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Responses of Silver Birch Saplings to Low Soil Temperature

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Two-year-old silver birch (*Betula pendula*) saplings were grown for a third growing season in controlled-environment rooms (dasotrons) at three soil temperatures (5, 10, and 20°C). All trees grew the first flush of leaves, but the growth of the second flush was almost completely inhibited at the two lower temperatures. The dry weight of the second-flush leaves was 50 times larger at 20°C than at 5 and 10°C, with about 100 times more nitrogen. Root growth was less affected than shoot growth.

Chlorophyll content, net assimilation rate and stomatal conductance were lower at low soil temperatures. The value of the cytoplasm resistance estimated from the electric impedance spectra was lower at 5°C than at 10 or 20°C. Leaf water potential was highest at the lowest soil temperature, and intercellular carbon dioxide concentration was only slightly lower in saplings growing in cooler soil.

We conclude that the effect of long-term exposure to cold soil on net assimilation and growth was not caused by stomatal closure alone. It is likely to be additionally mediated by the limited nitrogen acquisition at the low soil temperatures, and perhaps additionally by some other factor. As the growth depression of aboveground parts in response to low soil temperature was more significant in silver birch than what has earlier been found in conifers, the relative changes in air and soil temperature may eventually determine whether birch will become more dominant in boreal forests with climate change.

Keywords *Betula pendula*, biomass, electrical impedance, mineral nutrients, photosynthesis, soil temperature, stomatal conductance, water relations
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1 Introduction

Complex changes in the seasonal course of soil temperature can be expected to occur as a result of climate change. According to the "middle" climate scenario for Finland (Kuusisto et al. 1996) the mean air temperature in Finland will rise by 0.4°C (0.6°C in the winter) and rainfall by 1% (2% in winter) per decade during the next 100 years resulting in a warmer climate with slightly more precipitation. However, warmer wintertime air temperatures are not necessarily accompanied by warmer soil, because a reduction in snow cover will result in deeper and more long-lasting soil frost. In the present situation, the soil frost is commonly deeper in the southern and western part than in the eastern part of Finland because of differences in snow cover, even though the air temperature is higher in the south and west (Soveri 1986).

Silver birch (*Betula pendula* Roth) is one of the economically most important tree species in boreal Europe, and it has been predicted that the proportion of silver birch will increase relative to coniferous trees with the expected climatic warming (Talkkari 1998). However, these predictions are based on air temperatures, and it is not known whether they will be valid if the relationship between air and soil temperatures changes. Furthermore, the distribution of mountain birch (*Betula pubescens* ssp. *czerepanovii*) at the treeline has been suggested to be related to soil temperature (Karlsson and Nordell 1996).

The effect of soil temperature on the growth and photosynthesis of trees is generally difficult to study in the field because it is naturally confounded with the effects of air temperature and photoperiod. However, soil temperature is a crucial factor in determining the growth rate of plants. Low soil temperatures can decrease root growth and, consequently, formation of mycorrhizas (Kramer 1983; Domisch et al. 2001; 2002b) thereby reducing the surface area of roots for water and nutrient absorption. In addition to its direct effects on trees, low soil temperatures limit the nutrient availability by slowing down litter degradation. This is particularly important in boreal forests where nitrogen is a major factor limiting tree growth (e.g. Viro 1974).

Low soil temperature can affect growth by several different mechanisms. Low soil temperature affects water uptake in two ways: it decreases the permeability of roots to water by its effects on aquaporins and membrane fluidity (Lambers et al. 1998), and increases the viscosity of water, thus, slowing down its movement through both soil and roots (Kozlowski and Pallardy 1997). Lipids and proteins in root cell plasma membranes are altered by low temperature (Clarkson et al. 1988; Yoshida and Uemura 1989), which affects the transport properties of the plasma membrane (Iswari and Palta 1989). The resulting decrease in water uptake rate can cause reduction in photosynthesis by inducing partial stomatal closure (Farquhar et al. 1989) and a consequent decrease in intercellular carbon dioxide concentration (C_i) in leaves, decreasing the availability of CO_2 for assimilation. Meanwhile, decreased nutrient uptake can cause a reduction in the size of the photosynthetic machinery. The decreased uptake of water and nutrients may also affect electrical impedance properties of leaves, which have been shown to change as a result of various stress factors (Repo et al. 1994; Väinölä and Repo 2000; Repo et al. 2004).

Soil temperature is an environmental factor usually ignored in predictions of forest tree species distribution in changing climate. The general objective of our study was to assess whether this implicit assumption of lack of impact of soil temperature is justified or not. In addition, our aim was to determine what eco-physiological mechanisms are involved in the effects of soil temperature on tree growth. Our approach was to do a controlled environment experiment using saplings of a common broadleaf tree species. The specific aim of the study was to test the following hypothesis: Low soil temperature decreases photosynthesis in silver birch (*Betula pendula* Roth), which results in decreased shoot and root growth. This effect occurs because of slower inflow of water and/or mineral nutrients to the plant.

2 Material and Methods

2.1 Plants and Growing Conditions

The effects of soil temperature on the biomass, nutrient accumulation, and ecophysiological properties of silver birch saplings were studied in controlled environments under equal air temperature and photoperiod. The experiment was carried out in three dasotrons (Conviron RTR48, Controlled Environments Ltd., Winnipeg, Canada). Dasotrons are large phytotrons, designed for growing tree saplings, and they have been described in detail by Finér et al. (2001). The thermally insulated pots have coils at the top and bottom through which glycol brine circulates, allowing independent control of air and soil temperatures. To avoid heating of the topsoil by radiation and convection, the soil surface and upper coil were covered by a 45 mm thick plate of high density styrofoam. Saplings emerged through a hole in the center of the foam plate. The base of the stem was insulated from the cooling coil by a collar of 10 mm thick soft insulating foam. Although a gap around the edge of the cover and the hole in the centre allowed some air movement, the foam plate was removed daily for two hours for increased ventilation of the underlying soil.

Light was supplied by 250 W high-pressure sodium (Lumalux, Sylvania, U.S.A.) and 250 W Metal-halide (Metal/Rc, Sylvania, U.S.A.) lamps filtered through double walled polycarbonate (Lexan, General Electric Plastics, Pittsfield, MA, USA). Irradiance was continuously measured with quantum sensors (LI 190SB, Licor, Lincoln, Nebraska, U.S.A.) located at a height of 1.06 m above the soil surface. Initially the distance between the lamps and the quantum sensors was 1.5 m, but it increased when the lamp canopies were raised tracking the height growth of the saplings.

Twelve two-year-old silver birch saplings (central Finnish origin) were used, four in each of the three dasotrons. Before the experiment, the saplings had been grown at the forest tree nursery of the Finnish Forest Research Institute, Suonenjoki Research Station (62°05'N, 27°00'E, 130 m asl). At the beginning of the second growing season in the nursery the seedlings were transplanted to 10 dm^3 plastic buckets filled with fertilized peat, and they grew and overwintered outdoors in the nursery. The saplings reached a height of 1.5 m in the nursery. In the third spring, just before budbreak, the saplings were replanted into the dasotron pots, one per pot, without removing the peat originally in the buckets. The plastic pots (0.623 m^3) were filled with sand plus a 14 cm layer of topsoil from a birch forest, consisting mostly of organic matter. The saplings were transplanted before bud break, with an intact 10 dm^3 peat "plug" which minimized any possible planting shock.

After replanting, the saplings were kept in the dasotrons for 7 days under low light (approx. 100 µmol $m^{-2} s^{-1}$ PAR) and short photoperiod (8 h day) with air and soil temperature of 5°C. After this period, the third growing season with different soil temperatures but similar air condition started. During the growing season the air temperature was 20/15°C (day/night), relative air humidity was 60% for 35 days from replanting and thereafter 75%. The range of variation in room air temperature was ± 0.2°C and in air humidity, ± 3%. The photon irradiance (PAR) was about 450 µmol $m^{-2} s^{-1}$ at the top of the saplings, and 50 cm below the top, 270 µmol $m^{-2} s^{-1}$.

Soil temperature treatments of 5°C, 10°C and 20°C (one in each dasotron) started at the beginning of the growing season and continued for 14 weeks. Soil temperature was monitored with thermocouple sensors (105T, Campbell Scientific Ltd., Shepshed, England) at three locations in each pot, 5 cm and 10 cm down from the soil surface, and 10 cm above the coil at the bottom. The mean temperature was recorded with a datalogger (CR 10, Campbell Scientific Ltd., Shepshed, England) once per hour. Mean soil temperatures at a depth of 5 cm were 6.5, 11, and 20°C, with pots within each treatment differing from their mean by less than 0.5°C.

The saplings were watered once every two weeks, the first time with 7 litres of deionized water, and thereafter with 5 litres of deionized water per pot. The water was at soil temperature. The saplings were fertilized twice (45 and 59 days after replanting) during the experiment with 2.24 g (500 mg N and 90 mg P per sapling) of soluble fertilizer, dissolved in the irrigation water. The fertilizer was Superex 6 per plant (Kekkilä Oy,

Eurajoki, Finland), and it contained 223 mg g^{-1} of N (of which 30% NO_3^-, 62% urea, and 8% NH_4^+), 40 mg g^{-1} of P, 186 mg g^{-1} of K, 2 mg g^{-1} of Mg, 3 mg g^{-1} of S, 0.97 mg g^{-1} of Mn, 1.8 mg g^{-1} of Fe, 0.27 mg g^{-1} of B, 0.23 mg g^{-1} of Zn, 0.14 mg g^{-1} of Cu, 0.02 mg g^{-1} of Mo, and 0.01 mg g^{-1} of Co.

2.2 Measurements

Leaf water potential (ψ_l) was measured with a Scholander type pressure chamber (T. Pohja, Juupajoki, Finland) 43, 71 and 91 days after replanting. The measurements were done within one hour before midday. One fully expanded leaf near the top of each sapling was excised with a scalpel and transferred to the pressure chamber in a plastic bag. The whole procedure from severing the leaf to completing the measurement took less than 2 min.

Gas exchange was measured 36, 71 and 92 days after replanting, on three young fully expanded leaves from the upper part of the crown of each sapling. A portable photosynthesis system (LI-6400, LICOR, Lincoln, Nebraska, U.S.A.) with a standard broadleaf chamber, a blue/red light source (LICOR 6400-02B) and a CO_2 injector (LICOR 6400-01) was used. Transpiration rate, CO_2 net assimilation rate (A), stomatal conductance to water vapour (g_s), and intercellular CO_2 concentration (C_i) were computed by the same system. Measurements were done under light saturation conditions, with leaf temperature set to 22°C, and PAR photon irradiance (I^{PAR}) to 700 µmol $m^{-2}\,s^{-1}$. The CO_2 concentration at the chamber intake was set to 370 µmol mol^{-1}, and water vapour leaf to air difference was between 0.54 and 1.04 mmol mol^{-1} for all measurements. For most measurements air flow rate was set to 500 µmol s^{-1}, but when measuring leaves with very low photosynthetic rate, it was reduced to 250 µmol s^{-1}. Measurements were done within 4 h centred on midday. At 36 days after replanting also assimilation vs. C_i and I^{PAR} curves were measured. The light response curves (measured for the 20°C and 10°C treatments only, data not shown) were used to confirm that photosynthesis was light saturated at the measuring irradiance used.

Chlorophyll concentration was measured in three fully expanded leaves from the upper part of the crown of each sapling 44, 73 and 94 days after replanting. From each leaf a 6 mm diameter disk was cut with a paper punch. The three disks from each sapling were pooled, and extracted for two weeks in 4 ml of *N,N*-dimethylformamide at 5°C with continuous shaking. Absorbances were measured with a diode array spectrophotometer (HP 8453, Hewlett Packard GmbH, Waldbronn, Germany) and chlorophyll concentrations calculated with the equations given by Porra et al. (1989).

Ten first-flush leaves were sampled from each sapling 97 days after replanting for the electrical impedance spectroscopy (EIS). A leaf segment of 5 mm × 10 mm, was cut from each leaf and exposed to an impedance measurement in a measuring cell connected to an LCR meter (4284A, Agilent Technologies, Palo Alto, CA., U.S.A.) (Repo 1994, Repo et al. 2004). The measuring cell consisted of two Ag/AgCl electrodes (RC1, WPI, Sarasota, FL, U.S.A.) and electrode gel to improve the contact between cut surface of the leaf segment (5 mm surface) and the electrodes. An impedance spectrum (IS) of a sample was measured at 46 frequencies (between 20 Hz and 1 MHz). The partition of electrical current between extracellular and intracellular spaces depends on frequency and tissue properties, and when the tissue is represented by an electrical circuit model, tissue features can be quantified (see Repo and Zhang 1993). The parameters of the electric model, double shell model (Zhang and Willison 1991), were estimated by means of a complex non-linear least squares (CNLS) program LEVM v.6 (J.R. Macdonald, Department of Physics and Astronomy, University of North Carolina, Chapel Hill, NC, U.S.A.). The resistances of the model represent extracellular space, cytoplasm and vacuole, and the capacitances represent plasma membrane and tonoplast respectively. Specific resistance values as reported were calculated by multiplying the estimated resistances by the cross-sectional area of the sample and then dividing by the length of the sample. Specific capacitance values were calculated by dividing the estimated capacitances by the cross-sectional area of the sample and then multiplying by the length of the sample.

Growth curves were fitted to sequential measurements of leaf area during the first flush. Three leaves per seedling were measured on 17 occasions starting when they had an area of approximately 2 mm^2 until well after expansion ceased. A non-linear, mixed-effects growth model was fitted simultaneously to the natural logarithm transformed data from all leaves, saplings and treatments.

The growth function used was based on equation 3.1 in Prunty (1983). We use a form that allows for an asymptote different from one, and a turning point different from one:

$$y = a\,(1 + b(x + d) - \{[b(x + d)]^c + 1\}^{1/c})$$

where y is the natural logarithm of the area of a leaf, and x is time; $b = R/a$, with R the initial slope. For a growth curve, R is the initial relative growth rate. In the present case we have projected surface area of a leaf instead of weight, so R gives what is usually called relative expansion rate. The parameter a is the asymptote, so e^a is the final area of the leaf when growth ceases. The parameter d shifts the time axis, which reflects variation in the size of the leaves when measurement started, or more formally in the time when the extrapolated function predicted an area of 1 mm^2. Finally, c gives the sharpness of the transition between the initial slope and the asymptote. This function is piece-wise linear in the limit (when $c \to \infty$), but with a smooth transition otherwise.

Sapling and leaf were nested grouping factors. Fixed effects were fit for all four parameters in the equation: R, a, c, and d. As a non-linear function was used it is not appropriate to use R^2 for goodness of fit. The fit was done with the nlme function of the lme package version 3.1-45 (Pinheiro and Bates 2000) under R version 1.8.1 (R Development Core Team 2003). Probabilities from contrasts between parameter values estimated for the different temperatures were adjusted according to Holm's simple sequentially rejective multiple test procedure.

At final harvest, 97 days from the beginning of the treatments, all trees were cut at the soil surface. The leaves from the first flush and from the second flush were pooled into respective samples. The branches and main stems were separated into the part that had extended in the previous growing seasons (old stem), and the part extended during the second leaf flush (new stem). For root sampling, two segments were harvested on the opposite sides of the stem, each of which comprised 13% of the surface area of the pots, to the depth of 34 cm (organic layer + 20 cm mineral soil). The roots in the original peat plug (comprising both roots that had grown before the experiment and during the experiment) and in the soil outside it (only roots formed during the experiment) were treated as separate samples. All roots were separated from the segments by quickly immersing the roots in deionized water to remove the mineral soil particles attached on the root surface. Fine roots and coarse roots were separated at 2 mm, and the 2–5 mm roots were combined with the coarse root fraction. The stump and all coarse roots with diameter >5 mm were harvested. The biomass samples were first dried at 70°C, and then ground in a mill. The results were calculated for the whole pot surface area. The final dry weight and nutrient results were calculated taking into account the weight loss to 105°C, obtained from subsamples.

Nitrogen was analyzed with the Kjeldahl method and phosphorus spectrophotometrically with the molybdenum blue method.

2.3 Statistics

Significance of differences in leaf water potential, chlorophyll concentration, stomatal conductance, internal CO_2 concentration (C_i) and light saturated net assimilation rate (A^{max}) between the dasotrons and in time was assessed by multivariate analysis of variance, using a general linear model (GLM, Systat, SPSS Inc., Chicago, U.S.A.) procedure for repeated measures. Univariate ANOVA was subsequently done on these variables at each date only if the MANOVA showed a significant effect of the treatments. The variables related to biomass, nutrients, and the electrical impedance measurements were analyzed by one-way ANOVA as they were measured only at the end of the experiment. Multiple comparisons for all of the variables tested were done with Tukey's HSD test. Significant differences between dasotrons were assumed to be a response to the unreplicated soil temperature treatments. This is just an

assumption, but is likely to be true, as no differences in growth among dasotrons were detected in other tree saplings in no-treatment conditions (Lahti et al. 2005).

3 Results

3.1 Leaf Water Potential

The effect of soil temperature on leaf water potential (ψ_l) increased with time (Fig. 1). At the first measurement, 43 days after replanting, ψ_l was slightly, but not significantly, higher with lower soil temperature ($P = 0.24$). At the second measurement, 71 days after replanting, ψ_l was 0.33 MPa higher at 5°C than at 20°C ($P = 0.043$). After 91 days, ψ_l was 0.58 MPa higher at 5°C than at 20°C ($P < 0.001$), with that at 10°C in between. From day 43 to day 91, ψ_l at 5°C increased 0.2 MPa while that at 20°C decreased 0.1 MPa.

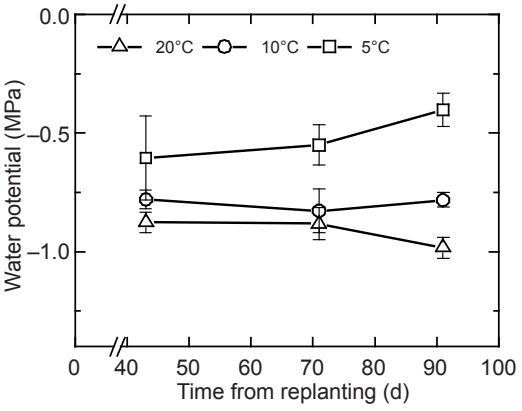

Fig. 1. Leaf water potential in leaves of silver birch saplings growing at different soil temperatures in the dasotrons. Means ± standard error (n = 4 saplings, 3 leaf samples from each).

3.2 Chlorophyll

Total chlorophyll concentration was the same with soil temperatures of 5°C and 10°C and slightly decreased in time (Fig. 2). In contrast, at 20°C it was much higher ($P < 0.001$) than in the other two temperatures. At first, 44 days after replanting, total chlorophyll concentration at 20°C was 64% higher than at 10 or 5°C. Seventy three days from replanting it was 3.2 times higher at 20°C than 10 or 5°C ($P < 0.001$) as it increased markedly in time. From day 73 to day 94 total chlorophyll concentration at 20°C decreased slightly but was still much higher than at 10 or 5°C ($P < 0.001$). Chlorophyll a/b ratio was not significantly different between the treatments (data not shown).

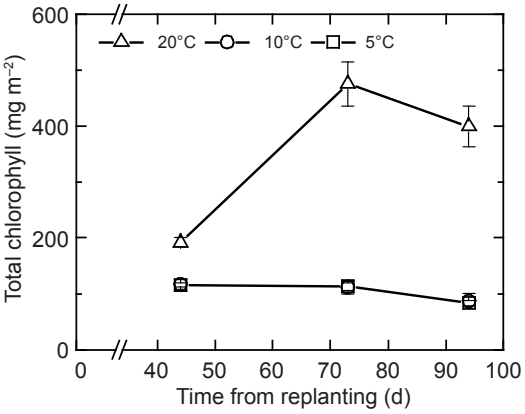

Fig. 2. Total chlorophyll mass per unit projected leaf area in silver birch saplings growing at different soil temperatures in the dasotrons. Treatment symbols, see Fig.1. Means ± standard error (n = 4 saplings, 3 samples from each).

3.3 Gas Exchange

Averaged over the different treatments, g_s and A^{max} changed significantly in time ($P < 0.05$) (Fig. 3). In the first time when measured, 36 days after replanting, g_s was higher at 20°C than at the two lower soil temperatures (5°C vs. 10°C, $P = 0.60$; 5°C vs. 20°C; $P = 0.001$; 10°C vs. 20°C, $P \leq 0.0103$). At the same time, there was no significant difference in C_i between treatments, but A^{max} was significantly higher at 20°C than 5 or 10°C ($P < 0.001$ and $P < 0.01$ respectively).

After 71 days from replanting g_s at 20°C was almost 5.5 times as high as at the date of the first measurement, g_s at 10°C being 2.5 times its initial value while g_s at 5°C decreased by 24% from its initial value. Stomatal conductance at 20°C was higher than at the two lower temperatures (5°C

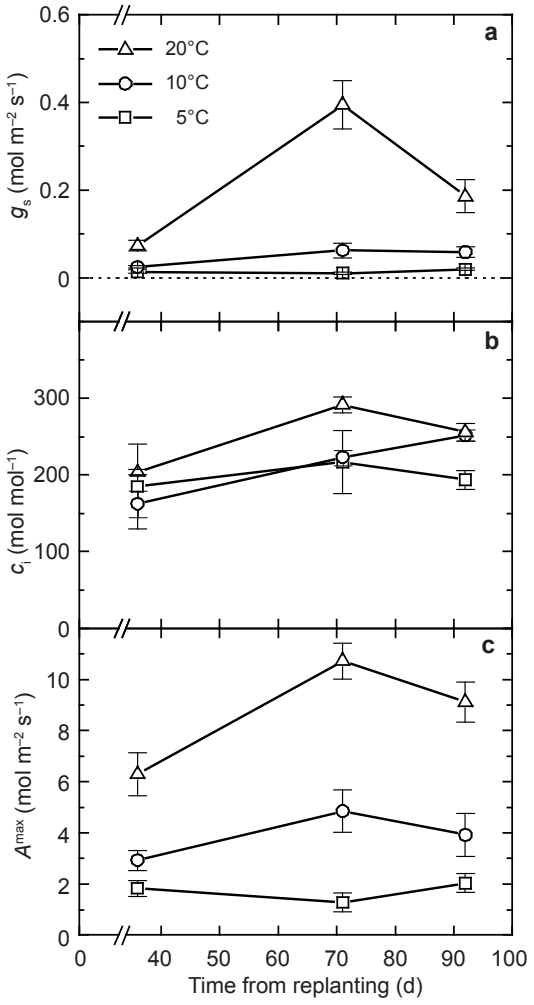

Fig. 3. Gas-exchange of leaves of silver birch saplings growing at different soil temperatures in the dasotrons. a) Stomatal conductance to water vapour, b) Intercellular carbon dioxide concentration, c) Light saturated net assimilation rate. Means ± standard error (n = 4 saplings, 3 samples from each).

vs. 20°C, $P<0.001$; 10°C vs. 20°C; $P<0.001$). At this time C_i at all soil temperatures had increased from its initial value. In contrast, A^{max} at 20°C and 10°C increased while A^{max} at 5°C decreased from its initial value. However, there were no significant differences in C_i between the treatments. Differences in A^{max} between all treatments were statistically significant (5°C vs. 20°C, $P<0.001$; 10°C vs. 20°C, $P<0.001$; 5°C vs. 10°C, $P=0.011$). At 20°C, A^{max} was 8.4 times higher than at 5°C.

Ninety-two days after replanting g_s at 20°C and 10°C was lower than three weeks earlier, while g_s at 5°C was higher than before. At this date, g_s was higher at 20°C than at the two lower temperatures (5°C vs. 20°C, $P=0.001$; 10°C vs. 20°C, $P=0.008$; 5°C vs. 10°C, $P=0.45$).

At 20°C and 5°C, C_i decreased, but at 10°C C_i increased, from its value three weeks earlier. Because of these changes, only C_i at 5°C was significantly lower than at the two higher temperatures (10°C vs. 20°C, $P=0.963$; 5°C vs. 10°C, $P=0.009$; 5°C vs. 20°C, $P=0.006$).

At 20°C and at 10°C A^{max} decreased but at 5°C A^{max} increased compared to three weeks earlier. Thus, A^{max} was higher at 20°C than at the two lower temperatures (5°C vs. 20°C, $P<0.001$; 10°C vs. 20°C, $P=0.001$; 10°C vs. 5°C, $P=0.191$).

3.4 Electrical Impedance

There was no significant difference in plasma membrane capacitance or in tonoplast capacitance between the treatments even though there was a tendency to decrease in both capacitances with increase in soil temperature (Fig. 4a). Extracellular, cytoplasm and vacuole resistance tended to be lowest at the lowest soil temperature (Fig. 4b). However, statistically significant differences were observed only for cytoplasm resistance ($P<0.05$ and $P<0.01$ for comparison of 5°C with 10°C and 20°C respectively).

The water content (mean±S.E.) in the leaves used for impedance measurements was 51.7±1.1%, 54.9±1.1 %, and 55.7±1.4% for the treatments 5, 10 and 20°C respectively.

3.5 Growth and Nutrients

The parameters of the leaf expansion curves (Fig. 5, Table 1) indicated that the final area, relative expansion rate, and the curvature were all significantly increased by 20°C treatment compared to 10 or 5°C, but only the relative expansion rate differed between 5 and 10°C.

At the final harvest, the old stem diameter

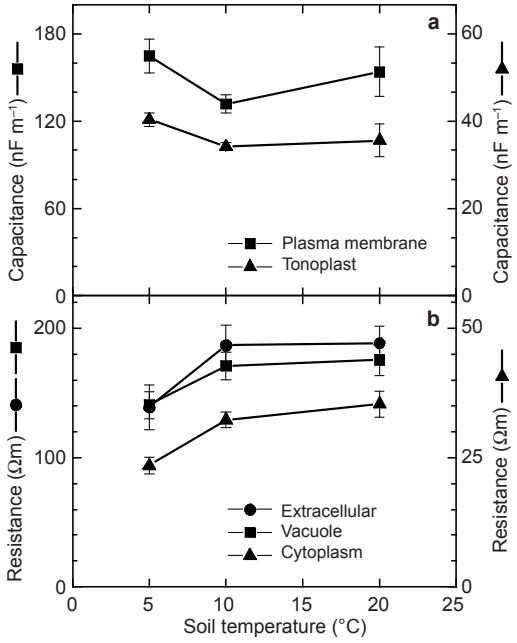

Fig. 4. Electrical impedance spectroscopic analysis of fully expanded leaves of silver birch at the end of the dasotron experiment. (a) Capacitances of plasma membrane and tonoplast. (b) Extracellular resistance, cytoplasm resistance and vacuole resistance. Means ± standard error (n = 4 saplings, 10 leaf samples from each).

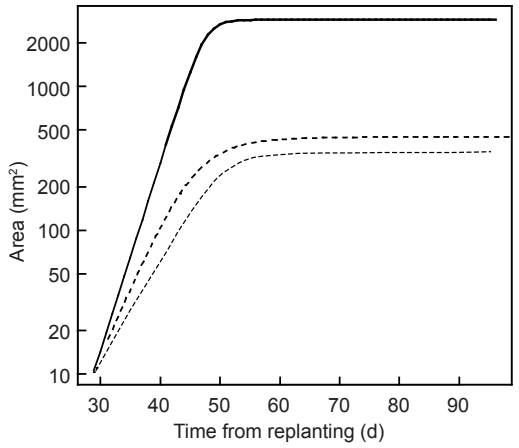

Fig. 5. Leaf area expansion during the first flush in silver birch in the dasotron experiment. Fitted curves. Curves shifted on time axis to equal area at the start of the measurements. From top: 20°C, 10°C, 5°C soil temperature.

(data not shown) and dry weight (Table 2) were considerably higher at 20°C than at the two lower soil temperatures, whilst the dry weight of the first flush of leaves was about the same in all treatments. However, the dry weight of the first flush leaves was very variable especially in the 20°C treatment, mostly because one tree had a lower dry weight in the first-flush leaves than other trees in this treatment. The 20°C treatment was the only one with significant growth of new stem and the corresponding second flush of leaves. The dry weights of the old stem, new stem, second-flush leaves and coarse roots were significantly lower at 5°C and 10°C than at 20°C, but no significant difference was found in the dry weights between the two lowest soil-temperature treatments (Table 2). The dry weight of fine roots was lower in the 5°C treatment than 10 and 20°C, but this difference was not significant. Moreover, the dry weight of those fine roots that were outside the original peat plug, was similar in all treatments, and comprised 26% of the total dry weight of all fine roots (data not shown). The total dry weight of the saplings was about twice as large in 20°C than 5 and 10°C. This was mostly due to the differences in the aboveground growth, and the root weight ratio was significantly lower at 20°C than 5 and 10°C.

At 20°C, the total amount of N in the second-flush shoot (leaves + stem) was 1424 mg, which was more than the N applied in the fertilizer (1000 mg). The N content of the first-flush leaves was higher at 10°C and 20°C than at 5°C, although this difference was not significant (Table 3). Moreover, the N content in the old stem was highest at 10°C and 20°C, suggesting that N had accumulated during the growing season. The amount of N in the second-flush shoot was 7.7 mg and 20 mg at 5 and 10°C respectively (Table 3). The N concentration in the second-flush leaves at 5°C was only 9.0 mg kg^{-1}, which indicates deficiency, whilst at 20°C, the N concentration was adequate, 23 mg kg^{-1}. The N concentrations at 5°C were quite low in old plant parts, 3.5 mg g^{-1} in old stems and 8.8 mg g^{-1} in first-flush leaves.

The correlation coefficient between the N concentration in first-flush leaves and chlorophyll concentration was 0.54 (P=0.072). This was due to the very low chlorophyll concentration

Table 1. Leaf area expansion during first flush in silver birch saplings. Larger values for the curvature parameter indicate a sharper transition to the asymptote. *P*-values from ANOVA are based on F with 2 and 477 degrees of freedom. Parameter values followed by different letters are significantly different (*P*-value adjusted with Holm's procedure < 0.05). See text for details of function fitted.

	5°C	10°C	20°C	P
Final area exp(a), mm^2	347 a	450 a	3041 b	<0.001
Rel. Expansion rate, R	0.165 a	0.215 b	0.301 c	<0.001
Curvature parameter, c	11.77 a	8.06 a	19.6 b	<0.001

Table 2. Dry weight (g) in different parts in silver birch saplings, and root weight ratio (root dry weight per total plant dry weight). n = 4, means ± s.e. The stem fractions include the branches, and the coarse roots include the stump. Within a column, means followed by the same letter are not significantly different ($P \leq 0.05$, Tukey´s test).

Soil temp. °C	Old stem	First-flush leaves	New stem	Second-flush leaves	Coarse roots	Fine roots	Total d.wt.	Root weight ratio
5	69.1±7.13a	9.5±0.66a	0.17±0.068a	0.79±0.304a	18.2±1.80a	40.3±7.33a	138±12a	0.42±0.02a
10	73.4±7.54a	11.8±1.67a	0.33±0.141a	1.21±0.373a	19.9±1.83ab	49.4±5.74a	156±16a	0.44±0.02a
20	127.2±6.52b	9.9±3.33a	32.17±5.28b	48.93±6.15b	30.0±4.3b	48.1±10.4a	297±17b	0.26±0.03b

Table 3. Nitrogen concentrations and contents in the different parts in silver birch saplings. The stem fractions include the branches, and the coarse roots include the stump. n = 4, means ± s.e. Within a column, means followed by the same letter are not significantly different ($P \leq 0.05$, Tukey´s test).

Soil temp. °C	Old stem	First-flush leaves	New stem	Second-flush leaves	Coarse roots	Fine roots
N concentration mg g^{-1}						
5	3.54±0.11a	8.84±1.22a	7.11±0.98a	9.01±0.83a	2.75±0.18a	7.47±0.32a
10	6.58±0.58b	13.13±1.22ab	12.35±1.21b	14.18±2.05a	5.61±0.55b	8.98±0.33b
20	5.94±0.40b	15.70±1.14b	8.05±0.54a	23.48±1.84b	3.73±0.44a	7.89±0.45ab
N content mg per plant part						
5	243±23a	84±10a	1.1±0.3a	6.6±2.3a	51±8a	299±53a
10	493±84b	160±35a	3.7±1.4a	16.2±5.1a	114±21b	445±59a
20	761±75b	152±50a	256.3±41.5b	1168±228b	117±30b	367±59a

Table 4. Phosphorus concentrations and contents in the different parts in silver birch saplings. P was not determined in stems. The coarse roots include the stump. n = 4, means ± s.e. Within a column, means followed by the same letter are not significantly different ($P \leq 0.05$, Tukey´s test).

Soil temp. °C	First-flush leaves	Second-flush leaves	Coarse roots	Fine roots
P concentration mg g^{-1}				
5	3.82 ±0.36 b	3.46±0.25 b	3.60±0.22 a	0.72±0.04 a
10	4.38±0.57 b	3.90±0.92 b	4.89±0.52 a	0.82±0.03 b
20	1.75±0.06 a	1.62±0.03 a	3.85±0.17 a	0.73±0.01 a
P content mg per plant part				
5	36.0±2.7 b	2.7±1.0 a	66.1±8 a	28.8±4.5 a
10	50.7±8.9 b	5.0±1.7 a	98.2±16 ab	40.9±5.7 a
20	17.7±6.4 a	78.8±8.6 b	113.7±11 b	34.9±7.5 a

at 10°C, whereas the N in the same leaves was intermediate.

By contrast to N, the P concentrations and the content in first-flush leaves were lowest at 20°C (Table 4). All P concentrations determined were highest at the intermediate temperature 10°C, although this was significant only in fine roots. However, the coarse-root P content was significantly highest at 20°C, the 10°C treatment being intermediate. The P content of the second-flush leaves and coarse roots was also evidently largest at 20°C. At 20°C, the P content of the second-flush leaves was no more than 79 mg, which may be compared to the amount applied with the fertilizer (180 mg).

4 Discussion

It has often been suggested that stomata close in response to low soil temperature primarily because water uptake by roots is limited and therefore the leaf water potential is lowered (Vapaavuori et al. 1992; Wan et al. 1999). However, in the present study, the water potential ψ_l was higher in leaves from saplings growing in soil at 5°C than at 20°C, in contrast to what Wan et al. (1999) observed in *Populus* in hydroponics. This indicates that low ψ_l was not causing stomatal closure, but rather the stomatal closure prevented the lowering of ψ_l. The result that C_i did not markedly decrease suggests that photosynthesis was only partly depressed by stomatal closure, and partly through some other mechanism, such as hormonal or nutritional effects of soil temperature.

Effects of soil temperature occurring over a period of weeks have been less frequently studied than those occurring over periods of several minutes to a few days. In short term experiments water uptake plays a very important role (e.g. Figs. 3 and 4 in Wan et al. 1999), but in the longer term other factors can become more important. Our results are compatible with the suggestion of DeLucia et al. (1991) that some other mechanism, such as a hormonal signal from root systems could induce stomatal closure at low soil temperatures, based on the behaviour of *Pinus sylvestris* seedlings. However, this hypothesis was not supported by the results of a short-term split-root experiment with *Pinus taeda* (Day et al. 1991), showing no evidence for a hormonal messenger: the stomata did not close if only half of the root system was cooled.

In the present experiment, nitrogen starvation is one likely mechanism behind the dramatic decrease in A^{max} with cool soil, as reflected by the low nitrogen and low chlorophyll concentrations at the lowest temperature. The N starvation may be a major reason for the stomatal closure as well because Dodd (2003) stated that "N deprivation invariably causes stomatal closure". However, this has been shown in a limited range of plant species and environmental conditions. The increase in net photosynthetic rate at 10°C compared to 5°C is better explained by the higher g_s, as the chlorophyll concentrations were equal at 5 and 10°C. The chlorophyll concentration was more depressed at 10°C than might have been expected from the leaf N concentration, which was intermediate. This accords with a study of *Picea abies* where a decrease in chlorophyll content per unit area was observed at low soil temperatures accompanied by a decrease in A^{max} but almost no change in needle nitrogen concentrations (Lahti et al. 2002). It appears that there might be an additional mechanism affecting chlorophyll synthesis at low soil temperature apart from N in the leaves. In this experiment, P did not explain the differences in photosynthesis, as the aboveground P concentrations were adequate at the two lower temperatures, but deficient at 20°C. It seems that the relative importance of several limiting factors for photosynthetic production varies at low soil temperature.

In the field, silver birch trees break their leaf buds when the air temperature increases, although the soil is still cold, and growth is initiated with the reserves of the previous year (Kozlowski and Pallardy 1997). Nevertheless, in this experiment, the expansion rate and the final leaf size of the first-flush leaves were affected by the soil temperature. Unlike the first flush, the second growth flush aboveground was almost completely inhibited by the low soil temperatures. The leaves of the first flush should be the source of photosynthate for the second-flush shoot growth, but as the photosynthesis was limited, the new growth was not possible. It has been suggested that as cell elongation is driven by turgor, the primary

effect of low soil temperature on growth would be mediated by the low water potential (Wan et al. 1999). However, in our study the water potential was highest at the lowest soil temperatures, and therefore the low water potential does not explain the depressed growth. It appears that decreased internal nutrient availability is the predominant effect of low soil temperature at leaf level in silver birch rather than water uptake, because water use is reduced in parallel with the decrease in water availability. One important mechanism of water use reduction was the decrease in leaf area (data not shown).

Here, silver birch root growth was much less affected by low soil temperature than shoot growth, which agrees with previous results on mountain birch (*Betula pubescens* ssp. *czerepanovii*, formerly *tortuosa*) seedlings (Weih and Karlsson 2001). Soil at 5°C completely inhibited both growth and nutrient uptake of mountain birch (Karlsson and Nordell 1996). In terms of aboveground growth, silver birch appears to be more sensitive to low soil temperature than the dominant conifers of the region, as the aboveground growth was not reduced by a treatment season of soil temperature of 9°C in Scots pine (*Pinus sylvestris*) and Norway spruce (*Picea abies*) (Domisch et al. 2001; 2002a, Lahti et al. 2005). In Norway spruce it was shown that this was not only due to predetermined shoot growth by the conditions of the year before the cold-soil treatment, as in a subsequent third growing season when all the treatments were again in equal conditions, the cold-soil treated spruce saplings grew better than those in other treatments (Lahti et al. 2005). Although root growth was affected by cold soil, the optimum soil temperature for Scots pine was 13°C (Domisch et al. 2001).

The likely reason for the inhibition of aboveground growth was that nitrogen limitation depressed photosynthesis and growth. The low nitrogen levels in the leaves at low temperatures are probably a sum of several factors. Active nutrient uptake is well known to be dependent on root temperature, and N assimilation and translocation may also be affected. N acquisition from soil was probably more affected by low temperature than translocation from roots to shoots, as the N concentrations in roots were not higher in the low temperature. In mountain birch, Karlsson and Nordell (1996) also concluded that inhibited nutrient uptake was a major factor controlling growth, as the availability of N in soil did not affect the growth response. By contrast, DeLucia et al. (1992) attributed the decreased A at low soil temperature to low foliar N and P, which they suggested was a result of inhibited nutrient transport from roots to shoots in the C_4 grass *Andropogon gerardii*. In the present experiment, the fine-root weight, including both old and new fine roots, was somewhat smaller at the lower temperatures (not significantly), which probably also had a role in the N uptake; however, considering that the P levels were higher at the lower temperatures, the quantity of roots and mycorrhizas probably was not one of the most important factors limiting total uptake. Furthermore, a large part of the readily available N in the fertilizer was nitrate, and it is possible that the low temperatures depressed root nitrate reductase activity. Microbial decomposition of the soil organic matter and fertilizer urea was presumably an additional source of N and other nutrients, still increasing the difference between the treatments.

P acquisition did not appear to limit plant performance even at 5°C soil temperature, although the 10°C treatment was more favourable for P accumulation. At 20°C, the P concentrations in the leaves were so low that P was presumably a growth-limiting factor in this treatment. The low P concentration in the first-flush leaves at 20°C suggests that P can be retranslocated from the first-flush leaves, but yet a large part of the P in these trees remained in the coarse roots. In Scots pine and Norway spruce, the relatively high P concentrations in seedlings with low soil temperature also suggested that P uptake may not be one of the most significant growth-limiting factors at low soil temperature (Domisch et al. 2002b; Lahti et al. 2005).

Electrical resistances of first-flush leaves were lower at 5°C than at the other two temperatures where they were at the same level. Possible explanations are changes in leaf water status, and in cell and vacuole sizes. For the first of these three possible effects there seems to be no evidence in the data as the three observations of extracellular resistance fall on a straight line when plotted against leaf water potential. Furthermore, lower water content at 5ºC than at 10

or 20ºC corresponded to lower electrical resistances, however, this is opposite to what could be expected. Therefore, some other explanations, e.g. changes in electrolyte concentrations (higher for lower resistance), cell membrane properties or cell/vacuolar sizes, are more probable but so far remain an enigma.

The light, soil and air temperature regimes of this study did not aim to mimic natural conditions. Instead in controlled environment experiments it was possible to break the correlation between soil and air temperature, and therefore to study temperature responses of plants as induced through roots. The soil temperatures used encompass those occurring in boreal forests, however. Further studies simulating natural conditions, with for example a specific decrease in snow cover and corresponding increase in soil frost, are necessary to assess the growth losses more precisely. Moreover, in competitive situations between birch and conifers, the tree canopies and the stand density affect the accumulation of snow, in a way that varies from tree species to another, and this has to be taken into account in prediction models as well.

The results presented here show that two-year-old silver birch saplings are highly sensitive to low soil temperature during the growth season; the second flush of shoot growth is more sensitive than root growth; and that nitrogen limitation is a major factor mediating the effects. This high sensitivity suggests that expected changes in soil temperature should be considered separately from changes in air temperature when analysing the possible effects of climate change on boreal forests. In some areas of the northern forests, soil frost may be deeper and last longer if there is less snow cover due to climate warming. Depression of growth of the now dominant coniferous trees by low soil temperature is less dramatic than in silver birch. Consequently, it is likely that the proportion of silver birch may not increase as a result of global warming as much as has been predicted based solely on the effects of air temperature.

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