_3$ treatment (6–9 mg l$^{-1}$) than in the other treatments (< 3 mg l$^{-1}$). In the NH$_4$Cl treatment, the slightly elevated NO$_3^-$ concentrations (< 3 mg l$^{-1}$) indicated that some nitrification has occurred (< 0.1 mg l$^{-1}$ in H$_2$O treatment).

Natural light in the greenhouse was supplemented with artificial lighting to give a photoperiod of 18/6 h and photosynthetically active radiation varying spatially from 280 to 330 µmol m$^{-2}$ s$^{-1}$ at the seedlings’ shoot level on cloudy days. On cloudy days, the mean daily and nocturnal air temperatures were 20 and 15 °C, respectively. Relative humidity varied from 30% to 50%. The seedlings were irrigated with the solutions twice per week, which kept the soil adequately moist and the solution available to the seedlings. After two months, the seedlings were harvested and measured for the same attributes as the sample seedlings had been prior to transplanting.

Further experiment (Exp. 2)

A total of 105 vigorous two-year-old seedlings of the same origin as in Exp. 1 with uniform height (mean = 33.9 cm, S.D. = 2.1) were selected for this experiment. Again, 15 seedlings were measured before transplanting and the rest were transplanted as in Exp. 1. Then 90 seedlings (2 × 3 × 15) were divided into 2 × 3 treatment groups, in which two soil-temperature and three N-solution treatments were applied (Table 1). After transplanting, the seedlings were grown from February to April 2003, after which they were harvested and measured.

Two soil temperatures (11.5 and 21 °C) were applied since they were considered to have a contrasting effect on N utilization by the seedlings (Gessler et al. 1998). Soil temperatures were adjusted with a water bath, in which the seedling pots were submerged so that their upper rim was ca. 5 cm above the water level. Seedling pots were covered with an insulating polyurethane layer with holes for the seedling shoots. During the experiment, both temperatures were applied simultaneously in two separate basins. Temperatures in the water bath, pots and ambient air were recorded with a datalogger (Table 2). The temperature basins were thus not replicated independently, but the possible dependency of observations was minimized by changing the seedling positions weekly within the temperature blocks and providing as homogenous lighting and seedling irrigation as possible for both basins.

Ammonium and nitrate solutions were applied to both temperature treatments as in Exp. 1. However, a combined solution (5 mg l$^{-1}$ N) of NH$_4$Cl and KNO$_3$ in the proportion 1:2 N was used as the control treatment, since similar ammonium-to-nitrate ratios have been found in the field (Smolander et al. 2000). In addition, in order to reduce possible nitrification and subsequent formation of NO$_3^-$, all N solutions were buffered with HCl to pH 4.2. Leachates from the pots were analyzed as in Exp. 1. N concentration in the leachates was higher in the KNO$_3$ and the combined solution treatment at a soil temperature 21 °C (< 4.5 and < 2.5 mg l$^{-1}$, respectively) than in the other treatments during the experiment (< 1.5 mg l$^{-1}$). In the NH$_4$Cl treat-

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**Table 1.** Solutions applied (5 mg l$^{-1}$ N) and prevailing mean temperature in the water bath in the treatments used in Exp. 2.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Solution</th>
<th>Temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amm_LT</td>
<td>NH$_4$Cl</td>
<td>11.5</td>
</tr>
<tr>
<td>Nit_LT</td>
<td>KNO$_3$</td>
<td>11.5</td>
</tr>
<tr>
<td>Mix_LT</td>
<td>NH$_4$Cl + KNO$_3$</td>
<td>11.5</td>
</tr>
<tr>
<td>Amm_HT</td>
<td>NH$_4$Cl</td>
<td>21.0</td>
</tr>
<tr>
<td>Nit_HT</td>
<td>KNO$_3$</td>
<td>21.0</td>
</tr>
<tr>
<td>Mix_HT</td>
<td>NH$_4$Cl + KNO$_3$</td>
<td>21.0</td>
</tr>
</tbody>
</table>

**Table 2.** Temperatures (°C) in the treatments used during Exp. 2.

<table>
<thead>
<tr>
<th>Position</th>
<th>Treatment</th>
<th>Mean</th>
<th>S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water bath</td>
<td>Lower temperature</td>
<td>11.5</td>
<td>1.4</td>
</tr>
<tr>
<td>Water bath</td>
<td>Higher temperature</td>
<td>20.9</td>
<td>2.0</td>
</tr>
<tr>
<td>Pots</td>
<td>Lower temperature</td>
<td>12.5</td>
<td>1.6</td>
</tr>
<tr>
<td>Pots</td>
<td>Higher temperature</td>
<td>20.6</td>
<td>1.9</td>
</tr>
<tr>
<td>Air</td>
<td>Both temperatures</td>
<td>19.3</td>
<td>2.4</td>
</tr>
</tbody>
</table>
ment, the slightly elevated NO$_3^-$ concentrations (< 0.4 mg l$^{-1}$) indicated that little nitrification had occurred.

**Results**

**Experiment 1**

At planting, mean total foliar N was 1.81% (or 18.1 mg g$^{-1}$). At harvesting, mean total foliar N was 0.82% in the pure water treatment, 0.90% in the NH$_4$Cl treatment and 0.95% in the KNO$_3$ treatment. In new needles at harvesting, foliar N was rather uniform in the treatments (mean = 0.83%). Height and diameter growth did not differ significantly between treatments (ANOVA: $P > 0.05$) (Fig. 1). Of the seedling attributes measured, only root-volume growth was significantly different between treatments

**Data analysis**

The seedlings in the three treatments in Exp. 1 were fully randomized, which was ensured by changing the pot positions weekly. Exp. 2 was a randomized block design in which the seedlings and treatments were positioned randomly within the two temperature treatments. Differences between treatments were analyzed with the analysis of variance (ANOVA) and Tukey’s test.
As compared with N application, irrigation with pure water resulted in decreased root growth (Tukey’s test: $P < 0.05$) (Fig. 2).

Experiment 2

Mean total foliar N was 1.27% at planting and 0.46%–0.56% at harvesting (in new needles 0.51%–0.57%). Height growth differed between N-source treatments at $P = 0.061$ (ANOVA) but not between root temperatures (Fig. 3 and Table 3). Diameter growth at the root collar was significantly affected by temperature ($P = 0.009$) and N source applied ($P = 0.041$) but, according to Tukey’s test, did not differ between individual treatments ($P > 0.05$). Initial diameter of the root collar at the time of planting was found to be a significant covariate for diameter growth ($P = 0.006$).

Shoot-volume growth did not differ among treatments (Fig. 4 and Table 3). Root-volume growth clearly differed between temperatures (ANOVA: $P < 0.0005$) but not between N-source treatments. The root-to-shoot ratio in terms of volume and dry mass at harvesting also differed significantly between the applied temperatures ($P < 0.0005$) but not between N sources ($P > 0.05$). No other attribute at harvesting differed significantly among treatments.

Discussion

Clearcutting and site preparation tend to increase soil temperature as well as alter moisture conditions and subsequently soil pH and nutrient conditions (Örlander et al. 1990, Kubin & Kempainen 1994). It has been suggested that in late-successional forest stands, the soil contains more ammonium than nitrate; while in the early successional stage, the situation is opposite due to increased mineralization and nitrification (Van Cleve et al. 1993, Kronzucker et al. 1997). The increasing ratio of soil ammonium to nitrate together with differing ammonium transport efficiencies in different tree species may be important forces in competition between species and

Fig. 3. Seedling growth in height and in diameter at root collar in the treatments used in Exp. 2 (mean + S.D.).

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>height</th>
<th>diameter</th>
<th>shoot vol.</th>
<th>root vol.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temp.</td>
<td>1</td>
<td>0.451</td>
<td>0.009**</td>
<td>0.487</td>
<td>&lt; 0.0005***</td>
</tr>
<tr>
<td>N source</td>
<td>2</td>
<td>0.061</td>
<td>0.041*</td>
<td>0.386</td>
<td>0.787</td>
</tr>
<tr>
<td>T × N</td>
<td>2</td>
<td>0.076</td>
<td>0.431</td>
<td>0.673</td>
<td>0.312</td>
</tr>
<tr>
<td>Diam.</td>
<td>1</td>
<td></td>
<td></td>
<td>0.006**</td>
<td></td>
</tr>
<tr>
<td>Error</td>
<td>83–84</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
in forest succession (Kronzucker et al. 2003). However, little data are available to test this hypothesis.

In practice, clearfelling and site preparation tend to increase the total N concentration in the soil solution (Smolander et al. 2000, Högbom et al. 2002, Piirainen et al. 2002). Furthermore, soil organic N can increase (concentrations below 3.5 mg l⁻¹) (Piirainen et al. 2002). In a clearcut Norway spruce stand, ammonium concentrations of ca. 0.5 and 2 mg l⁻¹ N have been found in the soil solution in unprepared mineral topsoil and in mounds, respectively (Smolander et al. 2000). Corresponding values for nitrate were somewhat higher, ca. 1 and 5 mg l⁻¹ N. Similar concentrations have also been found elsewhere (Högberg et al. 1986, Termorshuizen & Ket 1991, Nohrstedt et al. 1996, Högbom et al. 2002). On another clearcut Norway spruce stand (Piirainen et al. 2002), somewhat lower nitrate concentrations (below 0.5 mg l⁻¹) were found at locations without site preparation. In areas with higher N deposition, the concentrations of nitrate and ammonium in the soil solution are higher (Gessler et al. 1998, cf. Buchmann et al. 1995).

In the present study, the N levels available to seedlings were close to natural levels after outplanting to forest sites (e.g. Nohrstedt et al. 1996, Smolander et al. 2000). With respect to tree-nursery recommendations (Ingestad & Kähr 1985, Landis 1985, Rikala 2002), the foliar N concentrations were relatively low already at the start of the experiments. Therefore, the seedlings had suboptimal N availability as compared with the optimum nutrition; thus showing suboptimal growth rate. However, the use of nutrients in Finnish tree nurseries has traditionally been low, especially during the second year of seedling growth, since plantation establishment has been found to succeed reasonably well with these N-deficient seedlings (Rikala et al. 2004).

With the N concentrations used in this study, the source of inorganic N showed no clear effect on the growth of mycorrhizal Norway spruce seedlings planted in sandy soil at the two contrasting soil temperatures used (11.5 and 21 °C). Regardless of N application, the most pronounced effect was decreased root growth at the lower soil temperature (Exp. 2) and during irrigation with N-free pure water (Exp. 1). The sensitivity of the root-growth response to temperature and N availability is probably due to dependence on current photosynthesis, since shoot growth in conifer seedlings can utilize the previous year’s photosynthate reserves, while new root growth is more dependent on current photosynthates (van den Driessche 1987).

In general, high foliar N usually indicates better seedling growth during the second season (Malik & Timmer 1996, Nordborg et al. 2003). N uptake of planted Norway spruce seedlings depends on soil organic material, and on its mineralization, and increases with root growth (Kraske & Fernandez 1990, Örlander et al. 1990, Nordborg et al. 2003). According to the present study, restricted root growth due to limited N availability (Exp. 1) and low soil temperature (Exp. 2) during the first growing season after planting may suggest suboptimal preconditions for shoot growth during the next season. In practice, the use of site-preparation methods such as mounding, which increases soil temperature and
includes humus in the planting spot, is beneficial for reforestation with Norway spruce seedlings (Örlander et al. 1990).

Norway spruce has been found to take up more ammonium than nitrate but it has also been found to utilize mainly nitrate, which has better mobility in soil (Marschner et al. 1991, Lumme 1994, Buchmann et al. 1995, Gessler et al. 1998, Högbärg et al. 1998, George et al. 1999, Öhlund & Näsholm 2001). The presence of ammonium may decrease nitrate uptake by Norway spruce seedlings (Gessler et al. 1998). In pure nitrate solution, Norway spruce and Scots pine seedlings may show relatively minor growth reduction as compared with that in growing solutions with different nitrate-to-ammonium ratios (Ingestad 1979).

On the other hand, Norway spruce seedlings with natural mycorrhizae have been shown not to differ in their uptake of nitrate and ammonium (Lumme & Smolander 1996) nor in their uptake of N from inorganic and organic sources (Persson et al. 2003). In the present study, the seedling growth response suggests that mycorrhizae (Laccaria sp.) had no effect or affected the uptake of both N forms by seedlings similarly. In the nursery, Laccaria sp. mycorrhizae have previously been reported to retard seedling growth as compared with that of uncolonized control seedlings (Pennanen et al. unpubl. data). In general, mycorrhizal colonization of Norway spruce seedlings can cause increased uptake of N and elevated CO₂ assimilation, but uptake of ammonium (not of nitrate) may also result in an increased respiration rate (Eltorp & Marschner 1996, Brunner et al. 2000, Jentschke et al. 2001).

Prevailing soil temperatures in mature stands have been found to limit uptake of nitrate by Norway spruce more than that of ammonium (Gessler et al. 1998). Nitrate uptake by red maple has also been found to decrease with decreasing temperature (34–14 °C) (Adam et al. 2003). Also other soil conditions may affect the uptake of N (Stamp et al. 1997). With decreasing pH from 5.5 to 2.5, nitrate uptake by Norway spruce seedlings has been shown to decrease sharply (Peuke & Tischner 1991). In addition to low pH, ammonium and Al³⁺ can also decrease nitrate uptake (Peuke & Tischner 1991). With nitrate fertilization, Fe chelate can improve seedling growth (van den Driessche 1978). In a study with four birch species, only one showed a difference in growth depending on the inorganic N source applied; the effect of which was dependent on the light environment (Crabtree & Bazzaz 1993). In another study, silver birch seedlings differed only slightly in their uptake of both inorganic N sources; but biomass growth was higher with nitrate (Vuorinen et al. 1995).

In conclusion, most previous nutrient-uptake studies indicate that Norway spruce seedlings are able to utilize both inorganic N forms but usually prefer ammonium. This study, however, showed no clear effect of applying nitrate and ammonium with levels found in natural boreal conditions on the growth of mycorrhizal Norway spruce seedlings at either the low (11.5 °C) or high (21 °C) soil temperature. This discrepancy may be partly due to the lower, natural N application levels and temperatures used for the mycorrhizal seedlings growing in the soil in the present study, while previous studies have mostly focused on nutrient uptake in soilless cultures in controlled lab conditions. Therefore, the results of this study question the importance of N form on the actual growth of Norway spruce seedlings in boreal forests.

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