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Research

Long-term stem CO_2 concentration measurements in Norway spruce in relation to biotic and abiotic factors

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Summary

- Stem CO_2 concentrations (stem [CO_2]) undergo large temporal variations that need to be understood to better link tree physiological processes to biosphere–atmosphere CO_2 exchange.
- During 19 months, stem [CO_2] was continuously measured in mature subalpine Norway spruce trees (*Picea abies*) and jointly analysed with stem, soil and air temperatures, sap flow rates, stem radius changes and CO_2 efflux rates from stem and soil on different time scales.
- Stem [CO_2] exhibited a strong seasonality, of which over 80% could be explained with stem and soil temperatures. Both physical equilibrium processes of CO_2 between water and air according to Henry's law as well as physiological effects, including sap flow and local respiration, concurrently contributed to these temporal variations.
- Moreover, the explanatory power of potential biological drivers (stem radius changes, sap flow and soil respiration) varied strongly with season and temporal resolution. We conclude that seasonal and daily courses of stem [CO_2] in spruce trees are a combined effect of physical equilibrium and tree physiological processes. Furthermore, we emphasize the relevance of axial diffusion of CO_2 along air-filled spaces in the wood, and potential wound response processes owing to sensor installation.

Introduction

The net carbon balance of an ecosystem is the balance of carbon gain by assimilation and carbon loss by respiration. While assimilation can be clearly assigned to green plant tissues aboveground, respiration originates from both aboveground and belowground plant tissues, as well as soil organisms, and their dependences on physical and biochemical factors or their interactions are not well yet understood (Trumbore, 2006). Recent studies indicate that CO_2 release measured in a specific tissue does not always reflect local CO_2 respiration and that stem CO_2 efflux (EF_{Stem}), measured usually by chambers attached to the stem, does not necessarily reflect true stem respiration resulting from translocation of CO_2 dissolved in xylem water (Teskey *et al.*, 2008). Furthermore, Aubrey & Teskey (2009) stated that much of root-respired CO_2 enters the xylem stream, challenging the paradigm of root-respired CO_2 diffusing completely into the soil atmosphere and thus questioning the ability to derive root respiration rates by measuring soil CO_2 efflux (EF_{Soil}) only. Consequently, if part of root-respired CO_2 remains inside the tree, it can be refixed by chloroplast-containing tissues (Gansert & Burgdorf, 2005; Dima *et al.*, 2006; McGuire *et al.*, 2009) and might contribute to tree assimilation. Thus, common approaches to partition net ecosystem CO_2 fluxes into gross primary productivity and total respiration by response functions probably have to be revised.

Trees are an important link between the soil and the atmosphere, both in terms of water and carbon translocation. Stem internal CO_2 (stem [CO_2]) consists of CO_2 respired locally by different stem tissues, such as bark, phloem, cambium and living cells within the sapwood, and of CO_2 respired by roots or lower sections of the stem, dissolved in xylem water and being dislocated via sap flow (Moore *et al.*, 2008; Aubrey & Teskey, 2009). Hence, understanding tree internal CO_2 fluxes is essential when aiming at a better understanding of stem respiration and measured EF_{Stem}, but also of processes involved in soil respiration and links between forest ecosystem assimilation and respiration.

The contribution of respired CO_2 to either the CO_2 remaining inside the tree or to the CO_2 diffusing to the atmosphere is determined by respiration rates of individual tissues/organisms and by diffusion barriers (Sevanto *et al.*, 2011; Steppe *et al.*, 2012), all of which are a function of the actual phenological phase. For example, respired CO_2 in the bark travels through air-filled spaces when released to the atmosphere, whereas CO_2 respired by sapwood also has to cross water-saturated tissue and the nearly gas-impermeable cambium (Steppe *et al.*, 2007). Thus, translocation of CO_2 via sap flow is supposed to affect EF_{Stem} especially during day-time, while at night, when transpiration but also corticular and woody photosynthesis are close to/at zero, measured EF_{Stem} is assumed to better represent the actual stem respiration rate (McGuire & Teskey, 2004).

Tree internal CO_2 fluxes, such as import, export and recycling, could serve as one explanation for high spatial and temporal variability of previously reported EF_{Stem} rates (Ceschia *et al.*, 2002; Damesin *et al.*, 2002; Teskey & McGuire, 2005; Steppe *et al.*, 2007). Moreover, they might explain the documented temperature hysteresis or even decoupling of EF_{Stem} from temperature (Saveyn *et al.*, 2008a), which is in conflict with the commonly accepted theory that respiration should be related exponentially to temperature, as can be expected for purely biologically driven processes. Further factors have been identified that may also alter internal stem [CO_2] and EF_{Stem}, such as substrate limitations (Stockfors & Linder, 1998; Pruyn *et al.*, 2005), diurnal growth patterns (Daudet *et al.*, 2005) and changes in stem water status (Saveyn *et al.*, 2007b, 2008b). Together, these aspects make interpretation of respiration even more difficult.

Recently, Teskey *et al.* (2008) proposed that a correct estimate of stem respiration needs to account for internal CO_2 fluxes, which can be assessed by measuring CO_2 concentrations *in situ* in stems or branches of trees. Inserting a microelectrode (McGuire & Teskey, 2002) or a nondispersive infrared CO_2 sensor (Teskey & McGuire, 2007) into the stem allows for continuous and high-resolution measurements of stem [CO_2]. However, there are few studies that report continuously measured stem [CO_2] in trees and most of these were conducted in the glasshouse and/or were restricted to short periods of several days or weeks (Teskey & McGuire, 2002; Saveyn *et al.*, 2007b; Cerasoli *et al.*, 2009). The results of these studies vary widely in terms of flux magnitude and their interpretation (Bowman *et al.*, 2005; Teskey & McGuire, 2007; Saveyn *et al.*, 2008a). Furthermore, different relationships for internal CO_2 fluxes and EF_{Stem} have been reported. While Teskey & McGuire (2005, 2007) found EF_{Stem} to be directly proportional to stem [CO_2] in sweet-gum (*Liquidambar styraciflua*) and sycamore (*Platanus occidentalis*), EF_{Stem} of poplar trees was either uncoupled from stem [CO_2] or the two were inversely related to each other (Saveyn *et al.*, 2008b); no significant correlation could be found for rimu (*Dacrydium cupressinum*; Bowman *et al.*, 2005). Manipulations of xylem sap CO_2 concentrations in poplar and oak trees were reflected in EF_{Stem} (Teskey & McGuire, 2002) but crown removal and hence breakdown of the transpiration stream had no effect on EF_{Stem} of loblolly pine trees, leading to the conclusion that diffusion of CO_2 from the xylem to the stem surface is restricted in pine trees (Maier & Clinton, 2006). In agreement with this conclusion, Ubierna *et al.* (2009) could not detect any changes in the isotopic signal of EF_{Stem} of large conifer trees (*Abies grandis*, *Thuja plicata* and *Larix occidentalis*), neither after labelling their water source nor after crown removal. Hence, the abiotic and biotic drivers that affect the dynamics of stem [CO_2] and EF_{Stem} are not yet identified.

Thus, in this study, we combine ecophysiological measurements with continuous stem [CO_2] measurements that were conducted in a mature subalpine Norway spruce forest over 19 months (May 2009 to December 2010). We address the following objectives: to quantify the degree of daily and seasonal variations of stem [CO_2] explicable by pure physical equilibrium processes between CO_2 in gas and CO_2 dissolved in water, as described by Henry's law; to test interdependences of stem [CO_2] and stem, soil and air temperatures, sap flow rates and stem radius changes, as well as EF_{Stem} and EF_{Soil}; and to address the temporal consistency of such interdependences at seasonal and daily scales over the 19 months of study. To our knowledge, this is the longest time-series of continuous *in situ* measurements of stem [CO_2] and provides a deeper insight into the dynamic interdependences of the plant–soil system.

Materials and Methods

Study site

The Norway spruce forest Seehornwald Davos is located at 1639 m above sea level at 46°48′55.2″N, 9°51′21.3″E in the eastern part of the Swiss Alps. The site belongs to the long-term forest ecosystem monitoring network (LWF, run by the Swiss Federal Research Institute WSL, Switzerland), and several national and international research and monitoring networks. The mean age of the dominant Norway spruce (*Picea abies* (L.) Karst.) trees is 240 yr, reaching up to 450 yr. The patchy understory is dominated by dwarf shrubs of *Vaccinium myrtillus* L. and *Vaccinium gaultherioides* L., and moss mats (*Hylocomium splendens* (Hedw.) Schimp. and *Dicranum scoparium* Hedw.). Soils are acidic (pH 3.5–4.5, Jörg, 2008) and classified as rustic podzols and chromic cambisols (IUSS Working Group WRB, 2007). Mean annual precipitation is 1020 mm (1901–2008) with a distinct seasonality (*c.* 40% of annual rainfall occurs during the summer months June to August). Mean annual air temperature is 4.0°C. With a mean canopy height of 25 m and a leaf area index of 3.9 m^2 m^{-2}, the forest can be considered a moderately productive forest stand within this region of the Alps (Etzold *et al.*, 2011).

CO_2 concentrations in the stem

Continuous stem [CO_2] measurements were conducted during the years 2009 and 2010 on two mature spruce trees (tree 148 and tree 296) by inserting manufacturer calibrated nondispersive infrared (NDIR) CO_2 sensors (GMP221 Carbon dioxide probe 0…20 vol%; Vaisala Oyj, Vantaa, Finland) into the xylem, following instructions by McGuire & Teskey (2002) and Saveyn *et al.* (2008b). Briefly, we drilled 60 mm deep holes with a diameter of 20 mm into the stem, inserted the NDIR sensor and sealed the space between sensor housing and stem with rubber foam (Terostat-IX; Henkel AG & Co.KGaA, Düsseldorf, Germany). Seals were visually checked several times per year. The NDIR sensors were placed 25 cm above the respective stem efflux collars. Measurement precision of the sensor CO_2 reading is given as ± 0.15 vol% CO_2 (accuracy is ± 2% of the absolute CO_2 reading) by the manufacturer. Temperature dependency of the method was corrected according to the manufacturer (−0.1 vol %/°C). Stem [CO_2] is given in volume percentage (vol%, 1 vol % = 10 000 ppm).

In addition, CO_2 dissolved in the xylem sap (sap [CO_2], in mM) was calculated by applying Henry's law, extended by dissociation factors, according to Teskey *et al.* (2008) and McGuire *et al.* (2007):

$$\text{sap}[CO_2] = \left(1 + \frac{K_1}{10^{-\text{pH}}} + \frac{K_1 K_2}{(10^{-\text{pH}})^2}\right) K_H p CO_2 \qquad \text{Eqn 1}$$

where sap $[CO_2]$ is total dissolved inorganic carbon in solution, K_1 and K_2 are first and second acidity constants, K_H is Henry's constant, and pCO_2 is the partial pressure of CO_2 over the solution. Henry's law is based on the dependency of CO_2 dissolution in water on temperature, pH and partial pressure of CO_2: the dissolution of CO_2 in water increases with decreasing temperature and increasing pH. As sap pH can only be assessed by destructive measures, we assumed a constant pH of 5.6 as was given for loblolly pine (Carter & Larsen, 1965). Henry's law is only valid for temperatures above 0°C. Therefore, sap $[CO_2]$ is displayed for periods with stem temperatures above 0°C only. Stem temperature (T_{Stem}) was continuously measured 2 cm below the stem surface with copper-constantan thermocouples (type T). Stem $[CO_2]$ measurements were available from 1 April 2009, T_{Stem} measurements from 28 April 2009.

We calculated model stem $[CO_2]$ and sap $[CO_2]$ according to Henry's law under the assumption of a constant CO_2 concentration either in the liquid or in the gas phase, a constant pH of 5.6 and the measured T_{Stem}. Temporal variability of model stem and sap $[CO_2]$ values was thus solely attributable to the physical effect of temperature changes, as changes in the CO_2 pool were excluded. Using different sap pH input values (e.g. 5.6, 6.0 and 6.5) changed only the value of the respective model CO_2 concentrations, but not their temporal amplitudes. Differences between model and measured stem $[CO_2]$ were interpreted as net import or export of CO_2 into or out of the stem segment investigated. For the model stem $[CO_2]$ we assumed a constant sap $[CO_2]$ value of 3.5 mM (stem $[CO_2]_{sap=3.5}$), and for the sap $[CO_2]$ a constant stem $[CO_2]$ value of 4 vol% (sap $[CO2]_{stem=4}$), according to the annual mean values of our measurements.

Stem efflux measurements, EF_{Stem}

Stem CO_2 efflux (EF_{Stem}) was measured on four spruce trees (the two trees 296 and 148 with stem $[CO_2]$ measurements and two additional trees 147 and 411) growing in direct vicinity of each other. EF_{Stem} was measured campaign-wise with a custom-made chamber connected to a portable infrared gas analyser (Li-8100; Li-cor Inc., Lincoln, NE, USA), operating in closed mode. The chamber was made of a half cylinder of acryl glass (PMMA GS; Evonik Industries AG, Darmstadt, Germany), with the edges covered by neoprene. The chamber covered a stem surface area of 144 cm^2 and enclosed a volume of 770 cm^3. During the measurements it was placed and fixed on collars that were permanently installed at breast height on the south- and north-facing sides of each tree stem. Collars were sealed to the tree bark with rubber foam and Terostat-IX. In addition, the chamber was covered with aluminium foil to prevent stem photosynthesis and heating by direct radiation, and was equipped with a small fan, a temperature sensor and a pressure vent. The EF_{Stem} rates of all trees were measured consecutively with the same installation device (chamber connected to the IRGA). For one measurement cycle, each stem side (North, South) was measured twice within 10 min. Thus, EF_{Stem} per tree and at any point in time is given as an average of four measurements. All four trees were measured within 1 h. During the day (06:00–20:00 h), each tree was measured every hour, during the night (21:00–05:00 h) every 2 h (i.e. $n = 20$ for each tree per diurnal cycle). EF_{Stem} was measured during two campaigns in 2008 (6/7 August, 3/4 September), and three campaigns in 2009 (1/2 July, 22/23 July, 12/13 September). The EF_{Stem} measurements were fitted to temperature variations of each tree, using the following equation:

$$EF_{Stem.10} = EF_{Stem} * Q_{10}^{\left[\frac{10 - T_{Stem}}{10}\right]} \qquad \text{Eqn 2}$$

where $EF_{Stem.10}$ is EF_{Stem} estimated at 10°C, Q_{10} is the relative increase in EF_{Stem} for a temperature increase of 10°C and T_{Stem} is stem temperature (°C).

As sap flow is hypothesized to affect EF_{Stem} by translocation of CO_2, EF_{Stem} is assumed to represent stem respiration under low sap flow conditions only (McGuire & Teskey, 2004). In order to analyse the potential effect of sap flow on EF_{Stem}, we established tree-specific temperature response functions of EF_{Stem} for conditions when sap flow was almost zero, that is, during the night (18:00–6:00 h), using the following equation:

$$EF_{Stem} = a * e^{b * T_{Stem}} \qquad \text{Eqn 3}$$

Coefficients a and b are estimated by the model. Adjusted r^2 values of tree-specific models were 0.43, 0.73, 0.73 and 0.85 ($P < 0.001$) for trees 296, 148, 147, and 411, respectively. Assuming a comparable temperature dependence of respiration during the day, a theoretical EF_{Stem} was estimated for daylight conditions and compared with measured EF_{Stem} (dEF_{Stem} = measured EF_{Stem} − predicted EF_{Stem}).

Continuous stem radius changes

Stem radius changes (DR, difference in radius over time) were measured with automated point dendrometers (ZB06; natkon.ch, Hombrechtikon, Switzerland) mounted *c.* 1.5 m aboveground on the north-west side of each stem. The DR was measured every 10 s, averaged and stored every 10 min. The measured temperature sensitivity of the ZB06 of 0.27 µm $°C^{-1}$ was corrected using a linear temperature response function. The electronic resolution of the dendrometers (in combination with the logger used) was *c.* 1 µm. Variations in DR reflect tree water status on the short term (minutes to weeks) and wood growth in the long term (months to years; Steppe *et al.*, 2006; Zweifel *et al.*, 2006).

Sap flow

Sap flow of the sampled trees was continuously assessed with two Granier-type sap flow sensors (x600M; UP GmbH, Cottbus, Germany) per tree (Granier, 1985). One sensor was mounted at the north and one at the south exposed side of the stem, *c.* 1.5 m above the ground. For our analyses, we used the mean of both

readings per tree. Sensors were insulated against temperature and solar radiation fluctuations with Styrofoam and bubble wrap with a reflective surface.

Soil CO_2 efflux, EF_{Soil}

Continuous soil CO_2 efflux measurements (EF_{Soil}) were conducted during the snow-free period in 2009 and 2010, starting mid-May and ending mid-November. An automatically operating soil chamber (LI-8100; Li-cor Inc.) was installed *c.* 200 m away from the sampling trees, measuring soil CO_2 efflux every 30 min (Etzold *et al.*, 2011). Soil temperature (T_{Soil}) was measured in 5 cm soil depth. The continuous EF_{Soil} data were cross-checked with additional manual EF_{Soil} measurements on nine nearby plots during the growing period in 2009, using a portable closed chamber system (LI-8100 with LI-8100-103 chamber; Li-Cor Inc.). A tight and highly significant linear relationship between the continuous and the manual EF_{Soil} measurements confirmed the representativeness of our continuous EF_{Soil} measurements (continuous $EF_{Soil} = 0.60 \times$ manual $EF_{Soil} + 1.8$, adj. $r^2 = 0.95$, $P < 0.001$, $n = 6$; campaign averages as a function of daily-averaged continuous EF_{Soil} measurements). Manually measured data were not used for further analyses.

Definition of tree physiological phases

Tree physiological processes within 1 yr were assigned to different consecutive phases as follows (Table 1, Fig. 1): dormancy (period with hardly any transpiration of trees, mainly during winter time; mean daily sap flow rate < 10% of the summer maximum rate); spring (period with recovery from winter stem contraction by rehydration of depleted cell water reserves (Zweifel & Häsler, 2000) leading to radial stem expansion, but not yet to radial stem growth (as in Zweifel *et al.*, 2010)); wood growth (period of radial stem growth according to Zweifel *et al.* 2010); late summer (period after 97.5% radial stem growth is reached and mean daily sap flow > 10% of summer maximum rates). For further analyses, we separated dormancy into consecutive periods with air temperatures below and above 0°C.

Statistical analyses

All calculations and analyses were conducted with the R statistical software package 2.11.1 (R Development Core Team, 2011). For all tree physiological phases of the two study years we calculated correlations (Spearman rank) of stem $[CO_2]$ with T_{Stem}, T_{Soil}, T_{Air}, DR and sap flow, as well as cross-correlations with a maximum time lag of ± 3 d. We also smoothed the time-series of stem $[CO_2]$ and DR (smooth.spline function in the R statistical software) and calculated correlation matrices with the remaining residuals in order to eliminate the growth trend (stem $[CO_2]_{detrended}$ and $DR_{detrended}$). For cross-correlations, a positive time lag in days means that stem $[CO_2]$ lags behind the cross-correlation variable, a negative lag means that the cross-correlation variable lags behind stem $[CO_2]$. For each tree physiological phase, stem $[CO_2]$ was predicted by general linear models

Table 1 Correlation coefficients of mean daily values of stem CO_2 concentration and stem temperature (T_{Stem}), soil temperature (T_{Soil}), air temperature (T_{Air}), stem radius changes (DR), detrended stem radius changes ($DR_{detrended}$, correlated to stem $[CO_2]_{detrended}$) and sap flow for different tree physiological phases (see text for definitions), determined for Norway spruce (*Picea abies*) trees in the years 2009 and 2010

		T_{Stem}		T_{Soil}		T_{Air}		DR		$DR_{detrended}$		Sap flow	
Period	Days	*r*	r_{max}	*r*	r_{max}	*r*	r_{max}	*r*	r_{max}	*r*	r_{max}	*r*	r_{max}
Dormancy 2009	0–90	na	na	na	na	na	na	na	na	na	na	na	na
Spring 2009	90–147[†]	0.97*	=	0.89*	=	0.93*	0.96* (1)	−0.81*	=	−0.76*	=	0.71*	0.83* (1)
Wood growth 2009	147–222	0.67*	0.68* (1)	0.79*	=	0.63*	0.68* (1)	0.85*	0.90* (−3)	−0.38*	−0.60* (1)	0.24^{+}	0.40^{+} (2)
Late summer 2009	222–282	0.89*	=	0.94*	=	0.80*	0.92* (1)	0.33^{+}	0.52* (−2)	−0.07	0.53* (−2)	0.12	0.51* (2)
Dormancy > 0°C 2009	282–346	0.90*	0.93* (1)	0.86*	=	0.60*	0.67* (1)	−0.40*	−0.56* (2)	−0.63*	−0.73* (1)	0.19	0.62* (3)
Dormancy < 0°C 2009/10	346–77	0.42*	=	0.68*	0.75* (−3)	0.40*	=	0.69*	0.69* (1)	0.38*	0.40* (−1)	−0.20	−0.21 (−1)
Dormancy > 0°C 2010	77–110	0.17	=	0.24	0.51* (3)	−0.03	=	0.81*	0.88* (2)	0.04	=	0.20	=
Spring 2010	110–165	0.79*	0.85* (1)	0.37*	=	0.70*	0.83* (1)	−0.38*	−0.63* (2)	−0.59*	−0.79* (2)	0.38*	0.81* (2)
Wood growth 2010	165–228	0.91*	0.97* (1)	0.95*	=	0.81*	0.94* (1)	−0.17	−0.30* (2)	−0.58*	−0.88* (2)	0.33*	0.78* (3)
Late summer 2010	228–288	0.92*	0.95* (1)	0.97*	=	0.73*	0.83* (1)	−0.05	0.15* (−3)	−0.49*	−0.59 (1)	0.19	0.40* (2)
Dormancy > 0°C 2010	288–335	0.81*	0.93* (1)	0.92*	=	0.54*	0.82* (2)	−0.77*	=	−0.68*	=	0.22	0.67* (2)
Dormancy < 0°C 2010	335–365	0.75*	=	−0.38	=	0.82*	=	−0.01	−0.62* (3)	−0.07	−0.66* (3)	0.07	=
Year 2009	90–365	0.43*	=	0.59*	=	0.44*	0.46* (1)	0.67	0.71* (−3)	−0.32*	−0.48* (1)	0.21*	0.29* (2)
Year 2010	1–365	0.87*	=	0.83*	=	0.82*	0.84* (1)	0.15	0.21* (−3)	−0.39*	−0.52* (2)	0.63*	0.75* (2)

r, Spearman rank correlation coefficient; r_{max}, maximum correlation coefficient of time-lagged variables, with time lag in days given in parentheses. '=', r_{max} equals *r*; na, no data available.
Grey shadows indicate the highest *r* resp. r_{max} per time-period.
[†]T_{Stem} data available since Day 118 (28th of April).
*, Significant at $P < 0.001$; +, significant at $P < 0.01$.

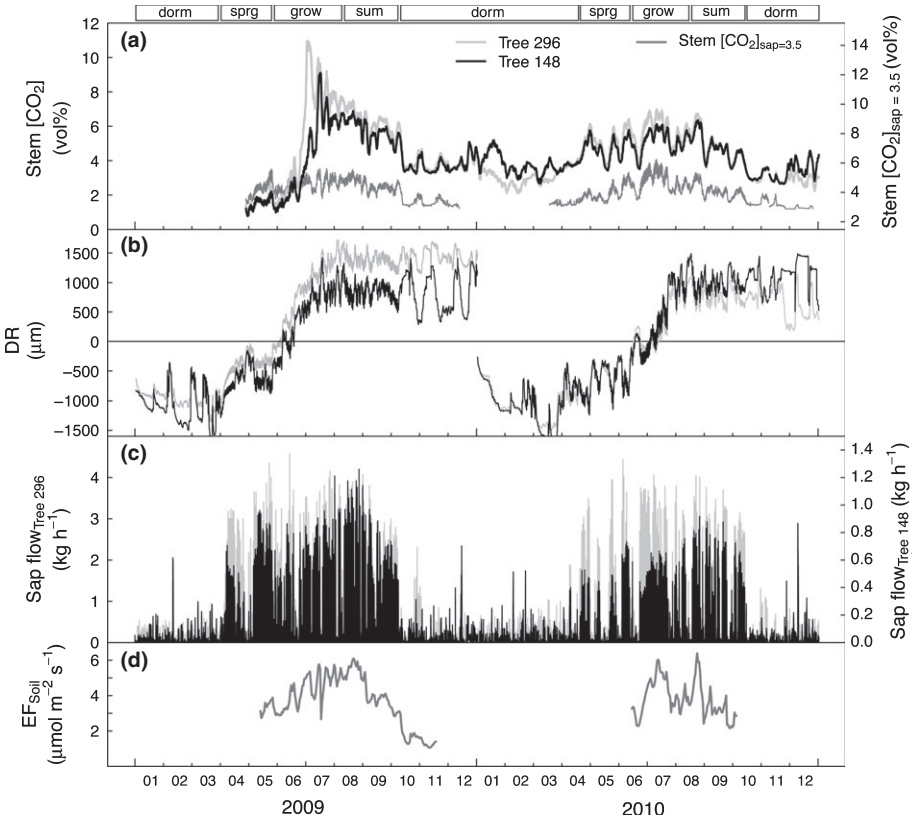

Fig. 1 Annual cycles of tree and soil physiological processes at a subalpine Norway spruce (*Picea abies*) forest in 2009 and 2010. (a) Stem CO_2 concentrations (stem [CO_2]) of two Norway spruce trees (trees 296 and 148) and a model stem [CO_2] curve calculated according to Henry's law, with the measured stem temperature and assuming a constant CO_2 concentration in the sap of 3.5 mM and a constant pH of 5.6 (stem $[CO_2]_{sap=3.5}$). (b) Stem radius changes (DR) for two Norway spruce trees (trees 296 and 148). (c) Sap flow rates for two Norway spruce trees (trees 296 and 148). (d) Efflux of CO_2 from the soil (EF_{Soil}). Tree physiological phases within a year are labelled: dorm, dormancy; sprg, spring; grow, wood growth; sum, late summer (as defined in the Materials and Methods section).

(GLM) with a minimum subset of required predictor variables (T_{Stem}, T_{Soil}, DR and sap flow). The best model was identified by a stepwise selection of variables in both directions with the step-AIC function from the MASS package in the R statistical software. For multiple regression models, we calculated the standardized beta coefficients in order to determine the relative explanatory power of the variables within each model.

For analyses on a diurnal time-scale, we excluded the long-term seasonality of the variables by normalizing hourly values by the respective daily maxima (in %). For all measures of absolute values (DR, stem and sap [CO_2], T_{Stem}), we also calculated the changes over each 30-min time-step (Δ).

Results

Seasonality of stem [CO_2]

Stem [CO_2] of mature Norway spruce trees increased as soon as cambial activity started in spring, was highest during July and August, and decreased slowly with the cessation of growth during late summer (Fig. 1a,b). Rather outstanding and nonrecurring in its magnitude was the stem [CO_2] peak during the early growth period in 2009 (Fig. 1a). The temporal course of modelled stem [CO_2], based on the assumption of a constant sap [CO_2] of 3.5 mM (stem $[CO_2]_{sap\ =\ 3.5}$), was closely related to measured stem [CO_2] during both vegetation periods (spring until late summer, 2009: adj. $r^2 = 0.31$, 2010: adj. $r^2 = 0.68$), but did not reflect the general increase of stem [CO_2] in the two wood growth phases. During winter 2009/10, especially from January to March 2010, stem [CO_2] showed large fluctuations and substantial differences between the two trees (Fig. 1a).

Stem [CO_2] and temperature conditions of air, soil and stem

Temperature as a main driver for respiration processes was measured in air (T_{Air}), soil (T_{Soil}) and stems (T_{Stem}; Fig. 2a). Stem [CO_2] was most closely related to T_{Stem} and T_{Soil}, whereas T_{Air} always explained less variation in stem [CO_2] than the other two temperatures (r_{max} in Table 1). T_{Stem} was more closely related to stem [CO_2] during the early active phases of the year (spring and wood growth), whereas T_{Soil} was more closely related in late summer and during dormancy (> 0°C). Interestingly, the correlation of stem [CO_2] to T_{Stem} and T_{Air} was best with a time lag of 1 d, whereas the correlation to T_{Soil} was best without a time lag (Table 1). Overall, > 80% of the seasonal variation in stem [CO_2] could be explained by T_{Stem} when excluding winter days with a mean daily T_{Stem} below 0°C and the growth period in 2009 (Fig. 3). Under freezing conditions, additional drivers seemed to affect the relationship between stem [CO_2] and T_{Stem}, resulting in less variance being explained (Table 1, Fig. 3). The exceptional increase and peak of stem [CO_2] in the wood growth period 2009 showed a weaker relationship with temperature than during the remaining vegetation period (Table 1, Fig. 3a), pointing towards a process affecting stem [CO_2] which did not occur in 2010.

With a linear regression model, we tested which combinations of two factors (out of our continuous long-term measurements:

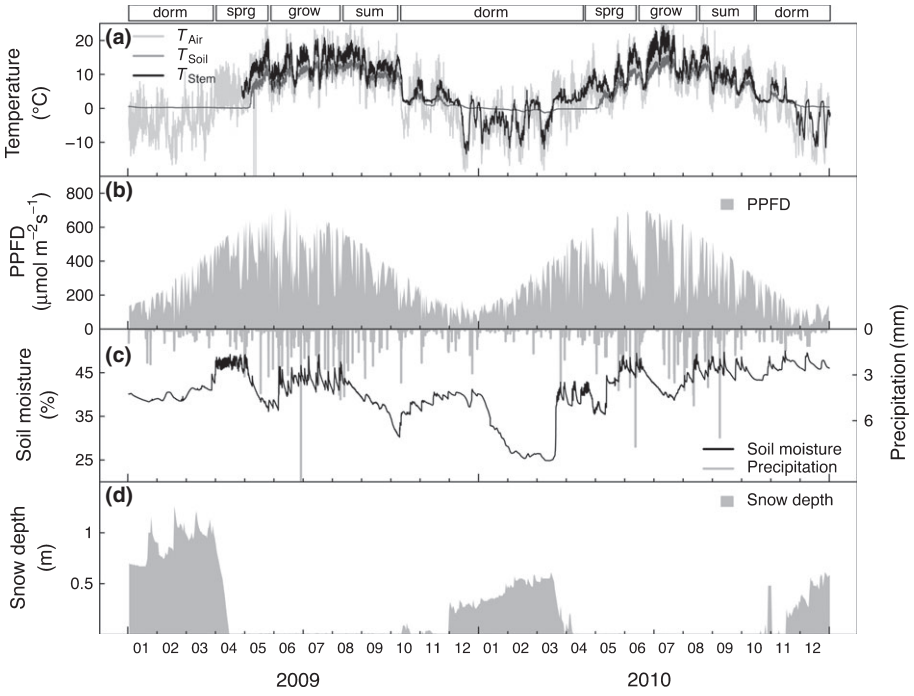

Fig. 2 Climate conditions at the Norway spruce forest during the two study years 2009 and 2010. (a) Air, soil and stem temperatures (T_{Air}, T_{Soil}, T_{Stem}). (b) Photosynthetic photon flux density (PPFD). (c) Soil moisture and precipitation. (d) Snow depth. Tree physiological phases within a year are labelled: dorm, dormancy; sprg, spring; grow, wood growth; sum, late summer; as defined in the Materials and Methods section.

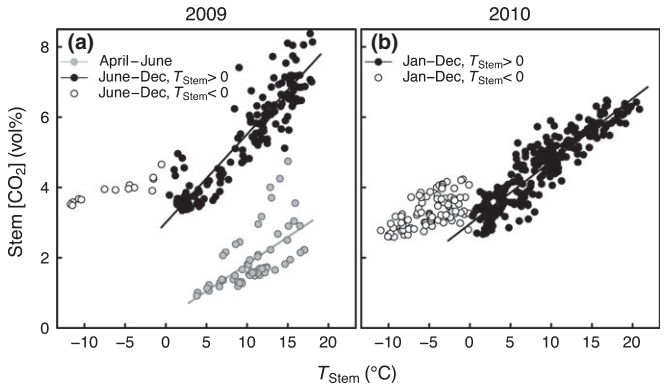

Fig. 3 Relationships between daily means of stem CO_2 concentrations (stem [CO_2], average of both trees) and stem temperatures (T_{Stem}) of Norway spruce (*Picea abies*) trees for the years (a) 2009 and (b) 2010. We distinguished the following time-periods: In 2009: April to June (stem [CO_2] = 0.15T_{Stem} + 0.28; adj. r^2 = 0.37, P < 0.001), June to December with T_{Stem} > 0°C (stem [CO_2] = 0.25T_{Stem} + 3.0; adj. r^2 = 0.85, P < 0.001) and June to December with T_{Stem} < 0°C (stem [CO_2] = 0.06T_{Stem} + 3.5; adj. r^2 = 0.40, P < 0.001). In 2010: January to December with T_{Stem} > 0°C (stem [CO_2] = 0.18T_{Stem} + 3.0; adj. r^2 = 0.84, P < 0.001), and January to December with T_{Stem} < 0°C (stem [CO_2] = 0.09T_{Stem} + 3.7; adj. r^2 = 0.33, P < 0.001).

T_{Stem}, T_{Soil}, DR and sap flow) explained the variation in daily mean stem [CO_2] best (Table 2). For almost all phases (except wood growth 2009, dormancy >°C 2010), T_{Stem} or T_{Soil} were the only or the main explanatory factors.

Stem [CO_2] in relation to stem radius changes and sap flow

Stem growth rates, and therefore DR dynamics, were different between the two observation years (Fig. 1b). The year 2009 was characterized by an early start of the vegetation period because of extraordinarily high temperatures in April and May (Fig. 2a) with plenty of melt water available to the trees (Fig. 2c,d). Thus, in 2009, most of the cambial activity took place during June and July (wood growth period; Fig. 1b). By contrast, the winter of 2009–2010 was extraordinarily dry (Fig. 2c), while the wood growth period of 2010 was characterized by large amounts of rainfall, low PPFD, and relatively low temperatures (Fig. 2). These climatic conditions led to low sap flow rates (Fig. 1c), frequently reduced cambial activity and thus reduced growth (Fig. 1b). Thus, the wood growth period started nearly 1 month later in 2010 than that in 2009, which is indicated by DR crossing the zero line in Fig. 1b. However, DR showed no consistent relationship with stem [CO_2] (Table 1). For example, during the most active period for stem physiological processes, the wood growth period, a very close positive relationship between DR and stem [CO_2] was found in 2009, but a negative relationship was found in 2010 (Table 1).

Eliminating the overall seasonal growth trend from the time series of DR and stem [CO_2] and thus strengthening the short-term, water-related information content of DR, led to a consistent negative correlation between stem $[CO_2]_{detrended}$ and $DR_{detrended}$ with a time lag of 1–2 d (except late summer 2009 and dormancy < 0°C 2009–2010; see Table 1), suggesting increasing stem [CO_2] with shrinking stems. In addition, daily means of stem [CO_2] were positively related to daily means of sap flow with a time lag of 1–3 d (Table 1), with highest correlations for the spring rehydration periods before cambial activity started.

When testing multiple variables for explaining the seasonal course of stem [CO_2], DR improved the multiple regression model (Table 2) in combination with temperature during wood growth 2009, late summer 2009 and 2010, and dormancy > 0°C

Table 2 Standardized beta coefficients and performance of general linear models (GLM) to explain mean daily stem CO_2 concentrations by mean daily T_{Stem}, T_{Soil}, DR and sap flow rates for different tree physiological phases (see text for definitions) for Norway spruce (*Picea abies*) trees in the years 2009 and 2010

Period year	Days	T_{Stem}	T_{Soil}	DR	Sap flow	r^2	r^2_{max} T_{Stem}
Spring 2009	90–147	1.46	−0.51	–	–	0.96*	0.94*
Wood growth 2009	147–222	0.40	–	0.70	–	0.86*	0.46*
Late summer 2009	222–282	0.92	–	0.42	–	0.96*	0.79*
Dormancy > 0°C 2009	282–346	0.98	–	–	−0.20	0.85*	0.86*
Dormancy < 0°C 2009/10	346–77	0.40	−0.67	–	–	0.61*	0.18*
Dormancy > 0°C 2010	77–110	0.31	–	0.86	–	0.75*	0.03
Spring 2010	110–165	1.24	−0.60	–	–	0.77*	0.72*
Wood growth 2010	165–228	1.14	–	–	−0.38	0.92*	0.94*
Late summer 2010	228–288	−0.29	1.26	–	–	0.96*	0.90*
Dormancy > 0°C 2010	288–335	–	0.78	−0.19	–	0.86*	0.86*
Dormancy < 0°C 2010	335–365	0.73	−0.34	–	–	0.68*	0.56*

r^2, coefficient of determination of the best GLM; $r^2_{max}T_{Stem}$, coefficient of determination derived from r_{max} of T_{Stem} given in Table 1 (i.e. including time lags); '–', not included in the GLM.
*, Significant at $P < 0.001$.

late 2010. Sap flow appeared as a second (negative) factor beside T_{Stem} in only two cases, but with marginal improvements of the respective models only (r^2_{max} T_{Stem} in Table 2).

Stem $[CO_2]$ and soil efflux EF_{Soil}

Daily mean stem $[CO_2]$ and daily mean EF_{Soil} showed consistent seasonal patterns and synchronous day-to-day fluctuations (Fig. 1a,d). T_{Soil} (> 1°C) as a potential driver for EF_{Soil} was closely linearly related to T_{Stem} ($T_{Stem} = 1.3 * T_{Soil} + 0.4$; adj. $r^2 = 0.92$, $P < 0.001$). The linear temperature dependency of stem $[CO_2]$ on T_{Stem} (Fig. 3) and the exponential temperature dependency of EF_{Soil} on T_{Soil} (data not shown; see Fig. 4 in Etzold *et al.*, 2011) resulted in close exponential relationships of the form 'stem $[CO_2] = a - b * \exp(-c \times EF_{Soil})$' for both years (Fig. 4, adj. $r^2 > 0.8$).

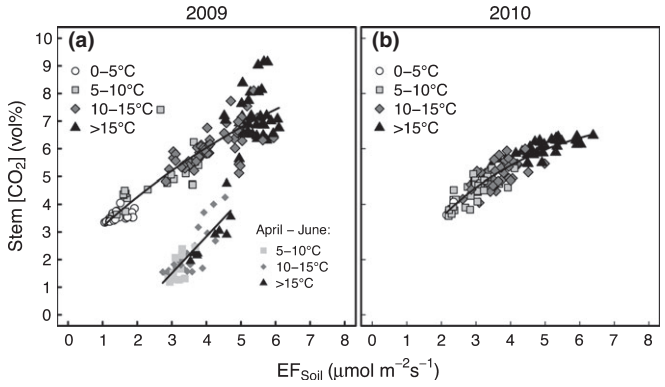

Fig. 4 Relationships between daily mean stem CO_2 concentrations (stem $[CO_2]$, average of both Norway spruce (*Picea abies*) trees) and daily mean soil efflux rates (EF_{Soil}) for four different stem temperature classes of the years (a) 2009 and (b) 2010 (2009: stem $[CO_2] = 12.36 - 10.37e^{-0.12EFSoil}$; adj. $r^2 = 0.84$, $P < 0.001$. 2010: stem $[CO_2] = 7.53 - 7.53e^{-0.32EFSoil}$; adj. $r^2 = 0.83$, $P < 0.001$). The nonlinear models include all days of the respective year for which EF_{Soil} was measured. The period from April to June 2009 (stem $[CO_2] = 1.33EF_{Soil} - 2.49$; adj. $r^2 = 0.72$, $P < 0.001$) is displayed separately and is not included in the model.

Confounding effects of temperature on stem $[CO_2]$ and sap $[CO_2]$

In order to amplify diurnal patterns, we selected sunny and warm summer days with a distinct diurnal cycle of tree physiological activity, which were defined by a mean day-time T_{Air} above 10°C and PPFD above 800 µmol m^{-2} s^{-1} (6:00–18:00 h; $n = 210$). At this time-scale, stem $[CO_2]$ dynamics were affected by physical equilibrium processes between water and gas-filled spaces in the wood owing to temperature changes according to Henry's law, as shown by the relationship between stem $[CO_2]$ and model stem $[CO_2]_{sap=3.5}$ (adj. $r^2 = 0.49$; $P < 0.001$). In general, the diurnal cycle of stem $[CO_2]$ lagged 3 h behind T_{Stem} and stem $[CO_2]_{sap=3.5}$ ($r = 0.96$, $P < 0.001$, Fig. 5a,b). The comparison of sap $[CO_2]$ calculated from measured stem $[CO_2]$ to that calculated from constant stem $[CO_2]$ of 4 vol% showed only small deviations (adj. $r^2 = 0.85$, $P < 0.001$, Fig. 5b).

However, the physical temperature effect could only explain about half of the diurnal courses of stem and sap $[CO_2]$ (Fig. 5a, b), leaving the remaining explanatory power to other tree physiological processes, for example sap flow and stem radius changes. Deviations between the measured and the modelled stem and sap $[CO_2]$ appeared most prominent during the onset (8:00–10:00h) and cessation (17:00–20:00h) of sap flow (compare Fig. 5a,b with Fig. 5f). During the onset of sap flow in the morning, all processes measured distinctly changed their dynamics, in agreement with the fast-changing stem and sap $[CO_2]$ (Fig. 5d,e): sap flow showed the sharpest increase (Fig. 5f), T_{Stem} reached its largest positive rate of change (Δ T_{Stem}, Fig. 5e), EF_{Soil} showed a small local efflux peak (Fig. 5f) and the stem radius experienced its fastest contraction per hour of the entire day (ΔDR, Fig. 5f). During the day, as long as sap flow was present until *c.* 20:00 h (Fig. 5f), stem $[CO_2]$ increased slower than stem $[CO2]_{sap=3.5}$ (Fig. 5a). During the night, stem $[CO_2]$ was constant, while stem $[CO_2]_{sap=3.5}$ decreased (Fig. 5a,d) and sap $[CO_2]$ increased faster compared with sap$[CO_2]_{stem=3.5}$ (Fig. 5b,e), indicating an accumulation of CO_2.

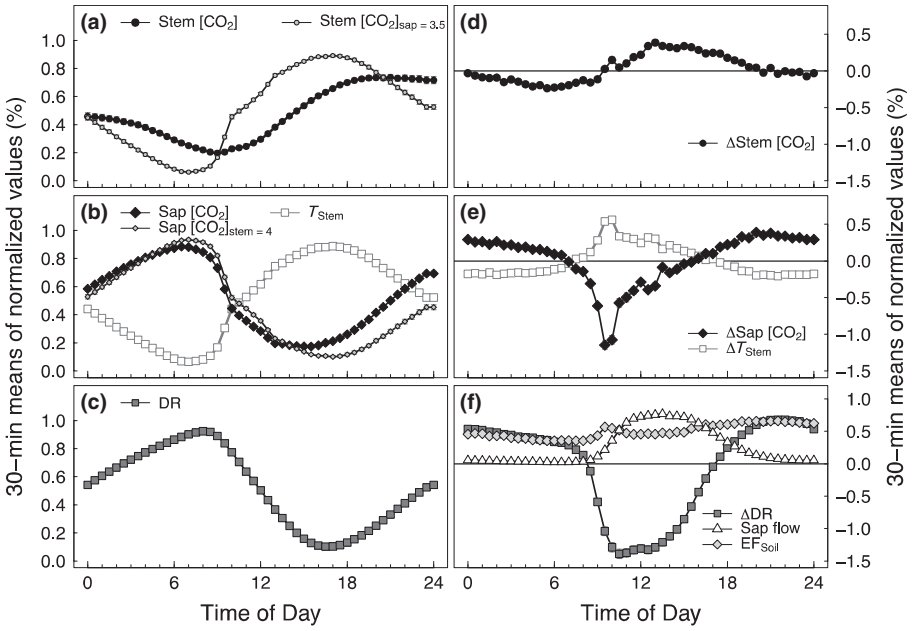

Fig. 5 Normalized diurnal courses of stem CO_2 concentrations (stem $[CO_2]$) and related measures of Norway spruce (*Picea abies*). Displayed are absolute values (a–c, f), rates of change (Δ) over 30-min time-intervals for stem $[CO_2]$), sap $[CO_2]$, T_{Stem} and DR as well as fluxes of sap flow and soil efflux (EF_{Soil}) (d–f). Values are normalized by the respective daily maxima. Only sunny days with a mean daytime (06:00–18:00 h) PPFD > 800 μmol m^{-2} s^{-1} (n = 210 d) are included. Means ± 1 SE are given. (a) Measured stem $[CO_2]$ and the corresponding model calculation with a constant $[CO_2]$ in the sap of 3.5 mM (stem $[CO_2]_{sap=3.5}$) according to Henry's law (with pH = 5.6). (b) Stem temperature (T_{Stem}), $[CO_2]$ in the sap calculated with measured stem $[CO_2]$ (sap $[CO_2]$) as well as with a constant stem $[CO_2]$ of 4 vol% (sap $[CO_2]_{stem=4\%}$; see the Materials and Methods section for details). (c) Stem radius changes (DR). (d) Rate of change of stem $[CO_2]$ (Δ stem $[CO_2]$). (e) Rate of change of sap $[CO_2]$ (Δ sap $[CO_2]$) and T_{Stem} (ΔT_{Stem}). (f) Rate of changes in DR (ΔDR), sap flow and soil respiration (EF_{Soil}).

Stem $[CO_2]$ and stem efflux (EF_{Stem})

EF_{Stem} normalized to 10°C ($EF_{Stem.10}$) followed a similar course to that of stem $[CO_2]$, both lagging behind T_{Stem} for *c.* 2–4 h (Fig. 6). However, stem $[CO_2]$ could only explain 47% of the daily variations in EF_{Stem} (P = 0.005), raising the question of whether EF_{Stem} is a representative measure of local stem respiration or whether EF_{Stem} is confounded by the translocation of CO_2 via sap flow. In order to quantify the potential effect of sap flow on EF_{Stem}, a theoretical EF_{Stem} rate was estimated from temperature response functions established during night under low sap flow rates that were then extrapolated to daylight conditions. Comparing the predicted vs the measured EF_{Stem} (dEF_{Stem} = measured EF_{Stem} − predicted EF_{Stem}) suggested that measured EF_{Stem} was smaller than expected from sole temperature response functions in most of the cases ($dEF_{Stem} < 0$; Fig. 7), indicating a dilution effect/net CO_2 export by sap flow of −1.1 to −1.7 μmol m^{-2} s^{-1} (i.e. slope of the regressions during the wood growth periods). However, this was only true for the wood growth period, as during late summer sap flow showed no effect on EF_{Stem}.

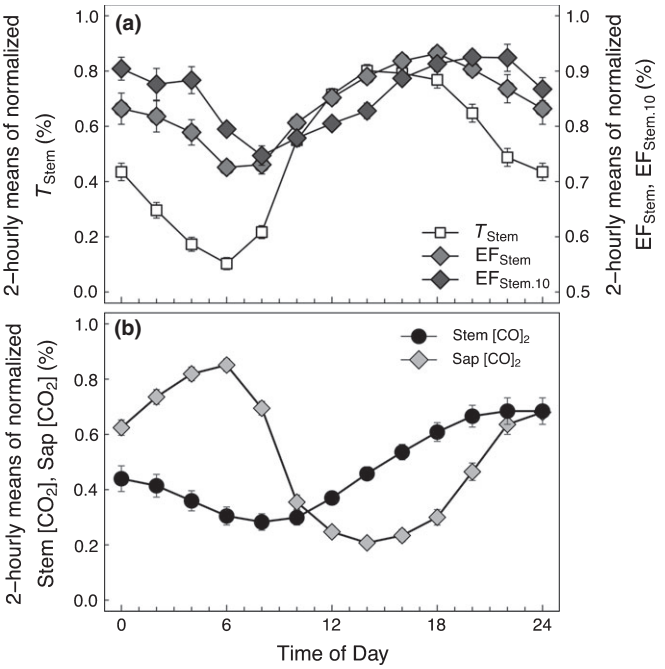

Fig. 6 Mean diurnal courses of (a) stem temperature (T_{Stem}), stem CO_2 efflux (EF_{Stem}) and stem CO_2 efflux corrected for a reference temperature of 10°C ($EF_{Stem.10}$) of four Norway spruce (*Picea abies*) trees. Data were collected during five measurement campaigns in summer 2008 and 2009. (b) Mean diurnal courses of stem $[CO_2]$ and sap $[CO_2]$, measured in two of the four sampling trees during the measurement campaigns in 2009 (trees 296 and 148). Values are normalized by the respective daily maximum. Two-hourly means ± 1 SE are given.

Discussion

Continuously measured stem CO_2 concentrations (stem $[CO_2]$) in Norway spruce at a subalpine site varied between 1 vol% and 10 vol% within the study period of 19 months (Fig. 1), which is consistent with previous reports on other tree species (Teskey

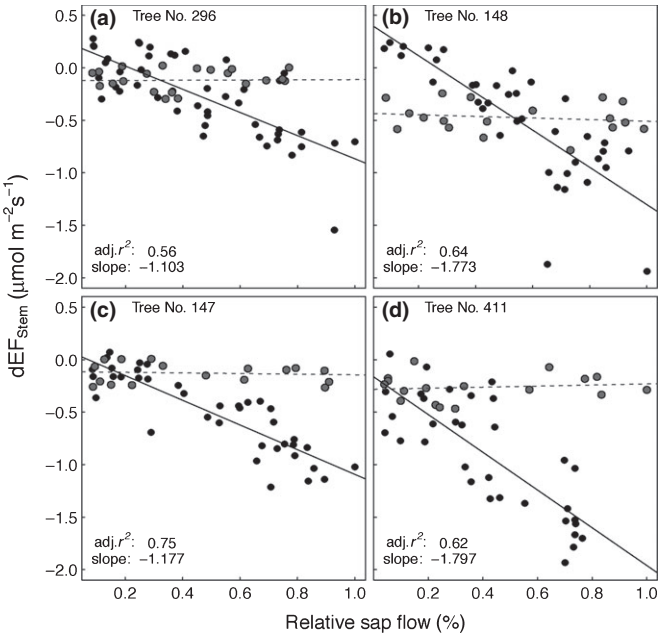

Fig. 7 Differences between predicted and measured stem efflux rates dEF_{Stem} (measured EF_{Stem} − predicted EF_{Stem}) in relation to relative sap flow rates. Data were collected on four Norway spruce (*Picea abies*) trees during five measurement campaigns in the wood growth and late summer periods (as defined in the Materials and Methods section) in the years 2008 and 2009. Predicted EF_{Stem} rates were derived from temperature response functions, established during night (18:00–08:00 h) under low sap flow rates and extrapolated to daylight conditions (see the Materials and Methods section for details). Relative sap flow values refer to the maximum sap flow rate of the respective tree. Regression lines for data collected in the wood growth period (solid line) and in late summer (tinted dashed line) are given. Adj. r^2 and slope of the regression line for the wood growth period are shown (for all four trees: $P < 0.001$). None of the regressions for the late summer period were significant.

et al., 2008). These concentrations are amazingly high compared with the CO_2 concentration in air (0.039 vol%), indicating a significant accumulation of CO_2 inside tree stems owing to high diffusion barriers (Sorz & Hietz, 2006; Sevanto *et al.*, 2011; Steppe *et al.*, 2012). Stem [CO_2] exhibited a strong seasonality (Fig. 1, Table 1), similar to the only other long-term study we are aware of (Eklund, 1990; Eklund & Klintborg, 2000). Based on our long-term data set, we had the unique opportunity not only to quantify variations in stem [CO_2] at different time scales (see later), but also to show the high consistency of relationships between stem [CO_2] and its potential drivers over almost 2 yrs (Tables 1, 2). In particular, T_{Stem} and T_{Soil} were strong driving factors for stem [CO_2] in both years. Furthermore, we found tight relationships between stem [CO_2] and EF_{Soil} during both vegetation periods, while other tree physiological measures (e.g. DR, sap flow) affected stem [CO_2] differently for different seasons in both years, helping us to identify possible cause–effect mechanisms.

Stem [CO_2] and its dependency on Henry's law

Henry's law describes the equilibrium process between all forms of CO_2 dissolved in water and CO_2 in air in relation to temperature, pH and partial pressure (Teskey & McGuire, 2002; Saveyn *et al.*, 2007a; Teskey *et al.*, 2008). The equilibrium dynamics driven by temperature changes have been found to play an important role when interpreting stem [CO_2] on seasonal (Fig. 1a) and diurnal scales (Fig. 5a, b). We found that 31% (2009) and 68% (2010) of the seasonal variability of stem [CO_2] (Fig. 1a), and 49% of the diurnal variability during summer (Fig. 5a), were attributable to physical effects of temperature changes (assuming constant CO_2 concentration and sap pH). Thus, equilibrium processes according to Henry's law need to be accounted for before CO_2 sink and source processes within a stem segment can be identified and interpreted in terms of tree physiology.

Although sap pH is known to have a substantial effect on the dissolution of CO_2 in water (Teskey *et al.*, 2008), we did not consider potential temporal variations of pH when calculating a model stem [CO_2]. However, seasonal and diurnal changes of sap pH in poplar trees were reported to be rather small, probably indicating a minor impact on changes of solubility of CO_2 in water over time (Aubrey *et al.*, 2011).

Stem [CO_2] on a seasonal time-scale

In spring and during both wood growth phases, measured changes in stem [CO_2] were distinctly larger than expected from Henry's law, that is, 80% larger during the 2009 wood growth period and 35% larger than the modelled stem $[CO_2]_{sap=3.5}$ in 2010. This is a clear indication that during these periods considerable amounts of CO_2 were produced, either because of local cambial respiratory activity or it was imported from belowground (Teskey *et al.*, 2008). Throughout most of the physiological phases during the 19 months of study, we found stem [CO_2] to be most closely related to T_{Stem} (Tables 1, 2). However, during the 2009 wood growth phase and late summer 2009 and 2010, T_{Soil} had a higher explanatory power than T_{Stem} (Table 1), indicating a potentially large influence of root-respired CO_2 transported up the tree. This coincides with root growth of Norway spruce that usually peaks in late spring and again during autumn when stem growth has already ceased (Puhe, 2003; Davidson *et al.*, 2006).

An additional indirect indication for a coupling of belowground and aboveground CO_2 is the close relationship between stem [CO_2] and the efflux of CO_2 from the soil (EF_{Soil}; Figs 1, 4). This relationship indicates either a coincidence of independent processes driven by closely related courses of stem and soil temperatures or a possible causality between them. EF_{Soil} was found to be exponentially related to T_{Soil} (Etzold *et al.*, 2011), whereas stem [CO_2] was linearly related to T_{Stem} (Fig. 3). Assuming stem [CO_2] is purely a function of local stem respiration, we would expect an exponential relationship to T_{Stem} and not a linear one. Furthermore, we would not expect a close relationship to EF_{Soil}. However, assuming a 50% or higher fraction of root-respired CO_2 transported via the xylem, we would expect a high correlation between EF_{Soil} and stem [CO_2], which was actually the case (adj. $r^2 = 0.83$). Thus, it seems to be most likely that a large amount of CO_2 produced in the roots is transported up the tree. Low sap flow rates seemed to increase stem [CO_2] (Fig. 5)

and EF_{Stem} (Fig. 7), whereas high sap flow rates seemed to induce a dilution effect and therefore a decrease in stem $[CO_2]$ (Fig. 7). Belowground growth phenology might not only affect changing explanatory powers of T_{Stem} and T_{Soil} on stem $[CO_2]$ during the year but also pinpoint to belowground sources of stem CO_2 during wood growth and late summer phases.

By contrast, during dormancy (> 0°C), sap flow was low (Fig. 1) and little CO_2 transport from belowground can be expected. Thus, local stem respiration should be the dominant source for stem $[CO_2]$ during this period. However, during these dormant periods, T_{Soil} still played an important role in explaining stem $[CO_2]$, either as single factor (Table 1) or in combination with T_{Stem} (only 2010; Table 2). At these times, T_{Stem} and T_{Soil} were less coupled than during the rest of the year as soils were insulated by a layer of snow, keeping T_{Soil} almost constant at *c.* 0°C, whereas T_{Stem} followed T_{Air} (Fig. 2a). Thus, in addition to local respiration, we hypothesize that CO_2 respired from the roots under the snow might diffuse up the stem along gas-filled spaces. With a diffusion coefficient of CO_2 in air of $1.6 \times 10^{-5}\ m^2\ s^{-1}$ (Nobel, 1991), changes in $[CO_2]$ in the roots could theoretically affect the stem section measured via air-filled spaces in the wood within 2–3 d. According to Sachs (1887), gas makes up to 17% of the xylem volume of fir trees (and we assume a similar value for spruce) and could build the necessary pathway for CO_2 diffusion. Thus, the origin of stem $[CO_2]$ during dormancy phases with temperatures > 0°C could be either root- or local stem-respired CO_2.

Furthermore, during dormancy with freezing conditions, large deviations between stem $[CO_2]$ of the two trees were observed (Fig. 1a). During these phases, temperature dropped below the threshold for frost-induced shrinkage of bark. Such conditions led to a (reversible) decrease in bark thickness of 1 mm or more (Fig. 1b), whereas xylem cells are reported to remain largely unaffected in size (Zweifel & Häsler, 2000). Such rapid and large frost-/freeze-induced variations in DR are known to induce pressure changes inside the tissues (Robson & Petty, 1993; Sevanto *et al.*, 2012), which might be responsible for changes in stem $[CO_2]$. These freeze/thaw processes may also explain the observed deviations in stem $[CO_2]$ between the two trees (Fig. 1a) and the differences in stem $[CO_2]$ levels, as it is well known that such processes do not occur simultaneously in all stems (Zweifel & Häsler, 2000). Overall, during winter stem $[CO_2]$ seems to be dominated by local respiration, but also affected by physically induced CO_2 concentration changes, such as pressure changes and/or diffusion processes.

Nonrecurring stem $[CO_2]$ peak during wood growth phase 2009

In the year 2009, T_{Stem} and DR could explain most of the variability of stem $[CO_2]$, during the wood growth phase, while in the year 2010, T_{Stem} and sap flow were the most important predictors (Table 2). Although this is partly explicable with different growth dynamics and thus different respiration rates in the 2 yrs, it does not satisfactorily explain the exceptional peak of stem $[CO_2]$ from April to June 2009. This period appears as an anomaly in all our analyses (Tables 1, 2, Figs 1, 3, 4) and could be induced by a physiological response to sensor installation, as the hole drilled into the stem to place the sensor was 2 cm diameter and 6 cm deep. Increased stem CO_2 efflux as wound response is a well-described phenomenon and usually interpreted as increased respiration owing to healing processes, such as cell repair, callus formation, lignification and suberization (Bloch, 1941; Uritani & Asahi, 1980; Schmitt & Liese, 1993; Levy *et al.*, 1999). The fact that the stem $[CO_2]$ peak occurred 3 months after installation might indicate a delayed wound response after tree dormancy ceased and radial stem growth started at the end of May (Dunn *et al.*, 1990; Schmitt & Liese, 1993; Dujesiefken *et al.*, 2005).

Furthermore, annual amplitudes of stem $[CO_2]$ signals seem to have decreased over the two study years (mean amplitude ± standard deviation of stem $[CO_2]$ within a 7-d window during vegetation period: 2009, 0.51 ± 0.50; 2010, 0.45 ± 0.26). However, data sets even longer than in this study (19 months) would be needed to reliably test potential sensor signal degradation, which might occur because of wound closure and thus increased diffusion resistances between sensor and measurement tissue of interest.

Stem CO_2 on a diurnal time-scale

On a diurnal scale, stem $[CO_2]$ in the morning (8:00–10:00 h) was found to increase more slowly than expected from Henry's law (Fig. 5a, only sunny summer days were analysed). One explanation could be that sap flow transports water with lower $[CO_2]$ into the measured stem section (Teskey & McGuire, 2007), thus acting as a dilution factor in the first hours after dawn (Fig. 5). Furthermore, water was withdrawn from the bark (as shown by shrinking stems, Fig. 5c,f), in parallel with the onset of sap flow. As bark water is reported to contain much lower $[CO_2]$ than xylem water (Cernusak & Marshall, 2000; Wittmann *et al.*, 2006), this could be a second dilution factor.

During the second half of the day, the increase in stem $[CO_2]$ was also slightly lower than expected according to Henry's law (Fig. 5a), meaning that stem CO_2 could have been diluted by sap flow, as also indicated by the analyses of sap flow in combination with EF_{Stem} (Fig. 7) and the findings of Teskey & McGuire (2007). With decreasing sap flow in the early evening, stem $[CO_2]$ remained constant, while modelled stem $[CO2]_{sap=3.5}$ decreased, suggesting an accumulation of locally respired CO_2 under low sap flow rates during night. The different effects of sap flow rates on EF_{Stem} in growth- and non-growth periods (Fig. 7) might have to do with the generally higher stem $[CO_2]$ during the growth period (Fig. 1). During this time, the stem (growth) respiration is assumed to be highest (Ryan, 1990) and the difference between locally produced CO_2 and imported CO_2 from belowground might be biggest.

Conclusions

Stem $[CO_2]$ was strongly related to stem and soil temperatures, which in turn affect physical equilibrium processes within the stem according to Henry's law but also affect different respiration

processes, making disentangling of single factors difficult. As the relationships of stem [CO_2] to tree physiological measures changed over time, long-term data sets are critical for the interpretation of stem [CO_2] dynamics. Clear indications were found for CO_2 translocation within the tree. However, whether these observations resulted from sap flow or axial diffusion, or a combination of both, remains unclear. Nevertheless, although the fate of stem [CO_2] is not yet known, it has huge implications on the partitioning of net ecosystem CO_2 exchange fluxes. If a large fraction of sap CO_2 is recycled internally, then gross primary productivity estimated based on light response curves as well as ecosystem respiration estimated based on temperatures will both be underestimated. Furthermore, as the potential for refixation of root-respired and translocated CO_2 changes strongly with phenophases, the underestimates might change during the year.

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