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Experimental vs. modeled water use in mature Norway spruce (*Picea abies*) exposed to elevated CO_2

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Rising levels of atmospheric CO_2 have often been reported to reduce plant water use. Such behavior is also predicted by standard equations relating photosynthesis, stomatal conductance, and atmospheric CO_2 concentration, which form the core of dynamic global vegetation models (DGVMs). Here, we provide first results from a free air CO_2 enrichment (FACE) experiment with naturally growing, mature (35 m) *Picea abies* (L.) (Norway spruce) and compare them to simulations by the DGVM LPJ-GUESS. We monitored sap flow, stem water deficit, stomatal conductance, leaf water potential, and soil moisture in five 35–40 m tall CO_2-treated (550 ppm) trees over two seasons. Using LPJ-GUESS, we simulated this experiment using climate data from a nearby weather station. While the model predicted a stable reduction of transpiration of between 9% and 18% (at concentrations of 550–700 ppm atmospheric CO_2), the combined evidence from various methods characterizing water use in our experimental trees suggest no changes in response to future CO_2 concentrations. The discrepancy between the modeled and the experimental results may be a scaling issue: while dynamic vegetation models correctly predict leaf-level responses, they may not sufficiently account for the processes involved at the canopy and ecosystem scale, which could offset the first-order stomatal response.

Keywords: dendrometer, DGVM, FACE, leaf water potential, sap flow, stomatal conductance, vegetation modeling

INTRODUCTION

Whether and if so how plants respond to increasing atmospheric CO_2 is critical for future ecosystem carbon and water cycling and largely depends on the response of the stomata that control both carbon (C) uptake and water loss. Several authors have suggested that water-related effects of elevated CO_2 (eCO_2) on stomatal closure might be or become more important than immediate effects on carbon uptake (Morgan et al., 2003; Holtum and Winter, 2010; Hartmann, 2011). One reason for this may be that while stimulated growth and biomass accumulation rarely persist over many years (Körner et al., 2005; Norby et al., 2010; Leuzinger et al., 2011b), but see McCarthy et al. (2010), stomatal responses tend to be sustained (see Holtum and Winter, 2010 for a review). For grassland, there is compelling experimental evidence that stimulated biomass production under eCO_2 is in fact a consequence of soil water savings resulting from reduced stomatal conductance (Niklaus et al., 1998; Morgan et al., 2004). Such a water-mediated CO_2-effect is expected to be more pronounced in water-limited ecosystems, although under extremely dry conditions it has not been observed (Housman et al., 2006). The experimental conditions (species composition tested, CO_2-administration method, laboratory vs. field experiment, ontogenetic stage of test plants) and particularly the experimental duration are fundamental for the interpretation of net water use under eCO_2 (Norby et al., 1999). For example, initial CO_2-induced increases in total leaf area per unit land area leaf area index, (LAI) in young, rapidly expanding systems will inevitably lead to increased stand transpiration, irrespective of the leaf-level stomatal response (Uddling et al., 2008; Tricker et al., 2009). Similarly, the long-term response (>10 years) often differs from measurements over only a few years (Körner, 2006). The scaling from first-order stomatal responses to stand water use therefore requires careful consideration of the interactions between the water- and the carbon cycle across temporal and spatial scales.

Overall, there is a striking lack of data from mature forests as most of the evidence for water savings under eCO_2 still comes from grass- or shrub ecosystems, or from branch bag experiments on mature trees (Roberntz and Stockfors, 1998; Pataki et al., 2000; Morgan et al., 2004). No eCO_2 experiments on tall trees exist in the tropical forests (Körner, 2009). Worldwide, there are only five experiments testing the effect of future CO_2 concentrations on entire tree crowns using the free air CO_2 enrichment (FACE) method (Körner et al., 2005; Norby et al., 2005), excluding whole tree chamber and agricultural experiments (Medhurst et al., 2006; Kimball et al., 2007). Although those five studies were conducted with different species and in a variety of biomes, three of them roughly agree on reduced total stand water use under eCO_2 of c. 10% (Warren et al., 2011). The remaining two were young, expanding systems where the transpiration response to CO_2 was dominated by a stimulation of LAI and therefore increased stand water use (Uddling et al., 2008; Tricker et al., 2009). Responses in leaf-level stomatal conductance (g_s)

to eCO_2 are less consistent in the five large forest FACE experiments mentioned (−4% to −44%), but approximately match findings from meta-analyses including experiments with woody plants in branch bag and greenhouse experiments (−21% Medlyn et al., 2001, −18% Ainsworth and Rogers, 2007, and no significant response from a much earlier review predominantly on seedlings and saplings, Curtis and Wang, 1998). At the Swiss Canopy Crane (SCC), where the present study was conducted, stomatal conductance was reduced around 10% in six deciduous tree species, and sap flow by 2–22%, resulting in a reduction of yearly stand transpiration of c. 10% (Cech et al., 2003; Keel et al., 2007; Leuzinger and Körner, 2007). Overall, water savings under eCO_2 tend to decline with the duration of the experiment (Medlyn et al., 2001; Leuzinger and Körner, 2010; Leuzinger et al., 2011b), with increasing woodiness (Ainsworth and Rogers, 2007), with increasing age of the studied plants (Medlyn et al., 2001), and from deciduous to coniferous trees (Ellsworth, 1999; Körner et al., 2007). Because the number of short-term experiments with herbaceous or young trees is disproportionately larger than the number of experiments with mature trees, we can assume that water savings under eCO_2 tend to be overestimated, particularly because the response is likely to diminish further when scaling up from the individual plant to the landscape level (McNaughton and Jarvis, 1991; Field et al., 1995; Leuzinger and Körner, 2010). A way to circumvent the necessity of scaling up CO_2-experiments with young, disturbed systems is $\Delta^{13}C$ analysis of tree rings to infer intrinsic water use efficiency (iWUE, Francey and Farquhar, 1982; Bert et al., 1997). Penuelas et al. (2011) in a recent review report that iWUE has increased by c. 20% over the past 40 years, with slightly larger responses in broad-leaved relative to coniferous trees. On the other hand, in a study on various species of oak, hornbeam and maple, no change in iWUE was reported based on only 2–3 leaf samples during the twentieth century (Miller-Rushing et al., 2009). Changes in iWUE may originate either from changes in the nominator (leaf-level photosynthesis) or the denominator (stomatal conductance), and thus cannot be used to fill in the lack of FACE experiments to estimate changes in stand transpiration under future CO_2 concentrations.

Experimental estimates of leaf-level and whole tree responses are also key to algorithms and their parameterization in dynamic global vegetation models (DGVMs) and earth system models, and therefore predictions of future vegetation responses and climate feedbacks (Moorcroft, 2006). Stomatal conductance models used in DGVMs predict substantial decreases of g_s with a doubling of atmospheric CO_2, fundamentally because the substomatal CO_2 concentration (C_i) is held approximately constant (Jarvis, 1976; Leuning, 1995; Haxeltine and Prentice, 1996). It is therefore little surprising that DGVMs predict global water savings by the vegetation of around 10–20% (Luo et al., 2008), which lead to increased runoff of mostly a few percent (e.g., Betts et al., 2007; Boucher et al., 2009; Long et al., 2010), matching estimates based on experimental data (Leuzinger and Körner, 2010). However, it is important to note that few of these model results can be validated due to a lack of data, and net responses largely hinge on the way the leaf-level response is scaled up to the canopy and landscape scale. The aim of the present study is to (1) provide novel data on water relations of fully grown Norway spruce (*Picea abies*) trees under approximately double pre-industrial CO_2-conditions, and (2) to discuss these findings in context of simulations of the experiment by the DGVM LPJ-GUESS (Smith et al., 2001; Sitch et al., 2003).

MATERIALS AND METHODS

STUDY SITE

The SCC is located in a mature, mixed deciduous forest 15 km south of Basel, Switzerland (47°28′N, 7°30′E, 550 m a.s.l.). The crane gondola allows access to all tree crowns located within the 30 m radius of the jib. The site has a mean January temperature of 2°C and mean July temperature of 19°C, long-term average annual precipitation amounts to 990 mm, two-thirds of which fall during the growing season. The oldest trees are c. 110 years old and reach heights of 35–40 m. Stand density is 415 trees ha^{-1} (trees >0.1 m breast height diameter), at a stem basal area of 46 m^2 ha^{-1} and a LAI of c. 5. The soil type is a Rendzic Leptosol (WRB) (Rendzina, FAO; Lithic Rendoll, USDA) with an accessible profile depth of at most 25 cm followed by rocky subsoil blending into the calcareous bedrock at 40–90 cm. The soil texture is a loamy clay (pH 5.8 in the top 10 cm). The species mixture includes deciduous trees (*Fagus sylvatica* L., *Quercus petraea* (Matt.) Liebl., *Carpinus betulus* L., and, less abundant, *Tilia platyphyllos* Scop., *Acer campestre* L., *Prunus avium* L.) as well as conifers (*Picea abies* (L.) Karst., *Larix decidua* Mill., *Pinus sylvestris* L., *Abies alba* Mill.). For more information on the site see (Pepin and Körner, 2002).

EXPERIMENTAL SET-UP AND FREE AIR CO_2 ENRICHMENT

Five Norway spruce (*P. abies*) individuals were selected for CO_2 enrichment (550 ppm), together with five control trees, only three of which were accessible with the crane gondola. The treatment was initiated on July 30, 2009. To simulate future atmospheric conditions, pure CO_2 was released through laser-punched irrigation tubes woven into the spruce trees with a central supply pipe running up the stem. Sample lines were connected to two infrared gas analyzers (LI-800, Li-Cor, Lincoln, NE, USA), in order to monitor and automatically adjust the amount of CO_2 supplied. CO_2 enrichment was discontinued when daily temperature maxima did not reach 6°C, or when above-canopy incoming radiation was less than 100 μmol m^{-2} s^{-1} (i.e., no night-time CO_2 enrichment). On average, the target CO_2 concentration of 550 ppm was achieved well: the mean across all sample lines during times of fumigation reached 563 ppm ± 94 s.e. in 2009 and 617 ppm ± 88 in 2010.

CONTINUOUS MEASUREMENTS AND MEASURING CAMPAIGNS

We continuously measured sap flow, stem radius changes, microclimate, and soil moisture. One heat dissipation sap flow sensor (TDP-30, Dynamax, Huston, TX, USA) was used per tree (5 treated, 5 controls), inserted directly into the stem after removing loose bits of bark. The sensors were oriented toward north, water-proofed with silicon paste and insulated with styrofoam and reflecting foil. They remained in the same position for both the 2009 and 2010 growing seasons. Stem radius changes (μm) were recorded with high-precision point dendrometers (ZB06, Natkon, Hombrechtikon, Switzerland), and, together with the sap flow signals, logged to two central data loggers (DL2e, Delta-T Devices Ltd., Cambridge, UK) in 10 min intervals (average over 30 s readings). Soil moisture was logged every 6 h at 0–10 cm depth using "ECH_2O Probes" (EC-10, Decagon Devices Ltd., Pullman, Washington, DC). Eleven sensors were distributed around the treated trees, 18 sensors around control trees, logging onto self-contained data loggers (Em50, Decagon). Measurements took place from day of year 134 to 297 (May 14–October 24) in 2009 and from day of year 134 to 267 (May 14–September 24) in 2010. Microclimate (temperature, relative humidity, incoming radiation, and precipitation) was logged above the canopy in 10-min intervals to a DL2e logger (Delta-T Devices Ltd.). Data gaps resulting from occasional logger failure were filled by interpolation based on climate recordings from a nearby weather station (2 km air-line distance).

On three cloudless days (July 29, August 6 2009, and July 14 2010), we measured daily courses (five measurements per tree pre-dawn to dusk) of leaf water potential and stomatal conductance on the five treated trees and the three controls that were accessible with the crane gondola. Two twigs per tree and time window were cut off with a razor blade, needles, bark, and phloem around the cut were removed, and their leaf water potential was measured subsequently using a pressure chamber (SKPM 1400, Skye Instruments, Powys, UK). Only plant material from the fully light-exposed top crown was selected to ensure comparable conditions. Stomatal conductance was measured on current and previous year's needles, removing the needles on two c. 2 cm wide bands to allow the gas-exchange chamber to close. A portable photosynthesis system (LI-6400 XT, LI-COR Biosciences, Lincoln, NE, USA) with a conifer chamber was used for this purpose. Readings were taken as soon as rates of net photosynthesis and stomatal conductance remained stable (<5 min). All measurements were taken at full sunlight ($>1000\,\mu$mol $m^{-2}\,s^{-1}$). Treated trees were measured at their target CO_2 concentration of 560 ppm, control trees at ambient CO_2 (390 ppm). Vapor pressure deficit (VPD) inside the cuvette was adjusted to ambient conditions. Stomatal conductance was calculated by multiplying the readings with the total leaf area of the samples (harvested at the end of the experiment). Because there were no systematic differences between current year and previous year needles, all analyzes presented here are based on the average values.

MODEL SPECIFICATION AND MODEL RUNS

We used the DGVM LPJ-GUESS, featuring an accurate representation of detailed plant physiological processes (Smith et al., 2001; Sitch et al., 2003), to simulate our experimental results. The model was run in cohort mode using daily climate data from an official Swiss weather station 11 km north of the study site (Basel-Binningen, Tank et al., 2002). Relative humidity, radiation and temperature data correlated well with weather data recorded at the experimental site (R^2-values equal to 0.86, 0.89, and 0.98 respectively), and specific correction factors were applied to simulate long climate time series at the experimental site as closely as possible. A 700-years spin-up period was allowed in order to equilibrate the various carbon pools with the background climate (data from 1901 to 1930 used repeatedly, CO_2 concentration at 296 ppm). Thereafter, we considered the period from 1901 to 2110. Atmospheric CO_2 concentration was altered in four different ways, all based on the actually measured mean values until 2010: (1) step change to 550 ppm in 2010, (2) step change to 700 ppm in 2010, (3) gradual change to 550 ppm until 2057, and (4) gradual change to 700 ppm until 2100. Because we only considered Norway spruce, only the plant functional type (PFT) "needle-leaved evergreen" was allowed to grow, all other PFTs were suppressed. Soil water storage capacity was set to 80 mm, which is the best estimate determined for a nearby site (see Walthert et al., 2004). Only one patch with a size of 1000 m^2 was computed, hence no competition took place in order to simulate the responses of mature coniferous trees. Because forest fires are extremely rare in this region, fire disturbance was suppressed. No further parameter changes or adjustments were made relative to the default version of LPJ-GUESS (Sitch et al., 2003).

Daily transpiration in LPJ is equal to the lower value out of daily water supply and daily water demand. If water supply < water demand, then the available soil water is transpired up to a maximum rate of 5 mm d^{-1} and thus independent of CO_2. On the other hand, if water supply > water demand, stomatal conductance decreases with increasing CO_2 because C_i/C_a (intercellular to ambient CO_2 concentration) is held constant and photosynthesis is stimulated (Haxeltine and Prentice, 1996). As a result, at a leaf temperature of 20°C, stomatal conductance is reduced c. 35% at 2× pre-industrial CO_2 concentration. Transpiration is then calculated from potential evapotranspiration, stomatal conductance and two (constant) scaling parameters.

DATA PROCESSING AND STATISTICAL ANALYSES

Stem water deficit was related to the individual's stem radius at the beginning of the experiment and expressed in per mille change from the initial value. The resulting time series were decomposed into radius changes due to changes in stem water storage and a growth component according to the method outlined in (Zweifel et al., 2005). Essentially, this method considers periods between stem radius peaks as stem water deficits (see **Figure 1** of Zweifel et al., 2005).

Millivolt signals of sap flow sensors were processed as follows to achieve most realistic conditions of zero-flow (R. Zweifel, personal communication): the maximum mV values were converted to temperature differences (ΔT) using a constant factor of 25. Then, ΔT maxima between 3 h and 8 h every night were connected with a straight line. This linearly changing baseline (ΔT_{max}) was used as the pre-nightly maximum value as in the standard transformation outlined in (Granier, 1985). Because sap flow signals are difficult to interpret as absolute mass flow densities, particularly when exact estimates of the sap wood width and the radial flow distribution are lacking (Leuzinger and Körner, 2007; Leuzinger et al., 2011a), we chose to use only relative sap flow values (**Figure 1**). Each sap flow time series was therefore standardized to its own pre-treatment maximum (mean of 20 largest values) resulting in time series between 0 and approximately 1, spanning both growing seasons (henceforth called "relative sap flow"). To test treatment-specific differences in daily courses of leaf water potential and stomatal conductance, we used mixed effects models (R package "nlme") with treatment and time

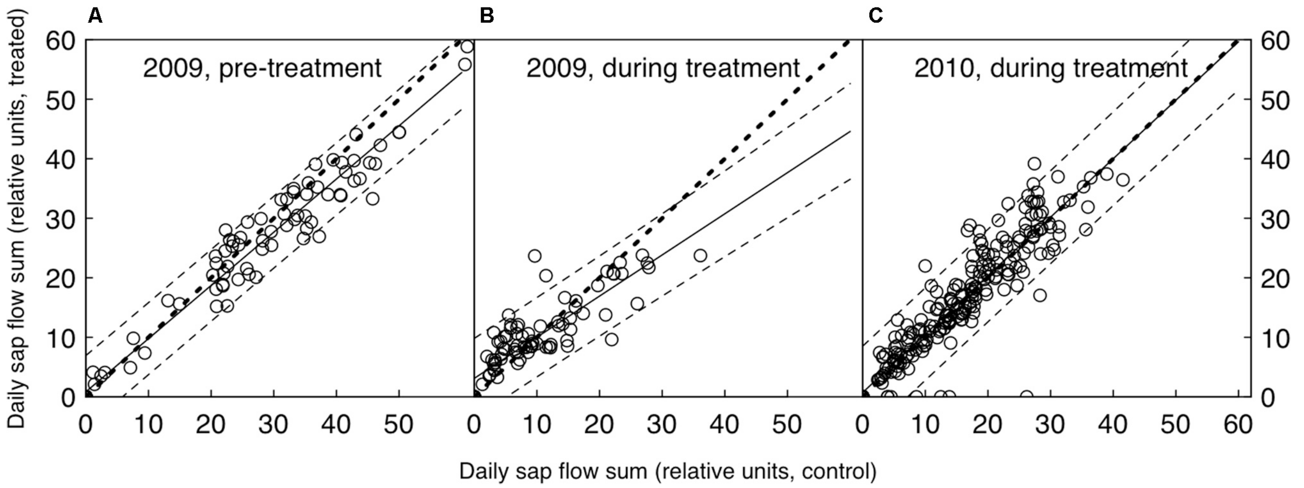

FIGURE 1 | Daily relative sap flow sums of the control and CO_2-treated trees, both before (A) and after the CO_2-treatment was initiated (B and C). Shown are the medians of each group ($n = 5$). The bold dotted line represents the 1:1 line, the solid line the linear fit (with dashed lines as 95% confidence intervals). The 1:1 line lies within the confidence intervals where data points are available, suggesting that there was no significant difference between treated and control trees in any of the three periods shown.

of day as fixed effects and tree as a random effect. Additionally, we used an autoregressive moving average (ARMA) correlation structure to model dependence among observations.

The relationship between stem water deficit (ΔW) and soil moisture (*sm*) was modeled individually for the pre-treatment and the two FACE periods (season 2009 and 2010) using a 2-parameter Michaelis-Menten-type hyperbola: $\Delta W = a \times sm/(b + sm)$, where "a" is the asymptote and "b" the soil moisture level at which stem water deficit reaches half of its asymptotic value (**Figure 3**).

Generally, we tested statistically significant differences between treatments by fitting models with common parameter estimates and varying parameter estimates for each treatment, followed by a comparison of the two models (**Figures 2, 4, and 5**). The CO_2-treatment was considered to affect the variable of interest statistically significantly, if the Akaike information criterion (AIC) was significantly lower in the more complex model (i.e., $\Delta AIC > 2$). To determine the envelope curves for the sap flow-VPD relationships, we calculated the 95th percentiles of relative sap flow (SF_{95}) for every 2 kPa VPDVPD bin and fitted the polynomial model $SF_{95} = a \times CO2 + b \times VPD + c \times VPD^2 + d \times VPD^3$, since all other attempts to fit a non-linear model failed. The factor CO_2 is the CO_2-treatment with the levels 1 (elevated) and 0 (ambient). The interaction term VPD $\times CO_2$ was not significant and was therefore dropped. All analyzes were carried out using R version 2.13.0 (R Development Core Team, 2011).

RESULTS

WATER RELATIONS OF *P. abies* UNDER ELEVATED CO_2

A comparison of the seasonal relative sap flow sums (median of both treated and control groups) did not show any change when the pre-treatment period was compared to the CO_2 enrichment period. (**Table 1**, **Figure 1**). The pattern did not differ when wet and dry periods were considered separately (both by soil moisture and VPD conditions). Before the treatment was initiated, the trees designated to be treated with eCO_2 tended to show less transpiration (Wilcoxon rank sum test, $p = 0.055$, **Table 1**).

A more detailed look at the daily courses of sap flow, leaf water potential, and stomatal conductance during bright sunny days before and after the start of the treatment confirmed that water consumption in *P. abies* remained unaffected by CO_2 enrichment. Daily courses of sap flow were not significantly different between treatments, neither before nor after treatment initiation (daily sums of relative sap flow, Wilcoxon rank-sum test, $n = 5$, p-values $= 0.90, 0.90, 0.79$ for left, center and right panel of **Figure 2A**). Similarly, leaf water potential and stomatal conductance differed with time of day (p-values < 0.0001 for both years and measurements) but not with the treatment: we employed linear mixed effects models with treatment, time and their interaction as fixed factors and tree individuals as random factors. Except for the leaf water potential measurements during the pre-treatment period (left panel of **Figure 2B**), where the interaction term was significant ($p = 0.022$), the treatment differences were not significantly different during any day (**Figures 2B,C**, p-values > 0.1). In both summers, low soil water availability resulted in low pre-dawn leaf water potentials around -1 MPa. During daytime, high VPD values exceeding 2 kPa caused leaf water potentials to drop to values between -1.5 and -2.0 MPa. Stomatal conductance peaked with light intensity and reached values about 100 mmol m^{-2} s^{-1} and declined in the course of the afternoon to values below 50 mmol m^{-2} s^{-1}.

Overall, sap flow was lower on July 14 2010 because of the very high VPD values (maximum of c. 3.5 kPa). All 3 days were cloudless, except for some haziness in the afternoon of July 14 2010. The dent in photoactive radiation (PAR) around 10 am is due to passing shadow from the crane top.

Point dendrometer data showed that growth was initiated in mid-April (day of year 135) and ended in mid-August (day of year 230) in 2009, with no systematic difference between

Table 1 | Comparison of measured and modeled transpiration under control and elevated CO_2 conditions, estimated from relative sap flow in *P. abies* in the experiment, and from total stand transpiration of evergreen needle-leaved trees in the dynamic global vegetation model LPJ-GUESS.

	Control ± s.e.	**Elevated CO_2 ± s.e.**	**Difference in %**
MEASURED			
2009 (pre-treatment)	2461 ± 223	2215 ± 162	−10.0% (n.s., $P = 0.056$)
2009 (during treatment)	1079 ± 175	1058 ± 171	−1.9% (n.s., $P = 0.095$)
2010 (during treatment)	3435 ± 452	3930 ± 449	+12.6% (n.s., $P = 0.55$)
MODELED			
2010 (step change to 550 ppm in 2010)	0.81 mm/d	0.73 mm/d	−10.0%
2010 (step change to 700 ppm in 2010)	0.81 mm/d	0.66 mm/d	−18.6%
2110 (step change to 550 ppm in 2010)	0.73 mm/d	0.66 mm/d	−9.0%
2110 (step change to 700 ppm in 2010)	0.73 mm/d	0.59 mm/d	−18.4%
2110 (gradual change to 550 ppm in 2057)	0.73 mm/d	0.66 mm/d	−8.7%
2110 (gradual change to 700 ppm in 2100)	0.73 mm/d	0.62 mm/d	−15.4%

Seasonal sum of sap flow (March–October, arbitrary units) and transpiration (modeled, mm/d) under control and treatment conditions are shown. The Wilcoxon rank-sum test is based on five replicates (tree individuals) each for control and treated trees.

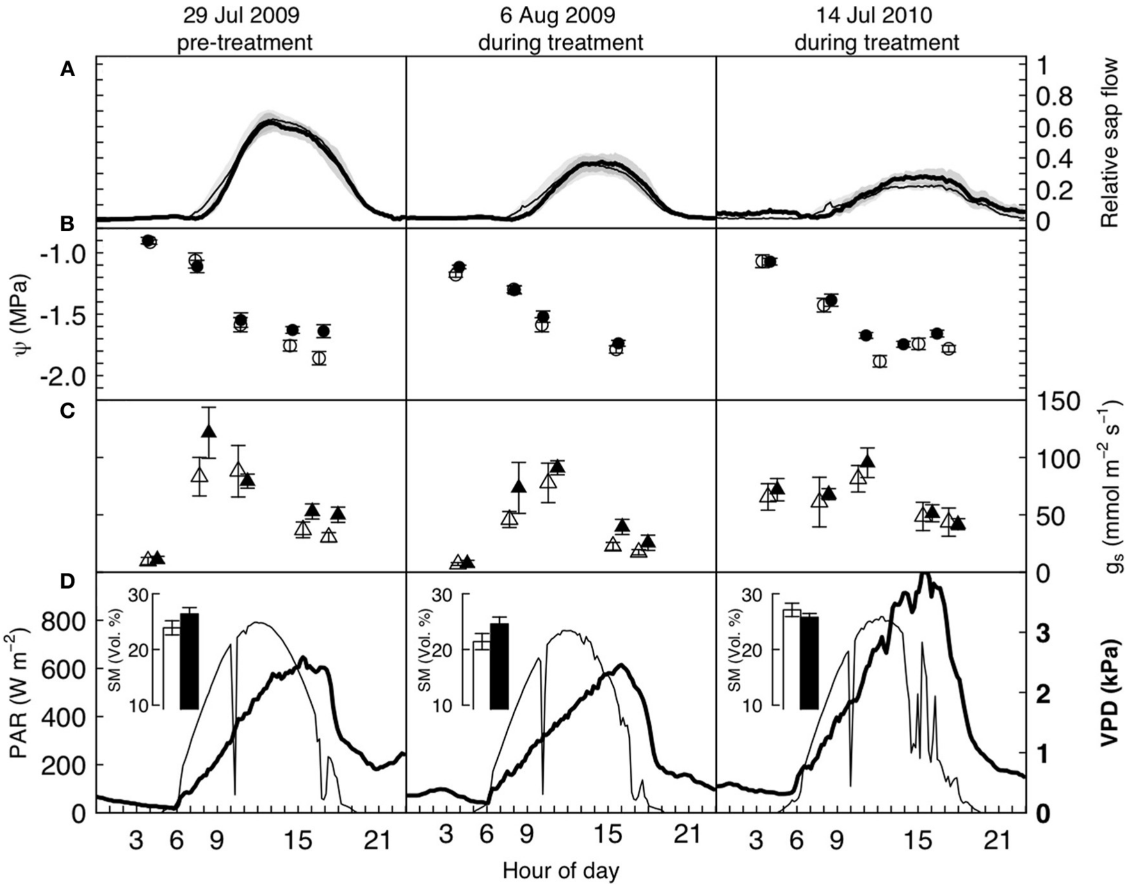

FIGURE 2 | Diurnal water relations data of mature *P. abies* trees under elevated (bold/filled symbols) and ambient (open symbols) atmospheric CO_2, before and after the treatment was initiated, on three cloudless summer days in 2009 and 2010. Panels **(A)** show relative sap flow (each tree standardized to its pre-treatment maximum, $n = 5$), panels **(B)** and **(C)** show mean leaf water potentials and stomatal conductance from pre-dawn to dusk, and **(D)** the photosynthetically active radiation (above the canopy, thin line, left hand side axis) and vapor pressure deficit (at canopy height, bold line, and font, right hand side axis). Shaded areas and bars represent one standard error. None of the differences between control and treated trees is significant on any of the 3 days (see text).

treatments (defined as the day when 10% of the yearly growth increment was reached, Wilcoxon rank-sum test, $p = 0.4$). Stem water deficit derived from dendrometer readings did not differ systematically between the two groups (**Figure 3A**). Soil moisture under CO$_2$-enriched trees tended to be higher than under control trees, but this difference was already present before the onset of the treatment in the first year and disappeared in the second year (**Figure 3B**). Decreasing mean daily soil moisture generally increased mean daily stem water deficit sharply, but as judged by the AIC the pattern did not change between the two groups, neither before nor after the start of the CO$_2$-treatment (**Figure 4**). Stem storage saturation occurred at a wide spectrum of soil moisture contents, but depleted stem water reservoirs only occurred at low soil moisture values (<25 Vol. %, **Figure 4**).

We also looked at the relative sap flow response to VPD according to experimental period (before and after treatment initiation). The 95th percentiles per 2 kPa bin did not differ between treatments, both before and after CO$_2$ enrichment, because the interaction between VPD and CO$_2$ in the polynomial model fits was statistically not significant (**Figure 5**).

COMPARISON TO MODELING THE CO$_2$-RESPONSE WITH LPJ-GUESS

Given the commonly implemented algorithms controlling leaf gas exchange in dynamic vegetation models, it can be expected that any PFT will show less water use under eCO$_2$ (Farquhar et al., 1980). To see whether and to what extent this is the case in the commonly used DGVM LPJ-GUESS, we simulated our experiment with local climate data. We suppressed all other plant functional types but "needle-leaved evergreen." The CO$_2$ concentration for the 700-years spin-up was kept at 296 ppm. After 1901, the four CO$_2$-scenarios outlined in the section "materials

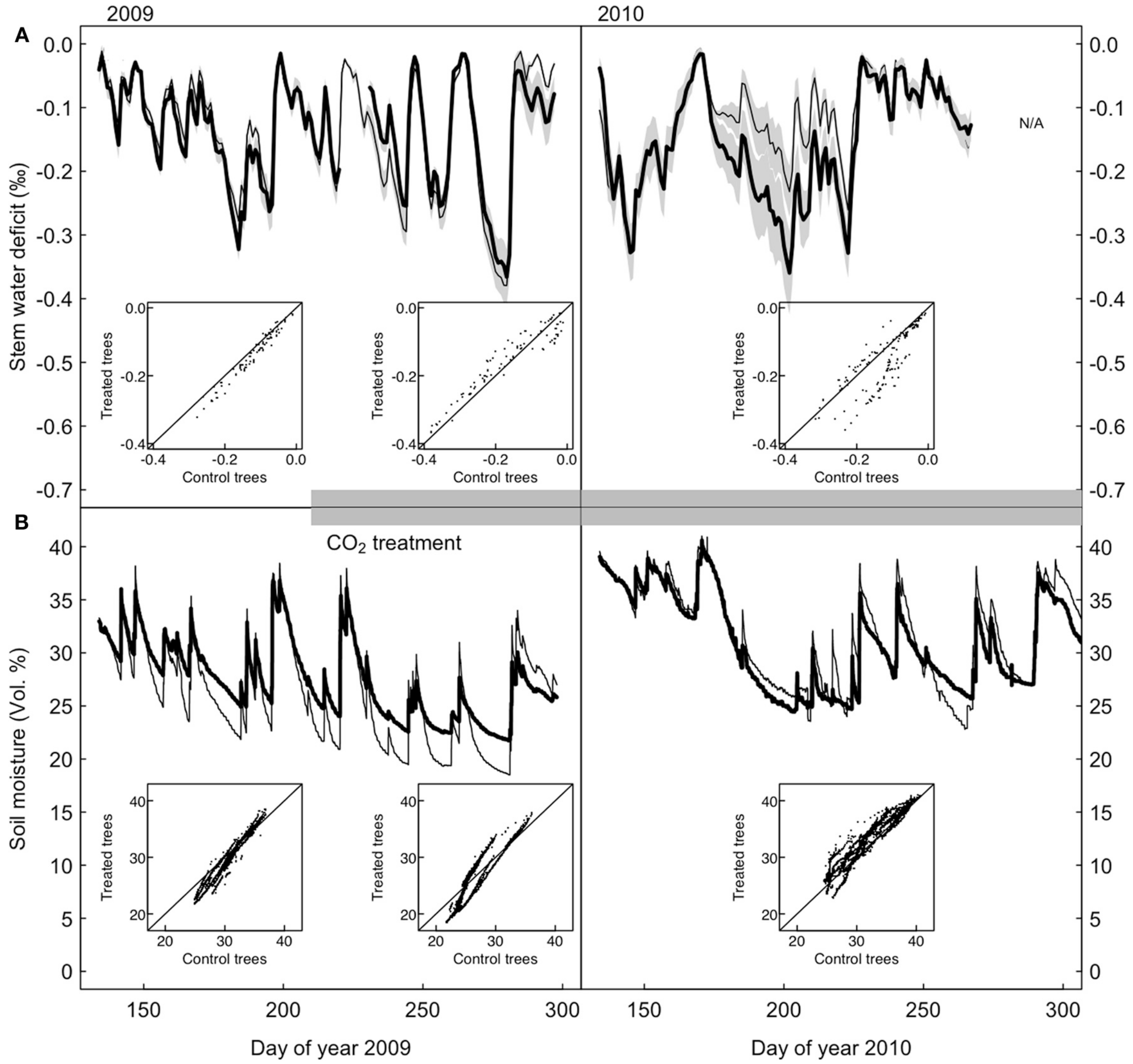

FIGURE 3 | Stem water deficit in control and CO$_2$-treated *P. abies* trees. (A) Standardized time series of stem water deficit in CO$_2$-treated (bold) and control (dashed line) trees, growth-trend corrected (see section "Materials and Methods"). **(B)** Soil water content measured in the 10 cm top soil layer for the control and treated area. The insets show that there are no systematic differences between CO$_2$-treated and control trees during any of the three periods.

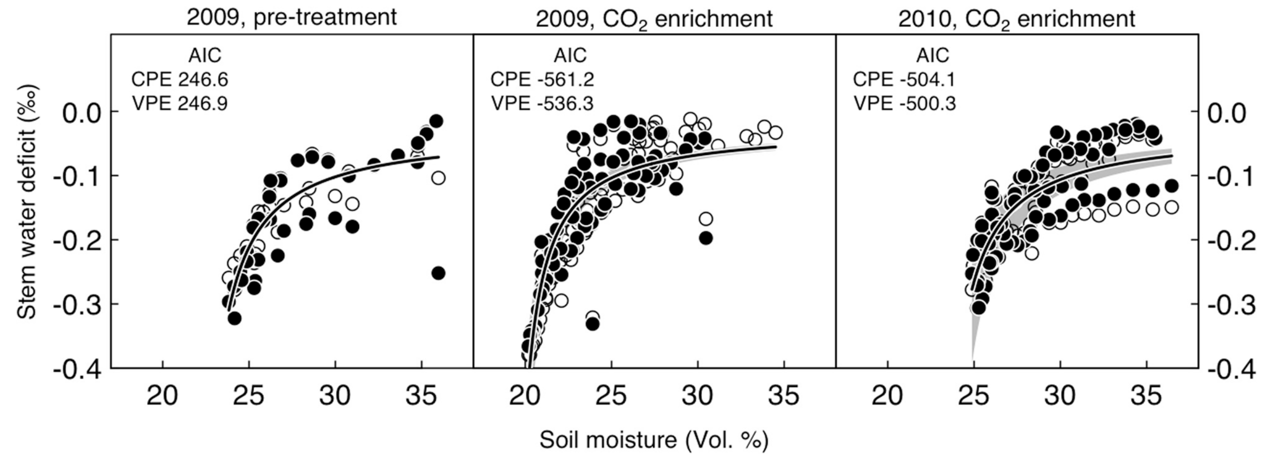

FIGURE 4 | Relationship between soil moisture (mean across ambient and CO_2-treated area) and stem water deficit in per mille for *P. abies* under ambient (open symbols) and elevated CO_2-conditions (filled symbols). Periods between day of year 175 and 250 were considered. AIC, Akaike Information Criterion; CPE, nonlinear model with common parameter estimates; VPE, nonlinear model with varying parameter estimates for each treatment. Soil moisture explained 77%, 82%, and 65% of the variation in stem water deficit during the pre-treatment period and the 2009 and 2010 CO_2 enrichment periods, respectively. The gray-shaded area around the regression line indicates the 95% confidence interval.

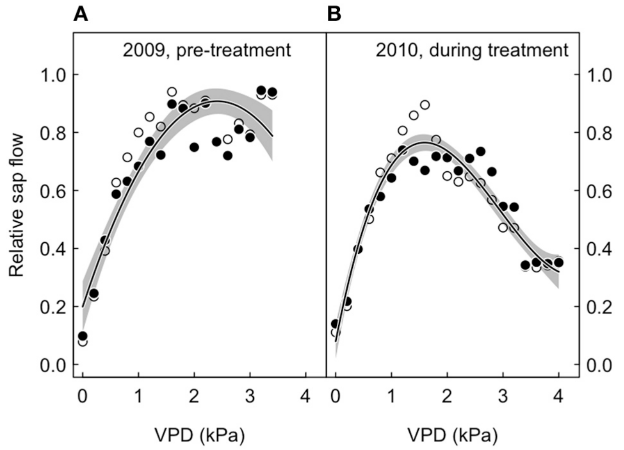

FIGURE 5 | Relative sap flow plotted against the vapor pressure deficit (VPD), according to treatment (open symbols, ambient; closed symbols, treated), before and after the initialization of the CO_2-treatment. Shown are 95 percentile values for 2 kPa bins, with non-linear fits (solid lines) and their 95% confidence intervals (gray-shaded area around the regression line). The merged model fits (treated and control trees) are shown. **(A)** 2009, before the start of the CO_2 treatment. **(B)** 2010, during the CO_2 treatment.

and methods" were imposed. The daily transpiration rates were sensitive to the final CO_2 concentrations reached (550 ppm or 700 ppm), but only little to whether a step or gradual change in the CO_2 concentration was used. Overall, there was c. 10% less transpiration in the 550 ppm scenario and c. 17% less transpiration in the 700 ppm scenario, regardless of how the concentration was reached or how long the new atmospheric conditions lasted (**Table 1**).

DISCUSSION

We aimed to assess the response of whole-tree water relations to future levels of atmospheric CO_2 in Norway spruce, one of the most abundant and economically important coniferous tree species in Europe. Using five different, fully independent approaches (sap flow-, dendrometer-, leaf water potential-, and soil moisture measurements), our experimental data shows that this species is unlikely to adjust its water use under atmospheric CO_2 concentrations anticipated to occur in c. 2050. This stands in contrast to the model results of the DGVM LPJ-GUESS, which predicts a c. 10% reduction in transpiration when simulating needle-leaved evergreen trees under CO_2 concentrations and site conditions matching those of the field experiment. Because of the wide distribution across Europe and Asia and its importance as a timber species the lack of a water use response of Norway spruce to elevated atmospheric CO_2 is fundamental for the parameterization and validation of DGVMs and fully coupled earth system models predicting the future water and carbon cycle. Further, it is central to our understanding of plant responses to eCO_2 and how they are scaled with ontogeny and the successional stage of the tested species or community.

Critical to the estimates of net plant water use under eCO_2 seem to be the developmental stage of the tested individuals, the species and PFT tested, the duration of the treatment, the method of CO_2 administration, and the nature and timing of the measured traits used as a proxy to estimate plant water use (**Table 2**). All these factors will have an impact on the net response of plant water use to eCO_2. Despite the large range of responses reported, the majority of studies tend to predict a decrease in stomatal conductance and therefore net water use under eCO_2 (e.g., Curtis and Wang, 1998; Medlyn et al., 2001). However, there are numerous examples that show no response or even an increase in water

Table 2 | Possible processes that contribute to the mitigation of the response of plant water use to eCO_2.

Observed process/characteristic of experiment	Stomatal conductance (g_s) or net water use/evapotranspiration per unit surface area	Example references
Duration of the experiment	Decrease with duration of experiment	Medlyn et al., 2001; Leuzinger et al., 2011b
Developmental stage of study plants, canopy closure	Decrease with increasing maturity/canopy closure	Medlyn et al., 2001; Uddling et al., 2009
Plant functional type	Decrease from herbaceous to woody plants and from deciduous to coniferous trees	Saxe et al., 1998; Medlyn et al., 2001; Ainsworth and Rogers, 2007
Combination with other global change drivers	Trend for decrease	Leuzinger et al., 2011b
Scaling from plant to canopy/landscape	Decrease with increasing scale	Field et al., 1995; Wullschleger et al., 2002
Upscaling from experimental period to yearly average response	Responses-dependent on weather conditions, average water use often lower than if extrapolated linearly from experimental period	Wullschleger and Norby, 2001; Cech et al., 2003; Leuzinger and Körner, 2007
Soil feedback	Decrease (through wetter soils)	Schäfer et al., 2002

The circumstances that lead to this decline can generally be associated with a more realistic scenario (young vs. mature stands, local vs. global scale response etc.)

use under eCO_2. For *P. abies*, the species tested in the present study, Roberntz and Stockfors (1998) found no effect on g_s using branch bags, and Barton et al. (1993), Kupper et al. (2006) and Uddling et al. (2009) all report an increase in water use under eCO_2 from glasshouse and branch bag experiments. For *Pinus taeda*, both a branch-bag and an open-top chamber experiment suggest that this species does not respond to eCO_2 in its water use (Teskey, 1995; Pataki et al., 1998). Contrary to grassland experiments, Domec et al. (2009) found reduced stomatal conductance under eCO_2 in tall, 28 years old *Pinus taeda* individuals only at high soil moisture, and no response during dry conditions.

In trees that are in an early (expanding) successional stage, increased LAI under eCO_2 may periodically (over-) compensate reductions in stomatal conductance (Li et al., 2003) or lead to an increase in total water use (Bobich et al., 2010). Increasing LAI following CO_2-treatment has been reported for a closed-canopy *Pinus taeda* stand growing at the Duke experimental forest (McCarthy et al., 2007). However, this canopy response to eCO_2 was determined by nitrogen availability patterns and additional N fertilization trials suggested that LAI stimulation is unlikely to occur at high fertility sites (McCarthy et al., 2007). Most other eCO_2 studies suggest that CO_2 will not cause an increase in LAI in mature systems (Bader et al., in preparation; Körner et al., 2005; Norby et al., 2005; Warren et al., 2011). Hättenschwiler and Körner (1997) even found a lower LAI under eCO_2 in a young, closed-canopy Norway spruce stand, similar to what tropical tree model ecosystems revealed after stand closure (Körner and Arnone, 1992). Furthermore, a LAI beyond c. 2.7 will not affect canopy conductance (Schulze et al., 1994). Given the age of our study trees (>100 years) and the nutrient-rich soil they thrive on, it seems improbable that CO_2 enrichment will enhance LAI in this stand.

We argue that the majority of the evidence underlying the existing reviews on plant water savings under eCO_2 experiments (Curtis and Wang, 1998; Medlyn et al., 2001; Ainsworth and Long, 2005; Ainsworth and Rogers, 2007) rests on short-term (sub-seasonal) experiments, predominantly on grasslands, tree seedlings or juveniles tested under relatively confined conditions (e.g., glasshouses). However, these test conditions tend to overestimate the effects of eCO_2 on stomatal conductance and/or water use and potential long-term alterations such as structural changes in the hydraulic pathway as have been reported for *Pinus taeda* at the Duke FACE site may remain undetected (**Table 2**; Domec et al., 2009).

Another source for overestimating water use under eCO_2 is temporal upscaling from an experimental period shorter than or non-representative of a whole year (Leuzinger and Körner, 2007, 2010). Often, the response strongly depends on the weather and soil moisture conditions (Wullschleger and Norby, 2001; Cech et al., 2003). Our study, together with others (Ellsworth, 1999; Schäfer et al., 2002; Bernacchi et al., 2003; Keel et al., 2007; Uddling et al., 2009), supports the view that near-natural conditions tend to yield a smaller or no response in annual water use to elevated CO_2.

Apart from the obvious absence of a CO_2-response in the water flux of our experimental trees, the patterns found (**Figure 2**) match with earlier reports on water relations of *P. abies* (e.g., Gross and Koch, 1991; Zweifel et al., 2001). The low pre-dawn shoot water potentials around −1 MPa resulted from a combination of soil water deficits and the hydrostatic water potential of c. 0.4 MPa. The diurnal courses of water relations also showed tight stomatal control over transpiration, preventing midday shoot water potentials from dropping below −1.9 MPa. This rather isohydric behavior allowed the maintenance of an adequate hydraulic safety margin from the critical threshold of −2.5 MPa that has been reported as turgor loss point (Gross and Koch, 1991) and as the level at which significant xylem cavitation occurs in branches of adult Norway spruce trees (Cochard, 1992; Lu et al., 1995). Compared to the first day of measurements (**Figure 2**, left panels), stomatal conductance decreased due to lower soil moisture or higher VPD, causing considerably less sap flow (**Figure 2**, middle and right panels). The incomplete night-time recovery of stem radius during times of low soil moisture (<30 vol. %) and high VPD suggests that internal water storage tissues could not be replenished and is thus indicative of a tree water deficit (Zweifel et al., 2005 **Figure 3**). We have no explanation for the high pre-dawn stomatal conductance measured in the morning of July 14 (**Figure 2**).

The modeled reduction in water use by coniferous trees for this site stands in contrast to our *in situ* measurements. The LPJ-GUESS dynamic vegetation model consistently predicted between 9% and 18% reduced transpiration, with the ambient CO_2 concentration (C_a, 550 ppm vs. 700 ppm) causing most of the sensitivity of the response and the mode of increase (step vs. gradual) as well as the duration of the new conditions (immediately after the increase vs. 100 years later) being rather insignificant. In LPJ-GUESS, but also in other dynamic vegetation models, the first-order response originates from the photosynthesis model through stomatal closure due to increased intercellular CO_2 concentrations (C_i). Because C_i/C_a is assumed constant, changes in C_a will result in proportional changes in stomatal conductance. However, in LPJ-GUESS this effect only manifests itself during ample water supply (see section "Materials and Methods"). Therefore, the CO_2-response does not increase with dry conditions, which has been found earlier (Hickler et al., 2008), although a carry-over effect from wet to dry periods (higher available soil moisture under eCO_2) is possible. This behavior certainly does not mirror observations from grassland (Niklaus et al., 1998; Morgan et al., 2004) and the drought × eCO_2 interactions in trees do not seem to be uniform (Beerling et al., 1996; Heath, 1998; Cech et al., 2003; Leuzinger and Körner, 2007 and others).

The key difference between photosynthesis models that are employed in dynamic vegetation models is essentially the formulation of C_i/C_a (Katul et al., 2000). Therefore, the range of modeled responses is relatively narrow (Luo et al., 2008) and foreseeable, unless model-specific feedback mechanisms dampen or enhance the initial signal. For example, increased LAI through altered carbon allocation patterns, soil moisture, or atmospheric feedback (in a fully coupled model) could all contribute to changing the initial response largely prescribed by the photosynthesis model. The fact that our modeled water relations response to eCO_2 seems largely-independent of the time the eCO_2–conditions are in place suggests that very little feedback mechanisms contribute to altering the first-order response, which seems to be stable across models and ecosystem types (Luo et al., 2008). Another important component potentially responsible for mitigating the CO_2-response are the leaf and canopy boundary layer resistances, which are in series with the stomatal resistance but not explicitly considered in LPJ-GUESS (McNaughton and Jarvis, 1991).

Clearly, there are limitations as to what conclusions can be drawn from a sample of five adult Norway spruce individuals treated with elevated atmospheric CO_2 over two seasons only. Such experiments struggle with the inherent trade-off between sample size and the realism of the experimental setting. While it may be more satisfying to get statistically more robust results on CO_2-responses with young trees, we make little progress if young trees respond differently to mature trees (Medlyn et al., 2001; Uddling et al., 2009; Leuzinger et al., 2011a,b). More data on water use under eCO_2 are needed from large-scale studies in mature systems to confirm our results. Equally important is the continuation of large-scale experiments over many years in order to alleviate some of the statistical shortcomings from originating from low replication.

In conclusion, we find a contrasting response between our experimental results with mature *P. abies* trees, and the model output from the global dynamic vegetation model LPJ-GUESS. Our experimental results are corroborated by evidence from studies on other plant functional types, and we argue that the more realistic the testing conditions, the smaller the water savings in response to eCO_2. The modeled water relations response to eCO_2 was strikingly robust both in this and previous studies. Because the modeled responses are closer to the leaf-level than to the ecosystem response in experiments, one explanation for the apparent discrepancy is that the propagation of the response from the leaf to the ecosystem is not captured appropriately in the currently available models. Attempts should be made to account for such processes in models potentially mitigating first-order CO_2-effects on plant water use.

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