ORIGINAL PAPER

A three-year increase in soil temperature and atmospheric N deposition has minor effects on the xylogenesis of mature balsam fir

Loïc D'Orangeville · Benoît Côté · Daniel Houle · Hubert Morin · Louis Duchesne

Abstract Tree growth in most boreal forests is strongly regulated by temperature and nitrogen (N) availability. The expected increases in soil temperature and N deposition over the next decades have the potential to affect the phenology of tree growth and xylogenesis. To test for these changes on xylogenesis of balsam fir (*Abies balsamea* (L.) Mill), 12 mature trees were subjected to a combination of experimentally increased soil temperature (+4 °C) with an earlier snowmelt (2–3 weeks) and N deposition (3 × ambient rain N concentrations using NH_4–NO_3 in artificial precipitation) over a 3-year period. Increased soil temperature and atmospheric N deposition had no significant effect on the number of tracheids produced (38–51), tracheid diameter (27.2–29.0 μm) and cell wall thickness (2.5–3.1 μm). For the 3 years of treatment, xylogenesis was initiated at minimum and average daily air temperatures of 0.6 ± 0.5 and 6.5 ± 0.6 °C, respectively, with inter-annual differences of 17 days in the onset of xylogenesis. The earlier snowmelt induced by soil warming did not hasten resumption of xylogenesis, and the time dynamics of xylogenesis was not affected by higher N deposition. Our results suggest that soil temperature and the timing of snowmelt have no direct influence on the breaking of cambium dormancy in balsam fir. The short-term effects of increased soil temperature and N deposition on xylogenesis of mature balsam fir appear to be small compared with the effects of air temperature and are likely to be associated with a persistent N limitation.

Keywords Radial/secondary growth · Climate change · Soil warming · Wood production · *Abies balsamea*

L. D'Orangeville (✉) · B. Côté
Department of Natural Resource Sciences, Macdonald Campus of McGill University, 21,111 Lakeshore Rd.,
Ste-Anne-de-Bellevue, QC H9X 3V9, Canada
e-mail: loic.dorangeville@mail.mcgill.ca

D. Houle · L. Duchesne
Direction de la recherche forestière, Forêt Québec, Ministère des Ressources naturelles du Québec, 2700 Einstein, Québec,
QC G1P 3W8, Canada

D. Houle
Ouranos Consortium, 550 Sherbrooke W., Montréal,
QC H3A 1B9, Canada

H. Morin
Département des sciences fondamentales, Université du Québec à Chicoutimi, 555 boul. de l'Université, Chicoutimi,
QC G7H 2B1, Canada

Introduction

Recent climate model simulations for the Eastern boreal forest of Canada predict a 3 °C temperature increase by 2041–2060 relative to 1971–1990 (Plummer et al. 2006) and a 1-month earlier snowmelt by 2050 relative to 1971–2000 (Houle et al. 2012). In N-poor ecosystems like the boreal forest, the plant response to climate change could be regulated by N availability (Magnani et al. 2007; Melillo et al. 2011). By increasing N mineralization rates and available N, warmer soils could promote growth and C sequestration in plants (Bronson et al. 2009; Jarvis and Linder 2000; Rustad et al. 2001; Strömgren and Linder 2002). Atmospheric N deposition is also increasing at the global scale and this trend is expected to continue in the next decades (+70 % by 2050; Galloway et al. 2004). Hence, atmospheric N deposition could contribute an increasing proportion of the N required for tree growth

(Magnani et al. 2007) and affect the tree response to climate change.

Understanding the interaction of additional N inputs from increased soil temperatures and increased N deposition is, therefore, of great importance to better predict forest growth. Results from the most recent studies on increased N availability suggest a positive effect on plant growth (Chapin et al. 1995; Hutchison and Henry 2010; Ma et al. 2011; Majdi and Öhrvik 2004; Mäkipää et al. 1999; Sager and Hutchinson 2005; Strömgren and Linder 2002; Zhao and Liu 2009). However, the mechanisms by which environmental factors affect wood production are not well understood (Savidge 2000). The volume of wood produced by a tree in a year is function of the length of growing season, the rate of cell division and the amount of cell expansion (Creber and Chaloner 1984). How much each of these components is affected by increasing soil temperature and N deposition is unknown, but clues can be found in studies on the separate effects of N availability and soil temperature on tree growth. The positive effect of N fertilization on the number of xylem cells produced annually is well established (Plavcová et al. 2013; Puech et al. 2000). Improving the N status of trees can also enhance the size of xylem cells due to increased photosynthetic rates and the associated increase in transpiration requirements (Beets et al. 2001; Mäkinen et al. 2002; Roderick and Berry 2001). Thus, the future increase in available N could lead boreal trees to produce larger xylem cells with thinner walls, which implies a reduction in the density and possibly the quality of the wood. Nitrogen fertilization can also affect the phenology of tree growth. Mitotic activity in the stem (Hawkins et al. 1995) and in the foliage (Rikala and Repo 1997) can be prolonged, and bud formation can be delayed following N fertilization (Bigras et al. 1996). Warmer soils, on the other hand, could have a negative effect on plant growth by inducing water stress through increased evapotranspiration (Rustad et al. 2001). Although plants could compensate with increased aquaporins activity in the roots (Maurel and Chrispeels 2001), this could lead conifers to produce smaller tracheids with thicker cell walls (Jyske et al. 2010). In addition to the above-mentioned potential effects of higher available N and warmer soils on various aspects of xylogenesis, warmer air temperatures could affect plant growth by inducing an earlier cambium reactivation in spring. Studies aimed at identifying the most important environmental factors affecting the onset of spring growth often found that air temperature was the most important factor (Begum et al. 2008, 2010; Dufour and Morin 2010; Gruber et al. 2010). Minimal temperature thresholds for cambial activity of many conifers were found to be ranging from 4 to 5 °C (Rossi et al. 2008). However, how temperature triggers cambium reactivation remains uncertain. Cell division proceeds extremely slowly at temperatures below 1–2 °C (Körner 2003). Air temperature could control the supply of some hormones (i.e. auxin, cytokinin) to the cambium (Fonti et al. 2007). Alternatively, some have hypothesized that snowmelt could be the starting signal. In some cold ecosystems, soil temperature is typically maintained at or below 0 °C during winter which inhibits water and nutrient uptake (Jarvis and Linder 2000). Experimentally delayed soil thawing has also been shown to affect the physiology of Norway spruce (*Picea abies* L. Karst.) needles (Repo et al. 2008). Several studies report cambial reactivation following snowmelt (Hoch and Körner 2003; Turcotte et al. 2009) and annual growth has been repeatedly correlated with the timing of snowmelt (Carrer et al. 2007; Jarvis and Linder 2000; Kirdyanov et al. 2003; Körner 2003; Vaganov et al. 1999). Delayed budburst has also been observed in trees subject to colder soils (Lopushinsky and Max 1990; Repo et al. 2007). However, recent results on the effects of experimental warming of forest soils suggest that soil temperature is not related to cambial reactivation in black spruce (*Picea mariana* (Mill.) Britton, Sterns & Poggenb.; Lupi et al. 2012a) and Norway spruce (Strömgren and Linder 2002). Whether other species of conifers e.g. *Abies* spp. and *Pinus* spp. respond similarly to soil warming remains unknown.

In this study, we experimentally increased soil temperature (+4 °C), hastened spring snowmelt (2–3 weeks) and increased atmospheric N deposition (+50 % of annual NH_4NO_3 in incoming precipitation during the growing season) during three years in a typical mature balsam fir stand (*Abies balsamea* (L.) Mill) of Eastern Canada. We hypothesized that (i) higher soil temperature and N deposition would increase xylem cell production, extend the duration of xylogenesis and increase the diameter of tracheids, and (ii) an earlier snowmelt would not affect the onset of xylogenesis.

Materials and methods

Study area

The study site is located in the Laurentian Mountains of eastern Québec, Canada (47°17′N; 71°14′O; 800 m above sea level). The 60-year-old even-aged stand is dominated by balsam fir with some paper birch (*Betula papyrifera* Marsh.) and white spruce (*Picea glauca* (Moench) Voss) as companion species. Stem density in 2008 was 2,024 trees ha^{-1} (diameter at breast height $\geq$9 cm) and the site index is 14 m at 50 years. The soil is a Orthic Humo-ferric podzol (spodosol) with a sandy loam texture. Annual N deposition ($NH_4 + NO_3$) averages 5.7 kg N ha^{-1} year^{-1} (Houle and Moore 2008), while mean annual air

temperature and precipitation between 1981 and 2006 averaged −0.3 °C and 1,535 mm.

Experimental design

In autumn 2008, three 12 m × 60 m blocks were laid out with the long axis perpendicular to the slope with a 12 m buffer strip in between. Each block was divided into two 24 m × 12 m subblocks separated by a 12 m × 12 m buffer zone. Each subblock was then divided into two 12 m × 12 m plots. The fertilization treatment was randomly distributed within each block, and the soil warming treatment was randomly nested within each subblock. Thus, the buffer zone within each block was set up to avoid contamination between fertilization treatments. Within each plot, a balsam fir tree was selected based on a visual estimation of good health and dominance or co-dominance in the canopy. The height and DBH of every selected tree were noted and ranged from 12.9 to 18.1 m and from 14.0 to 22.8 cm, respectively.

Around each experimental tree, heating cables (non-heating cables for the heating control) were buried in a spiral pattern 5–10 cm belowground (at the interface between the organic and the mineral layers) at 0.9–2.5 m from the base of the tree, with an average distance between cables of 30 cm. Great care was taken to avoid root damages and roots >5 mm in diameter were bypassed.

Soil temperature under the canopy of each tree was measured continuously with thermistors (precision: ±0.2 °C; model 107-L from Campbell Scientific Inc., Utah, USA) buried 5 cm belowground between cables. Dataloggers (CR1000, Campbell Scientific, Inc., Utah, USA) were used to control the activity of the warming cables and maintain an average difference of 4 °C on the basis of projections for the site for the 2070–2100 period (Houle et al. 2012). To evaluate potential effects of the burial of cables on the trees water and nutrient uptake, three nearby non-disturbed trees (control) were also monitored during the entire course of the experiment.

The rain solution used for the N fertilization treatment (fertilization control and N-enriched) was applied on the canopy using nozzles set up above each individual tree using a system of pumps and reservoirs, to simulate how atmospheric N is deposited on forest ecosystems. The composition of the artificial rain solution was based on the average ion concentration in incoming precipitation measured at the site in previous years. Deionized water in which were added Na (0.046 mg L^{-1}), Ca (0.089 mg L^{-1}), Mg (0.018 mg L^{-1}), K (0.026 mg L^{-1}), H (0.014 mg L^{-1}), Cl (0.07 mg L^{-1}) and SO_4 (1.01 mg L^{-1}) was used as rain solution. For the N treatment, 1.0 and 3.4 mg L^{-1} of NH_4^+ and NO_3^- were, respectively, added to the solution, which corresponds to approximately three times the concentrations measured in the local precipitation. Each year, from mid-June to mid-September, every tree was simultaneously subjected to a weekly artificial precipitation of 70 L, summing to 1.544 g N $tree^{-1}$ $year^{-1}$. On rainy days, watering was rescheduled to the next day.

Weather data

In addition to the soil temperature measured at the site, air temperature, and snow depth were measured in a clearing located approximately 200 m from the plots. Temperature sensors (HMP35CF, Campbell Scientific Inc., USA) recorded air temperature at a height of 3.3 m, and a sonic ranging sensor (SR-50, Campbell Scientific Inc., USA) was used to monitor the thickness of the snowpack. Measurements were made every 15 min and hourly averages recorded by the data logger (CR-1000, Campbell Scientific Inc., USA).

From the beginning of spring snowmelt until the first autumn frost, the average soil temperature in treated plots was maintained 4.0 ± 0.4, 4.1 ± 0.3 and 3.7 ± 0.9 °C higher than heating controls in 2009, 2010 and 2011, respectively (Fig. 1). In 2009, soil warming was started on April 27 with a remaining snowpack of 0.75 m. A system malfunction caused an early interruption of the soil warming in mid-August that year. In 2010, soil heating was started earlier (March 30) because of a warm spring, with a snowpack of 0.85 m. The treatment was stopped before the first freeze–thaw, on October 19. In 2011, the soil heating was started on May 5 (Fig. 1). The snowpack was then 0.60 m. Two consecutive system malfunctions reduced the temperature difference to +2.8 °C from May 17 to June 4 and to +0.7 °C from August 9 to August 23 that year. Heating was terminated on October 18 before the first freeze–thaw events.

Soil water content

Volumetric soil water content (SWC) in the plots was measured weekly during the snow-free period from 2009 to 2011. Soil water content was measured with a ThetaProbe ML1 (Delta-T Devices Ltd., Cambridge, England) in 2009 and with a FieldScout TDR-300 device with 20-cm rods and standard calibration (accuracy: ±3 % vol; Spectrum technologies Inc., Plainfield, USA) in 2010 and 2011. For each plot, nine measurements were randomly taken at a distance of 1.5 m from each tree and averaged.

Sample collection

The effect of treatments on xylogenesis was studied over 3 years (2009–2011) by extracting wood microcores (2.5 mm in diameter and 20 mm long) weekly from April
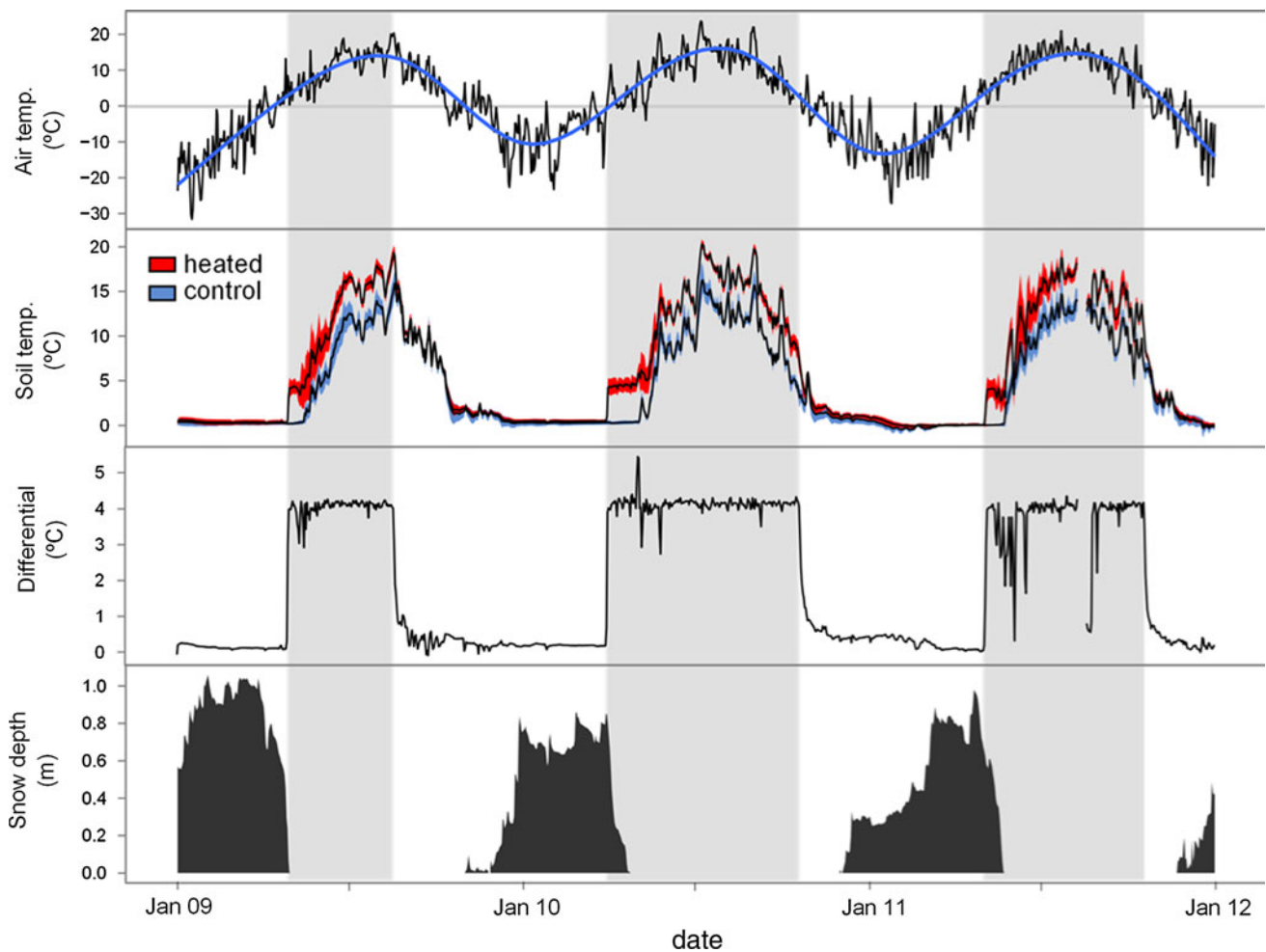
Fig. 1 Average air temperature, soil temperature and differential between heated and heating control plots, and snowpack in 2009, 2010 and 2011. *Colored ribbons* are standard error. The periods of soil warming are represented by the *shaded areas*

to October from each tree using a Trephor tool (Rossi et al. 2006). The sampling followed an upward spiral starting at 1.3 m aboveground. All samples were taken at least 10 cm apart to minimize the formation of resin ducts (Forster et al. 2000). The samples were stored at 4 °C in Eppendorf microtubes filled with a 10 % ethanol solution for tissue preservation.

Histological analyses

At the laboratory, the water from the microcores was removed through successive immersions in ethanol and Histosol™ and embedded in paraffin according to Rossi et al. (2006). Transverse sections were cut with a rotary microtome (6–10 µm in thickness), stained with cresyl violet acetate (0.16 % in water) after removal of the paraffin, and observed under visible and polarized light at a magnification of 400–500× to differentiate cells in the cambium and the differentiating xylem.

The number of cambial cells, enlarging cells, wall thickening cells and mature cells was counted along three radial files and averaged for every sample of each tree. Cambial cells were characterized by thin cell walls and less than half the diameter of enlarging cells. Both cambial and enlarging cells could be differentiated from other cell phases by the lack of secondary cell wall, a structure that shines under polarized light (Kutscha et al. 1975). Cells in the process of lignification and wall thickening showed violet and blue walls, the color blue revealing the deposition of lignin on the cell walls (Thibeault-Martel et al. 2008). Mature tracheids were characterized by completely blue cell walls.

An additional series of microcores was collected upon termination of xylogenesis, in mid-October of each year, and used for additional anatomical measurements. Following the standard preparation procedure described above, the sections were stained with safranin (1 %) and numerically photographed with a camera fixed on an

optical microscope at a magnification of 400×. Cell and lumen diameter as well as cell wall thickness were measured on three radial files per section, including pre-treatment year 2008, using Wincell software (Regent Instruments Inc.). Tracheids were classified as earlywood or latewood according to Mork's formula, where latewood cells are characterized by a lumen smaller than twice a double cell wall (Denne 1988).

Model fitting

The cumulative amount of cells produced throughout the growing season, calculated as the sum of enlarging, wall thickening and mature cells, was modelled with a Gompertz function (Rossi et al. 2003) for each tree (12) and each year (3), for a total of 36 models :

$$Y = A \exp\left[-e^{(\beta(\kappa-t))}\right]$$

where Y is the cumulative amount of cells produced, A is the upper asymptote, or annual amount of cells produced, β sets the rate of change, κ sets the x displacement, which is the date when maximal growth rate is reached, and t is the time computed in day of year (DOY). All regressions had $R^2 > 0.85$ and showed a normal distribution of the residuals (Motulsky and Ransnas 1987). The 'nlm' procedure from the R software was used with ordinary least square method for parameters estimation (R Development Core Team 2010). The beginning and ending of xylogenesis were determined as the day when the daily cell production $(Y_t - Y_{t-1})$ went above and below 0.1 cell day^{-1}, respectively. The duration of xylogenesis was calculated as the number of days between the onset and termination of cell production. Maximum growth rate was calculated as the maximum daily growth. Finally, we averaged the daily minimal and mean air temperature of the 7 days preceding the onset of xylogenesis for each tree and each year to estimate the threshold temperature for the onset of xylogenesis at the site.

Statistical methods

A linear mixed-model analysis of variance (ANOVA) with repeated measures was used to test for the effect of the warming treatment on soil water content, considering warming, year and sampling date as fixed factors and block as a random factor. A similar approach was used to detect any significant effect of treatments on the phenology of growth and cell characteristics, considering soil warming, fertilization and year as fixed factors and block as a random factor. Strong correlations of pre-treatment growth ring width (year 2008) were found with the onset of growth ($p = 0.06$) as well as with the total number of cells

produced ($p < 0.01$). Because pre-treatment growth was significantly smaller in heated-fertilized trees ($p = 0.02$; Table 2), it was used as covariate for the analysis of phenological and productivity parameters when significant at $p < 0.05$. As for the analysis of cellular morphology, no significant pre-treatment differences were detected, thus no covariates were included in the models. Similar ANOVA models were used to compare control trees with experimental trees, this time with cable burial and year as fixed factors. Data were tested a priori to meet the assumptions of ANOVA, and Tukey post hoc tests were used to identify which means differed from one another. Statistical analysis, modelling and plots were done using the R software (R Development Core Team 2010).

Results

The spring snowmelt was completed on May 16 in 2009 and on May 21 in 2011, but ended 3 weeks earlier in 2010 (April 27) because of warmer temperatures (Fig. 1). For the period of sampling (May to September), air temperature averaged 11.1 °C in 2009 compared with 12.5 °C in 2010 and 12.2 °C in 2011 (Fig. 1). In addition to its extended snow-free period, 2010 was characterized by a growing season with very low precipitations in August (38 mm compared to 122 mm in 2009 and 226 mm in 2011, data not shown). For the May to September period, 2011 was the wettest year with 747 mm in precipitation, compared to 585 mm in 2009, and 596 mm in 2010. Soil water content was significantly lower ($p < 0.05$) during the 2010 growing season (20.7 ± 0.2 %) relative to 2009 (28.1 ± 0.3 %) and 2011 (26.6 ± 0.2 %; Fig. 2). Soil water content tended to be higher in heated plots but the difference was not statistically significant ($p > 0.05$).

Growth dynamics

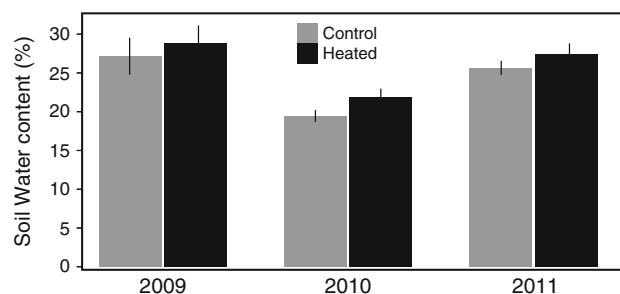For all treatment, the cambium averaged five to six cells prior to the onset of xylogenesis (Fig. 3). The number of

Fig. 2 Average soil water content in heating control and heated plots in 2009, 2010 and 2011. *Vertical lines* are standard error
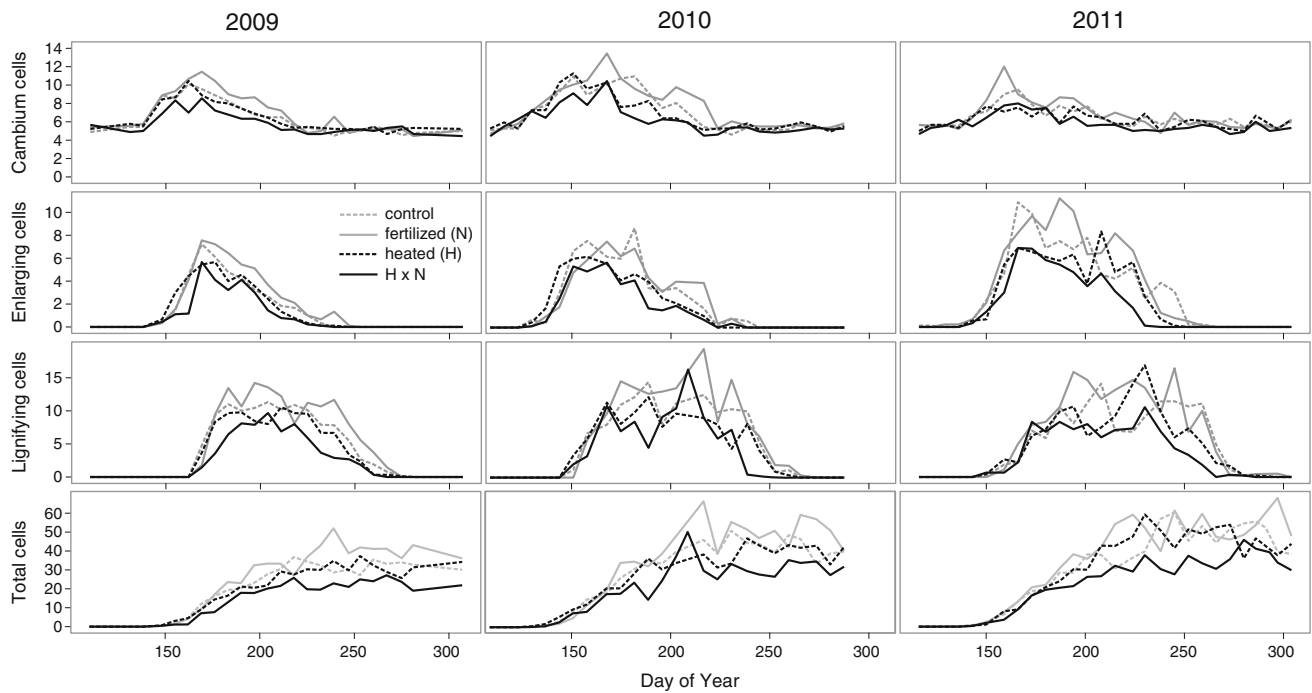
Fig. 3 Average number of cambial cells, enlarging cells, wall thickening-lignification cells and total number of cells in control, heated, fertilized and heated-fertilized balsam fir trees in 2009, 2010 and 2011

cambial cells averaged 11 ± 1 at the peak of mitosis in all treatments except fertilized-only trees with 14 ± 1 cambial cells. On average, up to 8 ± 1 and 11 ± 1 enlarging cells could be counted on a single sample in heated and non-heated trees, respectively (Fig. 3). The mean number of cells in the phase of wall thickening was 14 ± 2, 19 ± 2, 17 ± 2 and 16 ± 2 in control, fertilized, heated and heated-fertilized trees, respectively. Overall, fertilized trees produced the largest number of tracheids with an average of 68 ± 7 tracheids produced each year, followed by control (56 ± 6), heated (52 ± 5) and heated-fertilized (46 ± 5) trees.

The onset, ending and duration of xylogenesis were not significantly affected by the treatments (Table 1). The onset of cambial activity occurred significantly later in 2009 (May 29; $p < 0.01$) relative to 2010 (May 12) and 2011 (May 21; Table 1). Xylogenesis was initiated at a minimum daily air temperature of 0.8 ± 0.7, -0.5 ± 0.8 and 1.9 ± 0.8 °C in 2009, 2010 and 2011, respectively, and at average daily air temperatures of 7.4 ± 0.6, 5.3 ± 1.2 and 7.0 ± 0.8 °C in 2009, 2010 and 2011, respectively. In 2010, the maximum rate of growth was reached around June 14, 10 days earlier than in 2009 or 2011 ($p < 0.01$; Table 1). Cambial activity was maintained until September 4 in 2011, significantly later than in 2009 (August 10; $p < 0.01$) but similar to 2010 (August 29; Table 1). The overall period of cambial activity was significantly shorter in 2009 (73 days) relative to 2011

(106 days; $p < 0.01$) but similar to 2010 (108 days) due to the high variability measured that year.

Amount of tracheids produced and anatomical characteristics

There were no statistical differences in the number of tracheids produced between control trees and experimental trees ($p = 0.98$; Fig. 4). Furthermore, the average lumen diameter and wall thickness did not differ between the two groups ($p \geq 0.24$; Fig. 4). The annual production of xylem cells was not significantly different between years or treatments, with a mean annual production ranging from 38 to 51 tracheids (Table 2; Fig. 5). The average tracheid lumen diameter and wall thickness differed significantly between years ($p < 0.01$; Table 2; Fig. 5) with the average tracheid diameter displaying a similar trend ($p = 0.08$). When considering all treatments together, the tracheid lumen was significantly reduced in 2010 (21.1 ± 0.6 μm) relative to 2009 (23.5 ± 0.6 μm; $p < 0.05$) while the year 2011 did not differ from both previous years (22.9 ± 0.7 μm; Fig. 5). The average cell diameter in 2010 (27.2 ± 0.7 μm) also tended to be smaller than that of 2009 (28.6 ± 0.8 μm) and 2011 (29.0 ± 0.8 μm). The wall thickness of tracheids tended to be higher in 2011 (3.17 ± 0.03 μm) relative to 2009 (2.70 ± 0.02 μm; $p = 0.08$), while that of 2010 was intermediate (3.12 ± 0.03 μm; Fig. 5).

Table 1 Mean values (and standard error) for phenology parameters in control, heated, fertilized and heated-fertilized balsam fir trees measured in 2009, 2010 and 2011

	Onset of xylogenesis (DOY)	End of xylogenesis (DOY)	Duration (days)	Timing of maximum growth rate (DOY)
2009				
Control	147 (2)	219 (4)	72 (6)	172 (1)
Fertilized (N)	146 (2)	234 (9)	88 (11)	176 (1)
Heated (H)	144 (1)	228 (9)	84 (10)	174 (2)
H–N	159 (7)	206 (7)	47 (14)	176 (3)
2010				
Control	130 (3)	232 (10)	102 (12)	163 (1)
Fertilized (N)	133 (7)	235 (24)	102 (31)	165 (3)
Heated (H)	128 (3)	275 (45)	148 (48)	168 (4)
H–N	136 (3)	221 (13)	85 16)	164 (2)
2011				
Control	135 (2)	260 (14)	126 (16)	176 (1)
Fertilized (N)	141 (2)	239 (16)	99 (18)	174 (4)
Heated (H)	146 (2)	241 (7)	96 (6)	176 (3)
H–N	141 (7)	246 (14)	106 (20)	176 (3)
Statistical analysis				
Pre-Growth	**0.04**	0.48	0.29	0.22
Heating (H)	0.51	0.83	0.99	0.32
Fertilization (N)	0.17	0.31	0.23	0.88
H × N	0.90	0.26	0.28	0.57
Year (Y)	**<0.01**	**<0.01**	**<0.01**	**<0.01**
H × Y	0.63	0.69	0.66	0.92
N × Y	0.17	0.73	0.77	0.37
H × N × Y	**<0.01**	0.05	**0.02**	0.37

Probability values from mixed-model anovas. Significant p values at $p < 0.05$ are in bold. *DOY* day of year
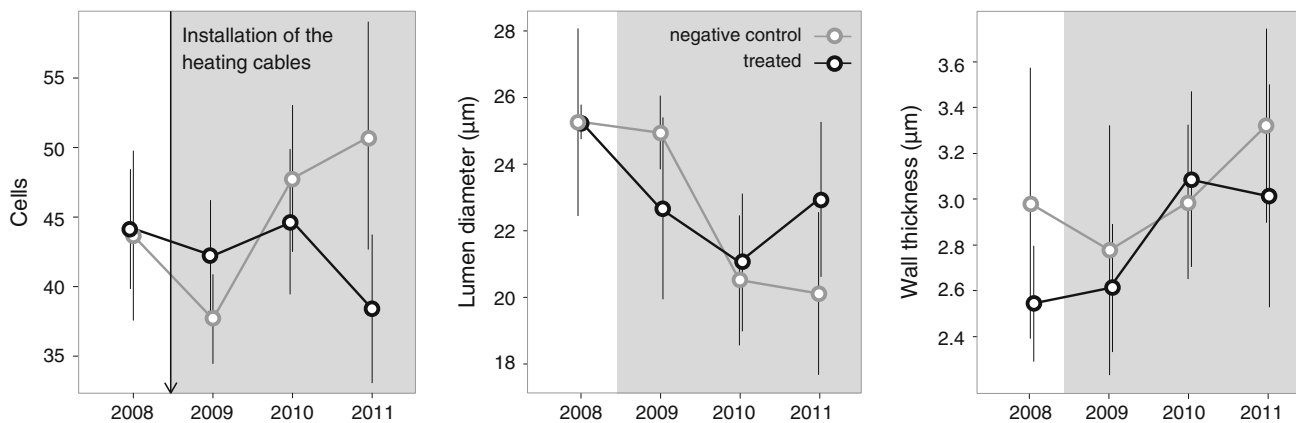
Fig. 4 Average number of tracheids, lumen diameter and wall thickness for control trees (*gray line* no cable burial) and experimental trees (*black line* cable burial) from 2008 to 2011. *Vertical bars* are standard error. The *shaded area* represents the period of treatment

The treatments had no significant effect on the average cell diameter, lumen diameter and cell wall thickness of tracheids (Table 2) or the ratio of earlywood to latewood (data not shown). The separate analysis of earlywood and latewood anatomical characteristics yielded similar results (data not shown).

Discussion

Initial root disturbance

Soil trenching was necessary to bury the heating and control cables in each experimental plot. The impact of the

Table 2 Probability values of the mixed-model anovas applied to the number of tracheids produced annually and their anatomical characteristics

	N cells	Cell diameter (μm)	Lumen diameter (μm)	Wall thickness (μm)
Previous	**<0.01**	–	–	–
Heating (H)	0.41	0.28	0.40	0.27
Fertilization (N)	0.75	0.89	0.93	0.62
H × N	0.12	0.39	0.55	0.34
Year (Y)	0.16	0.08	**<0.01**	**<0.01**
H × Y	0.99	**0.03**	**0.03**	0.51
N × Y	0.56	0.55	0.79	0.15
H × N × Y	0.95	0.61	0.51	0.68

For the analysis of the amount of tracheids produced, pre-treatment growth (2008) was used as covariate. Significant p values at $p < 0.05$ are indicated in bold
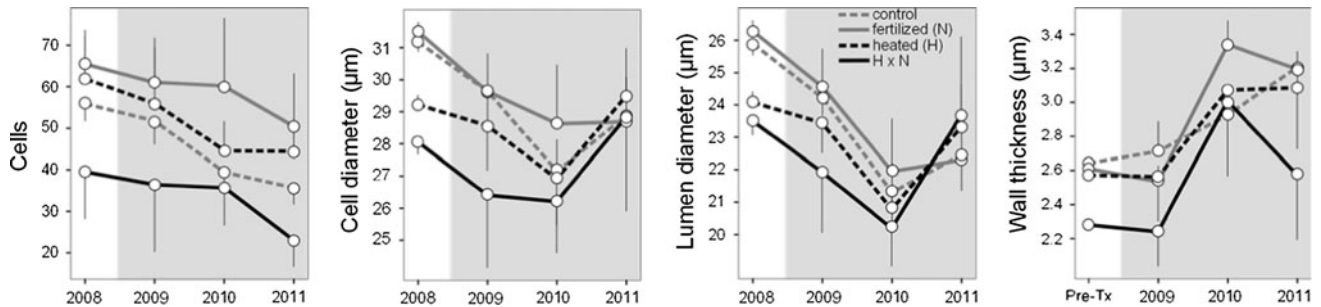

Fig. 5 Average number of tracheids, cell diameter, lumen diameter and wall thickness for control (*dotted gray line*), heated (*dotted black line*), fertilized (*full gray line*) and heated-fertilized balsam fir trees (*full black line*) from 2008 to 2011. *Vertical bars* are standard error. The *shaded area* represents the period of treatment

trenching on roots was minimized by running the cable below or above them. The effect of cable burial should, therefore, be mostly restricted to fine-roots. The average fine-root turnover rate in boreal forests is about 1.08 year^{-1} (Yuan and Chen 2010). Therefore, tree nutrient and water uptake could have been impaired during the first year of treatment. Since nutrient availability and xylem cell production are usually correlated (Plavcovà et al. 2013; Puech et al. 2000), the similar growth observed for our control trees and experimental trees suggests no significant impairment of nutrient and water uptake capacity. Cell lumen diameter and wall thickness of many conifers (Abe and Nakai 1999; Abe et al. 2003; Eilmann et al. 2009; Gruber et al. 2010; Jyske et al. 2010), including balsam fir (Rossi et al. 2009), are also known to respond to changes in water uptake. The fact that these two variables were not significantly different for our control trees and treated trees provides further evidence that the initial root disturbance did not interfere significantly with the response of trees to the experimental treatments.

Earlier snowmelt and the onset of xylogenesis

The lack of soil heating effect on the spring resumption of radial growth supports the hypothesis that soil temperature and the timing of snowmelt have no direct influence on the breaking of balsam fir cambium dormancy. Similar results

and conclusions were obtained for black spruce (Lupi et al. 2012a) and Norway spruce (Strömgren and Linder 2002) which suggests that the lack of response to earlier soil warming and rewetting on the onset of xylogenesis is likely generalized for conifers of the boreal forest. In our study, the thick snowpack generally maintained the soil slightly above the freezing point during the winter (Fig. 1). Other sites may, however, experience soil frost with concomitant effects on water availability (Jarvis and Linder 2000). Such conditions may require that snowmelt and soil thawing occur for xylogenesis to resume in the spring. If soil temperature and snowmelt are to be rejected as signals for cambium reactivation, air temperature remains the most likely triggering factor.

A strong argument for air temperature to be the main trigger of xylogenesis comes from experiments using artificial warming applied to parts of the main trunk of a tree. This treatment induced cell division and xylogenesis at various degrees for various evergreen conifer species even when a snow cover was present, (Barnett and Miller 1994; Gričar et al. 2006, 2007; Oribe et al. 2001, 2003). The preponderant effect of air temperature on spring growth onset over other environmental signals has been demonstrated in many studies (Begum et al. 2008, 2010; Deslauriers et al. 2008; Dufour and Morin 2010; Gruber et al. 2010). The recent analysis of cambial activity in conifers located at their latitudinal or altitudinal distribution limits

has revealed the existence of threshold air temperatures for radial growth of 5.6 and 8.5 °C for Swiss stone pine (*Pinus cembra* L.) and Norway spruce, respectively (Rossi et al. 2007), of 8.2 °C for Bosnian pine (*Pinus leucodermis* Antoine; Deslauriers et al. 2008), and minimal and average daily temperatures of 4–5 and 8–9 °C, respectively, for seven conifer species (balsam fir, European larch, Swiss stone pine, Scots pine, Bosnian pine, mountain pine and Norway spruce) at ten sites in Italy, Slovenia, Canada and Finland (Rossi et al. 2008). Relative to these last threshold values, xylogenesis at our site started at lower minimum and average daily temperatures of approximately 1 and 6 °C, respectively. These lower threshold temperatures could be explained in part by the different methodological approaches used to calculate the threshold values, as no logistic regressions were used in the current study to produce these numbers.

The comparison of naturally contrasting years in terms of temperature can give clues as to the growth phenology of a given tree species in a warmer world. In the current study, May 2010 was 1.1 °C warmer than the average for 1970–2008, while May 2009 was 2.2 °C colder. In 2010, cambial derivatives resumed differentiation 17 days earlier than in 2009. This result highlights the importance of air temperature for the onset of xylogenesis at our study site. Similar results have been obtained in Europe, where a warmer spring (+2.6 °C) induced a 20-day advanced onset of radial growth in Bosnian pine (Deslauriers et al. 2008).

The effect of increased soil temperature and N deposition

The average five to six cells measured in the dormant cambium in our study are consistent with the range of values observed in other balsam fir trees of Eastern Canada (Riding and Little 1986; Thibault-Martel et al. 2008). Upon cambial reactivation, trees from the current study produced 1.6–2 times the number of differentiating xylem cells than balsam fir trees growing in a colder and dryer boreal stand of Québec, Canada (Deslauriers et al. 2003), although the overall number of tracheids produced (38–51) was not higher than the mean number of tracheids observed in balsam fir trees from the colder site (Deslauriers and Morin 2005; Rossi et al. 2003). Because the cell division rates in the cambium are mainly driven by temperature (Körner, 2003), the warmer temperature at the study site could explain that a higher number of cells were differentiating simultaneously although the total number of cells produced was the same. The phenology of growth was also similar to previous studies, with xylogenesis starting in early to late May (Deslauriers et al. 2003; Thibeault-Martel et al. 2008) and ending in late August (Deslauriers et al. 2003).

Contrary to our hypothesis, neither treatment significantly affected the course of xylogenesis, the number of tracheids produced or their anatomy. The application of similar treatments to black spruce trees in Québec also yielded no effects on the number of tracheids produced although increases in cell wall thickness were noted (Lupi et al. 2012b). The combination of increased CO_2 and air temperature in a pine-birch forest of Norway also produced no significant effect on the number of cells (Rasmussen et al. 2002). It was suggested that the availability of N limited the response to increased CO_2 and air temperature. In our study, we hypothesized that the heating treatment would affect xylogenesis through its effects on N mineralization/availability and water availability. However, the effects of the treatments on soil inorganic N at our site were negligible (D'Orangeville et al. 2013). Although current forest carbon models predict positive effects from similar treatments on tree growth (Mäkipää et al. 1999), boreal forest soils are known to immobilize a large fraction of N inputs at the expense of the aboveground vegetation (Berg and Dise 2004; Friedrich et al. 2011; Houle and Moore 2008; Nadelhoffer et al. 1999). The existence of such N sinks at the study site was confirmed with a short-term ^{15}N isotopic pool dilutions approach, as almost all of the NH_4 and NO_3 made available in the forest floor was immobilized in less than a day (Ste-Marie and Houle 2006). Alternatively, balsam fir trees have been shown to intercept with their canopy a fraction of the nutrients deposited from the atmosphere, thereby avoiding soil competition (Houle et al. 1999). However, the lack of effects from 3 years of increased N deposition on xylogenesis suggests that this nutritional pathway has a limited effect on radial growth of balsam fir.

As for water, soil warming did not decrease its availability in the soil. The size of tracheids and the thickness of their wall usually respond to water stress (Beth Kirkham et al. 1972; Deri Tomos et al. 1989). Balsam fir is no exception, as the smaller lumen diameter and thicker walls of tracheids produced in 2010 are probably linked to the reduced water availability measured that year. However, no anatomical changes were detected in the tracheids wall thickness or lumen diameter of trees in heated plots, which confirms the absence of a water stress caused by the treatment. Although control trees did not display any signs of water deficiency in 2010 based on their xylogenesis, we cannot exclude that the additional irrigation applied evenly in all plots limited the negative impact of the 2010 water shortage as well as the higher soil temperature forcing on evapotranspiration rates.

Considering the realistic scenarios of increased soil temperature and N deposition used in our study and the lack of effect they had on the number of tracheids produced and their anatomical characteristics, the potential changes

in future xylogenesis—and hence on radial growth—due to higher N uptake could be smaller than expected for similar boreal forests. The absence of effects from the combination of increased soil temperature and N deposition in the current study does not exclude that effects could arise on the longer term in the presence of co-occurring changes in other climatic variables such as increased air temperature, reduced water availability and increased CO_2 concentrations (Bonan 2008). In addition, it should be noted that due to the high variability of the stand under study, our capacity to detect significant treatment effects would have benefited from a larger sample size.

Conclusions

The earlier warming and rewetting of soil in the spring did not hasten the differentiation of xylem cells of balsam fir. This study supports the hypothesis that air temperature is the main trigger of cambial cell differentiation in conifers. The limited response of xylogenesis of balsam fir to the realistic scenario of increased soil temperature and N deposition used in our study suggests that the impact of these two components of global change could be small for this species in the short-term. The influence of soil temperature on radial growth could, therefore, be limited to indirect effects on available soil nutrients which are more likely to respond significantly on more productive sites.

Conflict of interest The authors declare that they have no conflict of interest.

References

Abe H, Nakai T (1999) Effect of the water status within a tree on tracheid morphogenesis in *Cryptomeria japonica* D. Don. Trees 14:124–129

Abe H, Nakai T, Utsumi Y, Kagawa A (2003) Temporal water deficit and wood formation in *Cryptomeria japonica*. Tree Physiol 23:859–863

Barnett JR, Miller H (1994) The effect of applied heat on graft union formation in dormant *Picea sitchensis* (Bong.) Carr. J Exp Bot 45:135–143

Beets PN, Gilchrist K, Jeffreys MP (2001) Wood density of radiata pine: effect of nitrogen supply. For Ecol Manage 145:173–180

Begum S, Nakaba S, Bayramzadeh V, Oribe Y, Kubo T, Funada R (2008) Temperature responses of cambial reactivation and xylem differentiation in hybrid poplar (*Populus sieboldii x P. grandidentata*) under natural conditions. Tree Physiol 28:1813–1819

Begum S, Nakaba S, Oribe Y, Kubo T, Funada R (2010) Cambial sensitivity to rising temperatures by natural condition and artificial heating from late winter to early spring in the evergreen conifer *Cryptomeria japonica*. Trees 24:43–52

Berg B, Dise N (2004) Calculating the long-term stable nitrogen sink in northern European forests. Acta Oecol 26:15–21

Beth Kirkham M, Gardner WR, Gerloff GC (1972) Regulation of cell division and cell enlargement by turgor pressure. Plant Physiol 49:961–962

Bigras FJ, Gonzalez A, D'Aoust AL, Hébert C (1996) Frost hardiness, bud phenology and growth of containerized *Picea mariana* seedlings grown at three nitrogen levels and three temperature regimes. New Forest 12:243–259

Bonan GB (2008) Forests and climate change: forcings, feedbacks, and the climate benefits of forests. Science 320:144–1449

Bronson DR, Gower ST, Tanner M, Van Herk I (2009) Effect of ecosystem warming on boreal black spruce bud burst and shoot growth. Glob Change Biol 15:1534–1543

Carrer M, Nola P, Eduard JL, Motta R, Urbinati C (2007) Regional variability of climate–growth relationships in *Pinus cembra* high elevation forests in the Alps. J Ecol 95:1072–1083

Chapin FS, Shaver GR, Giblin AE, Nadelhoffer KJ, Laundre JA (1995) Responses of Arctic tundra to experimental and observed changes in climate. Ecol 76:694–712

Creber G, Chaloner W (1984) Influence of environmental factors on the wood structure of living and fossil trees. Bot Rev 50:357–448

Denne MP (1988) Definition of latewood according to Mork (1928). IAWA J 10:59–62

Deri Tomos A, Malone M, Pritchard J (1989) The biophysics of differential growth. Environ Exp Bot 29:7–23

Deslauriers A, Morin H (2005) Intra-annual tracheid production in balsam fir stems and the effect of meteorological variables. Trees 19:402–408

Deslauriers A, Morin H, Begin Y (2003) Cellular phenology of annual ring formation of *Abies balsamea* in the Quebec boreal forest (Canada). Can J For Res 33:190–200

Deslauriers A, Rossi S, Anfodillo T, Saracino A (2008) Cambial phenology, wood formation and temperature thresholds in two contrasting years at high altitude in southern Italy. Tree Physiol 28:863–871

D'Orangeville L, Houle D, Côté B, Duchesne L, Morin H (2013) Increased soil temperature and atmospheric N deposition have no effect on the N status and growth of a mature balsam fir forest. Biogeosciences (in press)

Dufour B, Morin H (2010) Tracheid production phenology of *Picea mariana* and its relationship with climatic fluctuations and bud development using multivariate analysis. Tree Physiol 30:853–865

Eilmann B, Zweifel R, Buchmann N, Fonti P, Rigling A (2009) Drought-induced adaptation of the xylem in Scots pine and pubescent oak. Tree Physiol 29:1011–1020

Fonti P, Solomonoff N, García-González I (2007) Earlywood vessels of *Castanea sativa* record temperature before their formation. New Phytol 173:562–570

Forster T, Schweingruber FH, Denneler B (2000) Increment puncher: a tool for extracting small cores of wood and bark from living trees. IAWA J 21:169–180

Friedrich UTA, Falk K, Bahlmann E, Marquardt T, Meyer H, Niemeyer T, Schemmel S, von Oheimb G, Härdtle W (2011) Fate of airborne nitrogen in heathland ecosystems: a ^{15}N tracer study. Glob Change Biol 17:1549–1559

Galloway JN, Dentener FJ, Capone DG, Boyer EW, Howarth RW, Seitzinger SP, Asner GP, Cleveland CC, Green PA, Holland EA, Karl DM, Michaels AF, Porter JH, Townsend AR, Vöosmarty CJ

(2004) Nitrogen cycles: past, present, and future. Biogeochem 70:153–226

Gričar J, Zupančič M, Čufar K, Koch G, Schmitt U, Oven P (2006) Effect of local heating and cooling on cambial activity and cell differentiation in the stem of Norway Spruce (*Picea abies*). Ann Bot 97:943–951

Gričar J, Zupančič M, Čufar K, Oven P (2007) Regular cambial activity and xylem and phloem formation in locally heated and cooled stem portions of Norway spruce. Wood Sci Tech 41:463–475

Gruber A, Strobl S, Veit B, Oberhuber W (2010) Impact of drought on the temporal dynamics of wood formation in *Pinus sylvestris*. Tree Physiol 30:490–501

Hawkins BJ, Davradou M, Pier D, Shortt R (1995) Frost hardiness and winter photosynthesis of *Thuja plicata* and *Pseudotsuga menziesii* seedlings grown at three rates of nitrogen and phosphorus supply. Can J For Res 25:18–28

Hoch G, Körner C (2003) The carbon charging of pines at the climatic treeline: a global comparison. Oecologia 135:10–21

Houle D, Moore JD (2008) Soil solution, foliar concentrations and tree growth response to 3-year of ammonium-nitrate addition in two boreal forests of Québec, Canada. For Ecol Manage 255:2049–2060

Houle D, Ouimet R, Paquin R, Laflamme JG (1999) Interactions of atmospheric deposition with a mixed hardwood and a coniferous forest canopy at the Lake Clair Watershed (Duchesnay, Quebec). Can J For Res 29:1944–1957

Houle D, Bouffard A, Duchesne L, Logan T, Harvey R (2012) Projections of future soil temperature and water content for three southern Quebec forested sites. J Clim 25:7690–7701

Hutchison J, Henry H (2010) Additive effects of warming and increased nitrogen deposition in a temperate old field: plant productivity and the importance of winter. Ecosystems 13:661–672

Jarvis P, Linder S (2000) Botany: constraints to growth of boreal forests. Nature 405:904–905

Jyske T, Hölttä T, Mäkinen H, Nöjd P, Lumme I, Spiecker H (2010) The effect of artificially induced drought on radial increment and wood properties of Norway spruce. Tree Physiol 30:103–115

Kirdyanov A, Hughes M, Vaganov E, Schweingruber F, Silkin P (2003) The importance of early summer temperature and date of snow melt for tree growth in the Siberian Subarctic. Trees 17:61–69

Körner C (2003) Carbon limitation in trees. J Ecol 91:4–17

Kutscha NP, Hyland F, Schwarzmann JM (1975) Certain seasonal changes in balsam fir cambium and its derivatives. Wood Sci Tech 9:175–188

Lopushinsky W, Max TA (1990) Effect of soil temperature on root and shoot growth and on budburst timing in conifer seedling transplants. New Forest 4:107–124

Lupi C, Morin H, Deslauriers A, Rossi S (2012a) Xylogenesis in black spruce: does soil temperature matter? Tree Physiol 32:74–82

Lupi C, Morin H, Deslauriers A, Rossi S, Houle D (2012b) Increasing nitrogen availability and soil temperature: effects on xylem phenology and anatomy of mature black spruce. Can J For Res 42:1277–1288

Ma LN, Lü XT, Liu Y, Guo JX, Zhang NY, Yang JQ, Wang RZ (2011) The effects of warming and nitrogen addition on soil nitrogen cycling in a temperate grassland, northeastern China. PLoS ONE 6:e27645

Magnani F, Mencuccini M, Borghetti M, Berbigier P, Berninger F, Delzon S, Grelle A, Hari P, Jarvis PG, Kolari P, Kowalski AS, Lankreijer H, Law BE, Lindroth A, Loustau D, Manca G, Moncrieff JB, Rayment M, Tedeschi V, Valentini R, Grace J

(2007) The human footprint in the carbon cycle of temperate and boreal forests. Nature 447:849–851

Majdi H, Öhrvik J (2004) Interactive effects of soil warming and fertilization on root production, mortality, and longevity in a Norway spruce stand in Northern Sweden. Glob Change Biol 10:182–188

Mäkinen H, Saranpää P, Linder S (2002) Wood-density variation of Norway spruce in relation to nutrient optimization and fibre dimensions. Can J For Res 32:185–194

Mäkipää R, Karjalainen T, Pussinen A, Kellomäki S (1999) Effects of climate change and nitrogen deposition on the carbon sequestration of a forest ecosystem in the boreal zone. Can J For Res 29:1490–1501

Maurel C, Chrispeels MJ (2001) Aquaporins. A molecular entry into plant water relations. Plant Physiol 125:135–138

Melillo JM, Butler S, Johnson J, Mohan J, Steudler P, Lux H, Burrows E, Bowles F, Smith R, Scott L, Vario C, Hill T, Burton A, Zhou YM, Tang J (2011) Soil warming, carbon-nitrogen interactions, and forest carbon budgets. Proc Nat Acad Sci 108:9508–9512

Motulsky H, Ransnas L (1987) Fitting curves to data using nonlinear regression: a practical and nonmathematical review. FASEB J 1:365–374

Nadelhoffer K, Downs M, Fry B, Magill A, Aber J (1999) Controls on N retention and exports in a forested watershed. Environ Monit Assess 55:187–210

Oribe Y, Funada R, Shibagaki M, Kubo T (2001) Cambial reactivation in locally heated stems of the evergreen conifer *Abies sachalinensis* (Schmidt) Masters. Planta 212:684–691

Oribe Y, Funada R, Kubo T (2003) Relationships between cambial activity, cell differentiation and the localization of starch in storage tissues around the cambium in locally heated stems of *Abies sachalinensis* (Schmidt) Masters. Trees 17:185–192

Plavcovà L, Hacke UG, Almeida-Rodriguez AM, Li E, Douglas CJ (2013) Gene expression patterns underlying changes in xylem structure and function in response to increased nitrogen availability in hybrid poplar. Plant Cell Environ 36:186–199

Plummer DA, Caya D, Frigon A, Côté H, Giguère M, Paquin D, Biner S, Harvey R, de Elia R (2006) Climate and climate change over North America as Simulated by the Canadian RCM. J Clim 19:3112–3132

Puech L, Türk S, Hodson J, Fink S (2000) Wood formation in hybrid aspen (*Populus tremula* L. x *Populus tremuloides* Michx.) grown under different nitrogen regimes. In: Savidge RA, Barnett JR, Napier R (eds) Cell & molecular biology of wood formation. BIOS Scientific Publishers Ltd., Oxford, pp 141–153

R Development Core Team (2010) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna

Rasmussen L, Beier C, Bergstedt A (2002) Experimental manipulations of old pine forest ecosystems to predict the potential tree growth effects of increased CO_2 and temperature in a future climate. For Ecol Manage 158:179–188

Repo T, Sutinen S, Nöjd P, Mäkinen H (2007) Implications of delayed soil thawing on trees: a case study of a *Picea abies* stand. Scand J For Res 22:118–127

Repo T, Lehto T, Finér L (2008) Delayed soil thawing affects root and shoot functioning and growth in Scots pine. Tree Physiol 28:1583–1591

Riding RT, Little CHA (1986) Histochemistry of the dormant vascular cambium of Abies balsamea: changes associated with tree age and crown position. Can J Bot 64:2082–2087

Rikala R, Repo T (1997) The effect of late summer fertilization on the frost hardening of second-year Scots pine seedlings. New Forest 14:33–44

Roderick ML, Berry SL (2001) Linking wood density with tree growth and environment: a theoretical analysis based on the motion of water. New Phytol 149:473–485

Rossi S, Deslauriers A, Morin H (2003) Application of the Gompertz equation for the study of xylem cell development. Dendrochronologia 21:33–39

Rossi S, Anfodillo T, Menardi R (2006) Trephor: a new tool for sampling microcores from tree stems. IAWA J 27:89–97

Rossi S, Deslauriers A, Anfodillo T, Carraro V (2007) Evidence of threshold temperatures for xylogenesis in conifers at high altitudes. Oecologia 152:1–12

Rossi S, Deslauriers A, Gricar J, Seo JW, Rathgeber CBK, Anfodillo T, Morin H, Levanic T, Oven P, Jalkanen R (2008) Critical temperatures for xylogenesis in conifers of cold climates. Glob Ecol Biogeogr 17:696–707

Rossi S, Simard S, Rathgeber C, Deslauriers A, De Zan C (2009) Effects of a 20-day-long dry period on cambial and apical meristem growth in Abies balsamea seedlings. Trees 23:85–93

Rustad LE, Campbell JL, Marion GM, Norby RJ, Mitchell MJ, Hartley AE, Cornelissen JHC, Gurevitch J, GCTE-NEWS (2001) A meta-analysis of the response of soil respiration, net nitrogen mineralization, and aboveground plant growth to experimental ecosystem warming. Oecologia 126:543–562

Sager EPS, Hutchinson TC (2005) The effects of UV-B, nitrogen fertilization, and springtime warming on sugar maple seedlings and the soil chemistry of two central Ontario forests. Can J For Res 35:2432–2446

Savidge RA (2000) Intrinsic regulation of cambial growth. J Plant Growth Regul 20:52–77

Ste-Marie C, Houle D (2006) Forest floor gross and net nitrogen mineralization in three forest types in Quebec, Canada. Soil Biol Biochem 38:2135–2143

Strömgren M, Linder S (2002) Effects of nutrition and soil warming on stemwood production in a boreal Norway spruce stand. Glob Change Biol 8:1194–1204

Thibeault-Martel M, Krause C, Morin H, Rossi S (2008) Cambial activity and intra-annual xylem formation in roots and stems of Abies balsamea and Picea mariana. Ann Bot 102:667–674

Turcotte A, Morin H, Krause C, Deslauriers A, Thibeault-Martel M (2009) The timing of spring rehydration and its relation with the onset of wood formation in black spruce. Agr Forest Meteorolog 149:1403–1409

Vaganov EA, Hughes MK, Kirdyanov AV, Schweingruber FH, Silkin PP (1999) Influence of snowfall and melt timing on tree growth in subarctic Eurasia. Nature 400:149–151

Yuan ZY, Chen HYH (2010) Fine root biomass, production, turnover rates, and nutrient contents in boreal forest ecosystems in relation to species, climate, fertility, and stand age: literature review and meta-analyses. Crit Rev Plant Sci 29:204–221

Zhao C, Liu Q (2009) Growth and photosynthetic response of two coniferous species to experimental warming and nitrogen fertilization. Can J For Res 39:1–11