Five-year-old Norway spruce (*Picea abies* (L.) Karst.) seedlings were subjected to three simulated growing seasons in controlled environment chambers. Plants were acclimated to a soil temperature of 16 °C during the first and third growing seasons, but were allocated at random to soil temperature treatments of 9, 13, 18 and 21 °C during the second growing season. Low soil temperature during the second growing season depressed stomatal conductance and photosynthetic rate (*A*) per unit of projected leaf area, although intercellular CO2 concentrations did not differ significantly between treatments. At all soil temperatures, total chlorophyll concentration first decreased and then increased, although the rate of increase and the final concentration increased with soil temperature, which may explain the effect of soil temperature on *A*. Neither chlorophyll a/b ratio nor leaf nitrogen concentration was significantly affected by soil temperature. Treatment differences disappeared during the third simulated growing season when plants were again acclimated to a soil temperature of 16 °C.

**Keywords:** chlorophyll a/b ratio, chlorophyll concentration, internal carbon dioxide concentration, nitrogen concentration, photosynthetic CO2 assimilation rate, *Picea abies*, stomatal conductance, transpiration rate.

**Introduction**

Plants exhibit seasonal and diurnal variations in physiological activity, which reflect both environmental conditions and the phases of plant ontogeny (Kozlowski et al. 1991, Grossnickle 2000). In conifers, photosynthetic capacity (Amundson et al. 1992, Vogg et al. 1998) and concentrations of chlorophyll (Linder 1972, 1980, Gond et al. 1999) and nitrogen (N) (Fife and Nambiar 1982, Helmisaari 1992, Millard and Proe 1992) vary seasonally according to species-specific patterns and environmental conditions. Soil temperature is an important factor regulating physiological activity (Linder 1980, Hansen et al. 1996, Ryypö et al. 1998, Wang and Zwiazek 1999). In general, low soil temperatures have a greater effect on seedlings than on adult trees, on growth than on photosynthesis (e.g., Häggren and Öquist 1990), and on the growth of roots than on the growth of other plant parts (Häggren and Öquist 1990).

Low soil temperatures can affect root growth and physiology in ways that impact the shoot through hormonal (e.g., Lösch and Schulze 1994) or hydraulic signals (Fuchs and Livingston 1996) or as a consequence of a carbohydrate imbalance between source and sink (Grossnickle 2000, Pregitzer et al. 2000). Through such effects, low soil temperature may also affect shoot gas exchange. Because large quantities of nitrogen (N) are required for the synthesis of photosynthetic enzymes and chlorophyll (Haynes 1986, Billow et al. 1994), there is often a positive relationship between foliar N concentration and net photosynthesis (e.g., Evans 1989, Grossnickle 2000). Soil temperature may, therefore, impact photosynthetic capacity through effects on N uptake or translocation.

In this study we investigated the responses to soil temperature of chlorophyll concentration, gas exchange and nitrogen concentration in 4- to 5-year-old Norway spruce (*Picea abies* (L.) Karst.) seedlings. We tested two hypotheses: (1) net photosynthetic rate decreases with soil temperature; and (2) the decrease in photosynthetic rate due to cold soil is accompanied by decreases in (a) stomatal conductance, (b) chlorophyll concentration and (c) foliar N concentration.

**Materials and methods**

**Plant material and growing conditions**

On August 7, 1998, 4-year-old Norway spruce seedlings of central Finland origin (Sv. 113, T3-92-0043), raised in the nursery at the Suonenjoki Research Station of the Finnish Forest Research Institute (62°40′ N, 27°03′ E), were transplanted to 10-l plastic buckets filled with peat. On October 8, the seedlings were transported to Joensuu where they were kept for 48 days in a growth chamber in a 6-h photoperiod (photosynthetically active radiation, PAR = 150 μmol m⁻² s⁻¹), a day/night temperature of 4 °C and relative air humidity of 90%.
November 25–30, 1998, each seedling was transplanted to a 0.46-m$^3$ cylindrical pot filled to a height of 100 cm with sand, topped by a 14-cm layer of organic soil from a Norway spruce forest and insulated by a 60-mm layer of expanded polystyrene. There were four pots in each of four controlled environment chambers. Each pot was equipped with two coils through which glycol brine was circulated at a controlled temperature (Finér et al. 2001). One coil was located on the top of the humus layer and the other was positioned 5 cm above the bottom of the pot. Total nitrogen concentration in the organic layer at the start of the experiment was determined by Kjeldahl analysis (Table 1). The concentrations of extractable macronutrients in the soil at the start of the experiment (Table 1) were determined by atomic absorption spectrometry (Ca and K) and spectrophotometry (P).

Both the soil and air temperatures in the controlled environment chambers were held constant at 4 °C, and the other environmental conditions were similar to the growth chamber conditions described above. Sixteen days after transfer to the controlled environment chambers, the first simulated growing season (GS I) was initiated. Figure 1 shows the time course of changes in soil temperature and daylength during the first and subsequent simulated growing seasons. During GS I and GS III, soil temperature was maintained close to 16 °C (range ± 0.4 °C), whereas during GS II, four plants were randomly assigned to soil temperature treatments of 9, 13, 18 and 21 °C. Air temperatures, relative air humidities, PAR and daylengths during the simulated growing seasons are given in Table 2. Between growing seasons, both air and soil temperatures were adjusted stepwise over 4 days to 4 °C.

During the experiment, soil water content was kept at field capacity (10% of volume) and varied little between treatments (range ± 0.2%). In GS I, the seedlings were fertilized six times with 4:1:4 N,P,K fertilizer plus micronutrients (see Figure 1). A total of 258 mg of N was supplied per seedling.

Measurements and sampling
Gas exchange was measured with an LI-6400 portable photosynthesis system (Li-Cor, Lincoln, NE) equipped with an LI 6400-05 conifer chamber (Figure 1). Leaf temperature was 22 °C, gas flow rate was 500 μmol s$^{-1}$, the ratio of the number of stomata on either side of the leaf (stomatal ratio) was 1, boundary layer conductance was 8 mol m$^{-2}$ s$^{-1}$ and the reference CO$_2$ concentration was 400 μmol mol$^{-1}$. Gas exchange was measured under the light conditions prevailing in the controlled environment chambers (Table 2).

Gas exchange was measured within 3 h of midday. Three branches of the main stem, located in the mid-crown, were chosen for analysis. Gas exchange of the tips (about 5 cm in length) of these branches, including both current (C) and 1-year-old (C + 1) needles, was measured. The same branches were measured throughout the experiment. Because of shoot growth, however, more C + 1 needles were measured at the beginning of the growing season, whereas at the end of each growing season mostly C needles were measured.

To express gas exchange per unit projected leaf area, needles enclosed in the chamber were counted. From each branch tip, the lengths and widths of 10 needles were measured. To estimate projected leaf area, 40 needles of different sizes were collected from additional plants. The dimensions of the needles were measured and the leaf area calculated with WinNeedle 3.0.1 (Regent Instrument, Québec City, PQ, and G. Aas 1995). The length x width of individual needles was plotted against leaf area and a second degree polynomial regression curve fitted ($R^2 = 0.92$). The equation was used to estimate the areas of the 10 needles measured; an average projected needle area for each branch tip was then calculated and multiplied by the number of needles enclosed in the gas-exchange chamber.

Needles from the same branch tips used for gas exchange measurement were sampled for chlorophyll analysis within 3 h of midday, on nine occasions (Figure 1). Two needles (one C and one C + 1 needle at the beginning, and two C needles in the middle and at the end of the GS) were taken from each of the three branches per seedling and pooled. The needles were weighed and their lengths and widths measured. Leaf area was estimated as described for the leaves used for the gas exchange measurements. The measured needles were placed in glass test tubes with 4 ml of N,N-dimethylformamide and extracted in

<table>
<thead>
<tr>
<th>Soil temperature (°C)</th>
<th>pH</th>
<th>Nutrient concentration (g kg$^{-1}$ DW)</th>
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<tr>
<td></td>
<td></td>
<td>Nitrogen</td>
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<tr>
<td>Organic layer</td>
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</tr>
<tr>
<td>21</td>
<td>3.95 (0.169)</td>
<td>3.93 (0.447)</td>
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<tr>
<td>18</td>
<td>3.86 (0.156)</td>
<td>5.03 (1.318)</td>
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<tr>
<td>13</td>
<td>3.84 (0.029)</td>
<td>4.74 (2.201)</td>
</tr>
<tr>
<td>9</td>
<td>3.95 (0.045)</td>
<td>4.65 (1.264)</td>
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<tr>
<td>Sand layer</td>
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<tr>
<td>21</td>
<td>5.72 (0.108)</td>
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</tr>
<tr>
<td>18</td>
<td>5.63 (0.046)</td>
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<tr>
<td>13</td>
<td>5.64 (0.018)</td>
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<tr>
<td>9</td>
<td>5.74 (0.034)</td>
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the dark at 5–7 °C with continuous shaking for 30 days (cf. Porra et al. 1989). Absorbances of the extracts were measured spectrophotometrically (HP 8453, Hewlett Packard, Waldbronn, Germany). Chlorophyll concentrations were calculated with the equations given by Porra et al. (1989).

Current, C + 1 and C + 2 needles were sampled for nitrogen analyses 14 times during the experiment (Figure 1). Each time, 15 needles of each age class were sampled evenly from the crown of each seedling. The needles were dried to constant mass at 40 °C. Dry needles were ground in a mortar with a pestle. For N analysis, 0.5 to 2.0 mg of sample was used and three subsamples were analyzed per sample. The standard used was acetanilide (N-phenylacetamide). Two standard samples were analyzed at the beginning of each set (49 samples) and a third standard was included for analysis with the experimental samples. Thereafter the standard was analyzed as every 10th sample. Nitrogen concentration was determined with an element analyzer (Carlo Erba Strumentazione, DP 110 PRC 200) with an accuracy of greater than 0.009 mg of the absolute N value for samples between 0.5 and 3.0 mg.

Statistical analysis

Data were evaluated by analysis of variance using the general linear model (GLM) procedure for repeated measures analysis of variance in the SPSS statistical analysis software package (SPSS, Chicago, IL). Soil temperature was a grouping factor and the sampling or measuring dates were treated as within-factors. The full factorial model was used to test treatment effects at \( \alpha = 0.05 \). Wilks’ Lambda given by GLM multivariate tests (for repeated measures) was used to evaluate treatment effects. When the sphericity (Mauchly’s test of sphericity) assumption was not fulfilled, the Huynh-Feldt correction was used to adjust the degrees of freedom for the tests of significance. For multiple comparisons, Tukey’s HSD test was used. Chlorophyll and nitrogen data were analyzed by type III sum of squares. For gas exchange data, however, type IV sum of squares was used because of missing values. In GS II and GS III, it is possible that needle development affected gas exchange. Therefore, we performed separate statistical analyses for these gas exchange measurements (i.e., for fully developed new needles) using GLM repeated measures. The chlorophyll and nitrogen concentrations and dry mass of the needles in the first and second dormancy periods (REST I and II in Figure 1) were compared by one-way analysis of variance.

Results

Gas exchange

During GS II, there was a statistically significant seasonal variation in all measured gas exchange parameters \( (P \leq 0.003) \) except intercellular CO2 concentration \( (C_i, P = 0.804) \). However, soil temperature had no significant effect on stomatal conductance \( (g_s) \ (P = 0.222, \text{Figure 2A}) \), and the effects of soil temperature on \( A \) (Figure 2B) and \( C_i \) (Figure 2C) were significant at the 10% level, but not the 5% level \( (P < 0.07) \). During GS II, \( A \) was slightly higher at 18 °C than at 9 °C \( (P = 0.053) \), but there were no treatment effects on \( C_i \). Gas exchange in GS III showed seasonal variation \( (P \leq 0.003) \), but no significant differences between treatments were found for \( g_s \). \( (P = \)
For GS II and GS III, when the final two sampling dates were analyzed separately, no effects of soil temperature on $g_s$ or $A$ were found. However, the main effect of soil temperature on $C_i$ over time in GS II was significant ($P = 0.036$) because $C_i$ was lower at 13 °C than at 9 °C ($P = 0.045$).

**Chlorophyll**

During GS II, there was a statistically significant seasonal variation both in total chlorophyll (chl) concentration on the basis of projected leaf area and in chl a/b ratio ($P \leq 0.006$). The treatments had significant effects on total chl concentration ($P = 0.004$, Figure 3A), but not on the chl a/b ratio ($P = 0.553$, Figure 3B).

During GS II, total chl concentration on a projected leaf area basis initially decreased to a low value, and thereafter increased more slowly at 9 °C than in the other treatments. Among treatments, total chl concentration at the end of GS II was significantly lower in the 9 °C treatment than in the 13 °C ($P = 0.039$) and 18 °C ($P = 0.003$) treatments, but the difference between the 9 and 21 °C treatments was significant only at the 10% level.

During GS III, total chl concentration and chl a/b ratio varied significantly ($P = 0.003$, $P < 0.001$, respectively). However, no significant treatment effects were found for total chl concentration ($P = 0.681$) or chl a/b ratio ($P = 0.115$).

For GS II, when the last two sampling dates were analyzed separately, the main effect of soil temperature on total chl concentration was highly significant ($P = 0.001$). Total chl concentration was significantly lower in the 9 °C treatment than in the other treatments ($P \leq 0.005$). For GS III, when the last two sampling dates were analyzed separately, no statistically significant effect of soil temperature on total chl concentration...
was found. There was no treatment effect on chl a/b ratio for the last two samplings for either GS II or GS III.

**Nitrogen concentration**

There were no significant differences in foliar N concentration (Figure 4) during the first rest period before the soil temperature treatments were imposed. During GS II, N concentrations in the C, C+1, C+2 and C+3 needles varied seasonally ($P \leq 0.002$), but treatment differences were statistically significant only for C+1 needles, where foliar N concentration was lower in seedlings in the 9 °C treatment than in the 18 °C treatment ($P = 0.035$).

During the second rest period, N concentrations of C+1 needles were slightly different ($P = 0.054$, Figure 4C) and, in C+2 ($P = 0.003$, Figure 4B) needles, significantly different between treatments. In C+2 needles, the treatment difference was due to the lower foliar N concentration in seedlings at 9 °C than at 13 °C ($P = 0.016$) and the lower foliar N concentration in seedlings at 21 °C than at 13 °C ($P = 0.005$) or 18 °C ($P = 0.045$).

During GS III, all needle age classes showed a similar statistically significant seasonal variation ($P \leq 0.021$) in N concentration as observed in GS II (Figure 4). Nitrogen concentration in C+2 needles ($P = 0.022$) differed significantly between treatments, the foliar N concentration of seedlings at 21 °C being significantly lower than at 18 °C ($P = 0.017$) and slightly lower than at 9 °C ($P = 0.073$). There were no statistically significant treatment differences in foliar N concentration for the latter two sampling dates.

**Needle dry mass**

When the last three samplings were analyzed separately, the main effect of soil temperature on dry mass of C+2 needles was significant ($P = 0.030$) because of the higher dry mass of needles at 9 °C than at 18 °C ($P = 0.024$). During GS III, dry mass of C needles differed significantly between treatments ($P = 0.020$) because of the higher needle dry mass of seedlings at 9 °C than at 18 °C ($P = 0.019$).

**Discussion**

We tested two hypotheses; namely, that (1) photosynthetic rate decreases in cold soil; and (2) the decrease in photosynthetic rate due to cold soil is accompanied by decreases in (a) stomatal conductance, (b) chlorophyll concentration and (c) foliar nitrogen concentration. Photosynthetic rates of 5-year-old Norway spruce seedlings were slightly depressed by the low soil temperatures during GSII, thus confirming Hypothesis 1, but the reduction was not accompanied by a significant decrease in stomatal conductance or nitrogen concentration, thus refuting Hypotheses 2a and 2c.

During GS II, chlorophyll concentration was strongly depressed at low soil temperatures, which probably accounts for the depression in photosynthetic rate, a finding that supports Hypothesis 2b. Chlorophyll concentrations in all treatments decreased in mid-season and then increased toward the end of the growing season. These results contrast with those reported by Linder (1980) for Norway spruce seedlings. In addition, the chlorophyll a/b ratios were high, which may be a result of the use of controlled environments (see, e.g., Linder 1980) or of the experimental conditions used. In particular, the light in the controlled environment chambers was deficient in the ultraviolet portion of the spectrum (wavelengths < 400 nm) (Finér et al. 2001, Figure 4), and this may have affected both needle chlorophyll concentration and the chlorophyll a/b ratio. A decrease in chlorophyll concentration could indicate lack of N (e.g., Solberg et al. 1998); however, because loss of chlorophyll is a nonspecific indicator of stress, it does not provide strong evidence of N deficiency (Linder 1980).

There were no aftereffects of soil temperature during GS II on gas exchange or nitrogen and chlorophyll concentrations during GS III, indicating that Norway spruce seedlings recovered rapidly from low soil temperatures.

**Acknowledgments**

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**Figure 4.** Effects of soil temperature on nitrogen concentrations of Norway spruce seedlings during the second (GS II) and third (GS III) simulated growing seasons. Panels: (A) C+3 needles during GS II; (B) C+2 needles during GS II and C+3 needles during GS III; (C) C+1 needles during GS II and C+2 needles during GS III; (D) C needles during GS II and C+1 needles during GS III; and (E) C needles during GS III. Values are means of four seedlings. Vertical bars indicate ± standard error. Day 1 is the beginning of the first simulated growing season (GS I). Abbreviations LD and SD refer to long and short day conditions, respectively.
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References


