Canadian Science Publishing | Canadian Journal of Forest Research

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Journal:	*Canadian Journal of Forest Research*
Manuscript ID	cjfr-2019-0190.R1
Manuscript Type:	Article
Date Submitted by the Author:	06-Aug-2019
Complete List of Authors:	Kilpeläinen, Jouni; Natural Resources Institute Finland (Luke), Natural Resources Domisch, Timo; Natural Resources Institute Finland (Luke), Natural Resources Lehto, Tarja; University of Eastern Finland Finér, Leena; Natural Resources Institute Finland (Luke), Joensuu Aphalo, Pedro; University of Helsinki Leinonen, Ilkka; Scotland's Rural College (SRUC) Ryyppö, Aija; University of Oulu Repo, Tapani; Natural Resources Institute Finland , Natural resources and bioproduction
Keyword:	Climate change, Fine root dynamics, Fine root survival, Minirhizotron, Picea abies
Is the invited manuscript for consideration in a Special Issue? :	Not applicable (regular submission)

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Root and shoot phenology and root longevity of Norway spruce saplings grown at different soil temperatures

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Abstract

Tree roots comprise a huge carbon pool. Their dynamics are driven by environmental factors and thereby affected by climate change. We studied the effects of soil temperature on root and shoot phenology and their linkages in Norway spruce (*Picea abies*). Saplings were grown in controlled-environment rooms for three simulated growing seasons (GS1, GS2, GS3). Soil temperature treatments 9, 13, 18 and 21°C were applied during GS2. Root growth was monitored with minirhizotrons.

Root growth commenced in all treatments simultaneously. Temporal growth patterns of short and long roots were usually bimodal. Root growth was very low in the coldest treatment during GS2 but increased during GS3 as an after-effect. Short root growth also continued later after colder than warmer treatments during GS3. Reduced sink strength of roots and increased carbohydrate accumulation into needles at 9°C during GS2 probably enabled compensatory root growth under restored temperatures in GS3.

Soil temperature did not affect shoot phenology, and root and shoot phenology varied between growing seasons; thus the linkage of root and shoot phenology was inconsistent. Root longevity was shorter and turnover rate higher in warmer than colder soil. This can further affect soil carbon dynamics and ecosystem carbon cycling in boreal forest ecosystems.

Keywords

Climate change, Fine root dynamics; Fine root survival; Minirhizotron; *Picea abies*

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Introduction

On the global scale, land and ocean surface temperature has increased ca. 0.9 °C since the late 19th century (IPCC 2014). For the boreal zone, climate change scenarios predict an increase of average surface temperature of ca. 1.5 °C to 7 °C from 1986-2005 to year 2100, with higher increases at higher latitudes (IPCC 2014). However, in spite of higher air temperatures leading to higher soil temperatures during growing season, a shorter period with insulating snow cover can result in more frequent freeze-thaw cycles and a lower soil temperature in future winters and springs (Halim & Thomas 2018).

Tree phenology is controlled by a combination of external drivers namely air and soil temperature, photoperiod, light quality and quantity, soil moisture and aeration, and nutrient availability and internal drivers i.e. resource allocation/competition within the tree (e.g. Way and Montgomery 2015, Radville et al. 2016b, Sloan et al. 2016). However, above- and below-ground parts of trees can react differently to changing climatic conditions. Contrary to shoots, tree roots probably do not have a rest phase during dormancy but at least temperate-region trees may grow around the year in suitable conditions (Radville et al. 2016b). Since as much as 2/3 of the net primary production of trees can be allocated to their roots (Helmisaari et al. 2002, Abramoff and Finzi 2015, McCormack et al. 2015), changes in root phenology can have important effects on tree and ecosystem functioning, and carbon allocation and sequestration.

Detailed information on root phenology is scarce. Temperature is the key driver of tree phenology (Hänninen 1990). Although air temperature and soil temperature are correlated, soil temperature is usually lower in the boreal growing season, and it changes with a considerable time lag compared to air temperature. Soil temperature can control the initiation and cessation of root growth; in temperate and boreal zones the threshold soil temperature for significant tree root growth is 2–6 °C (Lyr and Hoffmann 1967, Solfjeld and Johnsen 2006, Alvarez-Uria and Körner 2007). Shoot growth is also affected by soil temperature. Both the stem height growth

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and root elongation of Scots pine seedlings (*Pinus sylvestris* L.) were slower in soil at 5 °C compared to 9 °C, 13 °C and 17 °C during the first 9 weeks of the growing season, and also root biomass and number of root tips were lowest at the lowest soil temperatures (Domisch et al. 2001, 2002b). Relative biomass allocation between roots and shoots did not differ markedly in Scots pine seedlings grown at soil temperatures 5 °C for 0, 3, 6 or 9 weeks and then 9 weeks in 13 °C, although there were differences in plant total biomass (Domisch et al. 2002a). In contrast, low soil temperature (5 and 10 vs. 20 °C) reduced shoot growth more than root growth in silver birch (*Betula pendula* Roth) (Aphalo et al. 2006). Norway spruce, Scots pine and silver birch seedlings grew more roots during two months at soil temperatures 12-15 °C and 16-22 °C than at 7-12 °C but such difference in shoot biomass did not occur (Pumpanen et al. 2012).

Fine roots are the largest component in the belowground carbon cycle of forest ecosystems (Finér et al. 2011). Enhanced fine root growth at higher soil temperatures is associated with earlier root senescence resulting from increased root respiration (Eissenstat et al. 2000, McCormack and Guo 2014). In an experiment manipulating soil temperature in a Norway spruce (*Picea abies* (L.) Karst.) stand, root mortality was highest in the warmed soil (Majdi and Öhrvik 2004). In the same experiment, the total annual fine root elongation was highest in the control treatment while soil warming increased root growth between late autumn and spring from October to June (Majdi and Öhrvik 2004). Later measurements in the same experiment after an additional 12 years of treatments revealed that soil warming increased live root mass and the number of mycorrhizal root tips (Leppälammi-Kujansuu et al. 2013). In addition, soil warming decreased fine root longevity and thus increased root litter production (Leppälammi-Kujansuu et al. 2014b). Moreover, the longevity of the fine roots developed outside growing season was lower than in roots grown during the previous summer. A reason for this could be that more resilient roots are built during summer when there is more carbon available due to ongoing assimilation (Leppälammi-Kujansuu et al. 2014b). More roots were developed in warmed than in non-warmed soil during winter (Leppälammi-Kujansuu et al. 2014b) and this

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can contribute to the increased respiration (Eissenstat et al. 2000, McCormack and Guo 2014) explaining the shorter longevity of roots in warmed soil. Faster fine root turnover (inverse of longevity) means that more carbon is allocated to short-lived carbon pools; this may increase carbon losses to the atmosphere. However, if the faster turnover is compensated by increased photosynthesis (feedback to aboveground processes), then the long-term carbon input to soil may actually increase. The overall effect is still unclear and many interactions are involved. Anyhow, fine roots contribute significantly to the ecosystem carbon balance that depends on the above- and belowground carbon pools resulting from species composition, photosynthesis, respiration, assimilate allocation, litter production, decomposition and nutrient availability.

Observational studies in stands along latitudinal and elevational gradients have indicated that the growth allocated to fine roots is larger in colder than warmer climates. Fine root longevity and biomass per stand basal area were markedly higher in a Norway spruce stand located in northern than in southern Finland (Leppälammi-Kujansuu et al. 2014a). Both Scots pine and Norway spruce grew more mycorrhizal root tips in relation to foliage mass in northern than in southern Finland (Helmisaari et al. 2009). In Norway spruce, fine root biomass and the number of root tips per soil surface area increased towards north also in a gradient from Estonia to northern Finland (Ostonen et al. 2007). The biomass of ectomycorrhizas of Norway spruce in relation to stand basal area increased exponentially along a gradient from southern Germany to northern Finland (Ostonen et al. 2011). These findings may be related to lower soil temperature and lower nitrogen availability in colder climates. Apparently the foraging strategy of roots changes so that more absorptive roots are grown in colder than in warmer soil (e.g. Hertel and Schöling 2011, Ostonen et al. 2011).

The current study presents new results from a Norway spruce experiment in controlled-environment rooms where soil temperature was fixed to 16 °C during a growing season, manipulated to 9, 13, 18 and 21 °C during the next one, and then restored to 16 °C during a follow-up growing season. Results on aboveground growth and root growth as well as gas

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exchange, nutrient status, bud phenology, chlorophyll fluorescence, carbohydrate content and cold hardiness from this experiment were reported by Lahti et al. (2002, 2005) and Repo et al. (2004). The main findings of the previously published papers were that low soil temperature decreased root growth (Lahti et al. 2005), slightly reduced photosynthesis and chlorophyll concentration (Lahti et al. 2002), increased needle starch concentration and decreased dark-acclimated chlorophyll fluorescence whilst bud phenology was not affected (Repo et al. 2004). Significant effects on seedling biomass or total nutrient uptake were not observed (Lahti et al. 2005). Root and stem height growths were lower during low soil temperature treatment than during the follow-up season when the conditions were similar. During both treatment and follow-up seasons, root growth started earlier than shoot growth, it decreased during the rapid shoot elongation phase and then increased again when shoot growth decreased (Lahti et al. 2005) indicating that the allocation of carbohydrates between roots and shoots is an internal driver of phenology.

A combined reanalysis of part of these earlier reported data together with previously unpublished data on root growth and mortality based on minirhizotron imaging was done to investigate the patterns of shoot and root growth and to compare their phenology. Unlike in the earlier paper (Lahti et al. 2005), we analysed the growth, mortality, longevity and turnover of short (1st order) and long (higher than 1st order) roots separately, plus compared shoot phenology to root phenology in more detail. Changing soil temperatures as a result of global climate change are likely to affect the carbon dynamics of forest ecosystems where fine roots play a key role. The aim here was to assess the effects of different soil temperatures during a growing season and their after-effects during a follow-up growing season on fine root phenology (the timing of root growth initiation, maximum rate and cessation, temporal growth pattern and maximum standing length), growth and mortality, fine root longevity and turnover, and on the linkages between root and shoot phenology.

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Materials and methods

Plant material and growing conditions

Sixteen 5-year-old Norway spruce (*Picea abies* (L.) Karst.) saplings of central Finnish origin were assigned randomly to four controlled-environment rooms (RTR48, Conviron Ltd, Winnipeg, Canada), called dasotrons (Finér et al. 2001). Each dasotron had four 0.46-m^3 pots filled with one-meter-deep layer of sand and a 14 cm-thick layer of organic soil from a Norway spruce stand on the top of it. The soil temperature in the pots was controlled with two heat-exchange coils, one on top of the organic layer and another at the bottom of the pot. Each sapling was planted in one pot and was grown during three simulated growing seasons (GS). A GS included a 10-week long-day phase (LD) followed by a 3-week short day phase (SD) and there were 8-week dormancy periods (D1 and D2) between the growing seasons. In the first growing season (GS1), the saplings were acclimated to the dasotron conditions (Table 1). In boreal zone the rooting zone temperature ranges ca. 5-20 °C and averages ca. 10-12 °C during the growing season (Domisch et al. 2001) and soil temperatures colder and warmer than the average were selected here. The soil temperature treatments were 9 °C, 13 °C, 18 °C and 21 °C (abbreviated T9, T13, T18 and T21) in the second growing season (GS2) whereas their after-effects were studied during the third season (GS3) with 16 °C soil temperature that was similar as in GS1 (Table 1). The saplings were fertilised only during GS1 (Lahti et al. 2002). Air temperature sum with a 5 °C threshold was ca. 1150 degree days for each GS. Chilling unit accumulation for each dormancy period was about 57 CU (Hänninen 1990).

Measurements and calculations

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The stem diameter and shoot height were measured at 10-16-day intervals during GS2 and GS3. The *initiation day of stem diameter growth* was determined as the midpoint of the measuring interval between no change and the first time of increased stem diameter during a growing season. Stem diameter commonly increases at first in spring due to increase in stem water content but this could not be separated here from actual structural growth. The *day of maximum rate of diameter growth* was the midpoint of the measuring interval that included maximal diameter growth rate. The *cessation day of stem diameter growth* was the midpoint of the measuring interval when the maximum stem diameter was reached. Shoot elongation was determined to initiate at bud burst day and cease at bud set day (results reported by Repo et al. 2004). As shoot elongation occurred in the early part of GS and the number of measurements during this period was limited, an estimate for the *day of maximum rate of shoot elongation* was obtained by fitting cubic models and finding the maximum of the fitted curve (IBM SPSS statistics, Curve estimation regression). The days of initiation, peak and cessation of root growth were taken as midpoints of root imaging (see below) intervals.

Roots were digitally photographed (Bartz BTC-100X Camera System, Bartz Technology Company, Santa Barbara, CA) through the upper side of an acrylic minirhizotron tube, 60 mm in outer diameter, installed horizontally in each pot with the upper side 15-16 cm below soil surface. In each tube, 46 frames of 13 x18 mm^2 were photographed once during GS1SD and at approximately 1-week intervals during GS2LD and GS3LD+SD, once at the end of GS2SD and near the start and end of the dormancy period at each frame. In the image analysis the RootView software (Aphalo and Simonic 1999) was used to assess appearance, length, death and disappearance of the roots. First order roots without branching were defined as “short roots” and the higher order roots as “long roots”. If a particular root changed from the first to a higher order during the study it was changed into a long root retrospectively for all imaging sessions. This kind of order-based classification does not directly indicate the root functionality but generally the lower order roots function as absorptive roots and higher order roots as transport roots

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(McCormack et al. 2015). A root was considered to be "dead" when it seemed disintegrated in the image and "disappeared" when it was no more visible.

The following parameters were calculated separately for long and short roots. *Standing root length per unit image area* (l_{area}, m m^{-2}) was calculated by dividing the total length of live roots by the area of the image frames. The *net increment of* l_{area} (m m^{-2}) from a GS start was calculated by subtracting the l_{area} in the beginning of a GS from the l_{area} at each later imaging session. *Net root elongation rate per unit image area* (Δl_{area}, m m^{-2} d^{-1}) was the difference in l_{area} between two consecutive imaging sessions divided by the number of days in the interval. Both new root appearance and old root elongation were included in Δl_{area}. *Root mortality* per unit image area (m m^{-2}) was calculated by dividing the length of roots that died between two consecutive imaging sessions by the area of the image frames. Because the absolute root mortality depended on l_{area}, *cumulative proportion of dead root length* out of the sum of live root length and the cumulative dead root length at each imaging session were also calculated from the start of GS2 and GS3. Additionally, the *temporal patterns of root growth* (viz. distributed pattern, unimodal pattern or bimodal pattern with dominant growth peak in spring or autumn or equal peaks) as described by McCormack et al. (2014) were visually assessed from figures.

The *day of root growth initiation* (the appearance of new roots and/or the elongation of existing roots) was determined to be the midpoint of the imaging interval when Δl_{area} reached 5 % of the sum of positive Δl_{area} values of the growing season. Because some saplings grew some roots continuously also during dormancy period, the 5 %-threshold value was selected as the time of the start of significant root growth. The *day of maximum root standing length* was the day at the end of the interval when l_{area} reached its maximum during a growing season. The *day of maximum rate of root growth* was determined to be the midpoint of the imaging interval when the daily Δl_{area} reached its maximum during the growing season. The *day of root growth cessation* was determined to be the midpoint of the latter imaging interval when the Δl_{area} of two

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consecutive imaging intervals was less than 5 % of the sum of positive Δl_{area} values of the growing season (cf. Radville et al. 2016a). *Time difference between the peaks in root and shoot growth rates* was calculated as the difference in days when the maximum shoot and root growth rates were reached (equals the offset described by Abramoff and Finzi (2015)). The calculation of the time difference was based on the timing of the largest peaks only and was thus a rough estimate of the linkage of shoot and root phenology.

Fine root turnover (FRT) tells how many times the fine root population is replaced during a time period (here year, a). It was estimated with four different calculation methods: as 1) the inverse of median (FRT_{inv_med}) and 2) as the inverse mean root longevity (see survival analysis below) (FRT_{inv_mean}) (Majdi et al. 2005), and 3) and 4) as seasonal fine root net growth divided by average (FRT_{avg}) (Burton et al. 2000) and maximum fine root standing length (FRT_{max}) (McCormack et al. 2014), respectively. The FRT_{inv_med} and FRT_{inv_mean} were based on actual root longevity estimates during the whole study period (the unit is actual a^{-1}), whereas FRT_{avg} and FRT_{max} were based on the values during simulated growing seasons that are shorter than in natural conditions (the unit is simulated a^{-1}).

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Statistical analyses

General linear models were fitted to analyse the effects of soil temperature treatment, growing season (fixed factors) and their interaction on the timing of root growth initiation, peak root standing length, highest root growth rate, root growth cessation, shoot growth initiation, maximum shoot growth rate and shoot growth cessation as well as on the offset between the peaks in root and shoot growth. Although soil temperature is a continuous variable, it was treated as a factor in the analyses for comparison between the temperature treatments, because the changes in response variables were not always consistent with the temperature change. Growing season included both long day and short day periods.

The increments of l_{area}, root mortality and the proportion of dead root length were analysed separately for GS2 and GS3 with a linear mixed model using sapling as a subject variable and day of a growing season as a repeated variable (covariance type AR(1)). Fixed-effects model included soil temperature treatment (fixed factor), day of a growing season (covariate) and their interaction. Additionally for l_{area} the values at the start of growing seasons were included as a covariate, and l_{area} was a covariate for root mortality. Random-effects model included sapling (covariance type VC). Logarithmic (ln) transformations were applied for l_{area} and root mortality, and logit transformations for the proportions of dead root length prior the analyses. If the main effects or interactions were significant ($P \leq 0.05$) or nearly significant ($0.05 < P \leq 0.1$) in the general linear models, Bonferroni corrected post hoc test was applied for pairwise comparisons. These analyses were run with IBM SPSS Statistics 25 software.

Based on the minirhizotron images taken at different times, the roots are known to appear and die or disappear during certain time intervals. In this kind of interval censored data, time to the event (here root death) is within an interval. However, here the first imaging session was 136 days after planting and thus the time interval of root appearance observed in the first imaging session was wide. Some of these roots may have been in contact to minirhizotron tubes already at planting and the others grew later. To narrow the first interval for these roots, the appearance time and the start point of the interval for right-censored roots were set to the midpoint of this interval - a root was right-censored if it was alive at the end of the experiment. This differs from the method of Ding et al. (2019) who excluded the roots of the first imaging session from the analysis of their field study in which the birth time of the roots was not known. Here 225 short roots and 213 long roots were observed among all the saplings in the first imaging session at the end of GS1, i.e. 7.6 % and 24.9 % of all short roots and long roots, respectively. Disappeared roots (live during one session, disappeared in the next one) were classified as right-censored because most of them have been found to disappear for other reasons than decomposition (e.g. became covered by fungal mycelia, other roots or soil), and

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were thus presumably still alive at the time of disappearance (Leppälammi-Kujansuu et al. 2014b). The classification of disappeared roots as dead or censored affects the estimated survival time (naturally shorter time if dead). Here the effect was minor since this kind of disappearance took place only for 2.4 % of all short roots and 2.2 % of all long roots.

Nonparametric maximum likelihood estimation (NPMLE) of the survival function was carried out for the short and long roots and the survival was compared between different soil temperature treatments with asymptotic logrank k-sample test (permutation form, Sun's scores) using R 3.5.2 and the package interval 1.1-0.0 (Fay and Shaw 2010) through RStudio 1.1.463. In right-censored point data the NPMLE (called Kaplan-Meier estimator) is undefined after the largest right-censored observation because the NPMLE is not unique then. In interval-censored data the estimate is undefined due to non-uniqueness at certain intervals and the survival curves plotted with interval package are in these cases shown as descending slopes, and as step functions where they are defined uniquely (Fay and Shaw 2010). *Mean and median longevity* and their confidence intervals were estimated for short and long roots as by Repo et al. (2014). The median estimate is the first event time when the NPLME survival curve goes below 0.5 and the mean estimate is the integral of the survival curve. The descending slope of NPMLE curve was treated as an ad hoc estimate of survival in relation to time during the intervals where it was undefined. The 95 % confidence intervals were based on bootstrapping with 3000 resamples.

Results

Root growth initiation and cessation

The initiation of short and long root growth was not affected by soil temperature treatment ($P \geq 0.348$) (Table 2). Short and long root growth did not cease during GS2 (including SD phase), except long root growth ceased in T21 in one out of four saplings at 62 days since the start of

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305 GS2. During D2, short root growth ceased in one sapling in T9 and T21 and in two saplings in
306 T18 at 93 days since the start of GS2. Long root growth ceased during D2 in three saplings in
307 T13 and two saplings in T18 and T21 at 93 days since the start of GS2. Slow root growth
308 continued in the other saplings also during D2. During GS3, short root growth continued until
309 the end of the growing season in T9 and T13 while short root growth ceased in T18 and T21 (in
310 three saplings in the both treatments at 61±4 and 56±11 days (mean±SE), respectively). The
311 short root cessation was thus earlier in T18 and T21 than in T9 and T13 without cessation at all
312 ($P = 0.028$, pairwise $P \leq 0.029$; in statistical testing the trees that did not show root growth
313 cessation were conservatively marked to cease their root growth at the end of GS; in T18 and
314 T21 cessation at 68±8 and 65±12 days, respectively). Long root growth ceased in two saplings
315 in T9, T18 and T21 during GS3 (mean±SE as above: 78±8, 65±10 and 69±11 days,
316 respectively) while it continued until the end of growing season in T13 and the treatment
317 differences were not significant (testing as above: $P = 0.211$).
318
319 ***Patterns of standing root length and net root growth***
320
321 During GS2, the l_{area} of short roots increased with time, most in T21 and least in T9 (interaction
322 $P < 0.001$) (Fig. 1a). During the follow-up GS3, the l_{area} of short roots increased most after T13
323 and T21 and least in T18 (interaction $P = 0.041$). In short roots, l_{area} of the T9 saplings grew
324 clearly larger during GS3 when soil temperature was 16 °C (Fig. 1a).
325 During GS2, long root l_{area} remained lowest in T9 (interaction $P = 0.068$) (Fig. 1b).
326 During GS3, l_{area} of long roots increased more with time in T9 and T13 than in the two warmer
327 treatments (interaction $P < 0.001$).
328 The largest values of l_{area} occurred typically in the last imaging sessions of the growing
329 seasons, i.e. on day 86 since the start of GS2 and on day 79 since the start of GS3 (i.e. 226 days
330 since the start of GS2) (Fig. 1a-b). There was a trend for earlier l_{area} maximum in higher soil

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temperatures. Long root l_{area} peaked on average on day 70±16 in T21 during GS2. During GS3, l_{area} of short roots peaked on average on 65±9 and 45±18 days since the start of GS3 (212 and 192 days since GS2 start) in T18 and T21 which was slightly earlier in T21 than in T9 and T13 that peaked at GS end (interaction $P = 0.094$, pairwise $P = 0.018$ for both comparisons). During GS3, long root l_{area} peaked on 71±5, 66±10, 51±10 and 25±15 days since the start of GS3 (that are 218, 213, 198 and 172 days since the start of GS2) in T9, T13, T18 and T21, respectively, and earlier in T21 than in T9 and T13 ($P = 0.012$, pairwise $P = 0.019$ and 0.034) (Fig. 1b).

The lowest temperature of 9 °C decreased radically the fine root net increment rate (Δl_{area}), especially in short roots, during GS2 but the decrease changed to an enhanced growth during GS3 at 16 °C (Fig. 2a). Short root Δl_{area} generally had two peaks (bimodal root growth pattern) during both GS2 and GS3. However, Δl_{area} showed a distributed pattern in T9 during GS2 and a spring-concentrated unimodal pattern in T18 during GS3 (Fig. 2a,c; Table 3). During GS2, the bimodal pattern of short root Δl_{area} had equal peaks in spring and autumn in T13 while the pattern was autumn-dominant in T18 and T21 (Fig. 2b,c,d; Table 3). The bimodal pattern of short root growth was autumn-dominant in T9 and T13 and had equal peaks in T21 in GS3 (Fig. 2a,b,d, Table 3).

The Δl_{area} of long roots showed also a bimodal pattern during GS2 and GS3, with exceptions in T9 with a distributed (although increasing towards autumn) pattern during GS2 and in T18 and T21 with spring-dominant, unimodal patterns during GS3 (Fig. 2e,g,h; Table 3). The bimodal long root growth pattern was autumn-dominant in T18 in GS2 (Fig. 2g) and spring-dominant in T9 and T13 in GS3 (Fig. 2e,f) while having equal peaks in T13 and T21 during GS2 (Fig. 2f,h). The Δl_{area} of short roots peaked earlier in T18 than in T13 during GS3 (interaction $P = 0.019$, pairwise $P = 0.010$) (Table 3).

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355 ***Root mortality***

356

357 Cumulative short and long root mortalities per unit image area increased with time and more so
358 in T21 than in the other treatments during GS2 (interaction $P \leq 0.001$) (Fig. 3a-b). During GS3,
359 cumulative short and long root mortalities increased most in T21 and least in T9 (interaction $P =$
360 0.009 and $P = 0.007$ for short and long roots, respectively) (Fig. 3a-b).

361 The cumulative proportion of dead short root length out of total short root length
362 increased most in T21 both in GS2 and GS3 (interaction $P = 0.009$ and $P = 0.003$) (Fig. 3c). The
363 cumulative proportion of dead long root length increased most in T21 by the end of the GS2
364 (interaction $P = 0.004$) (Fig. 3d). There was a similar non-significant trend during GS3 (Fig. 3d).
365 Cumulative proportion of dead short and long roots increased first in the T9 in the beginning of
366 GS2 but levelled off later (Fig. 3c-d). Typically the dead root proportion was higher in short
367 than long roots (Fig. 3).

368

369 ***Linkages of root and shoot phenology and growth***

370

371 There were no treatment differences in the start ($P = 0.148$) or cessation of stem diameter
372 growth which continued until the end of each GS (Fig. 4a). During GS3, diameter growth of
373 most saplings was fast between the two first measurements and again later between the fifth and
374 the sixth measurement. The timing of the maximum diameter growth was on average more than
375 three weeks earlier in T9 than in T21 (15 vs. 39 days of GS3, i.e. 162 vs. 186 days since GS2
376 start) although not significantly so (interaction $P = 0.109$) (Fig. 4a). Soil temperature treatments
377 did not affect the timing of the maximum shoot elongation ($P = 0.743$) taking place on average
378 on day 42 during GS2 and day 38 during GS3 (185 days since GS2 start) (Fig. 4b).

379 The differences in the timing of peaks of shoot and root growth rates (offset values) did
380 not differ significantly between soil temperature treatments during GS2 (pairwise $P \geq 0.204$)

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(Fig. 5). During GS3, the growth rate of short roots peaked later than that of stem diameter in T9 and T13 and the both treatments differed significantly from T18 where short root growth rate peaked earlier than stem diameter growth (interaction $P = 0.002$, pairwise $P \leq 0.018$) (Fig. 5a). During GS3 the growth rate of short roots peaked later than that of shoot height in T13 while the opposite occurred in T18 and the treatments differed (interaction $P = 0.078$, pairwise $P = 0.028$) (Fig. 5c). During GS3 there was a similar difference in the timing of growth rate peaks of short vs. long root length between T13 and T18 (interaction $P = 0.034$, pairwise $P = 0.003$) (Fig. 5e). There was variability between growing seasons in the timing differences of shoot and root growth rate maxima (Fig. 5).

Survival analysis of roots

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Based on root data covering the whole study period from the start of GS1 to the end of GS3, short roots in T21 and T18 died earlier than in T13 and T9 ($P < 0.001$) (Fig. 6a). Similar significant difference was found for long roots that died earlier in the two warmer than in the two colder treatments ($P < 0.001$) (Fig. 6b).

Among the root cohort that appeared before GS2, short roots died later in T13 than in the others ($P = 0.005$). Some short roots in this cohort died earliest in T9 in the beginning of GS2; however, a higher proportion was dead after warmer than colder treatments at the end of the experiment (61-62 % vs. 81-90 %). The long roots of this cohort died earlier in T21 and T18 than in T9 and T13 ($P = 0.001$). In the root cohorts appearing during GS2 or after it, roots died earliest in T21 (long roots appearing during GS2 and short and long roots appearing after GS2) or in T21 and T18 (short roots appearing during GS2) ($P < 0.001$). The longevity of long roots in the three cohorts was 5-56 % higher than that of short roots.

Based on the data covering the whole study period, the longevity of short roots was around six months, the mean and median longevities being significantly higher in T13 than in

T18 and T21 (Table 4). The longevity of long roots was 2-3 months higher than that of short roots and the mean longevity was significantly higher in the two coldest treatments than in the two warmest treatments (Table 4). The longevities of short and long roots in T9 were near those of T13.

The root turnover estimates based on root longevity results were 2.0-2.3 a^{-1} in T9 and T13 which was lower than 2.6-3.1 a^{-1} in treatments T18 and T21 (Table 5). Among the turnover estimates based on root growth, FRT_{max} estimate was clearly lower than FRT_{avg} that was closer to the FRT_{inv} estimates based on root longevities (Table 5). FRT_{max} was not consistent with soil temperature treatments as it was largest in T9. The FRT_{avg} estimate of short and long roots was lowest in T13 and the difference was larger than in the estimates calculated from longevities (FRT_{inv}).

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Discussion

The commencement time for short and long root elongation (based on a 5 % threshold of the annual elongation) did not differ significantly between the soil temperature treatments during growing season GS2 or during the follow-up season GS3. This can be caused mainly by the simultaneous soil warming above the threshold soil temperature for the initiation of root growth that is lower than the lowest treatment temperature, 9 °C (e.g. Alvarez-Uria and Körner 2007). No differences were found during GS2 in root growth cessation either. However, as an after-effect, during GS3 short root growth ceased earlier in the treatments 18 °C and 21 °C than in treatments 9 °C and 13 °C. The saplings grown in cold soil with decreased root growth in GS2 might have taken full advantage of the more favourable growing conditions during GS3 when their roots grew well. Simultaneously with the increased root growth during GS3, there was a trend of stem diameter growth to peak earlier in case of cold than warm soil. This phenomenon coincides with the concept of competing plant sinks. Short root growth peaked 2-4 weeks later

than shoot height growth during GS2. This trend agrees with the review of 20 studies in the boreal region reporting that root growth peaked on average 48±8 days later than shoot growth (Abramoff and Finzi 2015). Our results also accord with an early study in which the shoot elongation of Norway spruce seedlings started later but peaked and ceased earlier than root elongation that continued nearly three months longer (Lyr and Hoffman 1967). Similarly, in a study with Scots pine seedlings in hydroponic culture, maximal fine root production took place at the end of the growing season when shoot growth had already slowed down (Iivonen et al. 2001). Thus, the trade-off among competing plant sinks is actualised in the timing of shoot and root growth peaks (e.g. McCormack et al. 2014, Radville et al. 2016b).

Soil temperature affected the growth rate (Fig. 2) of short and long roots, especially in the lowest soil temperature treatment (9 °C) where the patterns of short and long roots were distributed and root growth rate was low during GS2. However, the growth rate of long roots increased towards the end of GS2 in this treatment. In the other treatments the root growth patterns were mostly bimodal (two-peaked) but during GS3 only one prominent spring peak was observed in treatment 18 °C in both short and long root growth and in treatment 21 °C in long root growth. These unimodal root growth patterns were not noticed in colder treatments. The observed first peaks of root growth took place in early growing season before the fastest shoot growth phase. The second root growth peaks occurred late in the growing season when shoot elongation had already ceased but stem diameter growth still continued. No consistent treatment differences between the height of the first and the second peak were seen. Furthermore, besides the soil temperature during growing season, the timing of soil thawing in spring can have a large effect on root growth phenology. Experimentally delayed soil frost in a boreal Norway spruce stand inhibited the spring peak of fine root growth and the autumn peak also occurred later than in control plots (Repo et al. 2014).

Large inter-annual variation in root phenology is a common phenomenon (McCormack et al. 2014, Radville et al. 2016a). Also in our study there was remarkable phenological variation

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between growing seasons: short and long root growth initiated, peaked and ceased earlier, shoot height growth ceased earlier and stem diameter growth peaked earlier during GS3 than GS2. Here different soil temperature treatments but also the acclimation of trees to dasotron conditions may affect the variation between growing seasons. There can be also genetic differences between the saplings. Furthermore, Norway spruce has a predetermined growth pattern involving the elongation of preformed stem units during the next growing season (Pallardy 2008) and thus the conditions of previous season can have a larger effect on the current-year performance than in tree species having a free growth pattern. In shoot phenology, the interaction of photoperiod and temperature is known to be important and the conditions of a phase affect the next one in the annual cycle of trees (Hänninen and Tanino 2011). The budburst and growth onset can in most boreal and temperate tree species be accelerated by climate change, but growth cessation can be accelerated or delayed depending on species and ecotype (Hänninen and Tanino 2011). Probably the climate change effects on root phenology are not always straightforward either.

Here, some root elongation occurred in all the treatments during dormancy period when air and soil temperatures were 4 °C. However, root growth during the dormancy period was low in comparison to that during growing season. It seems evident that roots do not become dormant similarly as shoots, and many trees, such as Norway spruce, can produce some roots using stored carbohydrates during winter at soil temperatures only slightly above zero (Radville et al. 2016b, Wang et al. 2018). Soil temperature is the most important factor controlling the initiation and cessation of root growth if there is no water deficit or excess. In experimentally heated (from late April to mid-November) plots in a Norway spruce stand in northern Sweden, root elongation from early October to early June accounted for a quarter of the annual elongation in heated plots and significantly more than in non-heated plots (Leppälammi-Kujansuu et al. 2014b).

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In our study, particularly the growth of short roots but also of long roots was clearly reduced in the lowest soil temperature (9 °C). However, during the following growing season, when the air and soil conditions were similar in all treatments, the short roots and also long roots of 9 °C treatment showed clear growth enhancement compared with GS2 and the standing length of long roots grew more in the two colder than in the warmer treatments. Height growth of the saplings in 9 °C treatment was higher than in the other treatments in GS3 although it did not differ between treatments during GS2 (Lahti et al. 2005). The finding suggests that in suboptimal soil temperature, the photosynthetic rate can exceed the below-ground sink demand for carbohydrates. This will lead to the accumulation of carbohydrates in aboveground organs and subsequent depression of photosynthesis as reported earlier from this experiment (Lahti et al. 2002, Repo et al. 2004). However, the accumulated starch will boost growth during the next growing season if the growing conditions are favourable as in GS3 of our study (Repo et al. 2004). The starch accumulation may contribute to the compensatory growth of fine roots in Norway spruce if root mortality has increased e.g. due to soil frost (Repo et al. 2011, 2014). Similarly, Scots pine saplings grew more long and short roots as an after-effect of a waterlogging treatment compared to non-waterlogged controls (Roitto et al. 2019).

Root growth in cold soil is retarded leading to smaller root systems with less surface area and to a reduced potential for water and nutrient acquisition, and thus also decreasing above-ground growth according to a negative feedback. In addition, low soil temperature directly reduces the permeability of root membranes to water and increases the viscosity of water (Pallardy 2008).

Our results showed that the largest proportion of roots died in the highest soil temperature treatment both during the treatment and the follow-up growing seasons. Higher root mortality in warmer than in colder soil has been observed under field conditions, too (Tierney et al. 2003, Majdi and Öhrvik 2004). In our study, the high root mortality in the treatment with the warmest soil temperature was in accordance with the lowest observed root longevity. There was

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a clear leap in longevity from 18 °C to 13 °C both in short (median from 121 to 161 days) and long roots (mean from 225 to 289 days, median could not be defined). Longer lifespan of the roots in colder soil can be due to lower root respiration rate (lower maintenance costs) and reduced activity of soil organisms (less root herbivory) (Eissenstat et al. 2000, McCormack and Guo 2014).

Here the turnover rate based on the median of short root longevity was ca. 2.3 a^{-1} in treatments 9 °C and 13 °C and 3.0 a^{-1} in treatments 18 °C and 21 °C. Long root turnover was slower, ca. 1.3 a^{-1} in the two coldest (based on mean longevity) and 1.7 a^{-1} (based on median longevity) in the two warmest treatments. The inverse of longevity, i.e. root turnover shows how many times a root population is replaced annually. Our results show 30 % higher short and long root turnover in the two warmest treatments compared to the two coldest ones. In the experiment in northern Sweden, the root turnover estimate (based on the median longevity of short and long roots pooled) was 96 % higher in warmed (1.0 a^{-1}) than in non-warmed plots (0.51 a^{-1}) (Leppälammi-Kujansuu et al. 2014b). Indeed, tree root turnover estimates vary a lot, as they depend e.g. on tree species, stand developmental stage, studied soil layer, fine root diameter and different estimation methods (Lukac and Godbold 2010, Finér et al. 2011, Brunner et al. 2013). In a 47-year-old Norway spruce stand in eastern Finland, the turnover rate (based on median longevity) of short roots in combined data of topsoil and mineral soil was 1.2-1.3 a^{-1} and that of long roots (diameter ≤ 2mm) slightly lower (1.0-1.1 a^{-1}) (Repo et al. 2014). On the other hand, the turnover rates based on the median longevities of fine roots (diameter < 1mm) were 0.5-0.6 a^{-1} in the topsoil and mineral soil in mature Norway spruce stands both in northern and southern Finland (Leppälammi-Kujansuu et al. 2014a). In a southern boreal forest in Russia, fine root turnover of mature Norway spruces was as low as 0.10-0.24 a^{-1} (Lukac and Godbold 2010). According to a compilation of 11 studies in temperate and hemiboreal forests in Europe, the fine root turnover of Norway spruce varied between 0.26 and 1.77 a^{-1} with a mean 0.75±0.41 a^{-1} (±SD) (Brunner et al. 2013). An overall turnover estimate of tree fine roots based on data from

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30 boreal forest stands was on average 1.30±1.92 a^{-1} (±SD) (Finér et al. 2011). Therefore, our estimates based on the dasotron experiment fit in that range.

In conclusion, the root phenology of Norway spruce saplings was affected by soil temperature. As direct effects, root growth was decreased at 9 °C soil and the decrease was actualised as a more evenly distributed growth pattern of roots during the treatment growing season than in the other treatments that mostly had patterns with prominent root growth peaks in early and late growing season. As after-effects during the follow-up growing season, short root growth ceased earlier after the warm than cold soil treatments which can indicate that the earlier reported (Lahti et al. 2005) compensatory root growth is actualised also in an extended time frame. Simultaneously, the stem diameter growth peaked later in the warm treatments which can relate to trade-off between the competing sinks of roots and shoots. Root longevity was lower in warm than cold soil. The results add evidence for substantial increase in the fine root turnover rates of Norway spruce due to soil warming. Changing soil temperatures by global climate change are likely to affect the carbon dynamics of forest ecosystems. The carbon balance depends on the above- and belowground carbon pools as a result of changing species composition, photosynthesis, respiration, assimilate allocation, litter production, decomposition and nutrient availability. Fine roots are an important component in the carbon accumulation and respiration in soils as affected by temperature.

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Acknowledgements

We are grateful to recently deceased Prof. Hannu Mannerkoski for participating in the planning of the Joensuu dasotrons and in this experiment carried out in them. Thanks go to Mr. Markku Lahti for contribution in planning and running the experiment and to Ms. Eija Koljonen for root image analysis. We thank Dr. Seija Sirkiä for advising in the root longevity calculation. The

study was funded by the Academy of Finland (decision number 311455) and Natural Resources Institute Finland (project 41007-00004000).

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Tables

Table 1. Dasotron conditions during the experiment. Time indicates the number of days since the start of GS2 until each phase began. The duration of each phase is given in weeks. GS stands for growing season, LD for long day phase, SD for short day phase, D2 for dormancy period, RH for relative air humidity, and PAR for photon flux density of photosynthetically active radiation. GS1 and D1 preceding GS2 were similar to GS3 and D2, respectively.

Quantity	GS2LD	GS2SD	D2	GS3LD	GS3SD
Time, days	0	70	91	147	217
Duration, weeks	10	3	8	10	3
Air temperature (day/night), °C	20/15	20/15	4/4	20/15	20/15
RH (day/night), %	70/80	70/80	90/90	70/80	70/80
PAR, µmol m^{-2} s^{-1}	400	220	150	400	220
Photoperiod (day/night), h	18/6	8/16	8/16	18/6	8/16
Soil temperature, °C	9;13;18;21	9;13;18;21	4	16	16

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Table 2. The initiation time of short and long root elongation in days since the start of growing seasons GS2 and GS3, in the experiment with Norway spruce saplings with different soil temperatures (T) in GS2 (mean±SE, n = 4). The time in the beginning of growth is defined as the midpoint between two consecutive imaging sessions (interval of 5-9 days) where the net root growth exceeded 5 % of the total net growth during each GS. Different capital letters indicate significant ($P < 0.05$) pairwise differences between GSs within treatments and root types.

	GS2		GS3	
Soil T, °C	Short root, day	Long root, day	Short root, day	Long root, day
9	29.8±5.3^{B}	34.3±8.3^{B}	10.8±1.8^{A}	12.5±0.0^{A}
13	15.8±1.8	15.8±1.8	9.0±2.0	9.0±2.0
18	36.0±8.9^{B}	23.3±5.8	5.5±2.9^{A}	14.3±6.6
21	29.0±13.5^{B}	26.8±11.7	10.8±4.4^{A}	14.3±3.4

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Table 3. The time of peak net root elongation rate per unit image area in short and long roots since the starts of growing seasons GS2 and GS3 (mean±SE, n = 4). Days of overall peak (Overall), early season peak (1st) and late season peak (2nd) are presented. Day 46 was in the middle of a GS and divided GS to early and late season. Different capital letters indicate significant pairwise differences in overall peak days between GSs within treatments and small letters between soil temperature (T) treatments within GSs. The general root production pattern as assessed visually from Fig. 2 is indicated: B=bimodal, U=unimodal, S=spring-dominant, A=autumn-dominant, E=equal peaks, D=distributed.

		Peak of short root growth, days				Peak of long root growth, days			
	Soil T, °C	Overall	1st	2nd	Pattern	Overall	1st	2nd	Pattern
GS2	9	59±10	38±3	71±5	D	47±13^{B}	30±5	69±4	D
	13	54±13	18±0	65±4	BE	58±14	18±0	69±4	BE
	18	72±4^{B}	21±4	72±4	BA	55±8	23±6	58±6	BA
	21	60±6	23±9	60±6	BA	56±14	18±0	65±6	BE
GS3	9	33±12	16±4	59±3	BA	14±2^{A}	14±2	57±6	BS
	13	56±12^{b}	18±2	66±3	BA	45±15	18±2	68±3	BS
	18	7±3^{Aa}	7±3	59±6	US	28±11	21±4	56±4	US
	21	37±12	18±2	58±2	BE	40±12	20±0	61±4	US

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Table 4. Median and mean short and long root longevities (days with 95% confidence intervals (CI)) of Norway spruce saplings in the experiment with three growing seasons (GS) and two dormancy periods, with different soil temperatures (T) during the second GS. Different letters by longevity variables indicate significant differences between the treatments, i.e. no overlap in CI. *Median was not defined (cf. Fig. 6).

	Short root		Long root	
Soil T, °C	Median	Mean	Median	Mean
9	161±28[ab]	171±28[ab]	*	280±35[b]
13	161±15[b]	186±14[b]	*	299±17[b]
18	119±17[a]	136±13[a]	216±62[a]	235±24[a]
21	124±10[a]	139±8[a]	214±23[a]	216±13[a]

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Table 5. Mean short and long root turnover rates (a^{-1}) of Norway spruce saplings in the experiment with three growing seasons (GS) and two dormancy periods, with different soil temperatures during GS2. Turnover rates were calculated as inverse of median longevity (FRT_{inv_med}) and inverse of mean longevity (FRT_{inv_mean}) with data covering the whole study and as root length production during growing season divided by average (FRT_{avg}) and maximum (FRT_{max}) live root length, averaging GS2 and GS3. *Median was not defined.

	Short roots				Long roots			
Turnover	9 °C	13 °C	18 °C	21 °C	9 °C	13 °C	18 °C	21 °C
FRT_{inv_med}	2.3	2.3	3.1	2.9	*	*	1.7	1.7
FRT_{inv_mean}	2.1	2.0	2.7	2.6	1.3	1.2	1.6	1.7
FRT_{avg}	1.9	1.5	3.5	2.3	1.8	0.66	1.9	1.3
FRT_{max}	0.80	0.73	0.77	0.63	0.64	0.44	0.55	0.44

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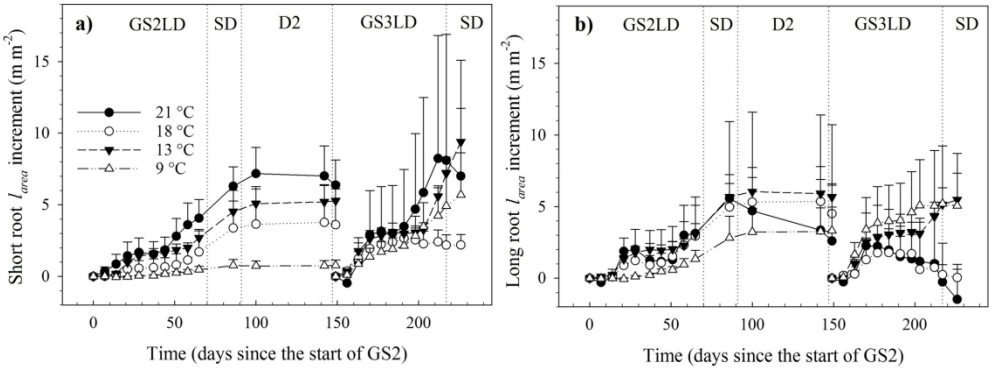

Fig. 1. Increment of standing length (l_{area}, length per unit image area) of short roots (a) and long roots (b) of Norway spruce saplings in the experiment with different soil temperatures during growing season 2 (GS2) with long day (LD) and short day (SD) phases, followed by dormancy period (D2) and growing season 3 (GS3) with similar air and soil conditions. Bars indicate standard errors (n=4).

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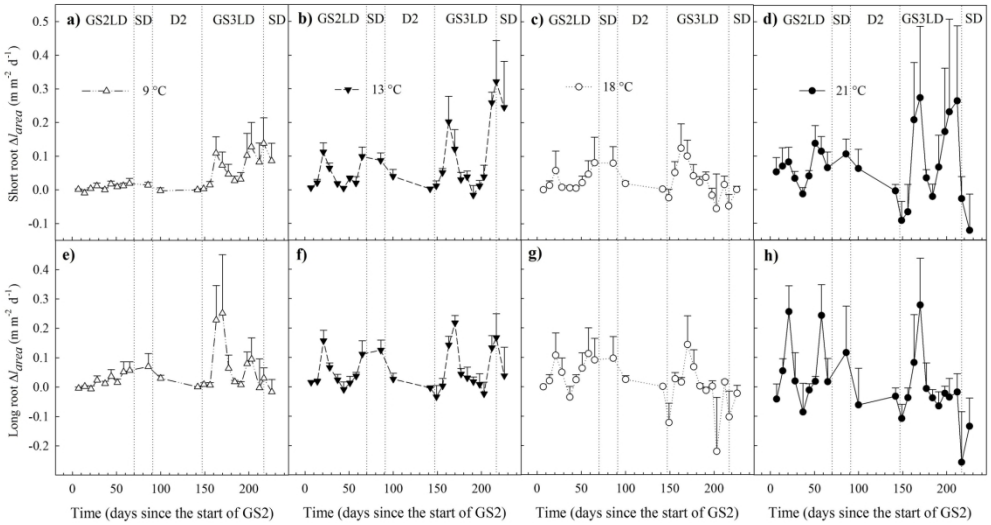

Fig. 2. Net root elongation rate per unit image area (Δl_{area}) of short roots (a-d) and long roots (e-h) of Norway spruce saplings in the experiment with different soil temperatures during growing season 2 (GS2) with long day (LD) and short day (SD) phases, followed by dormancy period (D2) and growing season 3 (GS3) with similar air and soil conditions. Bars indicate standard errors (n=4). The first imaging session of GS2 was on day 0.

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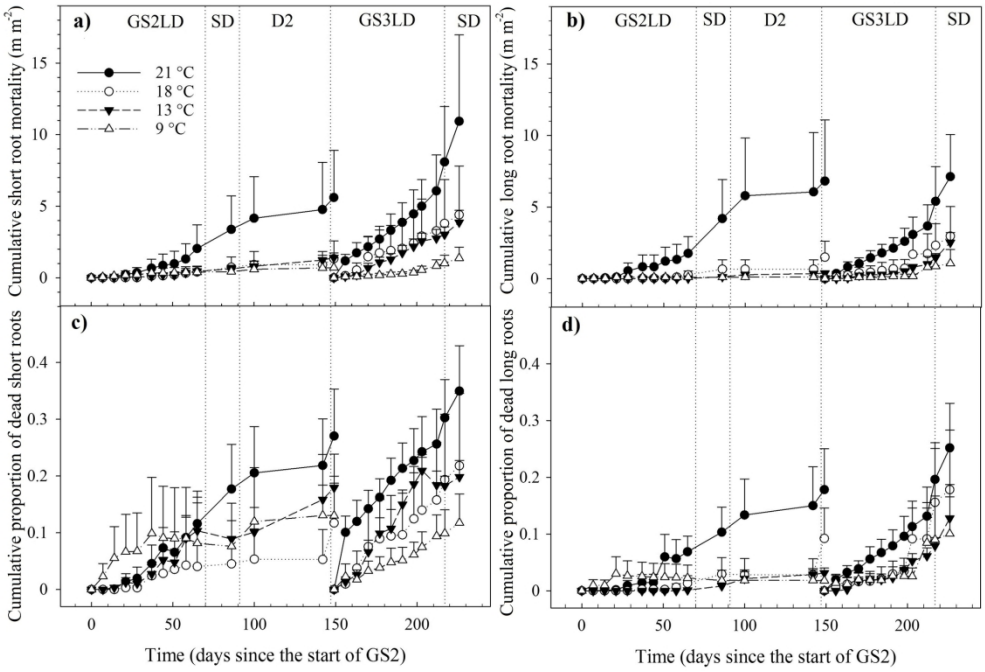

Fig. 3. Cumulative mortality (dead root length per unit image area) for short roots (a) and long roots (b) and cumulative proportion of dead root length out of the sum of live root length and the cumulative dead root length for short roots (c) and long roots (d) of Norway spruce saplings in the experiment with different soil temperatures during growing season 2 (GS2) with long day (LD) and short day (SD) phases, followed by dormancy period (D2) and growing season 3 (GS3) with similar air and soil conditions. Bars indicate standard errors (n=4).

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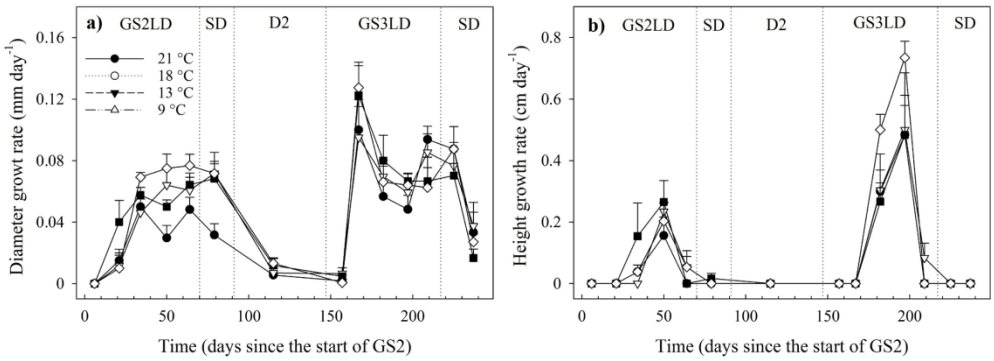

Fig. 4. Growth rate of stem diameter (a) and height (b) of Norway spruce saplings since the first measurements of growing seasons (GS) in the experiment with different soil temperatures during GS2 with long day (LD) and short day (SD) phases, followed by dormancy period (D2) and GS3 with similar air and soil conditions. Bars indicate standard errors (n=4).

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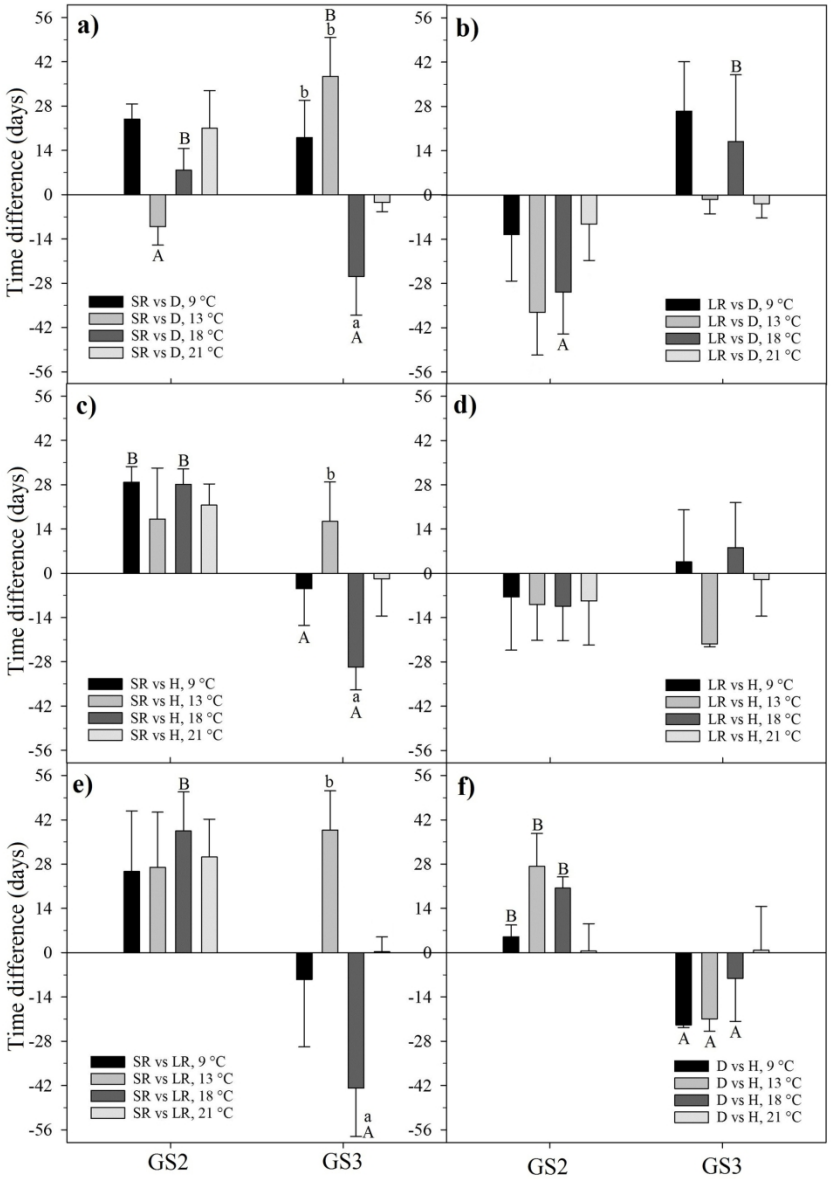

Fig. 5. Time difference in maximum rate of root growth [separately for short roots (SR) (a, c) and long roots (LR) (b, d)] and shoot growth [stem height (H) (a, b), stem diameter (D) (c, d)] in the experiment with Norway spruce saplings with different soil temperatures during the growing season (GS) 2 but similar conditions during GS3. The corresponding time differences between short and long roots (e) and between stem diameter growth and shoot elongation (f) are shown. For example in (c) a positive offset between the times of peak short root and shoot elongation indicates that the short root growth peaked later than shoot height growth. Different capital letters indicate significant pairwise differences between GSs within treatments and small letters between soil temperature treatments within GSs. Bars indicate standard errors (n=4).

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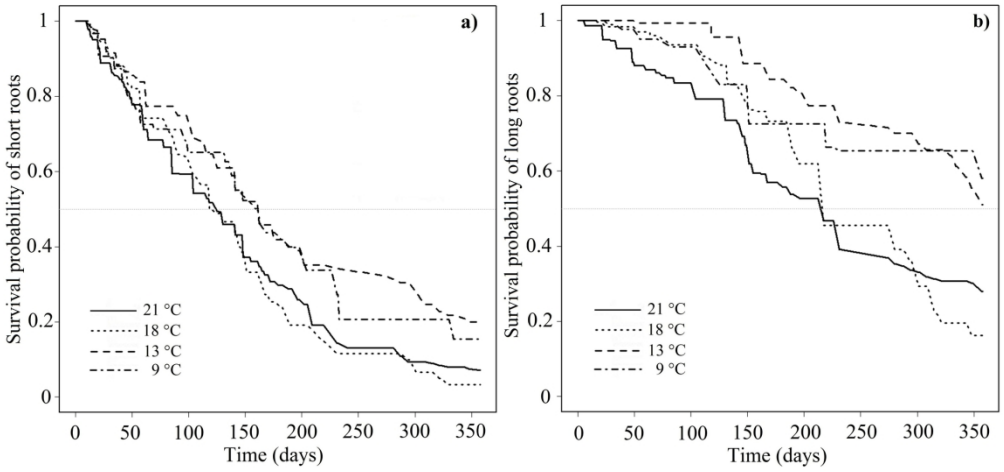

Fig. 6. Survival curves of short roots (a) and long roots (b) of Norway spruce saplings in the experiment with three growing seasons and two dormancy periods, with different soil temperatures in the second growing season. Each curve bases on four saplings. Median root longevity is obtained at survival probability of 0.5.

181x86mm (300 x 300 DPI)