Delayed soil thawing affects root and shoot functioning and growth in Scots pine

TAPANI REPO,1 TARJA LEHTO2 and LEENA FINÉR1

1 The Finnish Forest Research Institute, Joensuu Research Unit, P.O. Box 68, FI-80101 Joensuu, Finland
2 University of Joensuu, Faculty of Forest Sciences, P.O. Box 111, FI-80101 Joensuu, Finland

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Summary In boreal regions, soil can remain frozen after the start of the growing season. We compared relationships between root characteristics and water relations in Scots pine (Pinus sylvestris L.) saplings subjected to soil frost treatments before and during the first week of the growing period in a controlled environment experiment. Delayed soil thawing delayed the onset of sap flow or totally blocked it if soil thawing lagged the start of the growing period by 7 days. This effect was reflected in the electrical impedance of needles and trunks and in the relative electrolyte leakage of needles. Prolonged soil frost reduced or completely inhibited root growth. In unfrozen soil, limited trunk sap flow was observed despite unfavorable aboveground growing conditions (low temperature, low irradiance, short photoperiod). Following the earliest soil thaw, sap flow varied during the growing season, depending on light and temperature conditions, phenological stage of the plant and the amount of live needles in the canopy. The results suggest that delayed soil thawing can reduce tree growth, and if prolonged, it can be lethal.

Keywords: biomass, electrical impedance, minirhizotron imaging, phenology, REL, root morphology, sap flow, soil frost.

Introduction

Soil frost is common in northern latitudes. Its depth and duration depend on weather conditions in autumn, winter and spring, and on geographic location, soil texture, soil water content, vegetation and snow cover (Fahey and Lang 1975, Péwé 1979, Solantie 2000, Zhang et al. 2003). In a given location, the timing of soil thaw in spring may vary by weeks between years (Huttunen and Soveri 1993, Solantie 2003). In years with mild autumn temperatures and deep snow cover, soil may not freeze; therefore, soil temperature may increase soon after snow melt in spring. In contrast, freezing temperatures combined with little snow may lead to deep soil freezing. Under such conditions, the topsoil can thaw after snow melt in spring but the deeper soil layers can remain frozen for a long time, thus affecting water and nutrient uptake by roots and tree growth (Soveri and Varjo 1977). Predictions of future climatic conditions indicate that the occurrence of soil frost does not always decrease linearly with the increase in winter temperature because the protective snow cover may be reduced (Venäläinen et al. 2001).

Cold or frozen soil may inhibit water uptake in spring causing xylem cavitation, foliar injuries and reduced growth of shoots and roots (Tranquillini 1982, Larsen 1993, Bergh and Linder 1999, Cochard et al. 2001, Repo et al. 2005). In a root severing experiment conducted in winter, small snow-covered saplings of Engelmann spruce (Picea engelmannii Parry) were largely dependent on root water uptake to meet transpiration demands during late winter and early spring, whereas larger saplings were more reliant on water stored in stem sapwood (Boyce and Lucero 1999). Delayed soil thawing reduced the potential efficiency of Photosystem II in Scots pine in a controlled-environment chamber study (Repo et al. 2005) and in Norway spruce in the field (Repo et al. 2007). Delayed soil thawing also affected water potential and apoplastic electrical resistance in Scots pine needles and reduced growth of short and long roots and root tip formation (Repo et al. 2005). In a snow manipulation study, soil freezing to −4 °C increased fine root mortality in sugar maple (Acer saccharum Marsh.) and yellow birch (Betula alleghaniensis Brit.), but led to an earlier peak in fine root production during the subsequent growing season (Tierney et al. 2001). In another study, early snow melt and soil thawing in spring were followed by early initiation of trunk sap flow in Norway spruce (Picea abies (L.) Karst.) (Bergh and Linder 1999). However, few studies on the effects of soil frost have made concurrent measurements of root growth and trunk sap flow.

The aim of our study was to compare root and shoot growth dynamics, root morphology, trunk sap flow and other water-related attributes in Scots pine (Pinus sylvestris L.) saplings in response to delayed soil thawing. We hypothesized that delayed soil thawing reduces both water uptake by roots and trunk sap flow and results in injury to shoots and roots that leads to declines in growth.

Materials and methods

The study material consisted of 16 Scots pine saplings lifted from a forest plantation in eastern Finland (62°36′ N, 29°43′ E).
in autumn 2001. The stand had been established with 2-year-old container-grown seedlings of local origin in 1998, and the saplings were 5 years old at the time of lifting. The site was classified as a medium-poor Vaccinium type according to the Finnish classification system (Cajander 1949). The soil was fine-textured sand. The pH was 3.5 and 5.0 for organic and mineral soil, respectively. Total N concentration in the organic soil was 1.2%. Mean (± standard deviation) height and trunk diameter (15 cm above root collar) of the selected saplings were 89.4 ± 6.2 and 1.39 ± 0.11 cm, respectively.

After lifting, the saplings were planted in 0.46 m³ cylindrical containers (height 1.3 m, diameter 0.7 m) filled with mineral soil from the same stand. A 5-cm-thick organic layer from the same stand was placed on the surface of the forest soil. Four potted saplings were transferred to each of four growth chambers (RTR48 chambers, Conviron, Winnipeg, Canada) at Joensuu, in which air and soil conditions can be controlled independently (Finér et al. 2001). The study comprised two preconditioning annual cycles, i.e., dormancy (D1 and D2) and growing periods (GS1 and GS2), before the soil was frozen during D3. The soil thawing treatments began during D3 and GS3 (Table 1 and Figure 1) (cf. Repo et al. 2005). After the treatment periods, the saplings were grown for one annual cycle (D4 + GS4), under similar conditions as in D2 and GS2. In each annual cycle, the growing and dormancy periods lasted for 14 and 8 weeks, respectively. There was a 3-week short-day (SD) phase at the end of each growing period (Table 1). The saplings in each growth chamber were subjected to one of four soil thawing treatments. In treatments 1 (Trt1) and 2 (Trt2), soil thawing started 28 and 14 days before the start of GS3 when air temperature, photoperiod and photosynthetic photon flux were increased. In treatment 3 (Trt3), soil thawing started simultaneously with the start of GS3, whereas in treatment 4 (Trt4), soil thawing began 7 days from the start of GS3. Soil temperature (measured with a 105T thermocouple) and volumetric water content (measured with a ThetaProbe, ML2x, Delta-T Devices, Cambridge, U.K. and CS615, Campbell Scientific, Shepshed, U.K.) were monitored in the 5-cm-thick or-

Table 1. Chamber conditions during the preconditioning periods (D1, GS1, D2, GS2), during the periods when the soil thawing treatments started (D3, GS3) and during the post-treatment periods (D4, GS4). Abbreviations: D = dormancy period; GS = growing period; LD = long days; SD = short days; RH = relative air humidity; and PAR = photosynthetically active radiation.

<table>
<thead>
<tr>
<th>Variable</th>
<th>D1, D2, D4</th>
<th>D3</th>
<th>GS1–4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duration, weeks</td>
<td>8</td>
<td>8</td>
<td>11</td>
</tr>
<tr>
<td>Air temperature (day/night), °C</td>
<td>4/4</td>
<td>4/4</td>
<td>20/15</td>
</tr>
<tr>
<td>RH (day/night), %</td>
<td>90/90</td>
<td>90/90</td>
<td>70/80</td>
</tr>
<tr>
<td>PAR, µmol m⁻² s⁻¹</td>
<td>200</td>
<td>200</td>
<td>400</td>
</tr>
<tr>
<td>Photoperiod (day/night), h</td>
<td>6/18</td>
<td>6/18</td>
<td>18/6</td>
</tr>
<tr>
<td>Minimum soil temperature, °C</td>
<td>4</td>
<td>–2 to –3</td>
<td>15</td>
</tr>
</tbody>
</table>

Figure 1. (A) Mean daily air and soil temperatures in the organic layer, volumetric water content in the (B) organic layer and (C) mineral soil (at a depth of 12 cm) during soil thawing. Arrows on x-axis indicate the initiation of soil thawing by treatments during the dormancy (D3) and growing (GS3) periods. Periods GS2 and D4 refer to pretreatment growing period and post-treatment dormancy period, respectively. A 21-day short-day (6-h photoperiod) phase occurred at the end of the growing period. Soil thawing started 28 and 14 days before the start of GS3 in treatments Trt1 and Trt2, whereas the same event started simultaneously with the start of GS3 and following a 7-day delay in Trt3 and Trt4, respectively. Time indicates the running day.
gangic layer and in the mineral soil at a depth of 12 cm throughout the study. Soil water content was kept close to field capacity by irrigating twice-weekly during the growing seasons and at 2-week intervals during the dormancy periods. The frozen soil was not irrigated. The chemical composition of the irrigation solution corresponds to that of precipitation in southern Finland. Calculation of the temperature sum (degree days; dd) was based on the daily mean air temperature, with a threshold of 5 °C. The chilling unit (CU) accumulation followed a piece-wise linear function between daily mean temperatures of −3.4 and 10.4 °C (Sarvas 1974). A maximum rate of 1 CU day⁻¹ occurred at 3.5 °C, and 0 CU day⁻¹ beyond the temperature thresholds. Air temperature sum for the growing periods was 1230 dd and chilling units accumulation for the dormancy periods was 50 CU (Sarvas 1974, Hänninen 1990, Repo et al. 2005).

In all treatments, aboveground environment changed over a 6-day period from simulated winter to the target conditions of the growing periods. Root and shoot growth and trunk sap flow were monitored during the study. Root and shoot biomass and root morphology (total root length, root surface and projection area, number of root tips) were determined at the final harvest.

**Sap flow**

Sap flow was measured with a Dynamax Flow32 Stem-Flow Gauge system (Dynamax, Houston, TX) in two saplings per treatment. The Dynamax gauges (SGB-16 or SGB-19) were installed between the 3rd and 4th whorls from the top. The trunk surface was smoothed and sprayed with a Teflon liquid (TFE, Ease-Release Teflon) (van Bavel et al. 2000). A thin layer of silicon grease was spread on the inside surface of the Dynamax components, including the heater element, before the sensor was installed on the trunk. Power input was about 0.2 W. In the calculation of sap flow, the thermal conductance constant (K₀) was calculated as the mean value for the 2 h before the lights were switched off in the morning (van Bavel et al. 2000). The threshold for the temperature difference (ΔT) between the upper and lower thermocouples in the gauge was set to 0.5 °C. When ΔT ≤ 0.5 °C, the flow rate was defined as zero. Flow rate was logged at 15-min intervals and converted to daily rates.

Daily sap flow (g day⁻¹) was normalized to total live needle dry mass (DM) and to needle projected area (g day⁻¹ gDM⁻¹ and g day⁻¹ m⁻², respectively). Live needle biomass and projected area were determined by number of whorls and age fraction at the final harvest at the end of GS4 and used to estimate biomass at the end of GS3. The relationship between needle projected area and dry mass was determined by linear regression of sample needles, and this relationship was used to calculate total needle projected area based on total needle dry mass (3 days at 60 °C). The projected area of the sample needles was determined by scanning the needles (HP ScanJet 6100C/1, Greeley, Colorado) and analyzing the images with WinNeedle software (Régent Instruments, Quebec, Canada).

**Electrical impedance spectroscopy of needles and trunks**

Electrical impedance spectroscopy (EIS) can detect changes in tissue water balance and the existence of cell membrane injuries (Repo et al. 2002, 2005). Needles that developed during the preconditioning period GS2 (i.e., gs2-needles) were sampled on 10 occasions between GS2 and GS3 and analyzed by EIS as described previously (Repo 1994, Repo et al. 1994). On each sampling date, 10 needles were harvested per sapling and a 10-mm section was cut from the middle of each needle. The cut surfaces of the needle section were placed in the measuring cell between the electrode pastes (Signagel, Parker Laboratories, Fairfield, NJ). The pastes were connected by Ag/AgCl electrodes (RC1, WPI, Sarasota, FL) to the circuit analyzer (HP 4284A, Agilent, Palo Alto, CA) and the impedance spectrum (IS) (both real and imaginary parts) was measured at 46 frequencies between 20 Hz and 1 MHz. The extracellular resistance of the needles, which was calculated based on the estimated parameters of the distributed circuit element (single-DCE) model, corresponds to the threshold value of the real part of the IS when the frequency approaches zero (Repo et al. 1994). The extracellular resistance was normalized to the cross-sectional area and length of the sample to derive a specific number (Ω m).

The impedance spectra of the trunks were measured once before the soil thawing treatments in D3 and once after one week of GS3. Two silver needle electrodes (diameter 0.5 mm) were pushed into the trunk 15 mm apart to a depth of 2 mm at a position 15 cm above the root collar, and the IS was measured at 46 frequencies between 20 Hz and 1 MHz as described above.

**Electrolyte leakage of needles**

Electrolyte leakage of gs2-needles was tested to determine if delayed soil thawing caused cell membrane injuries. Eighteen needles were sampled from each sapling before (D3 phase) and after the start of the soil thawing treatments (GS3 phase). Eight-mm-long samples were cut from the middle of the needles, rinsed with distilled water and placed in 15-ml test tubes (six samples in each test tube, three replicate tubes per sapling). The tubes were stopped and kept overnight at +5 °C. Thereafter, 3.5 ml of distilled water was added to the tubes and they were placed in a shaker for 22 h at room temperature before conductivity was measured (C₁). The needle tissues were then heat-killed at 92 °C for 20 min and placed in the shaker for another 22 h before conductivity was remeasured (C₂). Relative electrolyte leakage (REL; %) was calculated as the ratio of C₂/C₁ multiplied by 100.

**Shoot and root growth dynamics**

The start and cessation of shoot elongation, trunk diameter growth, long (white and brown) root elongation, short root elongation and the cessation of needle elongation were determined in GS3. Measurements on shoots were made weekly and those on roots at 2- to 3-week intervals. The relative value of growth was denoted as 0% if there was no change, and 100% after growth ceased. Growth initiation and cessation were defined as the temperature sum when 10 and 90%, respectively, of the difference between the initial and final values were reached.
Root growth was monitored during GS2 and GS3 at 3- and 2-week intervals, respectively, with a minirhizotron imaging system (Bartz BTC-100X Camera System, Bartz Technology Company, Santa Barbara, CA). The acrylic minirhizotron tube (outer and inner diameters of 60 and 50 mm, respectively) was located horizontally at a depth of 5 cm in the mineral soil of each root container (Finér et al. 2001). Digital images of roots were taken in an upward direction along the entire extension tube in a total of 56 frames. The images were analyzed with RootView software (Aphalo and Simonic 1999). Live and dead root-tips were counted, and the total length of live and dead short roots, which were mostly mycorrhizal, and long dead root-tips were counted, and the total length of live and dead root-tips and the total length of the live and dead short and long roots in all frames were calculated for each root container at each imaging time. The number of root-tips and the length of the short and long roots at the end of D1 and in the middle of D2 were used as the reference when the cumulative number was calculated for each seedling during GS2 and GS3, respectively.

**Biomass of shoots and roots, root morphology**

Total biomass of stems and needles was determined by whorl and shoot age category at the final harvest at the end of GS4. Dry masses of live and dead stems and needles were determined (4 days at 60 °C).

Root biomass was determined for different soil layers in the root containers, i.e., organic layer and three 10-cm-thick mineral layers. The cylinder (24.6 cm in diameter) around the trunk, including the stump, was harvested with a soil corer. The soil outside this cylinder was sampled in two sectors by layers at the opposite sides of the trunk (the area of a sector was 385 cm²). Roots were separated from the soil and their length, surface area, projected area and the number of root-tips were determined by scanning (WinRhizo 3.1.2, Quebec, Canada). Roots were divided in three diameter categories (<2 mm, between 2 and 4.5 mm, >4.5 mm) and the dry mass in each category was determined. Specific root length (SRL) was determined as the ratio between root length and root dry mass in each diameter category (m g⁻¹). Root dry mass, length, area and number of root tips of the sector samples were used to extrapolate to the whole container. A root system was considered dead if the shoot was scored dead at the final harvest.

**Statistical analysis**

Treatment effects on extracellular resistance of needles, relative electrolyte leakage of needles, root growth and root tip formation were analyzed with the linear mixed model, with the assumptions necessary for the split-plot analysis of variance. The model was \( y = \mu + \text{treatment} + \text{time} + (\text{treatment} \times \text{time}) + \text{ replicate(treatment)} + \varepsilon \), where \( \mu \) is a constant. The treatment (i.e., different times of soil thawing) and time (i.e., sampling time) were considered fixed factors, and the replicate (i.e., tree) and \( \varepsilon \) were random terms. The analysis for extracellular resistance was performed on log-transformed data until sampling Day 318. After that time, the EIS readings for needles of the most affected saplings exceeded the measurable range and therefore they were omitted from the analysis. Statistical analyses of the IS data for trunks were performed for each measurement time based on the means for both the real and imaginary values at different frequencies. Then the mixed model analysis was performed as above. Trunk sap flow was analyzed with the linear mixed model in D3 and for different phases in GS3 by \( y = \mu + \text{treatment} + (\beta \times \text{time}) + \varepsilon \), where \( \beta \) is the regression coefficient of time and \( \varepsilon \) refers to the \( j \)th measurement for treatment \( i \) (i.e., different times of soil thawing) and j = 1 ... m). It was assumed that the distributions of the error vectors \( \varepsilon = (\varepsilon_1, \varepsilon_2, \ldots, \varepsilon_m) \) are independent multivariate normal distributions with homogeneous variance and first-order autoregressive covariance structure. Normality and homogeneity of the variance of the residuals were checked graphically and the selection of the covariance structure was based on Akaike’s information criteria. Differences between treatments at the start and cessation of growth and root mortality in GS3 were analyzed by the Mann-Whitney U-test. Treatment differences in shoot and root biomass were tested on mean values by ANOVA followed by Tukey’s test.

**Results**

**Root container conditions**

Air and soil temperatures were similar in the different treatments, except during the soil thawing periods in D3 and GS3 (Figure 1A). During the first week of cold acclimation in D3 before the soil thawing treatments, temperature in the organic and upper mineral soil layer decreased at a rate of 1.9 and 1.7 °C day⁻¹, respectively, followed by rates of 0.22 and 0.3 °C day⁻¹ in the next 9 and 12 days, respectively. Further decreases occurred at a rate of 0.04 °C day⁻¹ whereupon the soil became frozen for 17, 30, 44 and 51 days in Trt1, Trt2, Trt3 and Trt4, respectively (Figure 1). The minimum temperatures in the organic layer were –1.8, –1.9, –2.0 and –2.8 °C for Trt1, Trt2, Trt3 and Trt4, respectively. Volumetric water content decreased in the organic and mineral layers following soil freezing (Figures 1B and 1C), reaching minimum values of 8.2, 5.1, 5.2 and 5.0% in the organic layer in Trt1, Trt2, Trt3 and Trt4, respectively (Figure 1B). Soil thawing was observed by the increase in liquid water content and soil temperature. Water content in the organic layer increased in two days from the value of frozen soil to the maximum. The thermal capacity of the soil delayed soil thawing, the time required for the temperature to rise from <0 to 10 °C being on average 6 days.

**Sap flow**

In D3, with similar aboveground conditions in the four treatments (Table 1), there was detectable trunk sap flow in trees in the two earliest soil thawing treatments but treatment differences were not significant (Figure 2). Treatment and its interaction with sampling time had a significant effect on sap flow in GS3 \( (P < 0.01) \). Sap flow increased rapidly in saplings in Trt1 and Trt2 when aboveground conditions became favorable for growth at the beginning of GS3, but sap flow was inhibited.
partly or completely in saplings in the treatments with delayed soil thawing (Trt3 and Trt4). In Trt1 and Trt2 saplings, sap flow of about 500 g day\(^{-1}\) was reached within 10 days of the start of G3. After three weeks, sap flow continued to increase concomitantly with needle elongation and peaked at the end of the long-day photoperiod when needle elongation ceased (Figure 2). Immediately after the start of the short-day phase, sap flow dropped to 50% of the maximum. It decreased further to about 20 g day\(^{-1}\) at the beginning of D4 when air and soil temperatures decreased from 20/15°C (day/night) and 15 °C, respectively, to 4 °C. Sap flow of the living saplings did not differ significantly between treatments during the long- and short-day periods of G3.

When daily sap flow in G3 was normalized to the live needle biomass of the living saplings, no difference was found between treatments. The normalized sap flow was about 4.1 g day\(^{-1}\) g\(_{\text{DM}}^{-1}\) at the end of the G3 long-day period. There was a linear relationship between live needle projected area (\(y\); mm\(^2\)) and live needle dry mass (\(x\); g) \(y = 3821x + 609.95\); \(r^2 = 0.95\). Accordingly, daily sap flow expressed on a needle projected area basis averaged 1080 g day\(^{-1}\) m\(^{-2}\).

### Electrical impedance spectroscopy of needles and trunks

Sampling time and its interaction with treatment had significant effects on extracellular resistance (\(r_e\)) of needles (\(P < 0.001\) and \(P < 0.01\), respectively). The \(r_e\) values increased from about 70 Ω m at the end of G2 to between 90 and 110 Ω m at the end of D3 (Figure 3). In Trt4 saplings, there was a significant drop from 110 to 60 Ω m within 1 week from the start of GS3 (\(P < 0.01\)). After 3 weeks of GS3, \(r_e\) of the most affected saplings increased strongly and even exceeded the measurable range.

The magnitude of the real and imaginary parts and the shape of the impedance spectra (IS) were similar for saplings in all treatments during D3 (Figure 4A). The IS comprised one arc with a tail at low frequencies. After one week of GS3, the IS of Trt4 saplings were anomalous in shape and magnitude compared with the IS of saplings in other treatments (Figure 4B). At that time, the mean of the real part was significantly higher in saplings in Trt4 than in the other treatments (\(P < 0.0001\)).

### Electrolyte leakage of needles

Sampling time and its interaction with treatment had significant effects on relative electrolyte leakage (REL) of needles (\(P < 0.01\) and \(P < 0.05\), respectively). The REL was below 20% in D3 before the start of the soil thawing treatments (Table 2). After two weeks of GS3, REL increased in proportion to the delay in soil thawing, being significantly higher in Trt4 saplings than at the previous sampling time (\(P < 0.01\)).

### Shoot and root biomass, root morphology

Delayed soil thawing had significant effects on living shoot biomass (needles and stems) being significantly higher in Trt1 saplings than in Trt4 saplings at the end of the experiment (\(P < 0.05\)) (Figure 5). Three, two and one of the saplings survived in Trt2, Trt3 and Trt4, respectively.

Live root biomass declined significantly in response to the delay in soil thawing (\(P < 0.05\)) (Figure 5). However, delayed
soil thawing had no significant effects on total root biomass, root length, root surface or projected area and number of root tips which averaged 150 g, 900 m, 1.7 m², 0.5 m² and 180,000, respectively. When root dry mass was determined by soil layer, a declining trend by treatment was observed in the organic layer only, being 12.4, 12.4, 8.0 and 9.0 g for Trt1, Trt2, Trt3 and Trt4, respectively (central cylinder excluded).

Specific root length (SRL) was similar in all treatments. Mean SRL (roots of different diameters combined) was significantly lower in the organic layer than in the mineral layer (3.6 versus 7.7 m gDM⁻¹) (P < 0.001). The SRL decreased strongly with increasing root diameter (d) averaging 17.2, 2.1 and 0.19 m gDM⁻¹ for roots with d < 2 mm, 2 < d < 4.5 mm and d > 4.5 mm, respectively. In the organic layer, SRL was 20.4 and 0.87 m gDM⁻¹ for roots for d < 2 mm, 2 < d < 4.5 mm, respectively. The corresponding SRL values for the mineral layer were 15.6, 2.2 and 0.19 m gDM⁻¹ for d < 2 mm, 2 < d < 4.5 mm and d > 4.5 mm, respectively.

**Shoot and root growth dynamics**

There were no significant differences between treatments in the start and cessation of shoot elongation, trunk diameter growth and needle elongation for surviving saplings in GS3. Relative to air temperature sum, trunk diameter growth and shoot elongation started on average at 127 dd and 219 dd, respectively. Corresponding values for cessation of shoot elongation, trunk diameter growth and needle elongation were 467, 680 and 865 dd, respectively.

Time and its interaction with treatment had significant effects on long-root elongation (P < 0.0001 and P < 0.001), short-root elongation (P < 0.0001 in both cases) and root tip formation (P < 0.0001 and P < 0.05), respectively (Figure 6). Significant differences between the least effective (Trt1 and Trt2) and most effective (Trt3 and Trt4) treatments typically appeared toward the end of GS3. Some short-root elongation was observed in Trt1 before air temperature, photosynthetic photon flux and photoperiod increased at the beginning of GS3 (Figure 6B). Root growth and number of root tips were less in GS3 than in GS2, even in the treatments without delayed soil thawing (Figure 6). The total length of dead long brown roots tended to increase with increased delay in soil thawing in GS3 (Table 3).

**Figure 5. Effects of soil thawing on dead shoot biomass with litter (dead), live biomass of stems (stem) and needles (needle), live biomass of stumps (stump) and roots (root). A root system was considered dead if the shoot was dead at the final harvest. Biomass in each treatment was determined at the beginning (Start, roots excluded) and end of the experiment (n = 4).**

**Table 2. Relative electrolyte leakage (REL; ± SE) of Scots pine needles before (Day 269, D3 phase) and after (Day 310, GS3 phase) the start of the soil thawing treatments (n = 4). The P-value indicates the significance of the difference between sampling times.**

<table>
<thead>
<tr>
<th>Time (days)</th>
<th>REL (%)</th>
<th>Trt1</th>
<th>Trt2</th>
<th>Trt3</th>
<th>Trt4</th>
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</thead>
<tbody>
<tr>
<td>269</td>
<td>19.1 ± 0.6</td>
<td>14.2 ± 0.7</td>
<td>15.2 ± 1.5</td>
<td>15.3 ± 1.3</td>
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</tr>
<tr>
<td>310</td>
<td>11.9 ± 0.8</td>
<td>29.0 ± 15.9</td>
<td>40.1 ± 17.5</td>
<td>66.4 ± 10.7</td>
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</tr>
<tr>
<td>P-value</td>
<td>&lt; 0.10</td>
<td>&lt; 0.01</td>
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</tbody>
</table>
Discussion

Delayed soil thawing inhibited root growth and trunk sap flow in Scots pine saplings, causing needle injuries and declines in live shoot and root biomass. Even a mild soil frost (–1.8 °C) for 2.5 weeks reduced root growth during the subsequent growing period.

Sap flow in trunks, electrical impedance of needles and trunks, and relative electrolyte leakage of needles all indicated that saplings subjected to delayed soil thawing suffered from stress when the demand for evapotranspiration increased at the beginning of GS3 (cf. Figures 2, 3, and 4, Table 2). The decrease in extracellular resistance measured by EIS and the increase in REL of needles within the first week of GS3 both indicate loss of plasma membrane integrity resulting in leakage of ions from the cells into the apoplastic space (cf. Repo et al. 1994, 2005). As a result of loss of cell turgor and consequent drying, the extracellular resistance increased in the most affected needles. Anomalous features of the impedance spectra for trunks of Trt4 saplings at the beginning of GS3 indicate trunk drying, which was supported by the absence of sap flow and increased extracellullar resistance in needles. The responses of the saplings to delayed soil thawing were substantial, but with some differences between our study and a previous study by Repo et al. (2005). These differences may be associated with differences in soil frost conditions, origin of the saplings or the conditions before the treatments, i.e., two annual cycles in our study and one annual cycle in the previous study.

Initiation of trunk sap flow was dependent on the degree of soil thaw. This finding corroborates previous field studies with Norway spruce where early soil thaw was associated with early initiation of sap flow (Bergh and Linder 1999). Blockage of sap flow in frozen soil (Figure 2) could be caused by chemical or hydraulic signals, or both, from roots. Stomatal closure is probably the first reaction to shortage of water. Stomatal behavior is under hormonal control, with abscisic acid playing a central role in long-distance drought signaling in plants (Davies et al. 2002). If sap flow is reduced because of stomatal closure, the process should be reversible, i.e., the stomata should open and sap flow start in response to increased availability of water; however, this was not the case in our study. A more probable explanation for the absence of sap flow when soil is frozen is that irreversible changes occurred in the water transport and transpiration systems (e.g., xylem cavitation and loss of transpiring needle area) of the saplings in Trt3 and Trt4 at the early stages of GS3 (cf. Kramer and Boyer 1995). Reasons for these events might be low liquid water reservoirs in trunks and roots and poor recharge capability of the trunk because water uptake from cold soil is inhibited (Tranquillini 1982, Larcher 1985, Boyce and Lucero 1999).

Seasonal dynamics of trunk sap flow were affected not only by soil frost but also by aboveground conditions and the annual developmental stage of the saplings. Although in-depth analyses of diurnal sap flow (Perämäki et al. 2001, Čermák et al. 2007) and the effects of drought on the seasonal course of sap flow have been carried out, only a few studies have examined other factors that affect sap flow during the growing season, such as phenology, photoperiod, and air and soil temperature (Bergh and Linder 1999, Nadezhdina 1999). In our study, soil and aboveground conditions were kept constant in each period (Figure 1), enabling detailed analysis of the seasonal dynamics of sap flow.

When the soil thawed in D3 (Trt1 and Trt2), low sap flow

Table 3. Effects of soil thawing treatments on mean (± SE) total length (mm) of dead short and long brown roots and total number of dead root tips in GS3 assessed by minirhizotron imaging (n = 4).

<table>
<thead>
<tr>
<th>Trait</th>
<th>Trt1</th>
<th>Trt2</th>
<th>Trt3</th>
<th>Trt4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dead short roots</td>
<td>3.4 ± 1.8</td>
<td>2.9 ± 1.3</td>
<td>4.8 ± 1.1</td>
<td>3.3 ± 1.7</td>
</tr>
<tr>
<td>Dead long roots</td>
<td>2.0 ± 0.7</td>
<td>14.0 ± 2.6</td>
<td>15.9 ± 4.7</td>
<td>6.3 ± 3.1</td>
</tr>
<tr>
<td>Dead root tips</td>
<td>1.5 ± 0.5</td>
<td>2.0 ± 0.7</td>
<td>3.0 ± 2.0</td>
<td>2.3 ± 1.3</td>
</tr>
</tbody>
</table>

Figure 6. Effects of soil thawing on elongation of (A) long (white and brown) and (B) short roots, and (C) number of new root tips during the growing period GS2 (before the soil thawing treatments) and during the dormancy and growing periods D3 and GS3, respectively. Arrows on the x-axis indicate the initiation of soil thawing for treatments Trt1, Trt2, Trt3 and Trt4 (from left to right respectively, see Figure 1). Aboveground conditions became favorable for growth in all treatments at the beginning of GS3. Bars indicate standard errors (n = 4). Time indicates the running day.
Changes in root diameter distribution (Atkinson 2000). On average, the SRL was higher in mineral soil than in organic soil (20 to 40 g day\(^{-1}\)) was found despite unfavorable growth conditions above ground (Table 1). This low sap flow may meet the minimum demands for evapotranspiration in early spring, and thus contribute to the avoidance of water stress. During GS3, daily sap flow almost doubled after completion of shoot elongation and the start of needle elongation even though environmental conditions were kept constant throughout GS3. If we assume that transpiration of old needles was constant, then the development of new needles would explain the increased sap flow. The roles of irradiance and temperature as the driving forces of trunk sap flow became evident at the end of GS3. First sap flow dropped to half of the maximum in response to the short photoperiod with decreased photosynthetic photon flux but with no change in air and soil temperatures (cf. Čermák et al. 2007). Thereafter, sap flow declined to near zero as soil and air temperatures decreased to +4 °C.

Differences in trunk sap flow of living saplings in GS3 could be explained by differences in live needle area/biomass ratio in accordance with previous studies (Čermák et al. 2004). Such differences may have prevailed before the start of the experiment (cf. Trt1 and Trt2) or they may have developed during the treatments (cf. Trt3 and Trt4 with Trt1 and Trt2 in Figure 5). When daily sap flow was normalized to total needle projected area or to dry mass of live needles, the treatment differences (Trt4 excluded) disappeared. The normalized values for sap flow in our study (0.222 g h\(^{-1}\) gDM\(^{-1}\) and 61.1 g h\(^{-1}\) m\(^{-2}\)) were about one third of the values reported by Čermák et al. (2004) but they did not define the species or the annual stage of development of the trees from which the data were collected.

We predicted that delayed soil thawing would affect root morphology, i.e., dry mass, length, projection area and number of root tips, but only one such effect was observed. The expected response was found for root dry mass only and only in the organic layer suggesting that the stress caused by delayed soil thawing was strongest in this soil layer.

We found no difference in SRL among the soil thawing treatments. Instead, there was a strong dependence of SRL on root diameter (Ostonen et al. 2007), which limits the use of SRL for scoring root vitality because many factors may cause changes in root diameter distribution (Atkinson 2000). On average, the SRL was higher in mineral soil than in organic soil reflecting a difference in diameter distribution of roots between soil layers (Atkinson 2000, Engels et al. 2000).

Root growth was reduced during GS3 compared with the pretreatment GS2 period, even in Trt1 and Trt2 saplings that were not subjected to the stress of delayed soil thawing. The slow soil cooling at the beginning of the dormancy period allowed the roots to cold acclimate and so no direct root damage was expected during D3 (Ryyppö et al. 1998). Furthermore, by the time the roots had cold acclimated, conditions were similar in all treatments. Therefore, the differences in root growth between GS2 and GS3 cannot be explained by the conditions at the beginning of D3. Instead, the reduction in root growth was probably caused by decreased tolerance to long-term dehydration or mechanical stress, or both, as a result of soil freezing (cf. Sakai 1968, Tierney et al. 2001). An alternative explanation for the decrease in elongation of long roots between GS2 and GS3 could be a methodological limitation of the minirhizotron imaging (Johnson et al. 2001). It is possible that the long roots that grew well in the GS2 phase, and were observed by imaging, continued their growth outside the observation window of the tube in the subsequent GS3 growing season. Thus, they would not be recorded during GS3, which might result in underestimates of long-root growth. This kind of error would not occur in the analysis of short roots and root tips because of their smaller dimensions.

In conclusion, Scots pine saplings in frozen soils were adversely affected by elevated air temperature and photosynthetic photon flux because water uptake and consequent trunk sap flow were effectively blocked. The water shortage caused damage to the needles and decreased shoot and root growth. Stress reactions may occur even in species that are tolerant to frost and drought, such as Scots pine, when the soil remains frozen after the start of the growing season.

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References


