Research paper

Soil warming increased whole-tree water use of *Pinus cembra* at the treeline in the Central Tyrolean Alps

Gerhard Wieser[1,4], Thorsten E.E. Grams[2], Rainer Matyssek[2], Walter Oberhuber[3] and Andreas Gruber[3]

[1]Department of Alpine Timberline Ecophysiology, Federal Research and Training Centre for Forests, Natural Hazards and Landscape (BFW), Rennweg 1, A-6020 Innsbruck, Austria; [2]Ecophysiology of Plants, Department of Ecology and Ecosystem Management, Technische Universität München, Von-Carlowitz-Platz 2, 85354 Freising, Germany; [3]Institute of Botany, Leopold-Franzens-Universität Innsbruck, Sternwartestraße 15, A-6020 Innsbruck, Austria; [4]Corresponding author (Gerhard.Wieser@uibk.ac.at)

This study quantified the effect of soil warming on sap flow density (Q_s) of *Pinus cembra* L. at the treeline in the Central Tyrolean Alps. To enhance soil temperature we installed a transparent roof construction above the forest floor around six trees. Six other trees served as controls in the absence of any manipulation. Roofing enhanced growing season mean soil temperature by 1.6, 1.3 and 1.0 °C at 5, 10 and 20 cm soil depth, respectively, while soil water availability was not affected. Sap flow density (using Granier-type thermal dissipation probes) and environmental parameters were monitored throughout three growing seasons. During the first year of treatment, no warming effect was detected on Q_s. However, soil warming caused Q_s to increase significantly by 11 and 19% above levels in control trees during the second and third year, respectively. This effect appeared to result from warming-induced root production, a reduction in viscosity and perhaps an increase also in root hydraulic conductivity. Hardly affected were leaf-level net CO_2 uptake rate and conductance for water vapour, so that water-use efficiency stayed unchanged as confirmed by needle $\delta^{13}C$ analysis. We conclude that tree water loss will increase with soil warming, which may alter the water balance within the treeline ecotone of the Central Austrian Alps in a future warming environment.

Keywords: cembran pine, climate change, leaf conductance, sap flow, soil temperature manipulation.

Introduction

High-altitude treelines have raised concern as they may undergo significant ecological alterations through warming under climate change (Walther et al. 2005, Holtmeier and Broll 2007, Wieser et al. 2009). During the last century the global surface temperature has increased by about 0.6 ± 0.2 °C (Jones et al. 1988), and global change models predict further a increase by 1.4–5.8 °C for upcoming decades (IPCC 2013). Changes appear to become most pronounced at high altitude (Beniston et al. 1997, Diaz and Bradley 1997). At the treeline of the central Austrian Alps, the mean annual air temperature during the last 10 years (2000–10) was enhanced on an average by 0.8 °C (i.e., to 3.0 °C) when compared with the mean over the previous 38 years (2.2 °C). Temperature increase apparently was most pronounced during spring (by 1.5 °C) and summer (by 1.4 °C)

compared with autumn (by 0.6 °C) and winter (by 0.2 °C), implying early snow melt in spring. As a consequence, the growing season has increased by 4 ± 1 weeks during the last three decades (Wieser 2012). In contrast to temperature, however, annual precipitation has not changed significantly during the past 48 years.

Although effects of climate change on forest ecosystems are uncertain, Wieser et al. (2009) suggested that warming rather than the rising atmospheric CO_2 level will cause alterations in the structure and ecological functioning of the treeline ecotone in the Central Austrian Alps. Increasing air temperature in the snow-dominated regions of treelines in the Central Austrian Alps is expected to change ecosystem characteristics with associated biogeochemical and hydrological cycling (Barnett et al. 2005). In the Central Austrian Alps, summer is the main season of rainfall,

with it occurring every third to fourth day on average (Wieser 2012). Ample precipitation and moderate evaporative demand generally cause soil water content to be sufficiently high to meet the trees' water demand within the treeline ecotone (Tranquillini 1979). Because soil moisture at the treeline in the Central Austrian Alps seldom drops below 20% vol. (Wieser et al. 2009), treeline trees are rarely forced to restrict transpiration during the growing season (Tranquillini 1979, Mayr 2007, Matyssek et al. 2009). Under ample soil water availability upon climate warming, the water uptake capacity will possibly be enlarged through growth stimulation of the mycorrhizal root system and enlargement of the belowground water-absorbing surface. Additionally, water uptake will also be improved by rising soil temperature through lowered water viscosity (Kaufmann 1975), an increased permeability of root membranes (Goldstein et al. 1985, Wan and Zwiazek 1999, Wan et al. 2001) and aquaporin-mediated changes in root conductivity (McElrone et al. 2007, Ruggiero et al. 2007).

Although water fluxes between forest ecosystems and atmosphere have been studied intensively, the reasons for the intra-annual variability of transpiration and responses to extreme meteorological conditions such as during the dry summer of 2003 (Reichstein et al. 2002, Breda et al. 2006, Granier et al. 2007) still await clarification for conifers at the treeline. In situ manipulation of soil temperature is a common methodology for assessing potential effects of warming in forest ecosystems, making use of heating cables (Bergh and Linder 1999, Strömgren and Linder 2002), open-top chambers (Danby and Hik 2007), closed-top chambers (Kellomäki and Wang 1998) or greenhouses (Bronson and Gower 2010, Van Herk et al. 2011). Although experimental soil warming with heating cables or greenhouses provides a high degree of temperature control, such approaches cannot be used in remote areas of the treeline ecotone because of high energy requirements. Thus, soil roofing is a potential alternative to investigate the effect of soil temperature on tree-hydrological dynamics. We tested the hypothesis that a slight increase in soil temperature in the absence of other soil disturbance will result in increased canopy transpiration of *Pinus cembra* L. at the treeline in the Central Tyrolean Alps. Experimental soil warming was incited by roofing throughout three consecutive growing seasons while continuously monitoring sap flow density (Q_s) with thermal dissipation probes (Granier 1985). Findings are used to explore tree response in a future warmer environment within the treeline ecotone of the Central Austrian Alps.

Materials and methods

Study site

The study was carried out in a south-exposed *P. cembra* afforestation at the treeline above Haggen near St Sigmund in the Sellrain Valley, Tyrol, Austria at 2150 m above sea level (a.s.l.; 47°12′42″N, 11°05′04″E). Aspect and slope angle were SSW and 25°, respectively (Kronfuss 1997). During the study period

(2011–13) trees were ~25 years old. The stand formed a sparsely open canopy permitting dense understory vegetation comprising dwarf shrubs and herbaceous species. The stand density was 2117 trees ha^{-1} and the basal stem area was 13.7 m^2 ha^{-1}.

According to a 20-year record of meteorological data (1975–94) at a weather station nearby (1800 m a.s.l.; Kronfuss 1997), the mean annual temperature was 3.2 °C, with February as the coldest ($T_{mean} = -3.5$ °C) and July as the warmest month ($T_{mean} = 10.7$ °C). The mean annual precipitation was 909 mm with the majority of rain falling during the growing season from May through October (610 mm). Gneisses and mica schist dominated the geology at the study site. According to the World Base for Soil Resources (FAO 1998), the soil at the study site was classified as a podzolic cambisol (Neuwinger 1972). A humus layer of 5 cm thickness covered the loamy sand subsoil. The top 25 cm of the subsoil was enriched by $8 \pm 3\%$ of organic matter and the soil texture was dominated by sand ($71.3 \pm 1.4\%$) and silt fractions ($27.8 \pm 1.3\%$) with varying clay contribution ($1.0 \pm 0.1\%$). Hydraulic field capacity was 0.24 ± 0.08 m^3 m^{-3} at -0.033 MPa (sensu Blume et al. 2010), and the wilting point (at -1.5 MPa) was reached at 0.07 ± 0.01 m^3 m^{-3}.

Experimental design

Soil temperature manipulation was accomplished by roofing the forest floor according to the approach by Gruber et al. (2010). Roofs needed to withstand high wind velocity without considerably influencing the aboveground microclimate and soil water availability in the rooting zone. They consisted of frames anchored into the soil, covered with a 0.5-mm thick transparent polyvinyl skin. They were installed underneath the canopy at 15 cm above ground around the stems of six individual trees (hereafter 'warmed' trees). The area covered around each tree was 16 m^2 at least. An identical number of *P. cembra* trees served as control in the absence of soil temperature manipulation ('control' trees).

For practical reasons and to facilitate logistics of soil temperature and soil moisture monitoring (see below), three adjacent trees per treatment were assembled into a plot, resulting in four plots in total. Surface and slope run-off of water was allowed to penetrate the soil (in total 67% of precipitation; Neuwinger et al. 1988) during and after rainfall. Soil warming was operated in 2011 during July through October and continued throughout each snow-free period (May–October) during 2012 and 2013.

Environmental, sap flow density measurements and tree characteristics

Air temperature (T_{air}), relative humidity (CS215, Campbell Scientific, Shepshed, UK), solar radiation (R_s; SP1110, Campbell Scientific), wind velocity (v; A100R, Campbell Scientific) and precipitation (P; ARG100, Campbell Scientific) were monitored at 2 m aboveground. In order to determine seasonal differences in

soil temperature (T_{soil}) and volumetric soil water content (θ) between control and roofed plots, six soil temperature sensors (T 107 Probe, Campbell Scientific) and two soil moisture probes (EC-5, Decagon Devices, Inc., Pullman, WA, USA) were installed in each plot close to the trees used for sap flow measurements. While T_{soil} was measured at 5, 10 and 20 cm of soil depth (two sensors per plot and depth each), θ was measured at 10 cm. Data were transmitted to an AM16/32 multiplexer and recorded with a CR1000 data logger (both Campbell Scientific) programmed to record 30-min averages of measurements taken by 1-min intervals.

Sap flow density (Q_s) of the selected study trees was monitored by means of the heat dissipation approach according to Granier (1985), using three-channel battery-operated sap flow systems (M1 Sapflow Systems PROSA-LOG; UP, Umweltanalytische Produkte GmbH, Cottbus, Germany). Each system consisted of a three-channel PROSA-LOG datalogger, providing one constant source of electrical power (0.2 W) to heat three sensors each. Each sensor consisted of two probes of 20 mm in length and 2 mm in diameter each. The upper probe of each sensor included the heater, whereas the lower probe was unheated, remaining at trunk temperature for reference. The probes were inserted 15 cm apart from each other in the vertical direction into the outer 20 mm of the hydroactive xylem on the north-facing side of trees (Ewers and Oren 2000). Positions were below the transpiring foliage at ~25 cm aboveground to prevent sun exposure (Wieser et al. 2013). Probe positions, in addition, were fully insulated with a thick aluminium-coated foam cover to prevent any direct radiation while providing mechanical protection. The temperature difference between the upper heated probe and the lower reference probe was recorded every 30 min. Two car batteries (12 VDC, 90 Ah), which were recharged by means of an 80 W solar panel in combination with a charge controller each, provided power for all electrical equipment. For each tree Q_s (g m^{-2} s^{-1}) was calculated from the temperature difference between the two probes (ΔT) relative to the maximum temperature difference (ΔT_m), which occurred at times of zero flow according to the calibration equation by Granier (1987):

$$Q_s = 119 \times \left[\frac{\Delta T_m - \Delta T}{\Delta T} \right]^{1.231} \qquad (1)$$

Each night ΔT_m was determined and used as a reference for the following day. The underlying assumption of zero sap fluxes during the night is justified as vapour pressure deficits were mostly low. In addition, during most nights temperature time courses reached equilibrium between both sensors, suggesting complete refilling of tree-internal water storage. Environmental data and Q_s were monitored continuously throughout the growing seasons 2011 (17 June to 11 October), 2012 (15 May to 4 October) and 2013 (2 May to 8 October).

Tree characteristics, including stem diameter at height of sensor installation (D_s), projected crown area, 100-needle dry weight and specific leaf area (SLA) were determined in fall 2013. In *P. cembra*, heartwood and sapwood are clearly distinguishable on the basis of colour. Sapwood cross-sectional area was established from D_s based on measurements along increment cores (5 mm in diameter) taken at sensor height, according to the equation:

$$A_s = 7.89 \times \exp(0.223 \times D_s), \quad R^2 = 0.99 \qquad (2)$$

Gas exchange measurements

Needle CO_2/H_2O gas exchange was measured in situ with a portable measurement system (CIRAS 1, PP Systems, Hitchin, Hertfordshire, UK) equipped with a climate-controlled PLC6 leaf chamber, providing standardized conditions of 20 °C air temperature, 50% relative air humidity and 370 μmol CO_2 mol^{-1}. An LED light unit was used to maintain photosynthetic photon flux density of 1500 μmol m^{-2} s^{-1} at the needle surface. In each study tree gas exchange of current-year and 1-year-old needles of sun-exposed twigs was measured repeatedly during the growing season of 2012 (on 24 and 26 July, and 9 August). Gas exchange data were related to projected leaf area.

Stable carbon isotopes

Sun-exposed current-year needles were sampled from all study trees during late-August of 2012 and 2013 each for stable carbon isotope analysis. Needles were dried at 60 °C, ground to fine powder and 2.0 ± 0.05 mg of samples were each weighed into a tin capsule (3.5 × 5 mm, IVA Analysentechnik e.K., Meerbush, Germany) prior to combustion to CO_2 in an elemental analyser (Eurovector EA3000) that was connected to an isotope ratio mass spectrometer (Isoprime, GVI, Manchester, UK). Isotopic abundances were expressed using the δ-notation according to Farquhar and Richards (1984):

$$\delta^{13}C \, (‰) = \left(\frac{R_{sample}}{R_{standard}} - 1 \right) \times 1000 \qquad (3)$$

where R_{sample} is the mole fraction of $^{13}C/^{12}C$ in the sample, and $R_{standard}$ that of the international IAEA standard V-PDB for carbon. The analytical precision was <0.12‰ (expressed as standard deviation of the internal laboratory standard).

Data analysis

Sap flow density (Q_s) and environmental data were available at 30-min resolution. To prevent stem capacitance from biasing the analysis of the transpirational response to variations in environmental conditions (Oren et al. 1998, Ewers et al. 1999), we condensed diurnal courses of Q_s and environmental parameters to daily means. Finally, Q_s data were pooled over all the six trees per treatment. Single variable analyses were used to examine the response of Q_s to T_{soil}, R_s and D as these environmental

factors have often been found to be closely related to transpiration in conifers at the treeline in the Central European Alps (Anfodillo et al. 1998, Matyssek et al. 2009, Wieser et al. 2013). While relationships between Q_s and T_{soil} and Q_s and R_s were derived by linear regression analysis, the relationship between Q_s and D was analysed using an exponential saturation function:

$$T = a \times (1 - \exp(-b \times D)) \qquad (4)$$

where a and b are fitted parameters.

As daily mean T_{soil} and θ values in the two control and in the two warmed plots, respectively, did not differ significantly (all P values >0.1; one-way analysis of variance (ANOVA)), we used repeated-measures ANOVA (Moser et al. 1990) to test for differences in T_{soil} and θ between control and warmed plots ($n = 4$ per treatment). Differences in overall mean Q_s between control and warmed trees during the period before roof closure in 2011 and during the periods of roof closure in 2011, 2012 and 2013, respectively, were analysed by one-way ANOVA. One-way ANOVA was also used to test for differences in D_s, projected crown area, 100-needle dry weight, SLA, gas exchange parameters and carbon isotope signature between control and warmed trees. A probability level of $P < 0.05$ was considered as statistically significant. Statistical analyses were made with the SPSS 16 software package for Windows (SPSS, Inc., Chicago, IL, USA) and nonlinear curve fits were performed using FigP for Windows (BIOSOFT, Cambridge, UK).

Results

Environmental conditions

Daily mean solar radiation (R_s) varied between 18 (18 September 2011) and 347 W m^{-1} (27 June 2011), averaging 161 W m^{-2} in 2011, 170 W m^{-2} in 2012 and 161 W m^{-2} in 2013 (Figure 1). Daily mean air temperature (T_{air}) was 9.5 °C in 2011, 9.2 °C in 2012 and 8.1 °C in 2013, and varied between −4.2 °C on 16 May 2012 and 18.4 °C on 20 August 2012 (Figure 1). Daily mean vapour pressure deficit (D) was 0.33 kPa in 2011, 0.24 kPa in 2012 and 0.22 kPa in 2013, approaching zero on rainy days and reaching a maximum of 1.2 kPa on 23

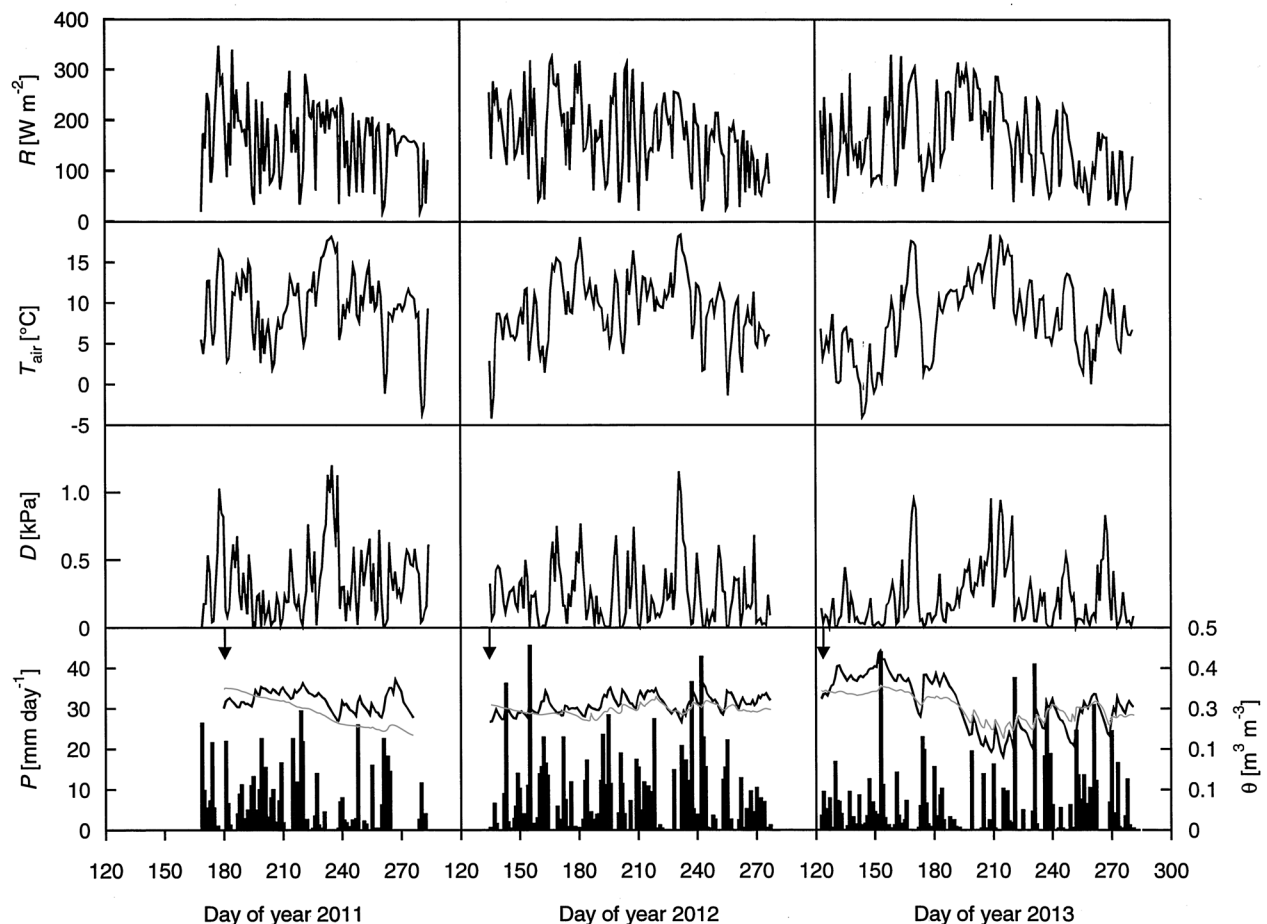Figure 1. Seasonal course of daily mean solar radiation (R), air temperature (T_{air}), vapour pressure deficit (D), daily sum of precipitation (P) and daily mean soil water content (θ) in 5–10 cm in the control (black) and the warmed (grey) treatment from 17 June to 11 October 2011, 15 May to 4 October 2012 and 2 May to 8 October 2013. Arrows indicate roof closure.

Table 1. Seasonal averaged soil water content (θ, m^3 m^{-3}) in 10 cm soil depth and soil temperature (T_{soil}, °C) in 5, 10 and 20 cm soil depth in control and warmed plots for the periods 2011 (2 July–11 October), 2012 (15 May–4 October) and 2013 (2 May–8 October). Values are the mean ± SE of four sensors per treatment. Differences in θ and T_{soil} between and control and warmed plots were analysed using repeated-measures ANOVA and are indicated as *P* values.

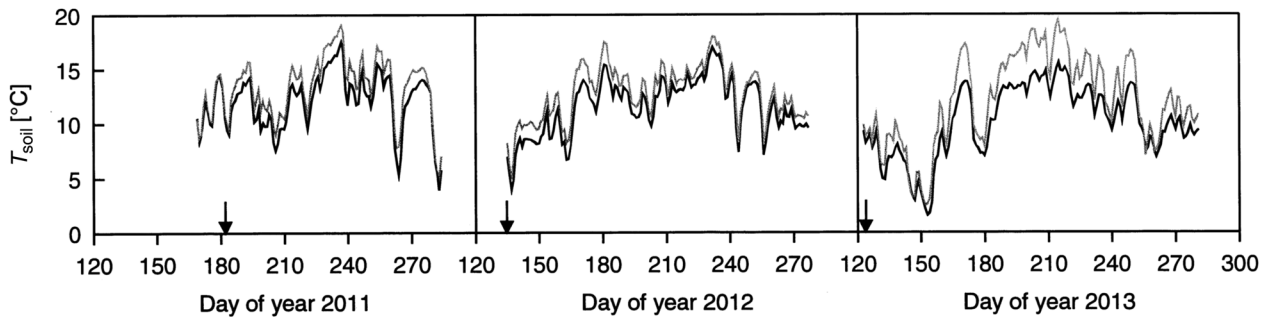| Year	θ		*P*	T_{soil} 5 cm		*P*	T_{soil} 10 cm		*P*	T_{soil} 20 cm		*P*
	Control	Warmed		Control	Warmed		Control	Warmed		Control	Warmed	
2011	0.32 ± 0.02	0.29 ± 0.02	0.53	11.6 ± 0.1	13.1 ± 0.1	<0.001	11.4 ± 0.2	12.5 ± 0.2	0.006	10.9 ± 0.1	11.9 ± 0.2	0.009
2012	0.31 ± 0.01	0.29 ± 0.01	0.30	11.3 ± 0.1	12.6 ± 0.3	0.006	10.9 ± 0.2	12.1 ± 0.3	0.009	10.4 ± 0.2	11.4 ± 0.2	0.014
2013	0.31 ± 0.01	0.30 ± 0.01	0.93	10.1 ± 0.4	12.1 ± 0.3	0.006	9.8 ± 0.4	11.4 ± 0.4	0.021	9.4 ± 0.3	10.5 ± 0.2	0.021

Figure 2. Seasonal course of daily mean soil temperature (T_{soil}) in 5 cm soil depth cm in the control (black) and the warmed (grey) treatment from 17 June to 11 October 2011, 15 May to 4 October 2012 and 2 May to 8 October 2013. Data are the mean of four sensors per treatment. Arrows indicate roof closure.

August 2011 (Figure 1). Daily mean wind velocity averaged 4.5 m s^{-1} throughout the 3-year study period (data not shown). Precipitation during the study periods 2011, 2012 and 2013 amounted to 602, 889 and 816 mm, respectively. Due to frequent rainfall over the years (Figure 1) soil water content (θ) in 10 cm soil depth varied between a maximum of 0.44 m^3 m^{-3} on 2 June 2013 and a minimum of 0.18 m^3 m^{-3} on 1 August 2013. Average θ over 2011, 2012 and 2013 did not differ significantly ($P \geq 0.30$ each) between control (0.31 m^3 m^{-3}) and warmed plots (0.29 m^3 m^{-3}, Table 1).

Before roof closure (DOY 169–182 in 2011) daily mean soil temperature (T_{soil}) did not differ significantly ($P = 0.68$) between control and treatment plots (Figure 2). During periods when roofs were installed, daily mean T_{soil} in 5 cm soil depth of the control varied between 1.5 °C on 1 June 2013 and 17.5 °C on 25 August 2011 (Figure 2). In parallel, roofing caused T_{soil} to average +1.6 ± 0.2, +1.3 ± 0.2 and +1.0 ± 0.1 °C above corresponding control levels at 5, 10 and 25 cm soil depth, respectively ($P < 0.021$ consistently; Table 1). Temperature differences between warmed and control plots were linearly correlated with *R* (Figure 3, during the study period of 2013).

Structural features of the study trees

In 2010, the year preceding the warming experiment, D_s did not differ significantly ($P = 0.66$) between control and warmed trees and averaged 8.1 ± 0.6 and 8.5 ± 0.5 cm, respectively. Soil warming had no effect on growth. After 3 years of treatment, D_s ($P = 0.65$) and A_s ($P = 0.80$) did not differ significantly between
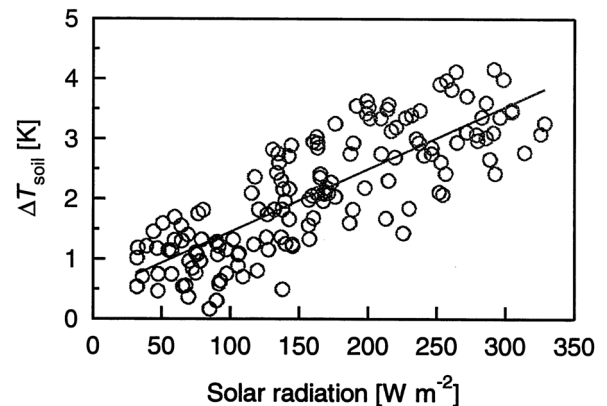

Figure 3. Daily mean soil temperature difference in 5 cm soil depth between warmed and control pots (ΔT_{soil}) as a function of solar radiation (*R*) obtained throughout the investigation period 2013. Points are mean values of four sensors per treatment. Points were fit by linear regressions: control: $y = 0.010R + 0.41$, $R^2 = 0.66$, $P < 0.001$.

control and warmed trees (Table 2). Soil warming during three growing seasons did not affect SLA or 100-needle dry weight. In 2013 current-year needle SLA was 43.3 ± 1.4 cm^2 g^{-1} in control and 43.2 ± 1.4 cm^2 g^{-1} in warmed trees ($P = 0.89$). Correspondingly, 100-needle dry weight was 1.79 ± 0.09 and 1.77 ± 0.06 g, respectively ($P = 0.88$).

Sap flow density and influencing factors

Seasonal courses in sap flow density (Q_s) in general followed those of *R* and *D* (Figure 4; cf. Figure 1). Before roof closure in

Table 2. Stem diameter at height of sensor installation (D_s), sapwood cross-section area (A_s) and sap flow density (Q_s) of *P. cembra* trees selected for sap flow measurements during the study periods 2011 (2 July–11 October), 2012 (15 May–4 October) and 2013 (2 May–8 October). A_s was estimated from D_s according to Eq. (2). Values are the mean ± SE of six trees per treatment. Significant differences ($P < 0.05$) between and control and warmed trees are marked in italics.

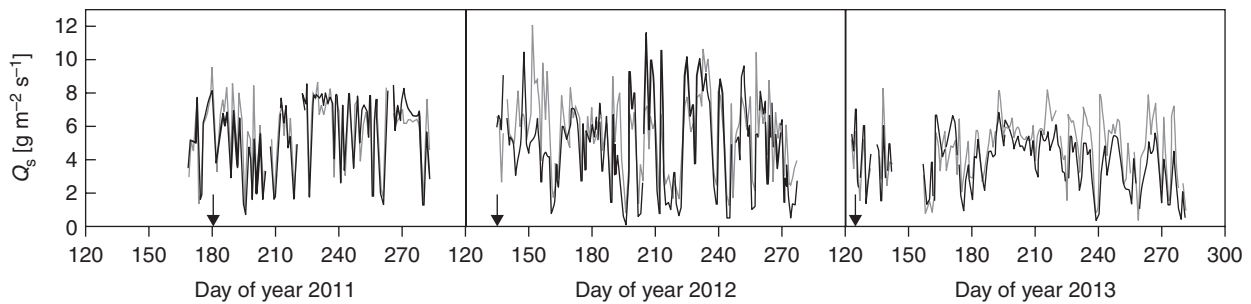| Tree	D_s (cm)			A_s (cm²)			Q_s (g m² s⁻¹)		
	2011	2012	2013	2011	2012	2013	2011	2012	2013
Control	8.6 ± 0.6	9.2 ± 0.7	9.8 ± .0.7	57.3 ± 9.9	66.5 ± 12.9	76.5 ± 15.9	5.13 ± 0.23	*4.93 ± 0.23*	*3.88 ± 0.13*
Warmed	9.0 ± 0.5	9.6 ± 0.5	10.2 ± 0.6	60.8 ± 6.5	70.1 ± 7.8	81.1 ± 9.5	5.26 ± 0.21	*5.49 ± 0.19*	*4.61 ± 0.15*

Figure 4. Seasonal course of daily mean sap flow density (Q_s) in the control (black) and the warmed (grey) treatment from 17 June to 11 October 2011, 15 May to 4 October 2012 and 2 May to 8 October 2013. Data are the mean of six trees per treatment. Arrows indicate roof closure.

2011 (DOY 169–182) daily mean Q_s did not differ significantly between control and warmed trees ($P = 0.84$; Figure 4) and averaged 5.37 ± 0.59 and 5.41 ± 0.60 g m⁻² s⁻¹, respectively. Even after roof closure on 2 July 2011 control and warmed trees resembled each other with respect to Q_s (Figure 4), in the absence of a warming effect during the first year of treatment (Table 2; $P = 0.68$). However, during the second (2012) and third year (2013), soil warming caused daily mean Q_s of warmed trees to increase significantly, on average, by 11 and 19% (both $P < 0.001$), respectively, above levels of control trees (Table 2).

Three years of soil warming did not modify the response of Q_s to T_{soil}, R_s and D. Examined at a daily timescale these results generally reflected positive correlations between Q_s and the three environmental factors. When correlating Q_s with T_{soil} in 5 cm soil depth, R and D throughout the study period of 2013, both in control and warmed trees a linear increase was found with T_{soil} ($P < 0.001$ each; Figure 5). Linear positive correlations also existed with R in control ($R^2 = 0.68$) and warmed trees ($R^2 = 0.50$; $P < 0.001$ each; Figure 6). With respect to D, Q_s increased sharply at low D, and tended to saturate at mean daily D values >0.2 kPa, in both control and warmed trees. D explained 62 and 67% of the variation in Q_s in control and warmed trees, respectively ($P < 0.001$ each; Figure 7).

Leaf-level gas exchange and carbon isotope signature

In July 2012, mean rates of net photosynthesis (P_n) and transpiration (E) along with stomatal conductance for water vapour (g_{H_2O}) of current-year and 1-year-old needles from warmed trees displayed an increase of 3–19% above respective levels of control
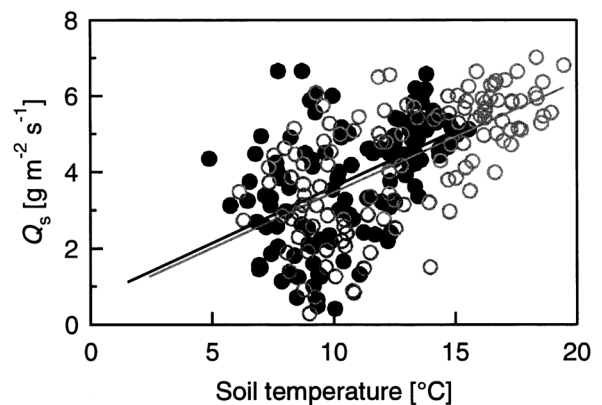

Figure 5. Daily mean sap flow density (Q_s) as a function of soil temperature in 5 cm soil depth in trees growing on control (black) and warmed (grey) plots obtained in the third year of soil warming (2013). Points are mean values of six trees per treatment. Points were fit by linear regressions: control: $y = 0.31R + 0.38$, $R^2 = 0.30$, $P < 0.001$; warmed: $y = 0.29R + 0.55$, $R^2 = 0.40$, $P < 0.001$.

trees (Table 3). Warming also caused an increase in instantaneous water-use efficiency (WUE) and intrinsic water-use efficiency (iWUE) in 1-year-old needles, which opposed the findings in current-year needles (Table 3). Such differences, however, failed to be statistically significant. Neither did δ¹³C of sun-exposed current-year needles yield a significant response in 2012, with −27.00 ± 0.14‰ in control and −26.69 ± 0.16‰ in warmed trees ($P = 0.24$). Corresponding δ¹³C levels in 2013 were −26.13 ± 0.16‰ and −26.18 ± 0.21‰, respectively ($P = 0.83$), reflecting some tendency to increase over the data of 2012.

Discussion

The experimental approach was appropriate to manipulate T_{soil}, enabling the clarification of tree water use in situ under a wide range
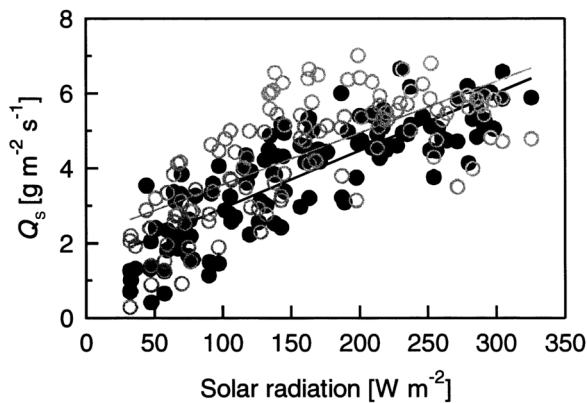

Figure 6. Daily mean sap flow density (Q_s) as a function of solar radiation (R) for the control (black circles and line) and the warmed treatment (grey circles and line) in the third year of soil warming. Points are mean values of six trees. Points were fit by linear regressions: control: $y = 0.015R + 1.39$, $R^2 = 0.68$, $P < 0.001$; warmed: $y = 0.014R + 2.18$, $R^2 = 0.50$, $P < 0.001$.

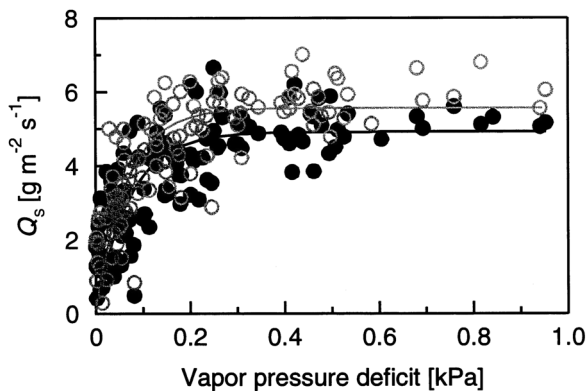Figure 7. Daily mean sap flow density (Q_s) as a function of vapour pressure deficit (D) for the control (black circles and line) and the warmed treatment (grey circles and line) in the third year of soil warming. Points are mean values of six trees. Points were fit by exponential saturation functions: control: $y = 4.19 \times (1 - \exp[-1.39D])$, $R^2 = 0.62$, $P < 0.001$; warmed: $y = 5.56 \times [1 - \exp(-1.53D)]$, $R^2 = 0.67$, $P < 0.001$.

of environmental conditions. Differences in T_{soil} between control and warmed plots largely depended on radiative warming, i.e., by incoming R_s (Figure 3). Moreover, treatment differences in daily mean θ stayed within the typical variation at the study site (Neuwinger 1972, 1980), which confirmed the employed roofing system to prevent any shortage in soil water availability (Figure 1; Table 1).

In our study, 3 years of soil warming did not cause any response in stem diameter increment of *P. cembra* (Table 2). As *P. cembra* exhibits determinate shoot growth, in any year growth is primarily impelled by the bud formation occurring in the previous year (Tranquillini 1979). Additionally, in alpine ecosystems, growth response to warming is mediated through a time lag by one or more years after initiation of warming (Danby and Hik 2007, Hagedorn et al. 2010). Responses during the extraordinary warm year of 2003 support our result, as the growth of *P. cembra* at the treeline in the Austrian Alps was hardly affected by a 4 °C warmer summer (Oberhuber et al. 2008). Neither was a growth response during 2003 observed in Swiss alpine and subalpine forests (Jolly et al. 2005).

Changes in Q_s observed in our study may also be related to treatment-induced alterations in leaf area. In *P. cembra* both cumulative projected foliage area (Af) and sapwood cross-sectional area (A_s) increase exponentially with stem diameter reflecting a stable Af to A_s ratio of 0.45 m² cm⁻² (Matyssek et al. 2009). Stable Af to A_s ratios have also been observed in other conifers (Waring and Running 1985, Waring and Schlesinger 1985) and reflect a long-term adjustment between water demand of the foliage and water supply through the trunk (Tyree and Zimmermann 2002). In our study however, we detected no soil warming effect on sapwood area (Table 2), implying that leaf area was also not affected.

Seasonal patterns in Q_s were similar to those reported from other *P. cembra* stands at the treeline in the Central Austrian (Wieser and Leo 2012, Wieser et al. 2013) and the northeastern Italian Alps (Anfodillo et al. 1998). We detected no significant treatment effects on Q_s during the first year of warming (Figure 4, Table 2). Similarly, Van Herk et al. (2011) reported that soil warming by 5 °C failed to affect Q_s in *Pinus mariana* in northern Manitoba, Canada, throughout one growing season.

Table 3. Net photosynthesis (P_n; μmol m⁻² s⁻¹), transpiration (E; mmol m⁻² s⁻¹), stomatal conductance for water vapour (g_{H_2O}; mmol m⁻² s⁻¹), instantaneous water-use efficiency (WUE; μmol CO₂ H₂O mol⁻¹) and intrinsic water-use efficiency (iWUE; μmol mol⁻¹), of current-year and 1-year-old needles of control and warmed *P. cembra* trees obtained in July 2012, and corresponding P values. Values are related to projected leaf area and are the mean of six trees ±SE per treatment.

	Current-year needles			1-year-old needles		
	Control	Warmed	P	Control	Warmed	P
P_n	14.20 ± 1.72	14.88 ± 1.33	0.75	$8.16 \pm 0.73.$	9.70 ± 0.76	0.16
E	4.45 ± 0.42	4.75 ± 0.35	0.61	3.30 ± 0.36	3.59 ± 0.26	0.53
g_{H_2O}	317 ± 31	374 ± 31	0.21	231 ± 14.90	248 ± 12.23	0.39
WUE	3.31 ± 0.27	3.18 ± 0.22	0.70	2.82 ± 0.37	2.85 ± 0.29	0.95
iWUE	0.044 ± 0.003	0.041 ± 0.002	0.34	0.037 ± 0.003	0.039 ± 0.003	0.55

During the second and third year, soil warming significantly increased Q_s (Figure 4, Table 2). Independent of the treatment Q_s was linearly correlated with T_{soil} as also observed in a snow cover manipulation experiment by Mellander et al. (2004). Hence, T_{soil} appears to determine water transportation along the soil–plant–atmosphere continuum, with low T_{soil} restricting water uptake (cf. Figures 2 and 4). In cold soils increased water viscosity and decreased root permeability are known to limit water uptake in conifers (Kramer 1940, 1942, Kaufmann 1975, Lopushinsky and Kaufmann 1984, Goldstein et al. 1985, Wan and Zwiazek 1999), contributing to low Q_s as observed in trees growing on our control plots (Figures 4 and 5). There is also evidence that the functioning of aquaporins (membrane-bound water channel proteins) is temperature dependent (Wan and Zwiazek 1999, Wan et al. 2001). Low temperature may inhibit root hydraulic conductivity due to reduced aquaporin performance, as root hydraulic conductance is positively correlated with leaf-level transpiration and stomatal conductance (Yang et al. 2011). In the absence of statistical significance, in our study soil warming appeared to stimulate foliar gas exchange (Table 3). Furthermore, Q_s was considerably higher in warmed than in control trees (Figures 6 and 7) as related to the same reference levels in R and D, emphasizing the influence of root zone temperature on aboveground metabolism (Havranek 1972, Gruber et al. 2010, Wieser et al. 2010).

Additionally important is the effect of T_{soil} on Q_s at high altitude as low soil temperatures limit fine-root growth (Häsler et al. 1999, Pregitzer et al. 2000, Oberhuber 2007). In *P. cembra* fine-root elongation rate peaks in July, increases with T_{soil} and ceases at rooting zone temperatures below 3–5 °C (Turner and Streule 1983). Thus, particularly in open stands, as was the case at the study site, soil warming might have enhanced root production in the uppermost soil layers during the second (2012) and third (2013) years of treatment, given that soil warming started immediately after each snow melt (Figure 2). In such a case, it is likely that the water uptake capacity became improved upon enlargement of the absorbing surface area belowground. Such an effect was probably absent during the first year of treatment, when soil warming was started in July, i.e., after the seasonal period of maximum fine-root elongation.

Conclusions

In accordance with our hypothesis, soil warming led to an increase in Q_s of *P. cembra* trees at the treeline in the central Austrian Alps. During the first year of treatment, no warming effect was detected on Q_s. In the second and third year, however, the effect appeared to result from warming-induced root production, a reduction in viscosity and perhaps an increase also in root hydraulic conductivity. Although radial stem growth and sapwood area increment did not reflect response to warming, T_{soil} was increased to an extent relevant for treeline water budgets.

Presuming a future environment with unchanged precipitation but increased surface temperatures, both tree-level water loss and carbon uptake may increase. Conversely, the response to warming may be transitory as advancing stand development may become resource limited. Such a development in net primary productivity emerged from the long-term FACE study reported by Norby et al. (2010). Consequently, further clarification of future treeline scenarios will require a combination of prolonged experimental soil warming and nutrient availability analysis. We conclude from the present study that tree water loss may increase with soil warming in a future environment, affecting the water balance within the treeline ecotone of the Central Austrian Alps.

Conflict of interest

None declared.

Funding

References

Anfodillo T, Rento S, Carraro V, Furlanetto L, Urbinati C, Carrer M (1998) Tree water relations and climatic variations at the alpine timberline: seasonal changes of sap flux and xylem water potential *in Larix decidua* Miller, *Picea abies* (L.) Karst. and *Pinus cembra* L. Ann For Sci 55:159–172.

Barnett TP, Adam JC, Lettenmaier DP (2005) Potential impacts of a warming climate on water availability in snow-dominated regions. Nature 438:303–309.

Beniston M, Diaz HF, Bradley RS (1997) Climate change at high elevation sites: an overview. Clim Change 36:233–251.

Bergh J, Linder S (1999) Effects of soil warming during spring on photosynthetic recovery in boreal Norway spruce stands. Glob Change Biol 5:245–253.

Blume HP, Brümmer GW, Horn R, Kandeler E, Kögl-Knabler I, Kretzschmar R, Stahr K, Wilke BM (2010) Scheffer/Schachtchabel: Lehrbuch der Bodenkunde. Spektrum Akademischer Verlag, Heidelberg.

Breda N, Huc R, Granier A, Dreyer E (2006) Temperate forest trees and stands under severe drought: a review of ecophysiological responses, adaptation processes and long-term consequences. Ann For Sci 63:625–644.

Bronson DR, Gower ST (2010) Ecosystem warming does not affect photosynthesis or aboveground autotrophic respiration for boreal black spruce. Tree Physiol 30:441–449.

Danby RK, Hik DS (2007) Responses of white spruce (*Picea glauca*) to experimental warming at a subarctic alpine treeline. Glob Change Biol 13:437–451.

Diaz HF, Bradley RS (1997) Temperature variations during the last century at high elevation sites. Clim Change 36:253–279.

Ewers BE, Oren R (2000) Analyses of assumptions and errors in the calculation of stomatal conductance from sap flux measurements. Tree Physiol 20:579–589.

Ewers BE, Oren R, Albaugh TJ, Dougherty PM (1999) Carry-over effects of water and nutrient supply on water use of *Pinus taeda*. Ecol Appl 9:513–525.

FAO, Isric lSSS (1998) World reference base for soil resources. FAO, Rome.

Farquhar GD, Richards RA (1984) Isotopic composition of plant carbon correlates with water-use efficiency of wheat genotypes. Aust J Plant Physiol 11:539–552.

Goldstein GH, Brubaker LB, Hinckley TM (1985) Water relations of white spruce (*Picea glauca* (Moench) Voss) at tree line in north central Alaska. Can J For Res 15:1080–1087.

Granier A (1985) Une nouvelle méthode pour la mesure du flux de sève brute dans le tronc des arbres. Ann For Sci 42:193–200.

Granier A (1987) Evaluation of transpiration in a Douglas-fir stand by means of sap flow measurements. Tree Physiol 3:309–320.

Granier A, Reichstein M, Breda N et al. (2007) Evidence for soil water control on carbon and water dynamics in European forests during the extremely dry year: 2003. Agric For Meteorol 143:123–145.

Gruber A, Wieser G, Oberhuber A (2010) Opinion paper: effects of simulated soil temperature on stem diameter increment of *Pinus cembra* at the alpine timberline: a new approach based on root zone roofing. Eur J For Res 129:141–144.

Hagedorn F, Martin M, Rixen C et al. (2010) Short-term responses of ecosystem carbon fluxes to experimental soil warming at the Swiss alpine treeline. Biogeochemistry 97:7–19.

Häsler R, Streule A, Turner H (1999) Shoot and root growth of young *Larix decidua* in contrasting environments near the alpine timberline. Phyton 39:47–52.

Havranek WM (1972) Über die Bedeutung der Bodentemperatur für die Photoynthese und die Transpiration junger Forstpflanzen und die Stoffproduktion an der Waldgrenze. Angew Bot 46:101–116.

Holtmeier F-K, Broll G (2007) Treeline advance—driving processes and adverse factors. Landsc Online 1:1–33.

Intergovernmental Panel on Climate Change (IPCC) (2013) Climate Change 2013: The Physical Science Basis. Cambridge University Press, Cambridge.

Jolly WM, Dobbertin M, Zimmermann NE, Reichstein M (2005) Divergent vegetation growth responses to the 2003 heat wave in the Swiss Alps. Geophys Res Lett 32:L18409.

Jones PD, Wigley TML, Folland CK, Parker DE, Angelli JK, Jebedeff S, Hansen JE (1988) Evidence of global warming in the last decade. Nature 332:791.

Kaufmann MR (1975) Leaf water stress in Engelmann spruce: influence of the root and shoot environments. Plant Physiol 56:841–844.

Kellomäki S, Wang K-Y (1998) Sap flow in Scots pines growing under conditions of year-round carbon dioxide enrichment and temperature elevation. Plant Cell Environ 21:969–981.

Kramer PJ (1940) Root resistance as a cause of decreased water absorption by plants at low temperatures. Plant Physiol 16:63–79.

Kramer PJ (1942) Species differences with respect to water absorption at low soil temperatures. Am J Bot 29:828–832.

Kronfuss H (1997) Das Klima einer Hochlagenaufforstung in der subapinen Höhenstufe - Haggen im Sellraintal bei St. Siegmund, Tirol (Periode 1975–1994). Schriftenreihe Forstl Bundesvers Wien, FBVA Bericht 100.

Lopushinsky W, Kaufmann MR (1984) Effects of cold soils on water relations and spring growth of Douglas-fir seedlings. For Sci 30:628–634.

Matyssek R, Wieser G, Patzner K, Blaschke H, Häberle K-H (2009) Transpiration of forest trees and stands at different altitude: consistencies rather than contrasts? Eur J For Res 128:579–596.

Mayr S (2007) Limits in water relations. In: Wieser G, Tausz M (eds) Trees at their upper limit. Treelife limitation at the Alpine timberline. Plant Ecophysiology. Vol. 5. Springer, Dorthrecht, The Netherlands, pp 131–143.

McElrone AJ, Bichler J, Pockman WT, Addington RN, Linder CR, Jackson RB (2007) Aquaporin-mediated changes in hydraulic conductivity of deep tree roots accessed via caves. Plant Cell Environ 30:1411–1421.

Mellander P-E, Bishop K, Lundmark T (2004) The influence of soil temperature on transpiration: a plot scale manipulation in a young Scots pine stand. For Ecol Manag 195:15–28.

Moser EB, Saxton AM, Pezeshki SR (1990) Repeated measures analysis of variance: application to tree research. Can J For Res 20:524–535.

Neuwinger I (1972) Standortuntersuchungen am Sonnberg im Sellrainer Obertal, Tirol. Mitt Forstl Bundesvers Wien 96:177–207.

Neuwinger I (1980) Erwärmung, Wasserrückhalt und Erosionsbereitschaft subalpiner Böden. Mitt Forstl Bundesvers Wien 139:113–144.

Neuwinger I, Wieser G, Winklehner W, Heiss G (1988) Soil water investigations in a high-level afforestation area near Haggen in the Sellrain Valley, Tyrol. Österreichische Wasserwirtschaft 40:57–61.

Norby RJ, Warren JM, Iversen CM, Medlyn BE, McMurtrie RE (2010) CO_2 enhancement of forest productivity constrained by limited nitrogen availability. Proc Natl Acad Sci USA 107:19368–19373.

Oberhuber W (2007) Limitation by growth processes. In: Wieser G, Tausz M (eds) Trees at their upper limit. Treelife limitation at the Alpine timberline. Plant Ecophysiology. Vol. 5. Springer, Dorthrecht, The Netherlands, pp 131–143.

Oberhuber W, Kofler W, Pfeifer K, Seeber A, Gruber A, Wieser G (2008) Long term changes in tree-ring-climate relationships at Mt. Patscherkofel (Tyrol, Austria) since the mid-1980s. Trees 22:31–40.

Oren R, Philips N, Katul G, Ewers BE, Pataki DE (1998) Scaling xylem sap flux and soil water balance and calculating variance. A method for partitioning water flux in forests. Ann For Sci 55:191–216.

Pregitzer KS, King JS, Burton AJ, Brown SE (2000) Responses of tree fine roots to temperature. New Phytol 147:105–115.

Reichstein M, Tehnunen JD, Roupsard O et al. (2002) Severe drought effects on ecosystem CO_2 and H_2O fluxes at three Mediterranean evergreen sites: revision of current hypotheses? Glob Change Biol 8:999–1017.

Ruggiero C, Angelino G, Maggio A (2007) Developmental regulation of water uptake in wheat. J Plant Physiol 164:1170–1178.

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.

Tranquillini W (1979) Physiological ecology of the alpine timberline. In: Tree existence at high altitudes with special reference to the European Alps. Ecological Studies. Vol. 31. Springer, Berlin, Heidelberg, New York.

Turner H, Streule A (1983) Wurzelwachstum und Sprossentwicklung junger Koniferen im Klimastress der alpinen Waldgrenze, mit Berücksichtigung von Mikroklima, Photosynthese und Stoffproduktion. Wurzelökologie und ihre Nutzanwendung. Internationales Symposium Gumpenstein, 1982. Bundesanstalt Gumpenstein, pp 617–635.

Tyree MT, Zimmermann MH (2002) Xylem structure and the ascent of sap. Springer, Berlin.

Van Herk IG, Gower ST, Bronson DR, Tanner MS (2011) Effect of climate warming on canopy water dynamics of a boreal black spruce plantation. Can J For Res 41:217–227.

Walther G-R, Beißner S, Pott R (2005) Climate change and high mountain vegetation shifts. In: Broll G, Keplin B (eds) Mountain ecosystems. Studies in treeline ecology. Springer, Berlin, pp 77–95.

Wan X, Zwiazek JJ (1999) Mercuric chloride effects on root water transport in aspen seedlings. Plant Physiol 121:939–946.

Wan X, Zwiazek JJ, Lieffers VJ, Landhäusser SM (2001) Hydraulic conductance in aspen (*Populus tremuloides*) seedlings exposed to low root temperatures. Tree Physiol 21:691–696.

Waring RH, Running SW (1985) Forest ecosystems. Analysis at multiple scales. Academic Press, San Diego, CA.

Waring RH, Schlesinger WH (1985) Forest ecosystems. Concepts and management. Academic Press, Orlando, FL.

Wieser G (2012) Lessons from the timberline ecotone in the Central Tyrolean Alps: a review. Plant Ecol Div 5:127–139.

Wieser G, Leo M (2012) Whole-tree water use by *Pinus cembra* at the treeline in the Central Tyrolean Alps. Plant Ecol Div 5: 81–88.

Wieser G, Matyssek R, Luzian R, Zwerger P, Pindur P, Oberhuber W, Gruber A (2009) Effects of atmospheric and climate change at thetimberline of the Central European Alps. Ann For Sci 66:402.

Wieser G, Oberhuber W, Walder L, Spieler D, Gruber A (2010) Photosynthetic temperature adaptation of *Pinus cembra* within the timberline ecotone of the Central Austrian Alps. Ann For Sci 67:201.

Wieser G, Gruber A, Oberhuber W (2013) Sap flow characteristics and whole-tree water use of *Pinus cembra* across the treeline ecotone of the central Tyrolean Alps. Eur J For Res 133:287–295.

Yang Q, Zhang F, Li F (2011) Effect of different drip irrigation methods and fertilization on growth, physiology and water use of young apple tree. Sci Hortic 129:119–126.