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ORIGINAL ARTICLE

Stomatal conductance in mature deciduous forest trees exposed to elevated CO_2

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Abstract Stomatal conductance (g_s) of mature trees exposed to elevated CO_2 concentrations was examined in a diverse deciduous forest stand in NW Switzerland. Measurements of g_s were carried out on upper canopy foliage before noon, over four growing seasons, including an exceptionally dry summer (2003). Across all species reductions in stomatal conductance were smaller than 25% most likely around 10%, with much variation among species and trees. Given the large heterogeneity in light conditions within a tree crown, this signal was not statistically significant, but the responses within species were surprisingly consistent throughout the study period. Except during a severe drought, stomatal conductance was always lower in trees of *Carpinus betulus* exposed to elevated CO_2 compared to *Carpinus* trees in ambient air, but the difference was only statistically significant on 2 out of 15 days. In contrast, stomatal responses in *Fagus sylvatica* and *Quercus petraea* varied around zero with no consistent trend in relation to CO_2 treatment. During the 2003 drought in the third treatment year, the CO_2 effect became reversed in *Carpinus*, resulting in higher g_s in trees exposed to elevated CO_2 compared to control trees, most likely due to better water supply because of the previous soil water savings. This was supported by less negative predawn leaf water potential in CO_2 enriched *Carpinus* trees, indicating an improved water status. These findings illustrate (1) smaller than expected CO_2-effects on stomata of mature deciduous forest trees, and (2) the possibility of soil moisture feedback on canopy water relations under elevated CO_2.

Keywords Biodiversity · Drought · Global change · Water relations · Temperate forest

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Introduction

Reduction of stomatal aperture is a common response of plants exposed to elevated CO_2 concentrations (Morison and Gifford 1984). In canopies well-coupled to the atmosphere, such reductions in stomatal conductance (g_s) result in a corresponding decrease in leaf transpiration. Hence, a large number of studies have been carried out during the last few decades to determine the effect of rising atmospheric CO_2 on g_s and water consumption of dominant forest tree species (for reviews see Curtis and Wang 1998; Saxe et al. 1998; Medlyn et al. 2001).

Most of the work examining stomatal responses to CO_2 in tree species has been confined to seedlings and saplings, with little research on mature forest trees. These experiments have demonstrated that g_s in young woody individual trees is generally reduced in response to elevated CO_2 (mean reduction of 21%, Medlyn et al. 2001). This has led to the prediction that water use in most forest trees will be reduced as the CO_2 concentration in the atmosphere increases. Similarly to herbaceous species, the stomatal responses of saplings exposed to elevated CO_2 were found to vary among species

and to be less pronounced under environmental conditions that reduce maximum g_s (e.g., drought, high temperature, high evaporative demand; Heath 1998; Wullschleger et al. 2002). Furthermore, there is now solid evidence that plant responses to CO_2 may change with experimental conditions such as the duration of exposure and plant age (Medlyn et al. 2001), soil characteristics (Bucher-Wallin et al. 2000) and biotic interactions (Körner 2002). Conifer species seem to make an exception, as stomata appear less responsive to CO_2 enrichment in this group of species (Teskey 1995; Ellsworth 1999). For all these reasons it is unrealistic to predict the long-term water relation responses of mixed forests to rising CO_2 concentrations from data obtained in saplings of certain species grown in artificial substrates for a short period of time. These uncertainties have led to a growing consensus that forest tree research must be carried out on mature individuals under natural forest conditions (Körner 1995).

Nevertheless, exploring the effects of elevated CO_2 on water relations of tall forest trees has always presented a considerable challenge given the large size and complex structure of their canopy. Hence, investigations conducted on trees growing near natural CO_2 springs (Jones et al. 1995; Tognetti et al. 1998, 1999) and experiments performed with branch-bags (Dufrêne et al. 1993; Teskey 1995; Roberntz and Stockfors 1998) have provided most of the data currently available on mature trees. Although branch-bags offer a useful alternative to growth chambers for the investigation of mature trees, there is little, if any, possibility of feedback effects from the soil because only a small portion of the whole crown is exposed to elevated CO_2. Based on sap flow measurements carried out in a mature forest in NW Switzerland, Cech et al. (2003) presented evidence that tree responses to elevated CO_2 may be influenced by soil moisture feedback. They reported that during a relatively dry period, CO_2-enriched trees showed increased sap flow density compared to control trees. This reverse CO_2-effect, where an increased rather than a reduced transpirational flux (and hence, g_s) was observed in response to elevated CO_2, suggests that daily water savings by CO_2-enriched trees may have contributed to an improved water status by the time when control trees fell short in soil moisture. These results highlight the importance of large-scale observations, in which coupled plant–soil systems are studied (Wullschleger et al. 2002).

Using free-air CO_2 enrichment (FACE) technology, major advances have been made towards a large-scale approach of studying forest trees under more realistic growth conditions (Ellsworth 1999; Wullschleger et al. 2002; Herrick et al. 2004). Yet, for reasons of practicability, long-term field CO_2 experiments with trees have mainly been conducted in relatively young (10–20 years) forest plantations with no (or little) natural interspecific competition for light and water. To our knowledge, no large-scale investigation has examined the effect of CO_2 enrichment on a natural, diverse forest. However, a study conducted on *Liquidambar styraciflua* L. trees emerging through gaps in the Duke *Pinus taeda* L. forest provides support to the significance of species identity (Herrick et al. 2004). While the stomata of *P. taeda* showed no significant CO_2 response (Ellsworth 1999), those of *L. styraciflua* do showed the response. Given the evidence that stomatal responses to elevated CO_2 are species-specific, it is essential to account for tree species diversity when investigating the responses of whole forest stands to elevated CO_2.

In this paper, we present stomatal data from the first FACE experiment exposing the canopy of mature broad-leaved trees from six different species in a natural temperate forest ecosystem to elevated CO_2 concentrations (ca. 540 ppm). Stomatal conductance was measured in all species on 11 days over the first full growing season of CO_2 exposure to test the hypothesis that stomatal aperture is reduced under elevated CO_2 and to determine whether CO_2 responses are species-specific. Further measurements were carried out during three additional growing seasons in the three dominant species only. Our main objective was to provide realistic experimental data on g_s of adult deciduous trees exposed to CO_2 enrichment for model-based predictions on the water consumption of forests in a high CO_2 atmosphere.

Material and methods

Site description

The experiment was conducted in a diverse forest stand located 15 km south of Basel, Switzerland (47°28′N, 7°30′E; elevation: 550 m a.s.l.). The forest is approximately 100 years old with canopy tree heights between 30 and 35 m. The stand has a stem density of 415 trees ha^{-1} (diameter $\geq$ 10 cm), a total basal area of 46 m^2 ha^{-1} and a leaf area index of approximately 5 in the experimental area. It is dominated by *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.) outside the CO_2-enriched area. Among the species included in the experiment, *Fagus* and *Quercus* contribute 24 and 18%, respectively, to the total basal area under the crane, whereas the other four species contribute less than 6%.

The climate is a typical humid temperate zone climate, characterized by mild winters and moderately warm summers. During the 4 years study (2001–2003, 2005), the growing season of deciduous trees lasted from the end of April to the end of October (ca. 180 days). Mean January and July temperatures are 2.1 and 19.1°C. Total annual precipitation

for the region averages 990 mm, of which two-thirds fall during the growing season. The soil is a silty-loamy rendzina and is characterized by a 15 cm deep rock-free topsoil and a 15–30 cm deep rocky subsoil (approximately 40% of the subsoil volume are stones) underlain by fragmented limestone bedrock. In the upper 10 cm, the soil has a pH of 5.8 (measured in distilled water extracts).

CO_2 enrichment system (web-FACE)

A 45-m freestanding tower crane equipped with a 30-m jib and a working gondola provided access to 62 dominant trees in an area of about 3000 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 roughly 550 m^2 were selected for CO_2 enrichment, whereof one slim individual of *Quercus* died. Control trees (3 *Fagus*, 2 *Quercus*, 2 *Carpinus*, 2 *Tilia*, 2 *Acer* and 1 *Prunus*) were located in the remaining crane area at sufficient distance from the CO_2 release zone (mainly in the SW area of the plot). CO_2-enrichment of the forest canopy was achieved by a free-air, pure CO_2 release system that consisted of a web of 4 mm plastic tubes (approximately 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. For a more detailed description, see Pepin and Körner (2002).

Stomatal conductance and meteorological measurements

Stomatal conductance to water vapour (g_s, mmol m^{-2} s^{-1}) was measured on upper canopy foliage of 13 trees in elevated CO_2 (ca. 540 ppm) and 12 trees in ambient CO_2 (ca. 375 ppm) during 11 sunny days in the summer of 2001 (12 June–25 August). In the subsequent years, measurements were restricted to *Fagus*, *Quercus*, and *Carpinus* (26 June, 8 July and 14 August 2002; 24 June, 22 July [all six species measured], 22 August 2003; 18 August 2005). Measurements of g_s were carried out in the morning (8:00–12:00) on three fully sunlit leaves per tree (1–4 trees per treatment and species) using a transient state diffusion porometer (AP4, Delta-T Devices, Cambridge, UK). The sampling procedure on each measurement day was designed to compare treatments under relatively similar weather conditions. Hence, a tree was randomly selected first, and then an individual of the same species but opposite treatment was randomly chosen. This procedure was subsequently extended to the other trees. In the drought summer 2003 (14 August, 20 August) predawn leaf water potential was measured with a pressure chamber (SKPM 1400,Skye Instruments, Powys, U.K.) from the canopy crane gondola on the same species where stomatal conductance was measured.

Occasional parallel studies with a steady-state photosynthesis system (LI-6400, Li-Cor, Lincoln, NE, USA) recalled consistently lower leaf conductances, a difference for which we found no explanation and which we consider intrinsic to the two devices. Since the LI-6400 system is based on measurements of mass flow and gas concentrations, whereas the AP4's conductance data rely on an indirect calibration procedure with pore plates and does not ventilate leaves, we rather trust the absolute LI-6400 readings. A comparison of g_s values by Körner et al. (1979) and results from as study on non-ventilated porometers by Verhoef (1997) point in the same direction. Concurrent measurements performed with the AP4 porometer and the LI-6400 gas exchange system under different environmental conditions indicated that readings from both instruments are linearly related ($g_{s(LI-6400)} = 0.623 \times g_{s(AP4)} - 2.09, R^2 = 0.972$). Hence, the difference is systematic and the AP4 produces signals proportional to the LI-6400. Subsequent measurements of g_s were, nonetheless, carried out with the AP4 porometer for its far better suitability for such a canopy survey, in which much of the 'true' precision comes from good coverage of the natural variability. Such canopy coverage requires rapid measurement in many leaves across all trees in a daily course. Perhaps even more importantly, the AP4 readings are so fast that they capture the momentary stomatal status in a leaf, whereas the time it takes to achieve readings with the LI-6400 will incur stomatal responses to conditions in the cuvette. Since we are exploring treatment differences rather than absolute values for their own sake, any systematic error would not affect our analysis.

Environmental data

Wind speed, photon flux density, rainfall, air temperature and relative humidity were measured above the tree canopy using a weather station located at the top of the crane (anemometer AN1, quantum sensor QS, tipping bucket rain gauge RG1, shielded temperature and relative humidity probe RHA 1, 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 was measured using time domain reflectometry (TDR). Six point probes were buried at approximately 10 cm depth, two of which in the CO_2 enriched area and four in the surrounding control area (ML2x, Delta-T, Cambridge, UK). Three additional probes were installed in 2004 and all probes were recalibrated. In addition, we used three profile probes to determine moisture content between 0 and 90 cm depth which provided some indication of relative moisture trends in the sub soil (MP-917 and probes PRB-F, Environmental Sensor Inc., Victoria, BC, Canada).

Data processing and statistical analysis

We analysed mean g_s per tree using a repeated measures analysis of variance (RM-ANOVA) with species and CO_2 treatment as fixed factor effects (type I sums of squares, factors in the same order as listed) and time (measurement day or year) as the repeated factor. Our tree sample consisted of 13 treated trees and 12 controls. The unreplicated species *Tilia*, *Acer* and *Prunus* were pooled and treated as 'other' species, hereafter referred to as 'TAP'. To test the effect of elevated CO_2 on g_s of all six species, a repeated measures ANOVA was carried out for the first year only (2001; in the following years, measurements have been performed on the three dominant species only). To determine whether CO_2-effects on g_s differed among years, a subsample of the three main species (*Carpinus*, *Fagus*, and *Quercus*) which were measured throughout the 4 years study was analyzed using averages for each tree and year. Furthermore, a RM-ANOVA was computed for each species separately. In the case of dominant species, a seasonal average was calculated for each tree and year, and analysed by a repeated measures ANOVA with treatment as a fixed factor and year as a repeated factor. A similar RM-ANOVA was also carried out for each species and year separately using measurement day as a repeated factor. Additionally, one-way ANOVAs with CO_2 treatment as a fixed factor effect were performed for each species and day separately and for all trees together. CO_2-induced reductions in stomatal conductance (in % of g_s under ambient conditions) were calculated for each species and year. A weighted average reduction was calculated for the years with no exceptional weather conditions (2001, 2002, and 2005) giving the first year a weight of 11/15, the second year a weight of 3/15 and the fifth year a weight of 1/15 based on the different number of measurement days (Fig. 2 "All"). In the case of 'TAP', the overall mean is identical with the first year mean, since no additional data were obtained in subsequent years with no exceptional weather conditions. All statistical analyses were computed using *R* version 2.0.1 with a level of significance of $P < 0.05$.

Results

Weather conditions

The years 2001, 2002 and 2005 were characterized by average weather conditions (Table 1), with the exception of a relatively dry period in August 2001 (Fig. 1). In the first 3 years there were only two TDR probes which produced inconsistent differences between the CO_2 enriched area and the control area, most likely due to the large spatial heterogeneity of soils. After reinstallation and calibration of the probes in 2004, soil moisture was slightly and consistently higher in the CO_2 enriched area. In the summer of 2003, central Europe experienced a severe drought with precipitation less than half of normal and air temperatures 2–4°C higher than the 10-year average (1989–1999). Towards the end of June, soil water content dropped to approximately 10% (no plant available moisture) in the top 30 cm measured at our study site and remained at this level throughout July and August (Fig. 1). Similarly low readings were recorded at 60–90 depth with the profile TDR probes during the peak of the drought in August. Hence, during this period, soils were desiccated down to 90 cm depth of the profile and trees depended on deeper moisture reserves (no ground water table on these slopes).

Table 1 Mean air temperature (T), and vapour pressure deficit (VPD) for each measurement day (8:00–12:00)[a]

Date	T (°C)	VPD (hPa)
12 June 01	12.2	5.1
13 June 01	15.6	6.9
21 June 01	18.5	11.0
26 June 01	21.5	12.1
27 June 01	23.0	12.9
4 July 01	19.0	9.1
26 July 01	21.3	9.5
28 July 01	22.3	7.2
31 July 01	–	–
12 August 01	14.7	5.8
25 August 01	22.2	7.8
26 June 02	18.1	7.6
8 July 02	20.8	8.9
14 August 02	16.8	5.7
24 June 03	23.4	12.6
22 July 03	22.0	10.4
22 August 03	20.5	12.2
18 August 05	18.5	2.8

[a]Due to instrument failure data of 31 July 2001 are missing.

Stomatal conductance

There was, over all six species, a tendency towards lower stomatal conductance in trees exposed to elevated CO_2 compared to trees under ambient CO_2 conditions (-10%, Table 2, Fig. 2). Conductances differed significantly among species and between measurement days leading to a lot of noise in the data set. Furthermore, a significant species $\times$ day effect indicated that different species responded differently to changing weather conditions, which added to the observed variation. Although we found species-specific reductions in g_s ranging from -4% in *Quercus* to -21% in *Carpinus*, the species $\times$ CO_2-treatment factor was clearly not significant (Table 2). Only on one single day in 2001 all species showed a slightly reduced g_s in elevated CO_2 (Fig. 2). To eliminate the large differences in g_s among species, the data were standardized with respect to the maximum daily aver-

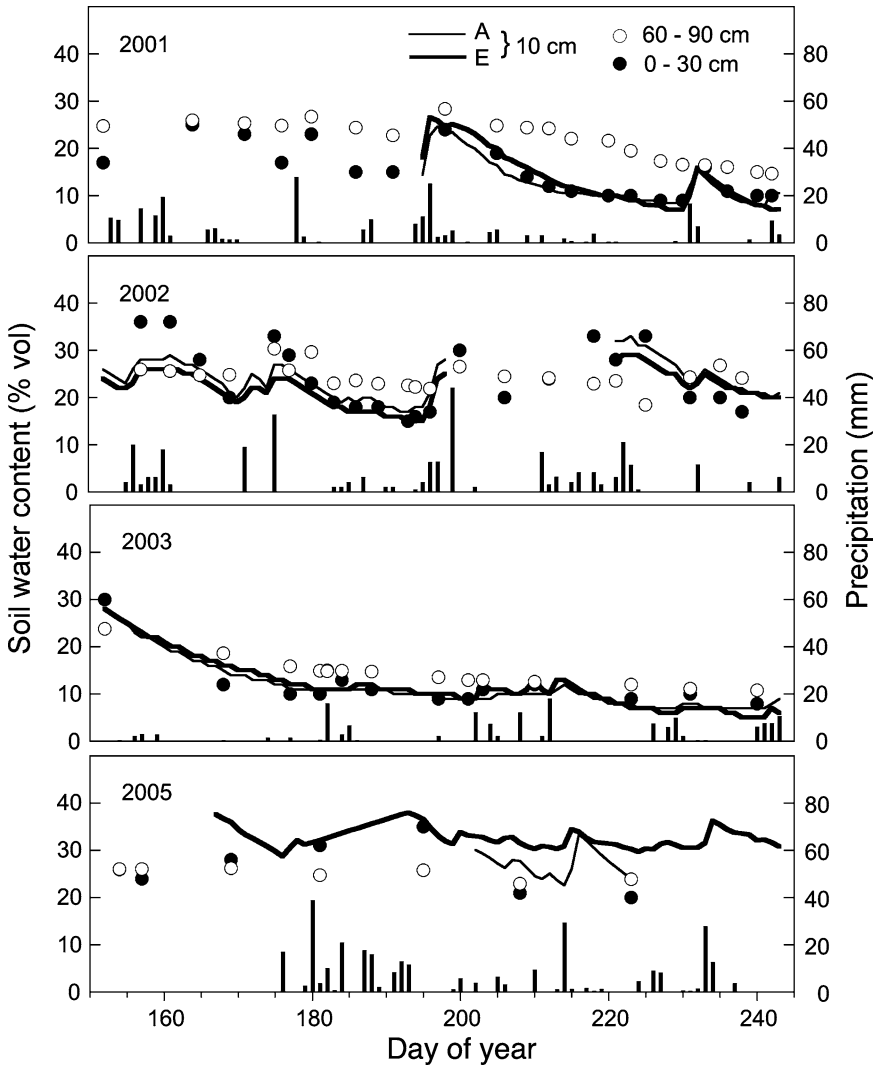

Fig. 1 Soil water content at 10 cm depth in the area exposed to ambient CO_2 (A, thin line, $n = 2$) and elevated CO_2 (E, thick line, $n = 4$–7), at 60–90 cm depth (*open symbols*) and 0–30 cm depth (*closed symbols*) both in the ambient CO_2 area ($n = 3$) and precipitation (*bars*) during four growing seasons.

age g_s of each tree. This, however, did not lead to a significant CO_2 effect either. We calculated the reduction of g_s which could still be detected with the given variation using a power *t*-test with a significance level of 0.05 and a power of 0.8. A one-sided power *t*-test was used since we did not expect an increase in g_s in response to elevated CO_2 under regular weather condition. The power test revealed that given the observed variation, a reduction of 25% in stomatal conductance in CO_2-enriched trees would be detectable across all species.

The dominant tree species (*Carpinus*, *Fagus*, and *Quercus*) were sampled over all four study years to examine whether stomatal responses to elevated CO_2 persisted in the long term. Results were very similar to those obtained in the first year when all six tree species were considered. There were no significant CO_2-effects on g_s, but significant differences in g_s among species and years (Table 3), the latter being driven by the lower conductances measured in 2003 during the exceptional drought. These differences in g_s between years were no longer statistically significant after exclusion of the drought year's data.

Table 2 Results of repeated measures ANOVA for stomatal conductance of six tree species (*Carpinus*, *Fagus*, *Quercus*, 'TAP' species (*Tilia*, *Acer*, *Prunus*)) exposed to elevated CO_2 over 11 measurement days from June to August 2001

Factor	df	*F*	*P*
Species	3	3.8	0.03
CO_2	1	2.4	0.14
Species × CO_2	3	0.2	0.91
Day	10	12.1	< 0.001
Species × Day	30	3.0	< 0.001
CO_2 × Day	10	1.0	0.47
Species × CO_2 × Day	30	0.5	0.98

Among species, *Carpinus* showed the largest and most consistent reduction in stomatal conductance in response to CO_2 enrichment which however was only statistically significant on two single days in the first experimental year

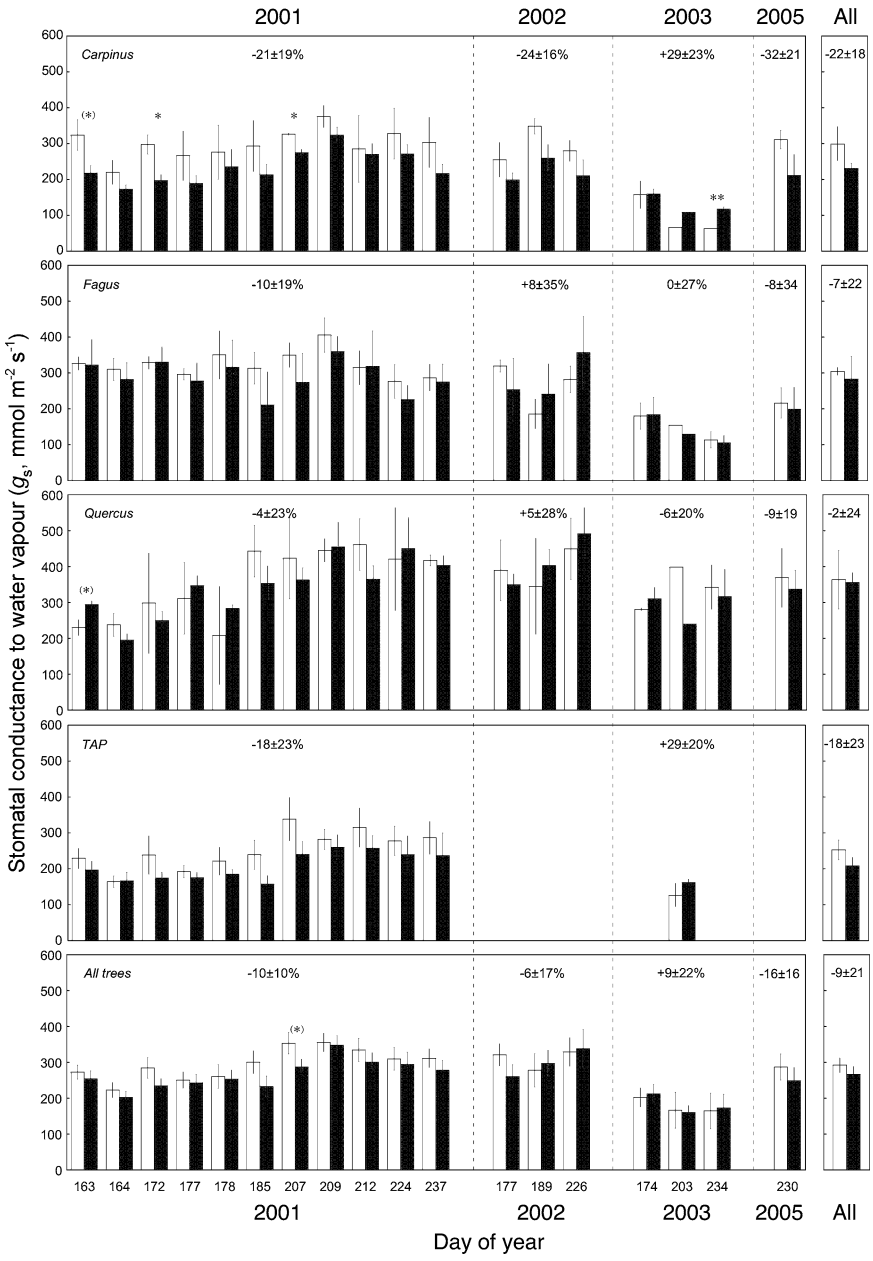

Fig. 2 Stomatal conductance (g_s, mean ± SE) of upper canopy foliage in six deciduous tree species ($n = 12$ trees) exposed to ambient (A, *open bars*; ca. 375 ppm) and elevated CO_2 (E, *dark bars*; ca. 540 ppm) during four growing seasons (8:00–12:00). 'TAP' refers to *Tilia, Acer*, and *Prunus*, three species that were pooled because they were not replicated (E, $n = 3$; A, $n = 5$). The 'All' column refers to average g_s for 3 years with no exceptional weather conditions (2001, 2002, 2005) weighted by measurement days per year. In the bottom panel, 'All trees' refers to six species in the year 2001 and on day 203 in 2003 (E, $n = 13$; A, $n = 12$) and the three main species (*Carpinus*, *Fagus*, *Quercus*) in 2002, 2003 and 2005 (E, $n = 10$; A, $n = 7$). Percent numbers represent mean CO_2 signals ± SE across season. The summer of 2003 was exceptionally dry. Note, the AP4 porometer produces somewhat high absolute g_s values, which however, does not affect differences between CO_2 treatments (see Material and Methods).$^{(*)}P < 0.1$; $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$

(Fig. 2). Over the four year period the CO_2 effect was not significant (Table 4). Test results were also not significant when considering the growing seasons with no extraordinary weather conditions only or each year separately. For the 'TAP' species, there were no significant differences between treatments (test results not shown). *Fagus* and *Quercus* showed very small (to negligible) and inconsistent stomatal responses (Fig. 2).

Stomatal responses to elevated CO_2 during a severe drought

During a prolonged drought period in the summer of 2003 (Fig. 1), g_s of CO_2-enriched trees and control trees were strongly reduced in most species compared to previous years (Fig. 2). There was, however, considerable variability in stomatal responses to drought among tree species. Mean seasonal g_s at ambient CO_2 during the drought year were only slightly reduced in *Quercus* (6%) compared to 2001, whereas in *Fagus*, the 'TAP' species and *Carpinus*, moderate to pronounced reductions were found (52–68%; see 'Year'-effect in Table 4).

Towards the end of this severe drought, we observed significantly higher conductances in CO_2-enriched *Carpinus* trees compared to control trees ($P = 0.004$, Fig. 2). A trend towards higher g_s values under elevated CO_2 was also observed in the 'TAP' species. At the same time, predawn leaf water potentials tended to be higher in CO_2-enriched *Carpinus* trees (-1.14 MPa in ambient CO_2 vs. -0.91 MPa in

Table 3 Results of repeated measures ANOVA for stomatal conductance of trees exposed to elevated CO_2 of three species (*Carpinus*, *Fagus*, and *Quercus*) during four growing seasons (2001, 2002, 2003, 2005)

Factor	df	*F*	*P*
Species	2	6.71	0.014
CO_2	1	0.50	0.49
Species × CO_2	2	0.13	0.88
Year	3	18.38	< 0.001
Species × Year	6	2.07	0.09
CO_2 × Year	3	0.93	0.44
Species × CO_2 × Year	6	0.64	0.70

Table 4 Results of repeated measures ANOVA for stomatal conductance of three species exposed to elevated CO_2 over four growing season (2001, 2002, 2003, 2005)

		Carpinus		*Fagus*		*Quercus*	
Factor	df	*F*	*P*	*F*	*P*	*F*	*P*
CO_2	1	3.11	0.18	0.02	0.91	0.02	0.89
Year	3	7.77	0.005	12.7	0.0005	2.73	0.11
CO_2 × Year	3	1.58	0.25	0.34	0.80	0.20	0.90

elevated CO_2, $P = 0.053$) but not in trees belonging to the 'TAP' species). In *Fagus* and *Quercus*, g_s was not altered by CO_2 enrichment under these dry soil conditions, in line with the data for other years with no extraordinary weather conditions when no significant CO_2 effect was detected on g_s.

Discussion

This study documents responses of stomatal conductance in a diverse natural forest to elevated CO_2. The 4 year data reveal that reductions in conductance in response to 540 ppm CO_2 are certainly smaller than 25%. Despite the large number of readings and 4 years of data collection, we were unable to demonstrate the occurrence of a statistically significant reduction of stomatal conductance and only found a tendency towards reduced g_s of around 10% when averaged across all species and trees. As can be seen in Fig. 2 *Carpinus* and the 'TAP' species (*Tilia*, *Acer* and *Prunus*) consistently show more pronounced reductions of g_s compared to other species which is in full agreement with sap flow data from the same trees in 2001 (Cech et al. 2003).

The size of the responses seen in *Carpinus* and the 'TAP' species over the 4 years, though not significant, are well in line with the mean 21% reduction reported by Medlyn et al. (2001) using a meta analysis for long-term experiments carried out in the field and are also consistent with measurements carried out on *Liquidambar styraciflua* in two FACE experiments (− 24%, Gunderson et al. 2002; − 28%, Herrick et al. 2004). The responses in *Fagus* and *Quercus* were much smaller and less uniform, hence CO_2 effects at the leaf level are much less likely even if we had more trees. In line with our findings Dufrêne et al. (1993), using branchbags in mature *Fagus*, also found no CO_2-effect on g_s in this most common European deciduous tree species. A number of full grown tree species, the noteworthy conifers, also have shown no stomatal response to elevated CO_2 (Teskey 1995; Ellsworth 1999).

Our data therefore support that stomatal responses to elevated CO_2 are most likely species-specific. The presence or absence of a certain species in a catchment would thus have hydrological consequences. Our data illustrate the risk of drawing general conclusions from a single species' response. In this sense, our results match studies conducted on saplings and potted trees, which also reported great species specificity in stomatal responses to CO_2 (Picon et al. 1996; Heath 1998).

The study of g_s in this natural, highly diverse, mature forest exposed to elevated CO_2 suffered from the common experimental and analytical difficulties when realistic test conditions come into play (Körner 2001). To comply with the height requirement of the study trees (between 30 and 35 m) and the complex structure of the canopy, a new CO_2 enrichment system was designed (web-FACE, Pepin and Körner 2002). However, the need of a canopy crane did not permit randomization of the treatment units (it would require several cranes) and therefore, we employed a detailed investigation of *a priori* differences in physiology and morphology between control trees and those later exposed to elevate CO_2. The analysis performed by Cech et al. (2003) revealed no systematic differences between the two groups of trees, hence the prerequisites for the experiment were fulfilled. Given the stature of the forest, the large size of the CO_2-enriched area exerted a further constraint, namely that not all species could be measured at the same time despite rapid crane operation. On the other hand, the area was still not large enough to permit replication in both treatments to a desirable extent. One way to handle this was to consider the CO_2 response of 'trees' only, irrespective of species (13 treated trees and 12 control trees). We performed such tests, but these revealed no significant CO_2 effect either, possibly due to the large variation in absolute g_s among species (at full stomatal opening g_s in *Quercus* is roughly 2 times that of the other species).

Despite these inevitable problems, it is still remarkable that the trend towards reduced stomatal conductance in these deciduous tree species exposed to elevated CO_2 was sustained throughout the summers of 2001, 2002 and 2005 (with no exceptional weather conditions as opposed to 2003). This is in agreement with earlier studies which have shown small but consistent responses in g_s of *Liquidambar styraciflua* trees over 3–4 years of CO_2 enrichment (Gunderson et al. 2002; Herrick et al. 2004). Bearing in mind that trees in this study are of considerable age, height, and size, the consistency of the responses over several years (particularly in

Carpinus) leads to our confidence in the data, although differences were rarely statistically significant. Very good correspondence between these leaf level stomatal conductance data and sap flow density carried out on the same trees in 2001 adds to this confidence. Reductions in mean daily sap flow density of CO_2-enriched trees averaged approximately 11% across all days of the growing season in 2001 (Cech et al. 2003), a value close to the non significant overall 10% reduction in g_s (mean of all six tree species) reported here for the first year, although the stomatal signal does not include a boundary layer component. Furthermore, the same species were found to be more responsive to elevated CO_2 (*Carpinus* and the 'TAP' species).

In plant systems with high stomatal control over transpiration, CO_2-induced reductions in g_s can lead to a decrease in water consumption and result in higher soil moisture content (Hungate et al. 2002), which was also found at our study site (Leuzinger 2006). During a period of relatively high evaporative demand and decreasing soil water content in 2001 summer, Cech et al. (2003) observed greater sap flow density in CO_2-enriched trees than in control trees. Although the differences in sap flow density between the two groups of trees were not statistically significant, treatment differences increased over time, providing support to the hypothesis that soil moisture savings in the CO_2-enriched area could reverse the effect of elevated CO_2 on stomatal conductance and transpiration. More recent soil moisture data from our site confirm this pattern of a reverse effect of soil drying during prolonged rainless periods (Leuzinger 2006). Based on these findings we expected similar soil moisture feedback to appear during the drought conditions of summer 2003 and indeed, we did observe such a reversal of CO_2 effects on g_s of *Carpinus* and a tendency in this direction in the 'TAP' species. Less negative predawn leaf water potentials in *Carpinus* under elevated CO_2 and drought adds a piece of evidence that water status in this species was improved compared to control trees and indicates that this species was more sensitive to CO_2 enrichment than the other study species.

These results suggest that small, CO_2-induced decreases in g_s at the leaf level which are hardly measurable, are sufficient to translate into a cumulative soil water enrichment in the area exposed to elevated CO_2. There are insufficient data for the rocky subsoil to verify this for roots at greater depth, but a higher predawn leaf water potential under elevated CO_2 and drought suggests water savings throughout all rooted soil horizons (Leuzinger et al. 2005). During the summer drought of 2003, these water savings must have occurred at much deeper soil layers, because soil moisture dropped to 10% at 60–90 cm depth (corresponding to air dry soil moisture). Manual TDR readings at high spatial frequency in 2002 confirmed that soil water content at approximately 10 cm was significantly increased in the CO_2-enriched area (Cech et al. 2003). Yet, during the extreme summer drought, this method was not applicable since the top soil was completely dry.

During the severe drought of summer 2003, g_s decreased considerably in all investigated species, with the exception of *Quercus* (a deep rooted, drought-tolerant genus which might have access to soil moisture at greater depth, Becker 1990; Epron and Dreyer 1993; Leuschner et al. 2001). Again, the results matched sap flow measurements showing nearly constant sap flow density in *Quercus* throughout the summer months, whereas in *Carpinus* and *Fagus* sap flow density decreased to half of the early summer maxima (Leuzinger et al. 2005).

In conclusion, we showed that stomatal responses to elevated CO_2 in these mature forest trees are certainly smaller than 25%, most likely in the range of about 10% across species. Globally, even small CO_2-driven reductions in stomatal conductance could have a significant impact on the water balance. Using gas exchange theory only, Gedney et al. (2006) speculated that such trends influenced run-off in the twentieth century in a continental specific way. But based on our and other field data (those for conifers in particular) and accounting for atmospheric feedback, we believe that theory based signals are likely to overestimate actual effects substantially. However in the long run, such species specific differences may lead to a change in species abundance driven by soil moisture (and nutrient) effects. Our results clearly demonstrate the need to account for biodiversity and both soil moisture and atmospheric humidity feedback on CO_2 responses of stomata in order to arrive at a realistic picture of the hydrological and biological consequences of ongoing atmospheric CO_2-enrichment.

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