CO$_2$ enrichment alters diurnal stem radius fluctuations of 36-yr-old *Larix decidua* growing at the alpine tree line

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Summary

- To understand how trees at high elevations might use water differently in the future, we investigated the effects of CO$_2$ enrichment and soil warming (separately and combined) on the water relations of *Larix decidua* growing at the tree line in the Swiss Alps.
- We assessed diurnal stem radius fluctuations using point dendrometers and applied a hydraulic plant model using microclimate and soil water potential data as inputs.
- Trees exposed to CO$_2$ enrichment for 9 yr showed smaller diurnal stem radius contractions (by $46 \pm 16\%$) and expansions ($42 \pm 16\%$) compared with trees exposed to ambient CO$_2$. Additionally, there was a delay in the timing of daily maximum (40 ± 12 min) and minimum (63 ± 14 min) radius values for trees growing under elevated CO$_2$. Parameters optimized with the hydraulic model suggested that CO$_2$-enriched trees had an increased flow resistance between the xylem and bark, representing a more buffered water supply system. Soil warming did not alter diurnal fluctuation dynamics or the CO$_2$ response.
- Elevated CO$_2$ altered the hydraulic water flow and storage system within *L. decidua* trees, which might have contributed to enhanced growth during 9 yr of CO$_2$ enrichment and could ultimately influence the future competitive ability of this key tree-line species.

Introduction

Understanding the impacts of increasing atmospheric CO$_2$ concentrations on plant water relations is an essential step in predicting future feedbacks between vegetation and environmental conditions and possible landscape-scale hydrological consequences (Betts *et al.*, 2007; Warren *et al.*, 2011b). Elevated CO$_2$ has led to reduced leaf-level stomatal conductance (g_s) in many studies, but the occurrence and extent of stomatal closure differ across plant life forms, species and age, as well as environmental conditions (Ainsworth & Rogers, 2007). In particular, low stomatal responsiveness to CO$_2$ enrichment has been reported for adult coniferous trees (Ellsworth, 1999). Additionally, stomatal closure responses to elevated CO$_2$ observed for single leaves might not lead to parallel changes in water relations at the whole-plant level or over longer temporal intervals (Wullschleger *et al.*, 2002). Regarding trees, sap flux measurements from large-scale free-air CO$_2$ enrichment (FACE) experiments have demonstrated reduced transpiration at the tree level in some forest ecosystems, with implications for CO$_2$-induced water savings at the stand scale (Wullschleger & Norby, 2001; Schäfer *et al.*, 2002; Leuzinger & Körner, 2007). However, increased above-ground biomass production, particularly increased leaf area, resulting from CO$_2$ enrichment means that larger scale transpiration responses are often smaller than g_s responses (Wullschleger *et al.*, 2002) and can even lead to increased stand-level water use under elevated CO$_2$ (Sonnleitner *et al.*, 2001).

Climate warming will mean higher vapour pressure deficit (VPD) and therefore greater evaporative demand in plants (IPCC, 2007). In the Alps, predictions of reduced summer precipitation as well as more rain instead of snow in winter could shift seasonal outflow patterns and enhance warming effects on plants (Beniston, 2006). In addition to influencing plant water relations independently, these climatic changes could alter the magnitude or direction of elevated CO$_2$ effects on plant water relations. CO$_2$-induced reductions in whole-tree-level transpiration became more pronounced under conditions of high VPD or reduced soil moisture in some larger scale field studies, indicating increased sensitivity to drier conditions under elevated CO$_2$ (Kellomäki & Wang, 1998; Sonnleitner *et al.*, 2001; Wullschleger & Norby, 2001; Warren *et al.*, 2011a). By contrast, a smaller CO$_2$ effect on sap flow under drier conditions was observed for mature broadleaf trees (Leuzinger & Körner, 2007). The potential interactive effects of elevated CO$_2$ and warmer, drier conditions on tree water relations remain unresolved, and few field experiments involving trees beyond the sapling stage have pursued this topic

through the simultaneous manipulation of CO_2 concentration and temperature or water availability (Kellomäki & Wang, 1998).

Compared with lowland forests, few studies have focused on the water relations of trees growing in high-elevation areas with a continental climate, probably because a continuous winter snowpack and relatively large amounts of summertime rainfall mean that soil moisture is generally high during the growing season (Körner, 2012). However, trees growing near their high-elevation limit in these regions are adapted to moist conditions and even moderate drought can lead to changes in their water status (Anfodillo et al., 1998). Even when soil water availability is high, water plays an important role in tree growth because turgor pressure drives irreversible cell expansion and contributes to the deposition of cell wall polymers (Proseus & Boyer, 2005). It is therefore important to understand the water relations of tree-line trees and how these trees might use water differently under increased atmospheric CO_2 concentrations and warmer, drier climatic conditions.

Continuously logging point dendrometers are minimally invasive tools that can help assess tree water status at a high temporal resolution (Drew & Downes, 2009). Changes in the stem radius at diurnal and longer time-scales are largely caused by a combination of radial growth and water-related shrinkage and swelling of the living bark tissue, where elastic cells contribute water to the transpiration stream during the day and are replenished at night and during periods of rain or fog (Molz & Klepper, 1972; Herzog et al., 1995). Along with leaves, bark tissue serves an internal water storage function, acting as a buffer that prevents extreme water potentials (which could result in cavitation) caused by rapid increases in transpirational demand (Herzog et al., 1995). While stem fluctuations are closely linked to transpiration rates in particular (Zweifel et al., 2001), the diurnal dynamics of stem radius fluctuations integrate all internal tree properties and external air and soil conditions that influence the flow of water within the soil–plant–atmosphere continuum (Molz & Klepper, 1973; Hsiao & Acevedo, 1974; Hinckley & Bruckerhoff, 1975). Dendrometers have been used in many studies of tree radial growth and water storage dynamics in natural environments, including studies on trees growing near their high-elevation limit (Deslauriers et al., 2007; Gruber et al., 2009; King et al., 2013). To our knowledge, however, a recently initiated FACE experiment in mature *Picea abies* trees in temperate lowland Switzerland (Leuzinger & Bader, 2012) is the only other study using dendrometers in a field-based CO_2 manipulation experiment.

We used point dendrometers mounted on 20 trees during the 2009 snow-free period to investigate how FACE, soil warming and the combined treatments influenced the amplitude and timing of diurnal stem radius fluctuations in 36-yr-old *Larix decidua* growing at the alpine tree line in the Swiss Alps. Our aims were based on previous results from the long-term experiment, where above-ground growth (in terms of ring width, stem diameter, and canopy size) of *L. decidua* was enhanced by 9 yr of elevated CO_2 (2001–2009) but not by 3 yr of soil warming (2007–2009;

Dawes et al., 2011b). Further, gas exchange and stable isotope measurements of *L. decidua* needles made in 2009 both indicated an enhanced photosynthetic rate under elevated CO_2, whereas g_s was not altered by CO_2 enrichment or soil warming (Streit et al., 2014). Given these findings, we asked (1) if increased canopy leaf area of trees exposed to several years of CO_2 enrichment without a substantial reduction in g_s led to greater transpirational demand at the whole-tree level, greater water transport through the tree, and therefore larger amplitudes of diurnal stem radius fluctuations (Steppe et al., 2006; Zweifel et al., 2007). (2) Regarding soil warming, we asked if soil drying associated with the treatment reduced water uptake and transport through the tree, and consequently led to smaller diurnal fluctuations. Further, we asked (3) if any CO_2-induced changes in diurnal fluctuation dynamics were dependent on climatic conditions and soil moisture, including our warming treatment. Finally, we applied a hydraulic plant model characterizing water relations of the investigated trees in response to high temporal resolution microclimate and soil matric water potential data (Steppe et al., 2006; Zweifel et al., 2007, 2012) in order to explore potential mechanisms explaining patterns observed in the dendrometer data.

Materials and Methods

Site and experimental design

The study site was located at Stillberg, Davos in the Central Alps, Switzerland (9°52′E, 46°46′N). The CO_2 enrichment and soil warming experiment covered an area of 2500 m^2 and was situated on a north-east-exposed 25–30° slope at 2180 m asl, slightly above the current tree line in the region (Barbeito et al., 2012). The site was located within a 5-ha afforestation research area where tree seedlings were planted into the intact dwarf shrub community in 1975 by the Swiss Federal Institute for Forest, Snow and Landscape Research (WSL). Soil types are sandy Ranker and Podzols (Lithic Cryumbrepts and Typic Cryorthods), derived from siliceous Paragneis parent material. There is a 5–20-cm-thick organic layer (Humimor according to Bednorz et al., 2000) dominated by an Oa horizon with a high water-holding capacity of 2.9 ± 0.2 g H_2O g^{-1} dry soil (F. Hagedorn, unpublished data).

Forty plots of 1.1 m^2 were established in early June 2001, 20 with a *Larix decidua* L. (European larch) individual in the centre and 20 with a *Pinus mugo* ssp. *uncinata* Ramond (mountain pine) individual in the centre (Hättenschwiler et al., 2002). A dense cover of understorey plants dominated by dwarf shrubs grew around the tree base in each plot. For the CO_2 enrichment experiment, plots were assigned to 10 groups of four neighbouring trees (two *L. decidua* and two *P. mugo* ssp. *uncinata* per group). Half of these groups were randomly assigned to an elevated CO_2 treatment, while the remaining groups received no additional CO_2. CO_2 enrichment was applied during daytime hours only throughout each snow-free period from 2001 to 2009 (details in Hättenschwiler et al., 2002; Dawes et al., 2011b). In 2009, the supplementary CO_2 was supplied from 4 June to 2 October and yielded a mean ($\pm$ 1 SD) CO_2 partial pressure of 545 ± 51 ppm

compared with *c.* 380 ppm in ambient CO_2 plots. The soil warming treatment was introduced to the experiment in spring 2007 using heating cables placed on the ground surface (details in Hagedorn *et al.*, 2010; Dawes *et al.*, 2011a). One plot of each tree species identity was randomly selected from each of the 10 CO_2 treatment groups and assigned a soil warming treatment. Each year, the heating system was turned on soon after snowmelt and turned off just before the site was covered in snow for the winter. In 2009, the heating system was active from 2 June to 11 October and resulted in a mean soil temperature increase of $3.1 \pm 0.4°C$ at 5 cm soil depth (mean $\pm$ 1 SE pooled across CO_2 treatments; $n = 10$; HOBO U23 Pro v2 dataloggers; Onset Computer Corp., Bourne, MA, USA). The warming treatment increased air temperatures near the ground surface (0.9°C at 20 cm height), but no temperature differences were detected within the tree canopy at heights > 50 cm (Hagedorn *et al.*, 2010).

Dendrometers were mounted on the stems of the 20 *L. decidua* trees during 2009. The study described here therefore consisted of 20 plots (each with one *L. decidua* tree) nested within 10 groups (each with one unwarmed and one warmed *L. decidua* plot). On average, *L. decidua* trees in the two CO_2 treatment groups were equivalent in size and vigour when treatment was initiated in 2001 (Dawes *et al.*, 2011b). The *L. decidua* trees were all 36 yr old in 2009 but were < 4 m tall and did not form a closed canopy.

Climate data and soil moisture measurements

Air temperature (°C), relative humidity (%), and the corresponding VPD (kPa), as well as precipitation (mm), incoming shortwave solar radiation (W m^{-2}; 310–2800 nm) and wind speed (m s^{-1}) data for 2009 were acquired from a WSL meteorological station located at Stillberg at 2090 m asl (Fig. 1). All measurements were made at 2 m above the ground and recorded every 10 min. Volumetric soil water content (%) at 0–10 cm depth was measured manually 13 times during the 2009 season at a fixed location within each plot using frequency domain reflectometry with a ThetaProbe ML2x probe (Delta-T Devices, Burwell, UK) inserted vertically into the soil. For the hydraulic plant model, we estimated daily values of volumetric soil water content in warmed and unwarmed plots from the manually sampled data by applying a coupled heat and mass transfer model (COUPmodel version 3; Jansson & Karlberg, 2004). Soil matric water potential (Ψ_{soil}; hPa) was measured throughout the 2010 snow-free period using tensiometers installed in a subset of the plots at depths of 5 and 20 cm below the surface. Values were slightly less negative at 20 cm but were similar at the two depths (Supporting Information Fig. S1a). As fine roots are located almost entirely within the top 10 cm of soil at the study site (F. Hagedorn, unpublished data), we used Ψ_{soil} data from 5 cm depth to establish a water retention curve relating water content to Ψ_{soil} for the organic
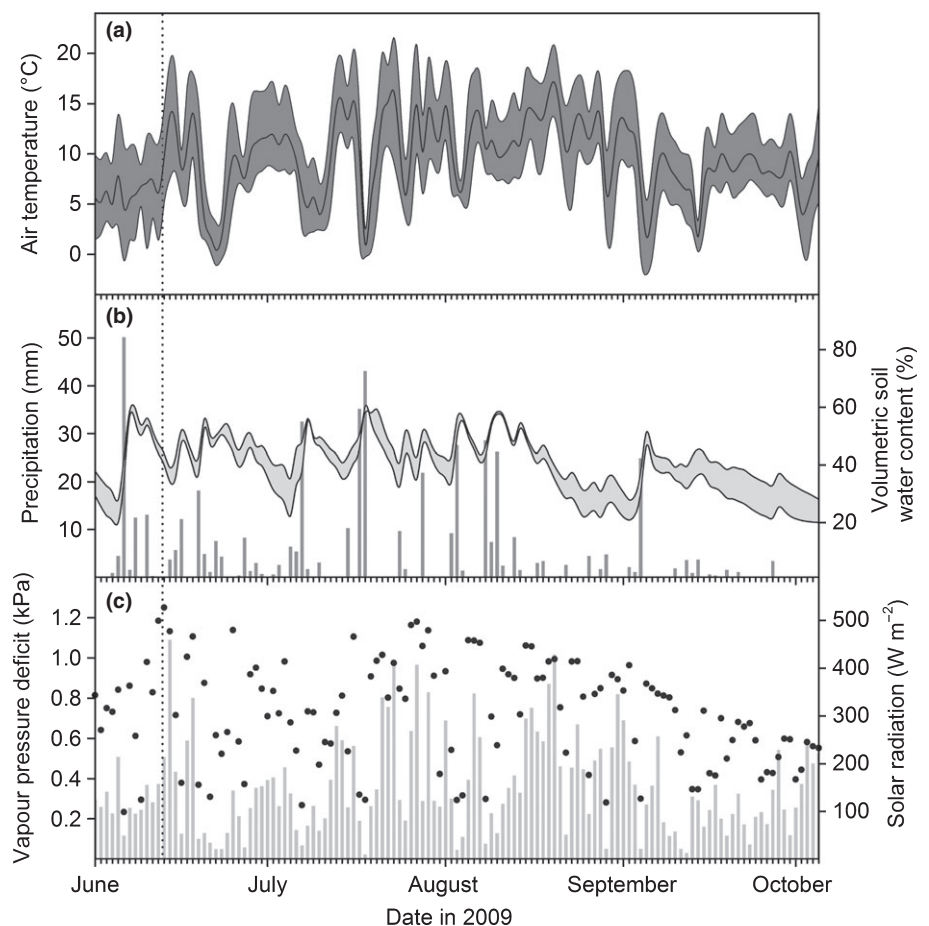

Fig. 1 Climate data during the 2009 snow-free period. (a) Daily (24-h) mean, minimum and maximum air temperatures measured at the Stillberg meteorological station, *c.* 100 m below the experimental plots. (b) Daily (24-h) summed precipitation (grey bars), measured at the meteorological station, and mean volumetric soil water content, measured at 0–10 cm depth in the experimental plots (upper line, unwarmed; lower line, warmed; shaded area, difference between warmed and unwarmed plots; $n = 4$). (c) Daily (daylight hours only) mean vapour pressure deficit (VPD; grey bars) and incoming shortwave solar radiation (black circles) at the meteorological station. The dotted vertical line designates the start of collection of dendrometer data on 13 June.

layer (Fig. S1b). This relationship was used to derive daily Ψ_{soil} values for 2009 needed as input for the hydraulic model.

Tree measurements

Point dendrometers (model ZB08-10; Natkon, Hombrechtikon, Switzerland) were used to continuously measure fluctuations in tree stem radius throughout the 2009 snow-free period. The dendrometers were installed on 6 June, c. 1 wk after snow had completely melted from all plots. For analyses in this paper, we considered data from 13 June to 5 October (1 wk before the first major snow event in autumn), with a gap from 23 to 30 July because of power failure after a lightning strike. Earlier dates were excluded to avoid any artefacts associated with settling of the instruments immediately after installation, and diurnal patterns on later dates were strongly influenced by freezing-induced bark contraction. One sensor was mounted on each tree stem just above the curved section at the stem base, 40–60 cm above the ground or at c. 20% of the total tree height (Table 1). Each sensor was mounted on a rigid, circular aluminium frame (10 cm diameter) surrounding the tree stem. The frame was fixed to the stem by three screws equally spaced around the frame circumference, which were screwed into the xylem to provide a stationary system (Ehrenberger et al., 2012). The instrument was positioned on the upslope side of the tree to avoid compression wood, and the sensing rod was pointed towards the centre of the stem. The outermost layers of dead bark (0.5–1 mm) were removed at the sensor contact point to minimize the influence of hygroscopic swelling and shrinkage of the bark surface (Zweifel & Häsler, 2001). The sensors were attached to a Campbell Scientific CR1000 datalogger (Campbell Scientific Inc., Logan, UT, USA), which recorded measurements every 10 s and averaged them over 10-min intervals. The electronic resolution of the dendrometers in combination with the logger was $< 4.0\,\mu m$ and the sensitivity of the dendrometers to temperature was $< -0.58\,\mu m\,°C^{-1}$. No temperature correction was applied because the temperature-induced measurement error over 24 h was less than the sampling resolution. We assumed that sensors on all trees were affected similarly by temperature changes, and therefore comparisons between experimental treatments were not biased by this slight sensitivity.

The 2009 time series dendrometer data were processed to determine diurnal phases of radius contraction and expansion according to Deslauriers et al. (2003; Fig. S2). We focused on the contraction phase (time from morning maximum to afternoon minimum) and the expansion phase (time from minimum to next morning's maximum). For each tree, we calculated the time of day when each diurnal maximum and minimum occurred as well as the amplitude (µm) and duration (min) of the contraction and expansion phases for the measurement period. These values were automatically extracted from the data using a program written in LABVIEW version 2009 (National Instruments Corp. 2012, Austin, TX, USA) and then manually corrected where necessary.

Preliminary analyses were made on the entire data period, but for statistical analyses of treatment effects we excluded 12 dates when all 19 trees showed zero diurnal contraction during and immediately after heavy rainfall. Accounting for the power failure in late July, the main data set included 95 of the 115 d in the measurement period. One tree was excluded from all analyses as a consequence of zero or very small diurnal fluctuations throughout the measurement period, probably because of poor placement of the sensor, yielding a replication of four for ambient CO_2, warmed plots and five for the remaining three treatments (Table 1). To assess how treatments affected stem radius dynamics during different parts of the measurement period, we divided the full data set into (Fig. 2): an early-summer period during which most stem radial growth occurred (period A, 13 June to 10 August), a post-growth period when the radius was more or less constant except for water-related expansion and contraction (period B, 11 August to 3 September), and an autumn period when most trees experienced a slight decline in radius (period C, 4 September to 5 October).

Trees with thicker bark have the capacity to produce larger diurnal fluctuation amplitudes, and a positive relationship between maximum daily stem size fluctuations and bark thickness has been reported for mature subalpine Picea abies located near our study site (Zweifel & Häsler, 2001). We therefore expressed fluctuation amplitudes relative to total (maximum) bark thickness, which was measured on microcores collected in autumn 2009 using a 2-mm-diameter increment puncher (TREPHOR; Università degli Studi di Padova, S. Vito di Cadore, Italy; Rossi et al., 2006). Two microcores were extracted from each tree, one from each side of the stem facing perpendicular to the slope, within 15 vertical cm from the dendrometer position (Dawes et al., 2011b). The (living) bark thickness of each core was measured using an MS5 stereomicroscope at ×40 magnification (Leica Microsystems GmbH, Wetzlar, Germany) and the mean thickness for each tree was used in analyses.

Table 1 Measurements made on Larix decidua trees in 2008 (canopy cover estimated using hemispherical photographs) or 2009 (all other variables), averaged for each of the four treatment combinations (± 1 SE)

Treatment	Replication	Total height (cm)	Stem radius (µm)	Bark thickness (µm)	Canopy cover (%)
Ambient CO_2, unwarmed	5	243.2 ± 20.9	25150 ± 2266	3371 ± 204	37 ± 4
Ambient CO_2, warmed	4	215.3 ± 35.1	24344 ± 3317	3499 ± 288	37 ± 7
Elevated CO_2, unwarmed	5	282.2 ± 34.7	33450 ± 3656	4237 ± 388	47 ± 6
Elevated CO_2, warmed	5	257.4 ± 20.6	27925 ± 1163	4209 ± 210	43 ± 5

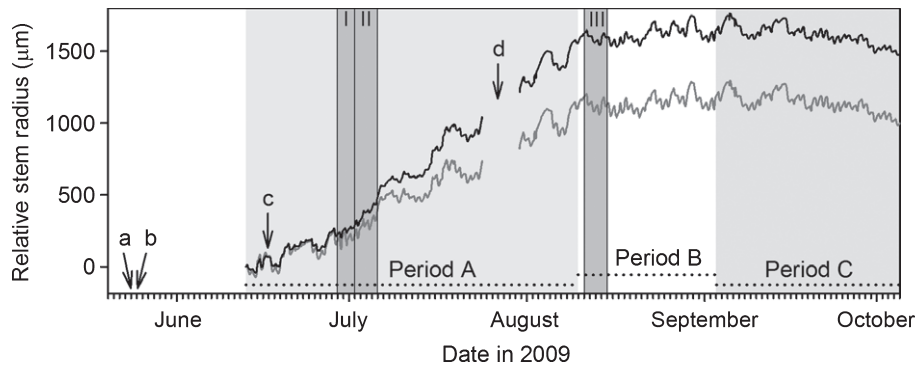Methodological details were given in Dawes et al. (2011b).

Fig. 2 Time series of *Larix decidua* stem radius dynamics obtained from dendrometers during the snow-free period in 2009. Measurements are given in μm and were set to zero at the start of data collection on 13 June. Mean values for each CO_2 treatment are shown, pooled across warmed and unwarmed plots (grey line, ambient CO_2, $n = 9$; black line, elevated CO_2, $n = 10$). Arrows show mean dates across all 19 trees for (a) snow completely melted from the plot (24 May); (b) bud burst on shoots formed in previous years (25 May, from Rixen *et al.*, 2012); (c) start of new shoot extension (17 June, from Rixen *et al.*, 2012); and (d) power failure after lightning strike (23–30 July). The season was visually divided into three segments: period A, main radial stem growth phase; period B, constant stem radius; and period C, slight decline in stem radius. The three short periods to which the hydraulic tree water relations model was applied are shown with darker shading (I, II and III).

Hydraulic tree water relations model

The hydraulic plant model HPMZ07 (Zweifel *et al.*, 2007) was applied to estimate values for specific physiological parameters of the individual trees in our experiment (Notes S1, Fig. S3). We then determined if these modelled parameters varied for trees exposed to different CO_2 and soil temperature treatments to explore potential physiological mechanisms for any treatment differences in the measured data. The HPMZ07 model was implemented using PHYTOSIM V1.2 software (PhytoIT, Ghent, Belgium), with inputs of 10-min resolution data for incoming shortwave solar radiation, VPD, wind speed, air temperature, and Ψ_{soil}, as well as the following tree-specific parameters: bark thickness (measurement methods described above); total tree height, leaf canopy size (calculated from hemispherical photographs) and stem diameter (measurement details in Dawes *et al.*, 2011b); and tree surface area, estimated from tree height and modelled tree crown envelopes according to Zweifel *et al.* (2002). Water potentials were measured on 1-yr-old mid-canopy shoots of a subset of the experimental trees during two warm, sunny days in 2011, yielding pre-dawn values above -0.6 MPa and minimum (midday) values above -2.2 MPa (details in Table S1). We therefore restricted the model to require calculated leaf water potentials above a threshold of -2.5 MPa.

The HPMZ07 model simulates tree-specific water potentials in the roots, xylem, bark and crown, the dynamic stomatal aperture of the crown, water flow and storage rates within the tree, and the related stem radius changes (Notes S2, Fig. S3). Modelled stem radius fluctuations are driven by functional relationships with turgor pressure-dependent growth (Steppe *et al.*, 2006) and with water tension-dependent depletion and refilling of the bark tissue (Zweifel *et al.*, 2001). Five parameters, each of which represented a specific tree physiological trait, were selected for optimization from a total of 18 parameters available in the model framework by using the combined model sensitivity and identifiability analysis tools integrated into PHYTOSIM (Fig. S3; De Pauw *et al.*, 2008). The selected parameters were: (1) the

water storage capacitance of the bark (C_bark); (2) the radial hydraulic resistance between xylem and bark (R_S); (3) an allometric parameter (b) for calculating the dynamically changing bark thickness in relation to the measured stem diameter, maximum bark thickness and tree height; (4) a growth parameter for radial wood growth (Phi); and (5) a parameter (Phi_bark) determining the stomatal responsiveness to tree water deficits (defined as decreases in stem radius, after Zweifel *et al.*, 2005). The term 'bark' in all model parameters represents elastic tissue external to the xylem, located in both above- and below-ground tissue.

The model was implemented separately for each tree in the experiment using an iterative optimization procedure (after Zweifel *et al.*, 2007) applied to the five parameters. This procedure minimized the deviation between the modelled and measured stem radius values, thereby reaching parameter values that provided the highest efficiency factor (EF) index for 'goodness-of-fit' (further details about EF calculations are provided in Notes S3). EF values > 0.25 indicated acceptable agreement between modelled and measured data (Hanson *et al.*, 2004; Zweifel *et al.*, 2007). We chose three periods of 3–4 d with low precipitation, and therefore clear diurnal fluctuation patterns, for applying the model. Run I (29 June to 1 July) and run II (2–5 July) were during the main seasonal stem growth period (A) but represented different tree growth rates, and run III (11–14 August) was in period B after most growth had occurred (Fig. 2). Further details about all aspects of the modelling application are given in Notes S1–S3, Figs S3 and S4, and Table S3.

Statistics

Effects of elevated CO_2 and soil warming on measured variables and on modelled parameters were tested with linear mixed-effects models fitted with the restricted maximum likelihood estimation method. For variables with repeated measurements, CO_2 and soil warming treatments, growing season period (A, B and C) or model run (I, II and III), and all two- and three-way interactions were included as fixed effects and tested for statistical significance

using Type I conditional F tests (Pinheiro & Bates, 2000). Climate variables and soil moisture were considered as potential co-variates in analyses of dendrometer measurements, as it is well documented that these conditions strongly influence tree water relations in general (Larcher, 2003) and the dynamics of radius fluctuations in particular (Zweifel et al., 2007). Interactions between climate variables and the experimental treatments were tested using likelihood ratio tests to determine if effects of CO_2 enrichment and soil warming depended on climatic conditions. The random effects structure respected our experimental design, with period or model run nested within plot nested within group. We applied a residual autocorrelation structure (autoregressive model of order 1, corAR1) to account for violation of independence of residuals from repeated measurements on a given tree, and we used a heterogeneous residual variance structure to allow differences among the three periods or model runs (Pinheiro et al., 2008). Variables measured only once were tested for effects of CO_2 enrichment, soil warming and their interaction, with group included as a random effect. Diurnal contraction and expansion amplitudes were square-root-transformed and modelled parameters were log-transformed to meet assumptions of homoscedasticity. For all statistical tests, effects were considered significant at $P < 0.05$. As a consequence of relatively low replication and therefore statistical power, we designated P-values ≥ 0.05 but < 0.10 as marginally significant. All analyses were performed using R version 2.15.2 (R Development Core Team, 2012) and mixed-effects models were fitted using the NLME package (Pinheiro et al., 2008).

Results

Climate and soil moisture

On average, snow was completely melted from the experimental plots on 24 May in 2009, with no significant differences between treatment groups (Fig. 2). During the dendrometer measurement period (13 June to 5 October), air temperatures at the meteorological station ranged from -2.0 to $21.6°C$ (mean $9.8°C$) and total precipitation was 425 mm (Fig. 1). Precipitation was relatively low after mid-August and led to declines in volumetric soil water content (Fig. 1b). The warming treatment led to somewhat drier soil: averaged over the season, volumetric water content was $35 \pm 4\%$ in unwarmed plots and $29 \pm 4\%$ in warmed plots (mean ± 1 SE, pooled across CO_2 treatments; Fig. 1b). CO_2 enrichment did not have a detectable effect on volumetric soil water content, and there were no interactive effects between CO_2 enrichment and soil warming. Despite drying associated with soil warming and seasonal precipitation patterns, the estimated Ψ_{soil} at 5 cm depth was always above -81 hPa (mean -16 hPa in unwarmed plots and -31 hPa in warmed plots), indicating very moist soil conditions throughout the snow-free period.

Tree stem radial growth

For all trees, the main period of stem radial growth started on 14–16 June (Fig. 2). Ninety-five per cent of the stem radial increment for the entire season was reached between 9 and 30 August, with no significant differences between treatments (Fig. 2, Table S2). The seasonal increase in radius tended to be greater for trees exposed to elevated CO_2 than for those exposed to ambient CO_2 (Fig. 2), although variation among trees was large and neither the treatments nor their interaction significantly influenced this value (Table S2).

Diurnal radius fluctuations

Throughout the entire measurement period, the amplitudes of the diurnal stem radius contraction ranged from 0 to 215 μm, with a mean (± 1 SE) of 50 ± 4 μm for all dates and all 19 trees. These values corresponded to 0–5.5% (mean ($\pm$ SE) $1.35 \pm 0.14\%$) of the total bark thickness and 0–1.5% (mean ($\pm$ SE) $0.38 \pm 0.04\%$) of the stem cross-sectional area. The timing of stem radius fluctuations varied widely throughout the season, with maximum values occurring between 00:00 and 16:40 h and minimum values occurring between 09:00 and 23:50 h. The duration of diurnal contraction and expansion was similarly variable, with values for both phases ranging from under 1 h to almost 24 h.

The 12 d with zero contraction amplitude, excluded from analyses of treatment effects, were rainy or at least cloudy and relatively cool (air temperature $< 10.6°C$; incoming shortwave solar radiation < 240 W m^{-2}; daytime VPD < 0.30 kPa) but showed no pattern regarding soil moisture. For the 95 d included in analyses, the amplitude and duration of diurnal contraction were positively related to mean daytime (shortwave) solar radiation and the daily maximum stem radius value occurred earlier on sunnier days. These relationships were stronger than those found with other measured climate variables, although solar radiation was positively correlated with VPD ($R^2 = 0.46$) and air temperature ($R^2 = 0.21$). We therefore included solar radiation as a covariate in analysis of treatment effects on these variables ($P < 0.0001$). Mean daily (24 h) air temperature had a stronger (positive) influence on the amplitude and duration of diurnal expansion and on the timing of diurnal minimum stem radius values (earlier minimum with higher temperature) than other climate variables. Consequently, we used mean air temperature instead of solar radiation as a covariate in the analysis of these measurements ($P < 0.0001$). Soil moisture did not show a significant influence on the measured variables. For all analyses, interactive effects between the covariate and the experimental treatments were not significant and were excluded from final statistical models.

Diurnal contractions expressed as a percentage of total bark thickness were smaller under elevated compared with ambient CO_2 (mean ($\pm$ SE) $-46 \pm 16\%$; $F_{1,8} = 8.85$; $P = 0.018$; Figs 3, 4a). The interaction between CO_2 and period was significant ($F_{2,30} = 5.56$; $P = 0.009$; Fig. 3), and separate analyses for each of the three growth periods indicated that the CO_2 effect was strongest during the main stem radial growth phase, period A ($-55 \pm 19\%$; $F_{1,8} = 12.49$; $P = 0.008$) and declined somewhat over periods B ($-42 \pm 16\%$; $F_{1,8} = 7.52$; $P = 0.025$) and C ($-36 \pm 15\%$; $F_{1,8} = 5.42$; $P = 0.048$). Diurnal expansion

Fig. 3 *Larix decidua* maximum diurnal stem contraction amplitude relative to bark thickness for each of the 95 d during 2009 included in the analysis, averaged for ambient (open circles; $n = 9$) and elevated (closed circles; $n = 10$) CO_2 treatment groups, ± 1 SE (pooled across soil warming treatments). Dotted vertical lines separate periods A, B and C. The inset figure shows the mean of all elevated-CO_2 trees divided by the mean of all ambient-CO_2 trees (E/A) for each day of the measurement period.
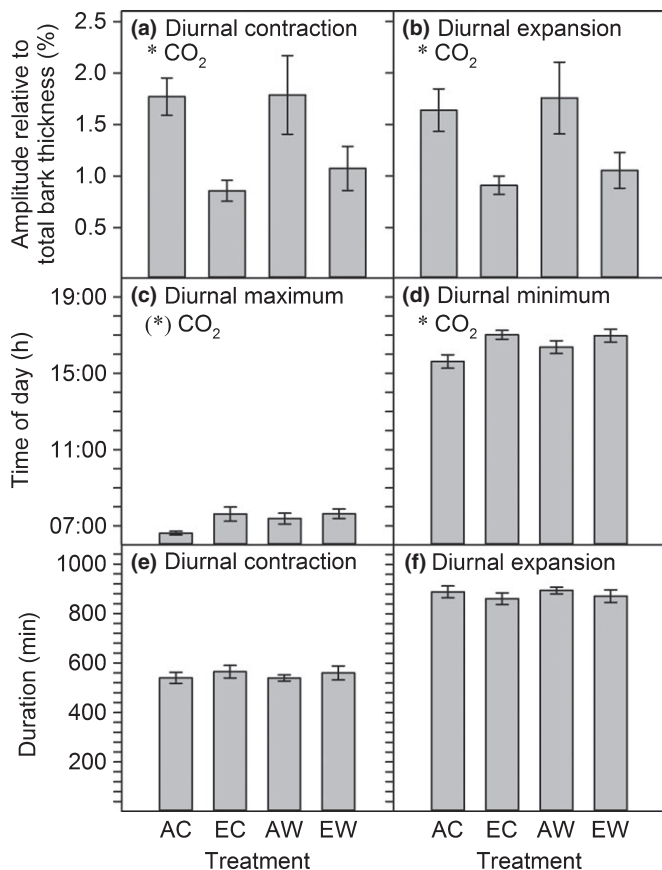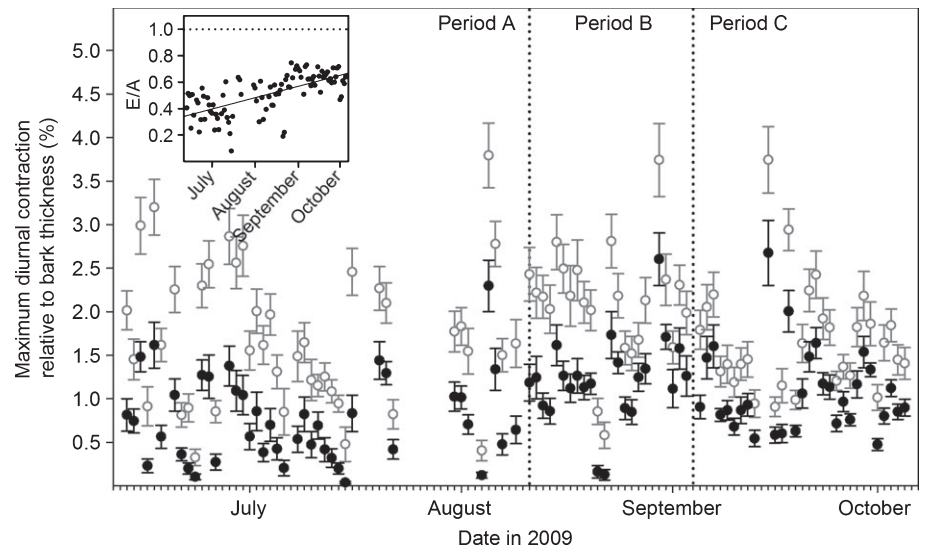

Fig. 4 *Larix decidua* diurnal stem radius fluctuations: amplitude of contraction (a) and expansion (b) as a percentage of total bark thickness; time of day of maximum (peak, c) and minimum (trough, d); and duration of contraction (e) and expansion (f) in min. The mean over the entire summer was determined for each individual tree before calculating the mean for each of the four treatment groups (± 1 SE). A, ambient CO_2; E, elevated CO_2; C, unwarmed soil; W, warmed soil; ($n = 5$ for AC, EC and EW; $n = 4$ for AW). CO_2 effects are indicated: *, $P < 0.05$ (significant); (*), $P < 0.10$ (marginally significant). There were no significant soil warming effects.

amplitudes were also reduced in trees exposed to elevated CO_2 ($-42 \pm 14\%$; $F_{1,8} = 7.63$; $P = 0.025$; Fig. 4b). The interaction between CO_2 and period was not statistically significant for this phase ($F_{2,30} = 2.24$; $P = 0.124$), but the CO_2 effect again declined somewhat over the season. Microcore measurements revealed that trees growing under elevated CO_2 had thicker bark ($+23 \pm 7\%$; $F_{1,8} = 5.49$; $P = 0.047$; Table 1). CO_2 effects were slightly weaker but still apparent when the absolute values of diurnal contractions ($-33 \pm 15\%$; $F_{1,8} = 4.91$; $P = 0.058$) and expansions ($-29 \pm 15\%$; $F_{1,8} = 3.31$; $P = 0.106$) were analysed, and interactive effects between CO_2 and period were unchanged.

In addition to reduced diurnal amplitudes, there was a slight delay in the timing of daily maximum (mean ($\pm$ SE) $+40 \pm$ 12 min; $F_{1,8} = 3.59$; $P = 0.095$, marginally significant) and daily minimum ($+63 \pm 14$ min; $F_{1,8} = 9.86$; $P = 0.014$) values for trees growing under elevated compared with ambient CO_2 (Fig. 4c,d). As with amplitudes, this shift in timing mainly occurred during periods A and B of the summer ($CO_2 \times$ period interaction: $F_{2,30} = 3.07$; $P = 0.061$ (daily maximum, marginally significant); $F_{2,30} = 5.13$; $P = 0.012$ (daily minimum)). Dendrometer height on the stem and the time of day when trees receive direct sunlight can influence the timing of diurnal peaks and troughs (Zweifel & Häsler, 2001), but neither of these values differed between CO_2 treatment groups. CO_2 enrichment did not affect the duration of the contraction or expansion phase ($P > 0.45$; Fig. 4e,f). Soil warming did not influence the amplitude, timing, or duration of contractions or expansions, and neither the interaction between CO_2 and warming nor the three-way CO_2–warming–period interaction was significant for any of the measured variables ($P > 0.14$; Fig. 4).

Modelled tree hydraulic parameters

Overall, the diurnal dynamics of tree water balance were simulated accurately with the model HPMZ07 for the available climate data and optimized tree-specific parameters (Table S3).

Model fit quality was considered unsatisfactory (EF value < 0.25) for one particular tree treated with elevated CO_2 and warmed soil, despite reasonable stem radius fluctuation data (Table S3). Values from this tree were excluded from statistical tests on the modelled parameters, although results were unchanged if these data were included. Comparisons of modelled stem radius fluctuations with the 10-min resolution dendrometer data for each individual tree investigated yielded a mean deviation between modelled and observed values of < 12 µm for run I, < 12 µm for run II, and < 17 µm for run III (example given in Fig. S4).

Statistical tests of treatment effects on the optimized model parameters indicated that the hydraulic xylem to bark resistance (R_S) was significantly greater for trees exposed to elevated CO_2 compared with those growing under ambient CO_2 (Table 2). There was a significant interaction between CO_2 and model run period for Phi_bark (stomatal responsiveness to tree water deficits) and C_bark (bark water storage capacitance), indicating differences between periods in the relative change in these parameters as a result of CO_2 treatment (Table 2). However, analyses of each model run separately did not yield significant CO_2 effects (data not shown). CO_2 treatment and interactions involving CO_2 were not significant for b (allometric parameter to calculate dynamic bark thickness) or Phi (growth parameter). The soil warming treatment and all other interactions were not significant for any parameter. All five parameters differed significantly over the three model run periods, irrespective of experimental treatment, with an increase in R_S and C_bark over the three runs, a decrease in Phi and Phi_bark, and the lowest values of b in run II (Table 2).

Discussion

Unexpectedly, diurnal stem radius fluctuation amplitudes in trees grown under elevated CO_2 for 9 yr were found to be smaller than values for trees exposed to ambient CO_2 (Figs 3, 4), despite having a larger canopy leaf area and therefore greater potential transpirational demand. Unlike our findings, mature *P. abies* in a lowland Swiss forest showed no change in water use, including stem water deficit derived from continuous dendrometer measurements, when exposed to elevated CO_2 in 2009 and 2010 (Leuzinger & Bader, 2012). We are not aware of additional CO_2 enrichment studies reporting stem radius change dynamics, and results for other proxies for tree water use indicate that results vary widely depending on tree developmental stage, species and the method used to assess water relations (Leuzinger & Körner, 2007; Warren *et al.*, 2011b).

Although reduced transpiration as a result of stomatal closure can lead to smaller amplitudes of diurnal stem radius fluctuations (Zweifel *et al.*, 2007), this physiological response alone would not systematically delay the timing of initial contraction or expansion as observed in our study. The evident lack of g_s response indicates that trees growing under elevated CO_2 did not transpire less in relation to tree size. This result is supported by data from gas exchange measurements made on the same trees in 2002 and 2003, where no difference in g_s was observed between CO_2 treatments (Handa *et al.*, 2005), and in 2009, where neither CO_2 enrichment nor soil warming altered g_s (Streit *et al.*, 2014). In the same 2009 study, $\Delta^{18}O$ isotope values (tissue enrichment with ^{18}O relative to source water; inversely proportional to g_s at a constant VPD) were measured in needles sampled throughout

Table 2 Average values and linear mixed-effects model results for five parameters optimized with the HPMZ07 hydraulic plant model for *Larix decidua* trees at the alpine tree line

Parameter	CO_2 treatment	Model run I Day of year 180–183	Model run II Day of year 183–187	Model run III Day of year 223–227	Linear mixed-effects model	
R_S	Ambient	0.20 ± 0.04	0.21 ± 0.04	0.32 ± 0.06	CO_2	$F_{1,8} = 6.31*$
	Elevated	0.30 ± 0.04	0.37 ± 0.04	0.44 ± 0.11	Model Run	$F_{2,28} = 7.42*$
					$CO_2 \times Run$	$F_{2,28} = 1.31$
C_bark	Ambient	59 ± 12	52 ± 10	187 ± 48	CO_2	$F_{1,8} < 0.01$
	Elevated	48 ± 11	63 ± 15	277 ± 43	Model Run	$F_{2,28} = 87.72*$
					$CO_2 \times Run$	$F_{2,28} = 6.47*$
b	Ambient	160 ± 46	146 ± 33	262 ± 46	CO_2	$F_{1,8} = 0.63$
	Elevated	188 ± 60	121 ± 33	165 ± 20	Model Run	$F_{2,28} = 4.93*$
					$CO_2 \times Run$	$F_{2,28} = 0.33$
Phi	Ambient	$1.28 \times 10^{-6} \pm 8.49 \times 10^{-7}$	$9.28 \times 10^{-8} \pm 2.40 \times 10^{-8}$	$2.60 \times 10^{-8} \pm 1.43 \times 10^{-8}$	CO_2	$F_{1,8} = 0.72$
	Elevated	$6.83 \times 10^{-7} \pm 4.07 \times 10^{-7}$	$3.36 \times 10^{-7} \pm 1.36 \times 10^{-7}$	$5.89 \times 10^{-8} \pm 2.18 \times 10^{-8}$	Model Run	$F_{2,28} = 9.45*$
					$CO_2 \times Run$	$F_{2,28} = 2.35$
Phi_bark	Ambient	$7.40 \times 10^{-3} \pm 1.23 \times 10^{-8}$	$6.50 \times 10^{-3} \pm 1.25 \times 10^{-3}$	$7.60 \times 10^{-4} \pm 1.01 \times 10^{-4}$	CO_2	$F_{1,8} < 0.01$
	Elevated	$4.37 \times 10^{-3} \pm 1.20 \times 10^{-3}$	$6.49 \times 10^{-3} \pm 1.27 \times 10^{-3}$	$7.41 \times 10^{-4} \pm 8.77 \times 10^{-5}$	Model Run	$F_{2,28} = 11.15*$
					$CO_2 \times Run$	$F_{2,28} = 2.95(*)$

R_S, hydraulic resistance between the xylem and bark (MPa s mg^{-1}); C_bark, water storage capacitance of the bark (g MPa^{-1}); b, allometric parameter to calculate the dynamic bark thickness (m^{-1}); Phi_bark, parameter determining stomatal responsiveness to tree water deficits (s^{-1}); Phi, parameter for radial wood growth (MPa^{-1} s^{-1}). Mean values ± 1 SE are shown for each CO_2 treatment group (ambient and elevated) and model run period (I, II and III), pooled across warmed and unwarmed plots ($n = 9$; excluding one tree with unsatisfactory model fit quality). For all parameters, statistical tests only indicated significant effects of CO_2 treatment, model run (I, II and III) and their interaction (F values and corresponding degrees of freedom shown; *, $P < 0.05$; (*), $P < 0.10$). Soil temperature and all other two- and three-way interactions were never statistically significant ($P > 0.10$; data not shown). Information about the HPMZ07 model fit quality is provided in Supporting Information Table S3 and Fig. S4.

the season and in tree rings. Consistent with gas exchange results, Streit *et al.* (2014) found no difference in $\Delta^{18}O$ between treatment groups in either tissue type.

Alternatively, changes in stem radius fluctuations can sometimes be attributed to reduced water uptake associated with altered fine root and/or mycorrhizal activity. As with reduced g_s, however, such decreases in water uptake would have altered transpiration and probably would not have influenced the relationship between the timing and amplitude of the diurnal radius changes, neither of which is consistent with our data. In support of this argument, fine root biomass and mycorrhizal mycelium production, measured in 2009 and in earlier years of the study, showed no response to CO_2 enrichment (Dawes *et al.*, 2013). Further, trees growing under ambient and elevated CO_2 did not differ in the timing of needle and shoot development (Rixen *et al.*, 2012) or in the timing of radial stem growth (Fig. 2, Table S2), so the observed changes in stem radius dynamics could not be attributed to a phenological shift. Altered xylem properties of conifers exposed to elevated CO_2 have been reported in some previous field experiments (Kilpeläinen *et al.*, 2007; Kostiainen *et al.*, 2009). Xylem properties determine the hydraulic resistance of the sapwood and thereby influence both the amplitude and timing of radius fluctuations (Steppe & Lemeur, 2007). For the trees in our study, however, measurements of tracheid characteristics made on microcore samples including the first 4 yr of CO_2 enrichment (2001–2004) indicated only a slight trend of larger tracheid lumen area in the latewood of *Larix* trees growing under elevated CO_2 (not statistically significant) and no change in the earlywood-to-latewood ratio (Handa *et al.*, 2006). Although detailed tracheid measurements were not made for later years of the study, these earlier findings suggest that properties of mature tracheids in the xylem were not responsible for the CO_2 effect observed with experimental data.

Instead, results from the hydraulic plant model suggest that trees exposed to elevated CO_2 had a larger flow resistance between the xylem and bark (R_S), representing a more buffered water supply system within the tree. This CO_2 effect was manifested as a reduced amplitude of diurnal radius fluctuations in combination with a greater time lag between sap flow in the xylem (onset of transpiration) and the contraction response of the bark. It has recently been found that radial hydraulic conductance is subject to short-term, even diurnal, changes caused by physiological shifts such as changes in xylem sap chemical composition and the expression of aquaporins (Steppe *et al.*, 2012). Therefore, it is possible that CO_2 enrichment influenced the abundance or activation status of xylem-expressed aquaporins controlling the exchange of water between xylem and bark. However, the close fit between modelled and measured stem radius changes over periods of 3–4 d indicated that such short-term modulations of R_S were not necessary for good model performance (Table S3, Fig. S4). Alternatively, longer term structural changes under elevated CO_2, such as altered abundance or distribution of ray tracheids and parenchyma, could have influenced radial conductance. In a 6-yr closed-chamber CO_2 enrichment

study of 20-yr-old *Pinus sylvestris*, Kilpeläinen *et al.* (2007) found a consistent, although weak, effect of fewer rays per mm^2 cross-sectional area in trees exposed to elevated CO_2.

While the close fit between model simulations and experimental data in our study provides a convincing interpretation of the observed CO_2 effect, we cannot rule out physiological explanations that were not captured in the hydraulic model. For example, enhanced photosynthetic rates in trees growing under elevated CO_2 (Streit *et al.*, 2014) might have led to greater fluxes of sugars within the phloem. Recent studies of deciduous tree species demonstrated that changes in the osmotic concentration in the phloem can cause detectable changes in the timing and amplitude of bark diameter variations (De Schepper *et al.*, 2010; Sevanto *et al.*, 2011; Mencuccini *et al.*, 2013). However, these same studies suggest that the osmotically driven contribution is relatively small compared with contributions from transpiration-induced xylem water potential, especially on sunny days with relatively high transpiration rates, which were the focus of our study.

As a consequence of differences in the use of stored water resources between larger and smaller trees, transpiration and stem radius changes vary as a function of tree size (Phillips *et al.*, 2003). In our study, the larger size of trees growing under elevated CO_2 for several years corresponded to larger stem diameters and thicker bark (Table 1), implying larger bark water storage and therefore an advantage in terms of water relations. As trees were only monitored with dendrometers in the ninth season of enrichment, we cannot determine if improved water relations associated with physiological changes contributed to growth gains before 2009. In contrast to water storage, the amplitude and timing of radial contractions and expansions were not related to bark thickness or tree size variables (data not shown). On average, elevated CO_2-treated trees with thicker bark actually had smaller fluctuations than ambient CO_2-treated trees when expressed in absolute terms. In addition, R_S was not dependent on tree size, which demonstrates that greater R_S values under elevated CO_2 were not simply a result of larger tree size and instead represent a physiological or anatomical change, as proposed above.

Soil warming did not lead to smaller diurnal fluctuation amplitudes (our second research question). This result suggests that evaporative demand primarily determines tree water relations in this wet system, whereas soil moisture is relatively unimportant. The consistently high Ψ_{soil} values at our study site (Fig. S1) and small effect of warming on soil moisture are related to the thick Oa horizon with a high water-holding capacity, frequent precipitation, and cool climate resulting in relatively low evaporative demand. In support of our results, Streit *et al.* (2014) found that g_s of the same study trees was sensitive to VPD but was not influenced by the soil warming treatment. Further, midday stomatal closure responses were observed for mature *P. abies* trees growing at a nearby site (1650 m asl) that is considered permanently wet and has Ψ_{soil} values similar to those at Stillberg (Zweifel *et al.*, 2002). In contrast to our results, King *et al.* (2013) reported decreasing diurnal fluctuation amplitudes in *L. decidua* as the number of days since the last rain event increased (soil moisture was not measured) at a drier site near the tree line in

southwest Switzerland. However, the positive effect of air temperature on diurnal expansion amplitude in our study is in agreement with observations by King *et al.* (2013), where monthly averages of *L. decidua* diurnal radius fluctuation amplitudes on days without precipitation were positively correlated with monthly air temperatures. In a study of intact cotton (*Gossypium hirsutum*) plants, Klepper *et al.* (1973) reported that low temperatures resulted in increased resistance between the xylem and elastic bark tissue and a reduced maximum water saturation level of stem tissues. Therefore, the positive correlation between air temperature and expansion amplitudes in our study could be related to negative effects of low temperature on radial conductance and stem tissue turgor.

Climatic and soil conditions, including our warming treatment, did not alter the CO_2-induced change in diurnal fluctuation dynamics. This result suggests that the somewhat drier soil and shorter days later in the summer did not contribute to the observed declining CO_2 effect over the course of the season (Fig. 3). It seems that the decline in CO_2 response size was instead related to different growth phases, also reflected in decreasing values for the growth parameter Phi throughout the season (Table 2), with the largest CO_2 effect occurring during the main growth phase in earlier summer. Large changes in other modelled parameters (R_S, +40%; C_bark, +320%; Phi_bark, −90%) from model runs I and II (during the main stem radial growth period) to run III (after most growth was complete) suggest that intra-seasonal shifts in these hydraulic traits might also have contributed to the reduced CO_2 response later in the season. Pronounced changes in hydraulic parameters over the growing season were reported for diffuse-porous *Fagus sylvatica* and ring-porous *Quercus robur* (Steppe *et al.*, 2008), but we are not aware of similar studies on conifers. Further studies spanning several years could determine whether the observed CO_2 effects are consistent over a wider range of climatic and soil moisture conditions, particularly warmer and drier summer conditions predicted for the Alps (Beniston, 2006).

Our study provides novel information about how the water relations of *L. decidua*, a key tree-line species in the Alps, function under current and predicted future atmospheric conditions, which in turn can influence the ability of this species to compete effectively with other tree species and with low-stature vegetation growing at the tree line. CO_2 enrichment clearly influenced the water supply system of these trees, apparent in altered diurnal stem radius fluctuation dynamics. Our exploration of potential mechanisms using a hydraulic plant model suggested that these CO_2 effects could be attributed to a change in specific traits influencing the water flow and storage system within the tree. Trees exposed to elevated CO_2 had larger stems and thicker bark, implying an enhanced water storage capacity, as well as a reduced amplitude and delayed timing of diurnal stem radius variations, which the hydraulic model proposed was attributable to increased radial resistance between the xylem and bark. In combination, these changes imply that trees growing under elevated CO_2 had a more buffered water supply system, which could have contributed to the increased growth rate of these trees observed during 9 yr of enrichment.

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Supporting Information

Additional supporting information may be found in the online version of this article.

Fig. S1 Soil matric water potential measurements from 2010.

Fig. S2 Stem radius fluctuation data divided into diurnal phases.

Fig. S3 Diagram of the hydraulic plant model HPMZ07 showing key functions and parameters.

Fig. S4 Example of measured and modelled stem radius fluctuations from one *Larix decidua* tree monitored with a point dendrometer.

Table S1 Water potential data for *Larix decidua* shoots sampled in 2011

Table S2 Amount and timing of stem radial growth in 2009 for *Larix decidua* trees with different CO_2 and soil warming treatments, as well as linear mixed-effects model results for these variables

Table S3 Efficiency factor (EF) used to assess how well the HPMZ07 model fitted measured data for each individual tree

Notes S1 HPMZ07 hydraulic plant model description.

Notes S2 HPMZ07 model parameterization procedure.

Notes S3 HPMZ07 model quality assessment.

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