ECOSYSTEMS ECOLOGY

Patrick G. Cech · Steeve Pepin · Christian Körner

Elevated CO$_2$ reduces sap flux in mature deciduous forest trees

Abstract We enriched in CO$_2$ the canopy of 14 broad-leaved trees in a species-rich, ca. 30-m-tall forest in NW Switzerland to test whether elevated CO$_2$ reduces water use in mature forest trees. Measurements of sap flux density (J_S) were made prior to CO$_2$ enrichment (summer 2000) and throughout the first whole growing season of CO$_2$ exposure (2001) using the constant heat-flow technique. The short-term responses of sap flux to brief (1.5–3 h) interruptions of CO$_2$ enrichment were also examined. There were no significant a priori differences in morphological and physiological traits between trees which were later exposed to elevated CO$_2$ (n=14) and trees later used as controls (n=19). Over the entire growing season, CO$_2$ enrichment resulted in an average 10.7% reduction in mean daily J_S across all species compared to control trees. Responses were most pronounced in *Carpinus*, *Acer*, *Prunus* and *Tilia*, smaller in *Quercus* and close to zero in *Fagus* trees. The J_S of treated trees significantly increased by 7% upon transient exposure to ambient CO$_2$ concentrations at noon. Hence, responses of the different species were, in the short term, similar in magnitude to those observed over the whole season (though opposite because of the reversed treatment). The reductions in mean J_S of CO$_2$-enriched trees were high (22%) under conditions of low evaporative demand (vapour pressure deficit, VPD <5 hPa) and small (2%) when mean daily VPD was greater than 10 hPa. During a relatively dry period, the effect of elevated CO$_2$ on J_S even appeared to be reversed. These results suggest that daily water savings by CO$_2$-enriched trees may have accumulated to a significantly improved water status by the time when control trees were short of soil moisture. Our data indicate that the magnitude of CO$_2$ effects on stand transpiration will depend on rainfall regimes and the relative abundance of the different species, being more pronounced under humid conditions and in stands dominated by species such as *Carpinus* and negligible in mono-specific *Fagus* forests.

Keywords Water relations · CO$_2$ enrichment · Free-air CO$_2$ enrichment · Global change · Stomatal conductance

P. G. Cech · S. Pepin · C. Körner
Institute of Botany, University of Basel,
Schönbeinstrasse 6, 4056 Basel, Switzerland

S. Pepin (✉)
Ministère des Ressources naturelles de la Faune et des Parcs,
Direction de la recherche forestière,
2700 rue Einstein, Sainte-Foy, Québec, G1P 3W8, Canada
e-mail: steeve.pepin@mrnfp.gouv.qc.ca
Tel.: +1-418-6437994 ext. 6616
Fax: +1-418-6432165

Introduction

Elevated CO$_2$ concentrations have long been known to reduce stomatal aperture in plants (Linsbauer 1916) and to decrease leaf transpiration (E) when stomatal conductance (g_s) is the dominant controller of E. The predicted doubling of atmospheric CO$_2$ concentration by the end of this century is thus expected to lead to a reduction in plant water use. Although there is strong evidence that g_s of herbaceous species is substantially reduced under elevated CO$_2$ (Woodward et al. 1991; Knapp et al. 1996; Wand et al. 1999), stomatal responses to CO$_2$ appear to be smaller in woody species (Saxe et al. 1998; Lodge et al. 2001).

Numerous observations of reduced g_s in response to elevated CO$_2$ have emerged from experiments with tree seedlings or saplings grown in controlled-environment facilities and field chambers (see, for example, the compilation by Field et al. 1995). Some exceptions to this apparent general response have been reported, particularly in coniferous species (e.g. Bunce 1992; Dixon et al. 1995; Picon et al. 1996). In addition to species-specific effects, a number of studies have demonstrated that stomatal responses to elevated CO$_2$ also vary with ambient humidity, CO$_2$ effects being greater under low evaporative demand (Will and Teskey 1997; Heath 1998; Wullschleger et al. 2002). Given the broad differences in responses and the uncertainties associated with the scaling of results from seedling studies to predict

the long-term responses of forest trees to elevated CO_2, experimental CO_2 research has been extended to larger-scale field studies during the last decade (Ellsworth et al. 1995; Wullschleger and Norby 2001).

The first field, albeit short-term, CO_2 experiment conducted at a higher scale reported a 30% reduction in the transpiration rate of 12-year-old eucalypt trees exposed to double ambient CO_2 concentrations (Wong and Dunin 1987). In longer-term experiments with coniferous species, CO_2 enrichment had no effect on g_s and J_S of *Pinus taeda* (L.) trees in a forest plantation (Ellsworth 1999; Schäfer et al. 2002), but significantly reduced (~14%) sap flux in 30-year-old *Pinus sylvestris* (L.) trees grown in large closed-top chambers (Kellomäki and Wang 1998). Recent studies conducted on a deciduous plantation reported a significant reduction in g_s of *Liquidambar styraciflua* (L.) trees exposed to elevated CO_2 (~22% across the whole growing season; Wullschleger et al. 2002), while sap velocity was, on average, 13% lower in CO_2-enriched plots than in control plots (Wullschleger and Norby 2001). Hence, responses in g_s were larger than actual signals in tree water fluxes. This is because above the scale of stomata, a number of processes such as leaf temperature or boundary layer conductance co-influence transpiration, causing the reductions in g_s under elevated CO_2 to have a less pronounced effect on stand transpiration (Field et al. 1995; Wilson et al. 1999). These factors highlight the importance including whole-tree measurements of transpiration in CO_2 experiments (Norby et al. 1999; Wullschleger et al. 2002).

Much of the current evidence of the effects of elevated CO_2 on tall mature trees comes from branch-bag experiments and studies conducted in the vicinity of natural CO_2 springs. For instance, CO_2-induced reductions in g_s were observed in mature *Quercus ilex*, *Q. pubescens* and *Arbutus unedo* trees exposed to elevated CO_2 concentrations throughout their lifetime as compared to control trees outside the spring area. Responses were, however, restricted to humid periods (Tognetti et al. 1998; 1999) or early spring (Jones et al. 1995). In contrast, there were no differences in g_s of European beech leaves and foliage of several conifer species when CO_2-enriched branches were compared to bagged control branches (Dufrene et al. 1993; Teskey 1995; Roberntz and Stockfors 1998; Barton and Jarvis 1999). Interestingly, the most recent meta-analysis compiling data from 13 long-term (>1 year) field studies, which ranged from seedlings grown in open-top chambers to branch-bag experiments, reported an overall 21% reduction in g_s associated with growth under elevated CO_2 conditions (Medlyn et al. 2001). The existing large volume of data on the responses of woody plants to rising atmospheric CO_2 clearly needs more relevant experimental data on dominant trees, which are key players in whether forests will reduce water use in a high CO_2 world.

In this study, we present data from the first free-air CO_2 enrichment experiment exposing the canopy of 14 mature broad-leaved trees (from six different species) in a temperate forest ecosystem to about twice the pre-industrial CO_2 concentration. Sap flux density was monitored prior to CO_2 enrichment and throughout the first full growing season of CO_2 exposure to test the hypothesis that water use of mature forest trees is reduced under elevated CO_2, and to determine whether there are differences among tree species. In addition to the analysis of the whole growing season data set, we examined the responses of trees growing under elevated CO_2 to a short-term return to ambient CO_2 concentrations. Such an experiment in a natural, mature forest with rough canopy aims at providing realistic experimental data on deciduous tree responses for model-based predictions on the water consumption of forests in a high CO_2 atmosphere.

Materials and methods

Site description

The study site is located in a highly diverse mixed forest stand ~15 km south of Basel, Switzerland (47°28′ N, 7°30′ E; elevation 550 m a.s.l.). The forest is 80–120 years old, with tree heights between 30 and 35 m, a tree density (diameter ≥0.1 m) of 415 trees ha^{-1} and a stem basal area of 46 m^2 ha^{-1}. Leaf area index (LAI) of the canopy in the experimental area is ~5. The stand used in this experiment is characterized by a dominance of *Fagus sylvatica* (L.) and *Quercus petraea* (Matt.) Liebl., with *Carpinus betulus* (L.), *Tilia platyphyllos* (Scop.), *Acer campestre* (L.) and *Prunus avium* (L.) present as companion species. In addition, the site has a strong presence of conifers [*Abies alba* (Mill.), *Picea abies* (L.), *Pinus sylvestris* (L.) and *Larix decidua* (Mill.)].

The climate is a typical humid temperate zone climate, characterized by mild winters and moderately warm summers. In the study year, the growing season of deciduous trees lasted from the end of April to the end of October (~180 days). Mean January and July air temperatures are 2°C and 19°C, respectively. Total annual precipitation for the region averages 990 mm, of which two-thirds falls during the growing season. Soils are of the rendzina type on calcareous bedrock (a silty loam with an accessible profile depth of ca. 30 cm and a pH of ~5.8 in the top 10 cm of the profile).

CO_2 enrichment system

Operations in the tree canopy were facilitated by a 45-m freestanding tower crane (installed by helicopter in an ~8 m tree gap) equipped with a 30-m jib. The working gondola provided access to 62 dominant trees in an area of ~3,000 m^2. A group of 14 adult broad-leaved trees (3 *Fagus*, 4 *Quercus*, 4 *Carpinus*, 1 *Tilia*, 1 *Acer*, and 1 *Prunus*), covering a canopy area of ~550 m^2 were selected for the CO_2 treatment. Control trees were located in the remaining crane area at sufficient distance from the CO_2 release zone, and additional control trees were selected outside the crane area. CO_2 enrichment of the forest canopy was achieved by a free-air, pure CO_2 release system (web-FACE, Pepin and Körner 2002) which consisted of a web of 4 mm plastic tubes (~0.5 km per tree) with 0.5 mm laser punched holes (spaced at 30-cm intervals) emitting pure CO_2 into the tree canopy. The rate at which pure CO_2 was injected into the tubing system and released in the tree crowns was set by 24 computer-controlled magnetic valves (~2 per tree). CO_2 concentrations in air samples drawn from 22 sampling lines distributed in the release zone were continuously measured and processed by a computer which adjusted the duration of injection pulses to maintain a target CO_2 concentration of 550 ppm as closely as possible. For a more detailed description, see Pepin and Körner (2002).

Due to fluctuating wind speed, CO_2 concentrations varied with patchy excursions to several hundred ppm above the target value

during complete calms or almost no deviation from controls during strong wind exceeding 10 m s^{-1}. However, 1-min averages of CO_2 measurements conducted at canopy height were within ±20% of the target concentration for 76% of the exposure time (2 May–28 October). The overall mean seasonal (2001) daytime CO_2 concentration within the canopy was 520 ppm. Since the pure CO_2 gas used to enrich the forest canopy carried a strong stable carbon isotope signal ($\delta^{13}C$ = ca. –31‰), the time-integrated mixing ratio of atmospheric and tank CO_2 in the canopy was recorded into new carbon assimilates. This signal was captured at 80 different canopy positions using C_4-grass [*Cynodon dactylon* (L.) grown in 50 ml of sand] phytometers and fully confirmed the gas analysis data (Pepin and Körner 2002). Time-integrated values of [CO_2] derived from the C isotope composition of *C. dactylon* leaves showed that the zone of CO_2 enrichment was constrained to the treated trees, except for a few down-wind trees situated in the E-SE end of the plot (prevailing wind direction NW).

Sap flow and meteorological measurements

Sap flux density within the xylem (J_S, m^3 H$_2$O m^{-2} sapwood area s^{-1}) was measured using the constant heat-flow technique described by Granier (1985, 1987). A sensor consisted of two 20-mm long and 2-mm diameter probes (each equipped with a copper-constantan thermocouple and coated with heating wire) inserted radially into the sapwood at breast height with a vertical spacing of ~15 cm (UP, Kolkwitz, Germany). The upper probe received a constant power of 200 mW while the lower reference probe remained unheated. During conditions of zero sapflow such as at night or during prolonged rain events the temperature difference, ΔT, between the two probes reached a maximum (ca. 9–15 K). Sap flux during the day caused a decrease in ΔT. J_S was calculated using an empirical relationship validated for several species by Granier (1985), and recently revalidated by Clearwater et al. (1999).

Measurements of J_S in 2001 were performed from 5 June to 1 October on 33 trees (14 in elevated CO_2 and 19 in ambient CO_2) using, in most cases, two sensors per tree (installed on opposite sides of the stem). In control trees, some individuals were equipped with only one sensor, allowing us to monitor more trees. Sensors were protected against rain and external thermal influences with aluminium covers filled with polyester wool. Readings were taken at 30 s intervals and recorded as 10-min means using multi-channel data loggers (CR10 and an AM416 Relay Multiplexer, Campbell Scientific, Utah, USA; DL2e, Delta-T, Cambridge, UK). The signals were averaged when there was more than one sensor per stem. Sap flux densities were also measured in year 2000 (prior to CO_2 enrichment; from 18 July to 13 September) on the same trees as in 2001 using the same equipment. Sensors were removed during the winter and re-installed in new positions in spring 2001, as recommended by Köstner et al. (1998).

Wind speed, photon flux density (PFD), rainfall, relative humidity, and air temperature were measured by a weather station located above the tree canopy at the top of the crane (anemometer AN1, tipping bucket rain gauge RG1, shielded temperature and relative humidity probe RHA1, quantum sensor QS, Delta-T, Cambridge, UK). Measurements were performed every 30 s (except for wind speed which was measured as wind run) and data were recorded as 10-min means using a data logger (DL3000, Delta-T, Cambridge, UK). Vapour pressure deficit (VPD) was calculated from 10-min averages of relative humidity and air temperature. Soil water content across a 90-cm profile was monitored every 3–7 days during the 2001 growing season at three locations in the control area and one location in the CO_2 enrichment zone using time domain reflectometry (MP-917 and probes PRB-F, Environmental Sensor, Victoria, BC, Canada). Additional soil moisture data were obtained at 10 and 20 cm depth from hourly measurements carried out in 2000 and 2001 using theta-probes (ML2x, Delta-T, Cambridge, UK) located in the vicinity of the CO_2 enrichment zone and connected to a data logger (DL2e, Delta-T, Cambridge, UK).

Off/on experiments

To investigate the short-term response of the trees growing under elevated CO_2 to a sudden return to ambient CO_2 concentrations, the enrichment system was switched off for 1.5 to 3 h around noon on 6 cloudless days. On 3 of those days, the interruption of CO_2 enrichment occurred during the period of ascending J_S and lasted until $J_{S max}$ had been reached. On the other three occasions switch off took place when J_{Smax} was about to be attained. CO_2 concentrations in the canopy decreased to ambient levels (ca. 365 ppm) within a few minutes after interrupting CO_2-supply. When CO_2 enrichment was resumed later during the day, the CO_2 concentrations reached the target concentration in <5 min following re-activation of the CO_2-supply system.

Data processing and statistical analyses

Daily means of J_S, VPD and meteorological variables were calculated over 13-h intervals starting at 0600 hours (true local time). Such intervals included more than 97% of the total daily dose of PFD.

The presence of a CO_2 effect onto daily mean J_S was tested by analysis of covariance (ANCOVA) with pre-treatment J_S values (i.e. year 2000 data) as covariate, and species and treatment as fixed factor effects (type I sums of squares, factors in the same order as listed). To prevent confounding between treatment effect and any possible seasonal effect, the analysis of data from both pre-treatment (ambient CO_2 concentration in 2000) and treatment year (elevated CO_2 concentration in 2001) was restricted to the same period during the growing season. An ANCOVA was computed for: (1) each day separately; (2) the mean of a given number of days according to three ranges of meteorological conditions; and (3) the mean of all measurement days (mid-July to mid-September). Two treated *Quercus* individuals had to be excluded due to sensor malfunction. In addition, one treated *Fagus* and one control *Quercus* were removed from analyses because their J_S values in 2000 were erratic and occasionally out of range (based on J_S data for trees of the same species). Hence, our tree sample consisted of 18 controls and 11 treated trees. Means across all trees for each group were calculated by giving *Quercus*, *Fagus* and *Carpinus* each a weight of 2, and the pooled trees of *Tilia*, *Acer* and *Prunus* (hereafter referred to as "TAP species") a weight of 1.

Off/on experiments were analysed using a set of control trees, so that for a given species, there was an equal number of trees in both control and treated group. In cases where a species had a greater number of control than treated trees, we selected individuals whose diurnal course of J_S was closest in relative terms to the treated trees. Our tree sample for this analysis consisted of 12 treated trees and 12 control individuals. Each day on which an off/on experiment was performed, was assigned to a nearby reference day (either just before or thereafter) with comparable meteorological conditions. Tree response to the resuming CO_2 enrichment was expressed as the percentage decrease in mean J_S calculated from measurements made over a 50-min period during the interruption of CO_2 enrichment, and over a 60-min period after CO_2 enrichment was re-initiated. In both cases, J_S values were averaged over periods that started at least 25 min after the new CO_2 environment was created. We considered the response to the re-start of CO_2 enrichment rather than the initial response to the discontinuation of CO_2-supply because of the large natural variation in mean J_S before switch off (trees were in the steeply ascending part of the daily course of J_S). The computed values were analysed with an ANCOVA for the mean response on the 6 days with a short-term discontinuation of CO_2 enrichment using the mean response observed on the 6 reference days as covariate and the two fixed factor effects species and treatment. A similar ANCOVA was also performed for each off/on day separately (using data of the assigned reference day as covariate). Additionally, one-way ANCOVAs with treatment as a fixed effect were computed for each species (or group of species) separately. All statistical analyses were computed using JMP 3.2.2 (SAS Institute, Cary, N.C., USA).

Results

Comparison of pre-treatment data

Analysis of all morphological and physiological data available from pre-treatment years (before 2001) indicated that there was no systematic difference between trees which were later exposed to elevated CO_2 and surrounding trees later used as controls (Table 1). Of particular interest was the absence of significant differences in J_S and g_s measured in the pre-treatment year (2000), indicating that the two groups of trees did not differ significantly in their water relation parameters prior to CO_2 enrichment. Furthermore, the strong linear relationship observed in 2000 between mean daily J_S of each respective set of trees also suggested that tree responses to varying environmental conditions were similar in both groups (Fig. 1).

Stem diameter did not differ significantly between the two groups of trees across all species (Table 1). However, treated *Fagus* and *Quercus* trees had smaller and larger diameter, respectively, than control trees of the same species. Irrespective of treatment, the variation in J_S parameters such as daily maximum J_S was explained mainly by differences among species rather than by the variation in stem diameter (two-factorial ANOVA, $P_{species} = 0.001$ and $P_{diameter} = 0.36$). Consequently, there were no significant differences in J_S parameters between treatment groups prior to CO_2 enrichment (Table 1), despite the differences in stem diameter for *Fagus* and *Quercus* trees.
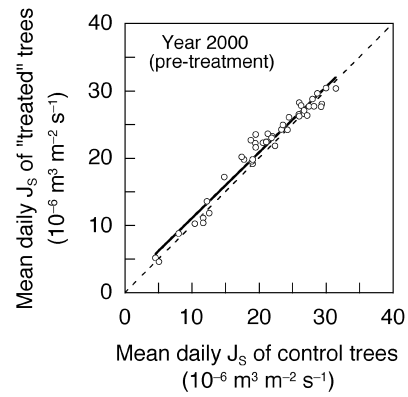

Fig. 1 Mean daily sap flux density (J_S) of trees that were later treated versus control trees in the pre-treatment year 2000. Each value represents the mean daily J_S of 18 control and 11 treated trees and was calculated by giving *Quercus*, *Fagus* and *Carpinus* each a weight of 2, and the pooled trees of *Tilia*, *Acer* and *Prunus* a weight of 1

Effect of elevated CO_2 on daily mean J_S

While control trees were showing relatively similar J_S values in both measurement years, trees that were exposed to elevated CO_2 in 2001 had slightly lower rates of sap flux in the treatment year compared to 2000 (Fig. 2). This trend was sharper at conditions of low evaporative demand (Fig. 2) and was, among the different species, most apparent in *Carpinus* and the TAP species (data not shown). When tested for each day separately, CO_2 effects on J_S were significant across all species only on a few single days (Fig. 3; 7 out of 60 days using $P < 0.05$). Differences in J_S between treatments were generally observed under conditions of low evaporative

Table 1 Pre-treatment comparison of traits from two groups of trees, of which one received CO_2 enrichment beginning in September 2000. Specific leaf area (*SLA*), light-saturated rate of photosynthesis (A_{max}), stomatal conductance at A_{max} ($g_{s\ max}$), foliar *N concentration* and C isotope abundance ($\delta^{13}C$) were measured in the upper canopy. Means, standard errors, and test results are indicated (two-factorial ANOVA, *P*-values for factor "species" are not indicated). See table note for the number of individuals per group

Tested variables	Control trees	Treated trees	$P_{treatment}$
Morphology			
Stem diameter (cm)[a]	42.4±2.5	37.2±3.1	0.220
SLA (cm^2 g^{-1})[b]	115±6	114±7	0.437
Photosynthesis, nutrition and $\delta^{13}C$			
A_{max} (µmol m^{-2} s^{-1})[b]	13.4±0.9	12.5±0.6	0.401
N concentration (% dry weight)[b]	2.7±0.1	2.7±0.1	0.373
$\delta^{13}C$ of leaves (‰)[c]	−26.8±0.2	−26.4±0.2	0.133
$\delta^{13}C$ of twigs (‰)[c]	−24.7±0.2	−24.4±0.2	0.219
Water relations			
$g_{s\ max}$ (mmol m^{-2} s^{-1})[b]	136±11	127±7	0.638
J_S (10^{-6} m^3 m^{-2} s^{-1}) on days with mean VPD <5 hPa and PFD <25 mol m^{-2} day^{-1}[a]	11.6±1.2	10.3±1.7	0.582
J_S (10^{-6} m^3 m^{-2} s^{-1}) on days with mean VPD >10 hPa and PFD >35 mol m^{-2} day^{-1}[a]	27.6±2.0	26.5±2.4	0.870
$J_{S\ max}$ (10^{-6} m^3 m^{-2} s^{-1}) on days with mean VPD >10 hPa and PFD >35 mol m^{-2} day^{-1}[a]	42.0±2.5	41.2±3.2	0.888

[a] Measurements were carried out in 2000 on 18 control trees and 11 treated trees; daily means of J_S, and VPD were calculated from 0600 to 1900 hours true local time
[b] Measurements were carried out in 2000 on 16 control trees and 11 treated trees
[c] Measurements were carried out in 1999 on the same 16 control and 11 treated trees as above
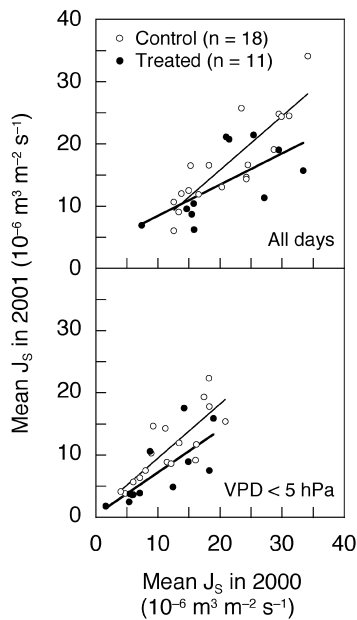
Fig. 2 Mean sap flux density (J_S) in 2000 (pre-treatment year) versus mean J_S in 2001 of 18 control trees (*open symbols*) and 11 trees receiving CO_2 treatment in 2001 (*filled symbols*). Each value represents the mean daily sap flux density of a tree averaged across all measurement days of both years and days with a mean daily (0600–1900 hours) VPD <5 hPa in each year
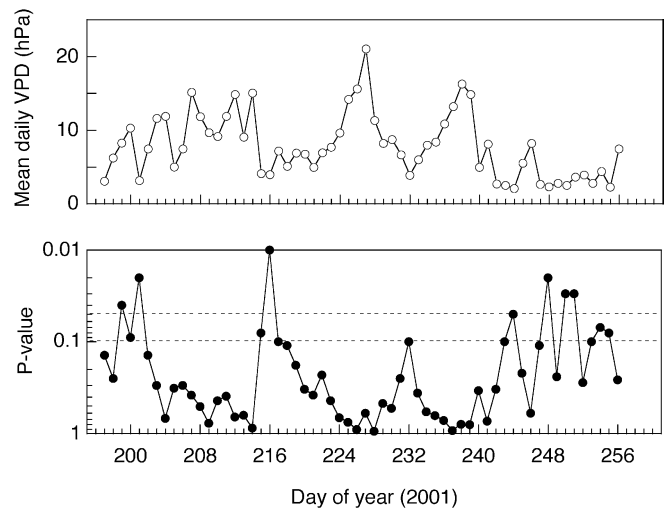

Fig. 3 Seasonal course of mean daily vapour pressure deficit (VPD, 0600–1900 hours), and P values from ANCOVA testing the effect of elevated CO_2 on daily mean J_S. Tests were computed for each day separately using pre-treatment J_S values (i.e. year 2000) as covariate, and species and treatment as fixed factors (type I sums of squares, factors in the same order as listed). P values of 0.05 and 0.10 are represented by *dashed* lines

demand (Fig. 3). This pattern of a significant treatment effect occurring at low VPD was confirmed when J_S data were stratified into three separate ranges of meteorological conditions. At low mean daily VPD (<5 hPa), we found a marginally significant reduction in mean J_S of 22% when comparing treated trees to control individuals (Table 2, $P=0.082$). Under conditions of higher mean daily VPD (>5 hPa), the reductions in mean daily J_S observed in treated trees were smaller and not statistically significant (Table 2, $P>0.129$).

To quantify the effects of elevated CO_2 on sap flux, we calculated the reduction in mean J_S of treated trees by comparing measured values of J_S and those expected had there been no CO_2 enrichment in 2001 (Table 2). The J_S of treated trees were computed for ambient CO_2 conditions (i.e. ~365 ppm, assuming there was no high CO_2) using J_S of control trees in 2001 and the relationship established between treated and control trees in the pre-treatment year (Fig. 1). Based on this approach, there was

a 7.9% reduction in mean J_S (Table 2; not significant, $P=0.304$) across all sampling dates for which there were corresponding J_S measurements in pre-treatment year 2000 (from mid-July to mid-September) and all weather conditions. When including all J_S measured during the period of CO_2 enrichment in 2001 (i.e. the entire seasonal time series), the reduction in mean J_S due to treatment effect averaged 10.7% (n.s., $P=0.189$). The same analysis performed for each species separately revealed marginally significant differences between J_S of treated and control trees in *Carpinus* only ($P=0.056$ for the whole season). However, the non-significance of test results in the other species is more difficult to interpret since the variation added by re-installation of the sensors in 2001 combined with a low number of replicates (compared to *Carpinus*) reduced the power of the analyses below 0.5.

Table 2 Reduction in mean daily sap flux density (J_S) of CO_2-enriched trees over three ranges of meteorological conditions and across the whole period of the year for which data from both growing seasons were available. The reductions were calculated by comparing measured values of J_S and those expected had there been no CO_2 enrichment in 2001. P values from tests on the effect of CO_2 enrichment are indicated (see text for details on ANCOVA)

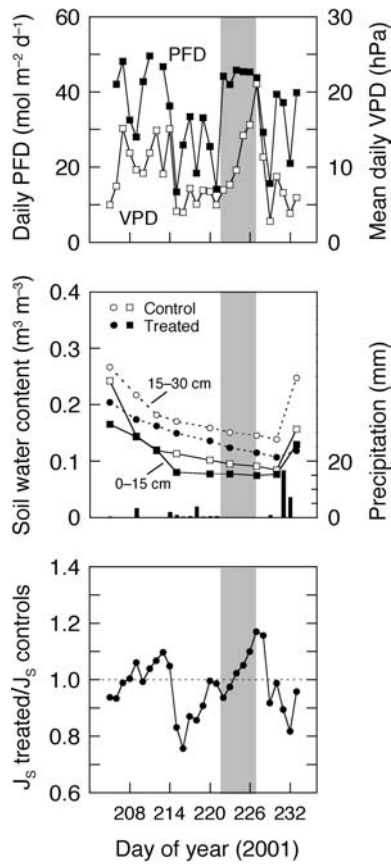| Meteorological conditions	Reduction in mean J_S	$P_{\text{treatment}}$ (ANCOVA)
Mean daily VPD <5 hPa and daily PFD <25 mol m^{-2} day^{-1}	22.1%	0.082
5< mean daily VPD <10 hPa and 25< daily PFD <35 mol m^{-2} day^{-1}	10.7%	0.129
10< mean daily VPD <15 hPa and daily PFD >35 mol m^{-2} day^{-1}	1.8%	0.553
All days mid-July to mid-September	7.9%	0.304
All days of growing season 2001	10.7%	0.189

Fig. 4 Mean daily vapour pressure deficit (VPD, 0600–1900 hours), daily photon flux density (PFD), soil water content at 0–15 cm and 15–30 cm depth, daily precipitation (*bars*), and ratio of mean daily sap flux density (J_S) of 18 control trees and 11 treated trees measured during a 4-week period in summer 2001. The *grey zones* indicate a rain-less period with continuously increasing daily VPD and high PFD

Fig. 5 Time courses of photosynthetic photon flux density (*PFD*), vapour pressure deficit (*VPD*) and normalized (relative) sap flux density (J_S) of *Tilia* trees when CO_2 enrichment was discontinued for 1.5 h near midday on 13 August 2001. The *open symbols* represent one control tree at ambient CO_2, *filled symbols* one treated tree exposed to elevated CO_2 (daily mean CO_2 concentration of 513 ppm). Values of J_S were normalized for each tree with respect to its daily maximum. The *grey zones* indicate the period when CO_2 enrichment was discontinued

Effect of elevated CO_2 during a dry period

In July/August 2001, there was a relatively dry period characterized by little precipitation and continuously decreasing soil water content, followed by 6 days with no precipitation and steadily increasing evaporative demand under high levels of solar radiation (Fig. 4; shaded area). A comparison of trees growing under elevated CO_2 and control trees showed different responses during the last 6 days of this period. Sharply rising levels of VPD over the first 3 days were paralleled by an increase in mean daily J_S of both groups of trees (data not shown). However, the changes in J_S were slightly more pronounced in trees receiving CO_2 enrichment. While the evaporative demand continued to increase over a few more days, the rates of sap flux started to decline in both groups of trees, with the reduction in J_S being greater in control trees. As a result, the ratio of mean daily J_S of treated trees to that of control trees continuously increased throughout this 6-day drying period (Fig. 4). Hence, the changes in the ratio over time reflected to some extent those in mean daily VPD.
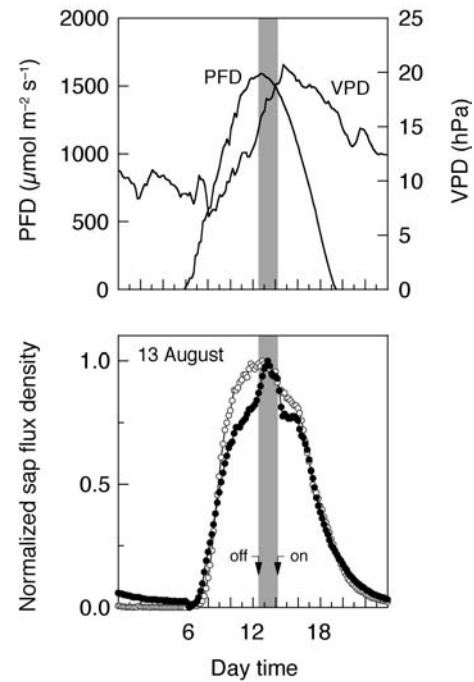
Off/on experiments

Short-term (1.5–3 h) decreases in atmospheric CO_2 concentration from ~520 ppm to ambient levels (~365 ppm) were associated with a rapid change in J_S of trees growing under elevated CO_2 which could not be observed in control trees. Soon after discontinuing the CO_2 supply, there was a sharp increase in flux rate exceeding the diurnal trend seen in controls (Fig. 5). This was followed, upon return of CO_2 concentration to the treatment level, by a rapid decline in J_S to values slightly lower than those expected based on the diurnal course depicted in controls. These changes in J_S resulted in a distinct "switch off hump" in the diurnal course of exposed trees (Fig. 5). Time lags between the changes in CO_2 concentration and the initial responses of stem sap flux were remarkably short (~15 min), given our temporal resolution of 30 s (recorded as 10-min averages) and the distance between sap flux sensors and canopy foliage (~25–30 m).

On reference days immediately before or after a switch off/on experiment, the diurnal changes in mean J_S of trees in the CO_2-enriched zone, calculated over the same time intervals as on switch off/on days, correlated very well with that of control trees ($R^2=0.94$, $P=0.002$; Fig. 6). Despite slight differences in the timing of off/on experiments and varying meteorological conditions among
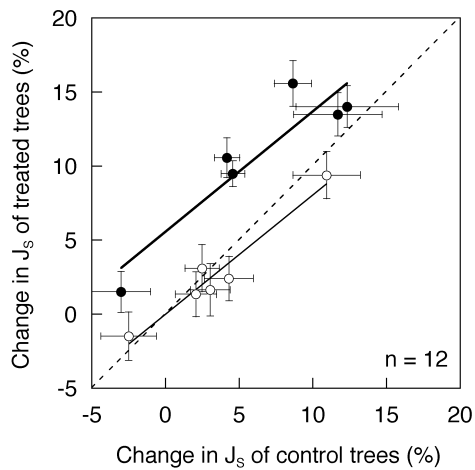
Fig. 6 Change in mean sap flux density (J_S) of 12 control and 12 treated trees during a short-term discontinuation of CO_2 enrichment. Each value represents the mean changes in J_S calculated from measurements made over a 50-min period after CO_2 enrichment was interrupted, and over a 60-min period after CO_2 enrichment was resumed. *Open symbols* are for reference days (when CO_2 enrichment was not discontinued) and *filled symbols* are for off/on days (when CO_2 supply was interrupted for 1.5–3 h). Each group included the same number of individuals from six different species. For the calculation of the mean response *Quercus*, *Fagus* and *Carpinus* were given a weight of 2, and the pooled trees of *Tilia*, *Acer* and *Prunus* a weight of 1. *Bars* indicate ± SE

reference days, the calculated relative temporal changes in J_S were similar in both control and high CO_2 trees (data points all very close to the 1:1 line; open symbols, Fig. 6). This suggests that our test parameter was appropriate to evaluate short-term responses in off/on experiments. In cases where CO_2 enrichment was discontinued for 1.5–3 h during the day, there was also a significant relationship between the changes in mean J_S of control trees (due to diurnal variation) and treated trees (due to switch off/on and diurnal variation) (R^2=0.85, P=0.008). However, there was an overall shift towards greater responses in treated trees as indicated by data points falling above the regression line for the reference days (filled symbols, Fig. 6). Although this shift was observed in all species, it was most pronounced in *Carpinus* and TAP species, smaller in *Quercus*, and weakest in *Fagus* (Fig. 7).
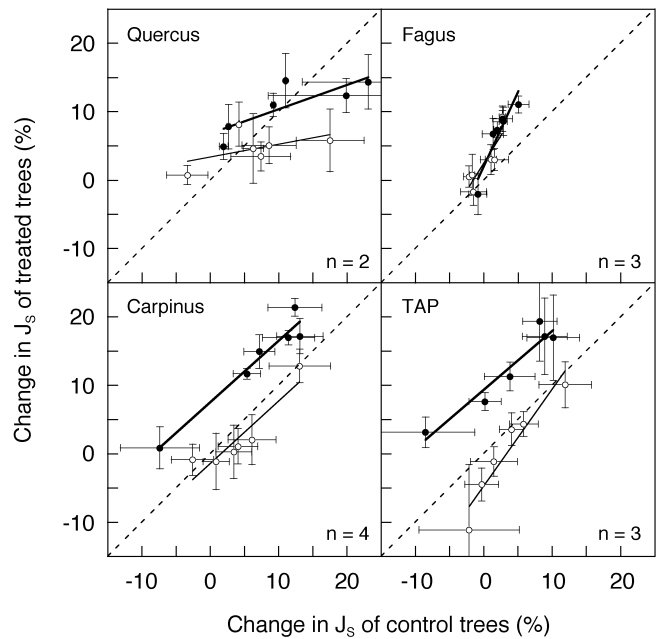

Fig. 7 Change in mean sap flux density (J_S) of different tree species during a short-term discontinuation of CO_2 enrichment. Each value represents the mean change in J_S calculated from measurements made over a 50-min period after CO_2 enrichment was interrupted, and over a 60-min period after CO_2 enrichment was resumed. *Open* and *filled symbols* are for reference days (when CO_2 enrichment was not discontinued) and off/on days (when CO_2 supply was interrupted for 1.5–3 h), respectively. *N values* indicate the number of individuals in each group of trees (in the case of the pooled species *Tilia*, *Acer* and *Prunus*, there is one individual per species and treatment group). *Bars* indicate ± SE

Changes in J_S of treated trees to be expected on off/on days (over the 1.5–3 h test period) if there had been no discontinuation of CO_2 supply were predicted using the responses of control trees and the regressions obtained on reference days (Figs. 5, 6). The short-term effects of a discontinued CO_2 enrichment on J_S were then calculated by comparing the predicted and measured responses of treated trees. Across all trees and all six off/on experiments, the discontinuation of CO_2 enrichment caused J_S to increase by 6.6% (P<0.001; Table 3). Moreover, the effect was significant on all 6 days when an off/on experiment was performed, with P values ranging from

Table 3 Effect of a short-term (1.5–3 h) discontinuation of CO_2 enrichment on sap flux density of CO_2-enriched trees. *P* values from ANCOVAs per off/on-day and across all days are shown for each species and for all trees pooled together. Mean (± SE) changes in sap flux density (ΔJ_S) during CO_2 discontinuation were calculated across all trees by giving *Quercus*, *Fagus* and *Carpinus* a weight of 2, and the pooled trees of *Tilia*, *Acer* and *Prunus* a weight of 1. Number of trees per treatment group is in parentheses

Comparison level	Day of year						All days	Mean ΔJ_S during discontinued CO_2 enrichment (%)
	172	207	213	225	235	236		
Fagus (3)	0.228	0.085	0.619	0.004	0.975	0.034	0.353	0.9±0.8
Quercus (2)	0.648	0.684	0.475	0.240	0.593	0.827	0.689	6.1±1.3
Carpinus (4)	0.096	0.009	0.130	0.024	0.041	0.084	0.010	10.4±1.0
TAP (*Tilia*, *Acer*, *Prunus* pooled, 3)	0.024	0.031	0.170	0.055	0.155	0.210	0.123	13.5±1.6
All trees (12)	<0.001	<0.001	0.028	<0.001	0.027	0.049	<0.001	6.6±1.0

<0.001 to 0.05 (Table 3). Although the magnitude of the effect varied among off/on days, the ranking of the species according to their response remained the same (*Carpinus* and TAP species were most responsive and *Fagus* least responsive; data not shown). At the species level, the increase in J_S of treated trees following an interruption of the CO_2 supply was significant on single off/on experiments in *Fagus*, *Carpinus* and TAP species (Table 3). Across all 6 switch off/on days, the increases in J_S were statistically significant only in *Carpinus* trees (Table 3). The non-significant *P* values for *Fagus* (*n*=3), *Quercus* (*n*=2) and TAP species (*n*=3) were merely due to low statistical power since significant effects of CO_2 discontinuation across all six experiments were found when any two of the above-mentioned species (or species groups) were pooled for statistical tests (*n*=5 or 6, data not shown).

Discussion

Our study indicated that CO_2 enrichment of mature deciduous trees resulted in a 2–22% reduction in J_S of treated trees compared to controls (Table 2), and that this reduction in J_S averaged 10.7% over a 4-month field season, with more pronounced effects during humid weather conditions. CO_2 effects on J_S could be documented in the very short term (hours) and over a complete growing season. However, the signal strongly depended on species. Short-term interruptions of CO_2-supply resulted in clear and immediate CO_2-response signals of the sap flux in these tall trees, similar in magnitude to those observed over the whole season.

Trees in the natural forest ecosystem under investigation differed in various parameters such as diameter, height and age. Consequently, the absolute values of J_S varied a priori in sampled trees (not significantly across all species; Table 1). Data were thus obtained prior to the treatment to account for a priori differences. Furthermore, all data analyses were based on relative changes observed in trees exposed to CO_2 enrichment versus those not receiving any treatment. Hence, our measurements of J_S in the outer 20 mm of the xylem should be representative of that occurring over the entire sapwood area, unless elevated CO_2 is significantly altering the radial pattern of xylem sap flow (an effect for which there is currently no evidence).

For the first time, the web-FACE CO_2 enrichment system permitted the exposure of entire tree canopies in a natural, mature forest to elevated CO_2. The large size of trees and dimensions of the experiment did not permit a randomization of the treatment and control units, a common statistical requirement that precludes selection bias. However, such treatment comparisons can be validated, provided there exist no (unknown) systematic effects between experimental units (Neter et al. 1996). Therefore, it is key to this analysis of tree responses to determine a priori differences which might occur between treated and control trees. The analysis of all morpholog-ical and physiological data collected prior to the start of CO_2 enrichment indicated that there were no such differences between the trees later exposed to elevated CO_2 and the trees later used as controls (Table 1). A distribution-free, simple permutation test also confirmed the absence of systematic effects between groups of trees (D. Spinnler, unpublished data), further validating the results of our experiment.

The reduction in mean daily J_S of treated trees in response to CO_2 enrichment was much greater on days with low VPD, and almost negligible on days with a high evaporative demand (Table 2). The overall CO_2 effect on J_S tended to be less during the drier mid-July to mid-September period (an average 7.9% reduction; n.s.) than that observed over the whole season (an average 10.7% reduction across all 2001 sampling dates; n.s.). This was likely due to the generally higher VPD values during the July–September period as compared to early summer and autumn (included in the whole season mean reduction).

A 10.7% decrease in seasonal J_S observed across all tree species in our study is comparable to the 13% reduction in sap velocity found over a 5-month growing season in a plantation of *Liquidambar styraciflua* exposed to free-air CO_2 enrichment (Wullschleger and Norby 2001). In the same study, Wullschleger and Norby (2001) reported significant CO_2 effects on stand transpiration only on days when mean VPD >10 hPa and mean daily radiation >400 J m^{-2} s^{-1}. However, their parallel measurements of g_s showed a general trend toward a less pronounced effect of elevated CO_2 on g_s as VPD increased (Wullschleger et al. 2002), similar to what we found with our measurements of J_S.

The contributions of the different species to the overall reduction in J_S of 10.7% across the whole season varied substantially, with *Carpinus* and TAP species showing the most pronounced reductions, whereas responses in *Quercus* and *Fagus* trees were small. On a species level, large variation in measured rates of sap flux among individual trees and low number of replicates for treated trees within each species decreased the power of statistical tests below that necessary to detect a significant CO_2 effect of 10% with reasonable probability. Re-installation of the sensors into new xylem positions in 2001 (approx. same height, but at sufficient distance from the previous year's position) introduced additional variation (year-to-year). There is also substantial variability of J_S within tree trunks, which may be caused by a patchy xylem activity around the trunk. In this study, variation within trees (i.e. variation due to the position of the sensors) accounted for ca. 30% of the total variation in mean daily J_S between trees. A spatial variation in J_S of similar magnitude and a lack of systematic pattern within the trunk have also been found in Mango trees (Lu 1997; Lu et al. 2000) and *Laurus azorica* (Jimenez et al. 1996). Given all this natural variability, it is encouraging that the pre-treatment sap flux rates of the two groups of trees yielded no significant deviation from a 1:1 relationship (Fig. 1).

Since the ratio of leaf area to sapwood area has so far not been shown to increase in response to CO_2 enrichment

(Gielen et al. 2001; Norby et al. 2001), any reduction in J_S must be associated with lower g_s (assuming no changes in VPD in the short-term). Indeed, concurrent measurements of g_s carried out on clear sunny days in 2001 indicated an overall reduction in g_s of 14% in foliage exposed to elevated CO_2 (across all species; S. Keel, unpublished data). The close correspondence between measurements of J_S and g_s suggests that the decoupling coefficient (Ω; Jarvis and McNaughton 1986) of trees at the study site is likely to be low and similar across species. This is consistent with results from a study in a 100-year-old *Fagus* plantation in Germany that estimated Ω at 0.2 (Herbst 1995), and a review by Wullschleger et al. (1998) reporting low Ω values for sites with wind speeds exceeding 2 m s^{-1} (average wind speed at our site was 2.4 m s^{-1}). Thus, tree foliage in this study can be considered as being well coupled to the atmosphere, and canopy transpiration as being controlled in large by g_s (e.g. *Fagus* and *Prunus* produce very open crowns).

A continually reduced whole-tree transpiration in CO_2-enriched trees must lead to a less depleted soil moisture, which at some point could reverse the CO_2 effect on g_s. Such water savings being advantageous under drought conditions have previously been found in grasslands (for references see Niklaus et al. 1998; Volk et al. 2000) and more recently in a scrub-oak shrubland (Hungate et al. 2002). Our measurements of soil water content were, however, spatially too variable to detect any significant effect between treatments, but a higher spatial resolution in soil moisture measurements carried out in the following season (2002) revealed a clear moisture signal in the CO_2-enriched zone (Fig. 8; t-tests: $P>0.131$ in 1999 and $P<0.001$ in 2002). Moreover, we observed during a relatively dry period with rising evaporative demand that treated and control trees did not adjust their J_S at the same rate. Such behaviour resulted in an increase in the ratio of J_S of treated trees to J_S of control trees reaching values >1 (Fig. 4). This was in contrast to values of J_S generally found to be higher in control trees throughout the 2001 growing season. Even though there were no significant differences in J_S between the two groups of trees, the constant trend of increasing J_S ratio suggests that reversal of the CO_2 effect via a soil moisture feedback might have occurred when evaporative demand was highest. Thus, water savings by CO_2-enriched trees on humid days may become at least partly used up during drier conditions, thereby reducing the whole season effect of CO_2 enrichment on sap flux.

Short-term discontinuation of CO_2 enrichment confirmed that trees exposed to elevated CO_2 transpire less than control trees, and the ranking of species' responsiveness was fully consistent with that suggested by the analysis of whole growing season J_S data and by the porometer measurements. Additionally, there was a very good correspondence between increased J_S (~6.6%) of treated trees upon a short-term return to ambient CO_2 concentrations (Table 3) and CO_2-induced reduction in mean daily J_S (~7.9%) estimated for the entire period of mid-July to mid-September (Table 2).
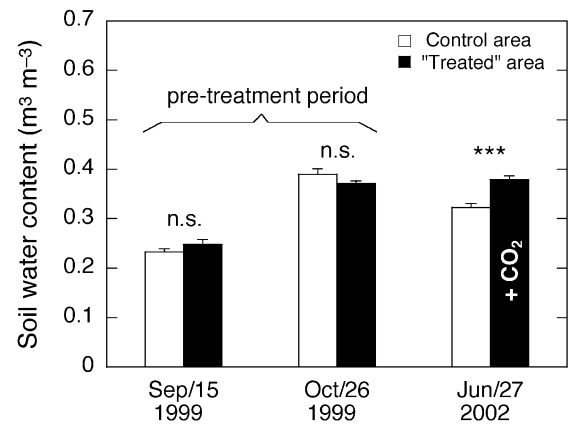

Fig. 8 Comparison of volumetric soil water content of the upper 15 cm soil layer in control area and the area where trees were later exposed to elevated CO_2. Measurements were carried out at 56 locations during fall 1999 ($n=43$ and $n=13$ in control and treated area, respectively) and at 78 locations in summer 2002 ($n=39$ in each zone) using time domain reflectometry (TRIME-FM2, IMKO, Ettlingen, Germany). *Error bars* indicate ± SE

There is very little information to compare our data with in terms of species responses to CO_2 enrichment in large trees. In our study, the least responsive species to elevated CO_2 was *Fagus sylvatica*, in full agreement with a branch-bag study that reported no significant CO_2 effect on g_s of beech trees (Dufrêne et al. 1993). Hence, our results demonstrate that the transpiration of mono-specific *Fagus* forests is likely not to be affected by elevated atmospheric CO_2. The magnitude of an effect of elevated CO_2 on stand transpiration will depend on the relative abundance of the different species, being more pronounced, for example, in stands dominated by species such as *Carpinus*. In the long term, differential changes in water-use among tree species may lead to changes in species composition, especially where drought exerts a selective pressure. There is indeed evidence that the adjustment of whole-tree transpiration varies considerably among different species (Phillips and Oren 2001). However, a species that reduces its water use under elevated CO_2 or benefits from water savings achieved by other species may become more abundant in its habitat or disperse to new ones.

Another consideration when interpreting the effects of CO_2 on plant water use, is whether the observed reductions in J_S will be compensated or enhanced by morphological adjustments in canopy density. Although currently available evidence suggests a reduction or no change in steady-state LAI or leaf area density (Körner and Arnone 1992; Hättenschwiler et al. 1997; Norby et al. 1999; Gielen et al. 2001; Norby et al. 2001; Schäfer et al. 2002), this has not yet been fully resolved for adult tree canopies. Hence, allometric adjustments in response to CO_2 enrichment, if they occur, will likely further reduce tree transpiration rather than lessen signals as shown here. An overall reduction in water vapour fluxes from forest vegetation could lead to a drier atmosphere, particularly

in continental areas, which in turn would stimulate vapour loss from trees at the landscape level (Amthor 1995). Such feedbacks would be smaller though in areas under strong influence of oceanic air masses such as our study site.

Conclusion

This study clearly documents that species presence and abundance will determine the effect of atmospheric CO_2 enrichment on the regional water balance. Our data underpin the notion that biodiversity is a key component of ecosystem responses to global change and that we have to anticipate significant influences of rising atmospheric CO_2 concentrations on hydrology via biota, irrespective of other consequences elevated CO_2 might have. That the effects of CO_2 enrichment on J_S in mature deciduous forest trees were more pronounced during moist periods, points at the possibility of enhanced runoff during high rainfall periods. On the other hand, should the reductions in sap flux translate into water savings as suggested by our results, CO_2-enriched trees would enter drier periods with a higher soil moisture capital, permitting prolonged gas exchange (for a few days), but direct (stomatal) CO_2 effects on water flux during such dry periods seem to be negligible. The degree to which direct stomatal effects (humid periods) and CO_2-induced soil moisture effects (initial part of dry periods) will affect the whole season water balance will be dictated by temporal rainfall patterns and soil properties. Overall, our data suggest greater hydrological impacts of elevated CO_2 in humid areas/periods.

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