Planta (2010) 232:1115–1125
DOI 10.1007/s00425-010-1240-8

ORIGINAL ARTICLE

Sustained enhancement of photosynthesis in mature deciduous forest trees after 8 years of free air CO_2 enrichment

Martin Karl-Friedrich Bader · Rolf Siegwolf · Christian Körner

Received: 12 June 2010 / Accepted: 22 July 2010 / Published online: 11 August 2010

Abstract Carbon uptake by forests constitutes half of the planet's terrestrial net primary production; therefore, photosynthetic responses of trees to rising atmospheric CO_2 are critical to understanding the future global carbon cycle. At the Swiss Canopy Crane, we investigated gas exchange characteristics and leaf traits in five deciduous tree species during their eighth growing season under free air carbon dioxide enrichment in a 35-m tall, ca. 100-year-old mixed forest. Net photosynthesis of upper-canopy foliage was 48% (July) and 42% (September) higher in CO_2-enriched trees and showed no sign of down-regulation. Elevated CO_2 had no effect on carboxylation efficiency (V_{cmax}) or maximal electron transport (J_{max}) driving ribulose-1, 5-bisphosphate (RuBP) regeneration. CO_2 enrichment improved nitrogen use efficiency, but did not affect leaf nitrogen (N) concentration, leaf thickness or specific leaf area except for one species. Non-structural carbohydrates accumulated more strongly in leaves grown under elevated CO_2 (largely driven by *Quercus*). Because leaf area index did not change, the CO_2-driven stimulation of photosynthesis in these trees may persist in the upper canopy under future atmospheric CO_2 concentrations without reductions in photosynthetic capacity. However, given the lack of growth stimulation, the fate of the additionally assimilated carbon remains uncertain.

Keywords Elevated CO_2 · Global change · Photosynthetic acclimation · Swiss Canopy Crane

Abbreviations

A_{growth}	Light-saturated net photosynthesis measured at growth CO_2 concentration (ambient CO_2: A^a_{growth}; elevated CO_2: A^e_{growth})
A_{550}	Light-saturated net photosynthesis measured at 550 ppm leaf chamber CO_2 concentration
A_{380}	Light-saturated net photosynthesis measured at 380 ppm leaf chamber CO_2 concentration
C	Carbon
E	$A^e_{growth}/A^a_{growth}$
E'	A_{550}/A_{380}
FACE	Free air carbon dioxide enrichment
J_{max}	Maximal photosynthetic electron transport rate (a proxy for ribulose-1,5-bisphosphate regeneration)
LAI	Leaf area index
N	Nitrogen
PPFD	Photosynthetic photon flux density
SLA	Specific leaf area
NSC	Non-structural carbohydrates
SCC	Swiss canopy crane
SE	Standard error of the mean
V_{cmax}	Maximal carboxylation rate of Rubisco
ALVPD	Air-to-leaf vapour pressure deficit

M. K.-F. Bader (✉) · C. Körner
Institute of Botany, University of Basel, Schönbeinstrasse 6, 4056 Basel, Switzerland
e-mail: Martin.Bader@unibas.ch

R. Siegwolf
Paul Scherrer Institute, 5323 Villigen PSI, Switzerland

Introduction

Every year, the burning of fossil fuels and dramatic changes in land use feed vast amounts of CO_2 to the

atmosphere (Le Quéré et al. 2009). The uptake of CO_2 from the atmosphere through photosynthesis and its recycling through respiratory processes represent the largest fluxes in the global carbon (C) cycle (Schimel 1995; Sabine et al. 2004). If the C assimilated by plants is not completely recycled and some remains stored in the biosphere for prolonged periods, this could mitigate atmospheric CO_2 enrichment. The knowledge of the long-term response of photosynthesis to elevated atmospheric CO_2 is key to understanding such future ecosystem responses. Under the current atmospheric CO_2 concentration that exceeds the pre-industrial level by nearly 40%, photosynthesis in C_3-plants is still CO_2 limited (Farquhar et al. 1980; Tans 2008). Hence, photosynthetic rates increase in response to elevated CO_2, because the increased substrate availability stimulates Rubisco (ribulose-1,5-bisphosphate carboxylase/oxygenase) carboxylation whilst competitively inhibiting the oxygenation process (Drake et al. 1997; Ainsworth and Rogers 2007). In trees that harbour ~90% of the terrestrial biomass carbon (Roy et al. 2001) and account for 50% of the terrestrial net primary production (Bonan 2008), elevated CO_2 has been reported to increase leaf photosynthesis by 30–60%, regardless of tree age (Gunderson and Wullschleger 1994; Curtis and Wang 1998; Saxe et al. 1998; Medlyn et al. 1999; Norby et al. 1999; Nowak et al. 2004; Ainsworth and Long 2005). The magnitude of this stimulation is species dependent and modulated by environmental factors such as light, temperature, soil water and nutrient supply (Curtis and Wang 1998; Nowak et al. 2004).

Sustained photosynthetic stimulation is one of the prerequisites for growth stimulation under elevated CO_2. However, there is no direct translation of CO_2 uptake per unit leaf area into plant growth (Körner 2006). In fact, long-term exposure to elevated CO_2 may affect morphological, biochemical and physiological plant properties that feed back to both photosynthesis and net carbon incorporation into the plant body (Gunderson and Wullschleger 1994; Egli et al. 2001). Photosynthetic acclimation to elevated CO_2 commonly occurs at the biochemical level through decreases in Rubisco carboxylation (V_{cmax}) resulting from reductions in Rubisco concentration and, less importantly, through declines in the maximal electron transport rate (J_{max}; Drake et al. 1997; Moore et al. 1999; Stitt and Krapp 1999; Ellsworth et al. 2004; Ainsworth and Rogers 2007). Imbalances in sink–source relations due to reduced or insufficient sink capacity often result from nutrient limitations (e.g. growth at low fertility sites or progressive nitrogen limitation) and lead to accumulation of photosynthates in leaves (Körner and Miglietta 1994). This sugar signal triggers a response mechanism that targets the small subunit of Rubisco and eventually leads to selective down-regulation of Rubisco (Rogers and Ellsworth 2002; Long et al. 2004; Ainsworth and Rogers 2007). Non-selective down-regulation on the other hand emerges from a loss of total leaf N or the dilution of leaf N by non-structural carbohydrates (NSC), which indirectly affect Rubisco concentration (Ellsworth et al. 2004). Early observations of photosynthetic down-regulation could be largely attributed to experimental constraints such as pot size (restricted rooting volume; Drake et al. 1997). However, also in field experiments, where the spatial constraints on the root system had been overcome, down-regulation of photosynthesis occurred in CO_2-enriched trees, resulting mainly from reduced carboxylation capacity due to leaf N dilution (on mass and area basis) by NSC accumulation, i.e. from a non-selective, indirect effect on Rubisco (Medlyn et al. 1999; Ellsworth et al. 2004). When photosynthetic down-regulation occurs, the stimulative effect of elevated CO_2 is diminished but not completely eliminated, and photosynthesis is still enhanced under elevated CO_2 even after several years of enrichment (Saxe et al. 1998; Crous et al. 2008). In a meta-analysis comprising CO_2 fertilisation experiments other than free air carbon dioxide enrichment (FACE), photosynthesis of (mostly young) European forest trees declined over time by 10–20% under elevated CO_2, but was still stimulated by 51% relative to control trees (350 vs. 700 ppm CO_2; Medlyn et al. 1999). In FACE experiments, down-regulation of photosynthetic capacity was observed in the aggrading Aspen-FACE stands; however, this was a transitory effect that disappeared after steady-state LAI had been reached (Ellsworth et al. 2004; Uddling et al. 2009). A strong sugar-mediated, selective down-regulation of Rubisco occurred in old needles of mature *Pinus taeda* growing in an N-limited steady-state system at the Duke-FACE facility (Rogers and Ellsworth 2002; Crous et al. 2008).

Despite the bulk of literature on the effects of elevated CO_2 on photosynthesis in trees, very few studies have addressed the responses of mature dominant trees that have reached steady-state canopy development (constant leaf area index). The FACE experiment at the Swiss Canopy Crane (SCC) is the only study worldwide where several species of hardwood trees growing in a near-natural, mature mixed forest were exposed to elevated atmospheric CO_2. We evaluated the long-term CO_2 response of photosynthesis and associated leaf traits in five tree species during the 8th year of canopy CO_2 enrichment. Our objective was to assess the magnitude of photosynthetic enhancement and potential down-regulation under elevated CO_2. Gas exchange, biochemical and morphological leaf parameters were measured in sunlit foliage during the mid and late growing season to account for seasonal sink–source dynamics. Given the lack of persistent growth stimulation above and below ground in the CO_2-enriched trees of this stand (Körner et al. 2005; Asshoff et al. 2006;

Bader et al. 2009), we anticipated that reduced sink capacity would feed back to photosynthesis, thereby partially offsetting the CO_2-induced stimulation of leaf-level C uptake. Such a decline in photosynthetic enhancement would involve: (1) a reduction of the maximal rate of leaf photosynthesis; (2) a decline in V_{cmax} and/or J_{max}; (3) increased foliar non-structural carbohydrate (NSC) concentration; (4) diminished leaf nitrogen concentration (mass based); and (5) reduced specific leaf area (SLA).

Materials and methods

Study site

The Swiss Canopy Crane (SCC) facility is located in a species-rich forest 15 km south of Basel, Switzerland (47°28′N, 7°30′E, 550 m a.s.l.). The ca. 100-year-old stand grows on a gentle NNW-exposed slope and reaches canopy heights from 30 to 35 m. The leaf area index (LAI) is around 5, tree density is 415 trees ha^{-1} (breast height diameter ≥0.1 m) and stem basal area amounts to 46 m^2 ha^{-1}. The forest is dominated by *Fagus sylvatica* L., *Quercus petraea* (Matt.) Liebl. and *Carpinus betulus* L., accompanied by less abundant tree species such as *Tilia platyphyllos* Scop., *Acer campestre* L., *Prunus avium* L. and four species of conifers (*Picea abies* (L.) Karst., *Larix decidua* Mill., *Pinus sylvestris* L., *Abies alba* Mill.). The soil type is a Rendzic Leptosol (WRB) (Rendzina, FAO; Lithic Rendoll, USDA) with an accessible profile depth of maximal 25 cm followed by rocky subsoil merging into the calcareous bedrock at depths of 40–90 cm. The soil texture was classified as loamy clay with a pH of 5.8 in the top 10 cm of the profile.

The climate in this temperate region is distinguished by mild winters and moderately warm summers with mean air temperatures in January and July of 2 and 19°C, respectively. Long-term mean annual precipitation in the study region is 990 mm. Approximately two-thirds of the yearly precipitation falls during the 6-month growing season (Pepin and Körner 2002).

Free air CO_2-enrichment system

Future CO_2 levels in the tree canopies were established by applying a novel free air CO_2-enrichment (FACE) technique called web-FACE (Pepin and Körner 2002). Briefly, pure CO_2 was pulse released through a fine web of perforated tubes plaited into tree crowns with the help of a construction crane. Canopy CO_2 supply was governed via computer-controlled magnetic valves to maintain the target CO_2 concentration of 550 ppm as accurately as possible. CO_2 concentration in the canopy served as the main control signal and was monitored by an air sampling system consisting of multiple suction heads per tree feeding canopy air through sampling lines into infrared gas analysers. CO_2 release was confined to daylight hours (photosynthetic photon flux density, PPFD > 100 μmol m^{-2} s^{-1}) of the growing season and was disengaged from the time of leaf shedding to bud break (end of October to mid-April). Twelve deciduous trees growing in the 60 m operating range of the crane were selected for CO_2 enrichment (three *Fagus sylvatica*, three *Quercus petraea*, three *Carpinus betulus*, one *Tilia platyphyllos*, one *Acer campestre,* one *Prunus avium*) and received elevated atmospheric CO_2 since autumn 2000. In 2006, the individual *Prunus avium* tree suffered from storm damage and was therefore excluded from the CO_2 enrichment. An adequate number of control trees were accessible in the remaining crane area at sufficient distance to the CO_2-enriched zone.

Leaf gas exchange measurements

In early July and mid-September of 2008, instantaneous rates of CO_2/H_2O gas exchange were measured with two identical portable photosynthesis systems (LI-6400, LI-COR Biosciences, Lincoln, NE, USA) on 25 trees of five species. Light-saturated net photosynthetic rates (PPFD = 1,200 μmol m^{-2} s^{-1}, LI-COR 6400-02 LED light unit) were determined between 8:30 and 12:30 h at ambient and elevated CO_2 concentrations (i.e. at 380 and 550 ppm) at 25°C leaf temperature and air-to-leaf vapour pressure deficit (ALVPD) of 1.18 ± 0.01 and 1.47 ± 0.02 kPa (means ± SE) in June and September, respectively. Measurements were taken on eight mature leaves per tree from different sunlit branches. Leaves that initially showed low stomatal conductance <70 mmol m^{-2} s^{-1} were replaced by leaves exceeding this threshold. By means of the adjustable leaf chamber CO_2 supply, gas exchange was first measured at 380 ppm CO_2 and subsequently, on the same leaf, at 550 ppm CO_2, designated hereafter as A_{380} and A_{550}, respectively. The light-saturated net photosynthesis measured at growth CO_2 concentration is termed A_{growth}. Recordings were taken as soon as the net rate of photosynthesis and stomatal conductance (g_s) remained constant. Individual measurements did not exceed 5 min. The instantaneous photosynthetic enhancement ratio (E') was calculated as the leaf-intrinsic ratio of A_{550}/A_{380} for any tree in ambient and elevated CO_2. The enhancement ratio that compares A_{growth} of CO_2-enriched trees (A^{e}_{growth}) with A_{growth} of control trees growing under ambient conditions (A^{a}_{growth}) is denoted by E. We calculated E using weighted species means to account for the varying number of trees available in each species (less weight assigned to *Acer* and *Tilia* that occurred with only one tree individual in each CO_2 treatment). Both photosynthetic enhancement ratios, E' and E, were

expressed as percentages (A_{550}/A_{380} or $A^{e}_{growth}/A^{a}_{growth} - 1 \times 100\%$). In addition, A/C_i curves of the replicated species (*F. sylvatica*, *Q. petraea*, *C. betulus*) were recorded on two to four leaves per tree. Gas exchange rates were first recorded at a leaf chamber CO_2 concentration (C_a) of 400 ppm CO_2, before C_a was stepwise reduced to 300, 200, 100 and 40 ppm, subsequently C_a was returned to 400 ppm (to check if the initial rate could be restored) and then increased to 600, 800, 1,000, 1,500 and 2,000 ppm. Individual response curves were completed within 25–35 min. A/C_i curves were analysed using a Farquhar-type photosynthesis model (Long and Bernacchi 2003). Nonlinear least squares regression was applied to estimate V_{cmax}, the maximal carboxylation rate of ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco), J_{max}, the maximal rate of electron transport, and photosynthetic limitation due to triose-phosphate utilisation (TPU-limitation).

Leaf properties

At peak season (July 2008), we also measured leaf thickness (SM 112, Teclock, Nagano, Japan, precision 0.01 mm) and foliar chlorophyll content non-destructively (CCM-200, Opti-Sciences, Tyngsboro, MA, USA) on 30 leaves per tree canopy. A different set of ten leaf samples per tree was collected with a leaf puncher for determination of SLA, leaf N and NSC concentrations. Immediately after sampling, leaf discs (1.2 cm^2) were dried at 80° C for at least 48 h and then weighed for biomass quantification. Then, samples were ground and all samples from one tree were pooled for determination of leaf N concentration (mg N g^{-1} dry mass) using a CHN-analyzer (Vario EL *III*, Elementar Analysensysteme GmbH, Hanau, Germany). Non-structural carbohydrates (NSC = starch, sucrose, glucose and fructose) were analysed applying an enzymatic starch digestion followed by a spectrophotometric glucose test after invertase and isomerase addition (Körner and Miglietta 1994).

Statistical analysis

Gas exchange parameters were analysed using a linear mixed-effects model. We fitted the model using restricted maximum likelihood (REML) and included the fixed factors 'species', 'FACE-treatment' and 'leaf chamber CO_2'. 'Leaf chamber CO_2' was also nested within the random factor 'tree individual'. The photosynthetic enhancement ratios E and E' were analysed in three-way repeated measures ANOVAs with the fixed factors 'FACE-treatment', 'species' and 'date'. CO_2 effects on leaf properties were analysed with two-way ANOVAs with the fixed factors 'species' and 'FACE-treatment'. All statistical analyses and graphics were performed using R version 2.9.0 (http://www.r-project.org).

Results

Leaf gas exchange

In the early and late growing season during year 8 of CO_2 enrichment, the instantaneous enhancement of light-saturated net photosynthesis by elevated CO_2 ($E' = A_{550}/A_{380}$) was similar in trees growing under ambient and elevated

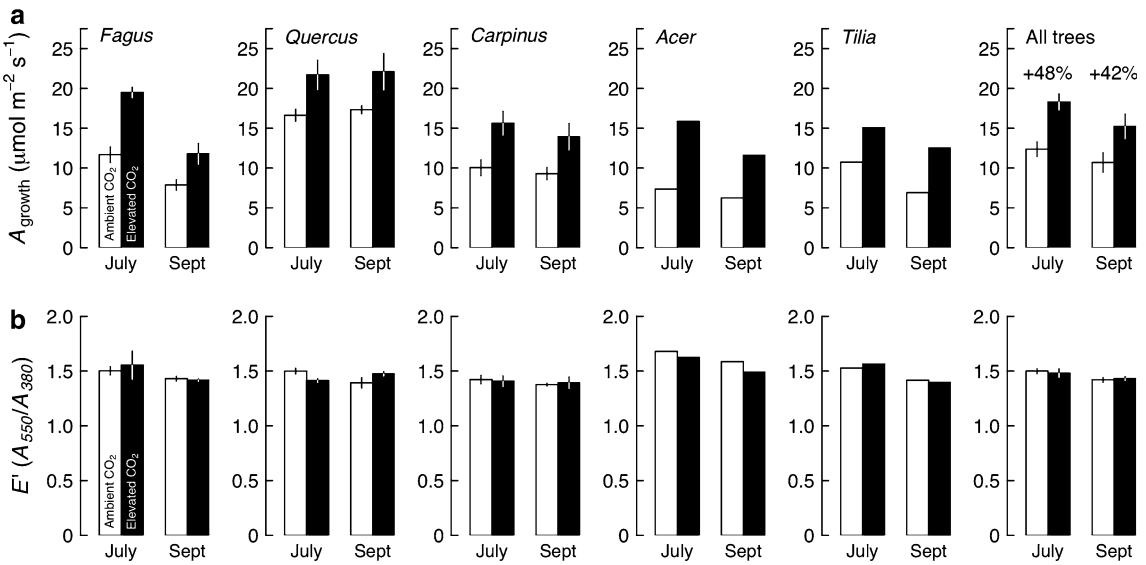

Fig. 1 CO_2-induced enhancement of light-saturated leaf photosynthesis of five deciduous tree species measured in the mid and late growing season of 2008 at the SCC FACE site, Switzerland. Trees were growing in a near-natural stand under ambient (*white*) or elevated CO_2 (*black*). **a** Light-saturated net photosynthesis measured at growth CO_2 concentration, A_{growth}. **b** Instantaneous leaf-intrinsic photosynthetic enhancement, $E' = A_{550}/A_{380}$. Means ± SE, $n = 11$ (elevated CO_2), $n = 14$ (ambient CO_2)

Table 1 Results of a three-way ANOVA on the effects of species identity, FACE and sampling date (peak and late growing season) on the instantaneous leaf-intrinsic photosynthetic enhancement (E') involved with a switch in leaf chamber CO_2 from 380 to 550 ppm

Factor	*Df*	*F*	*P*
Species	4, 15	4.380	<0.010**
FACE	1, 15	0.005	0.947
Date	1, 15	8.030	<0.010**
Species × FACE	4, 15	0.177	0.948
Species × date	4, 15	0.535	0.710
FACE × date	1, 15	0.603	0.444
Species × FACE × date	4, 15	1.032	0.407

Df degrees of freedom (numerator, denominator); *F* *F*-value
** $P < 0.01$

Table 2 Linear mixed-effects model results on the effects of species identity, FACE and leaf chamber CO_2 on photosynthetic capacity of five deciduous tree species growing under ambient and elevated CO_2 (FACE = CO_2 effect, CO_2-LC = leaf chamber CO_2 concentration)

Factor	*Df*	*F*	*P*
Species	4, 15	17.838	<0.001***
Date	1, 781	190.947	<0.001***
FACE	1, 15	0.008	0.929
CO_2-LC	1, 19	1045.290	<0.001***
Species × FACE	4, 15	0.833	0.525
Species × date	4, 781	44.540	<0.001***
Species × CO_2-LC	4, 19	1.281	0.312
FACE × CO_2-LC	1, 19	0.047	0.830
FACE × date	1, 781	0.398	0.528
Date × CO_2-LC	1, 781	5.421	0.020*
Species × FACE × date	4, 781	3.865	0.004**

Df degrees of freedom (numerator, denominator); *F* *F*-value
*** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$

CO_2 (Fig. 1; Table 1). Averaged across all trees, the short-term switch in CO_2 supply to the leaf chamber from 380 to 550 ppm led to a photosynthetic enhancement of 49% in early July and declined significantly to 42% in September (Fig. 1; Table 2). As a consequence of this strong CO_2 stimulation, the rate of light-saturated net photosynthesis measured at growth CO_2 concentration (A_{growth}) was significantly higher in CO_2-enriched trees compared to ambient controls ($E = A^{e}_{growth}/A^{a}_{growth}$) reaching 48% in July and 42% in September (Fig. 1). There was significant variation amongst the study species with *Quercus petraea* showing the highest rates of net photosynthesis under both treatments and at both measured leaf chamber CO_2 levels, whilst the lowest rates were seen in *Acer campestre* (Table 2). The significance of the species × FACE × date interaction (indicating species-specific down-regulation over the growing season, Table 2) was caused by the single *Tilia* tree and when this

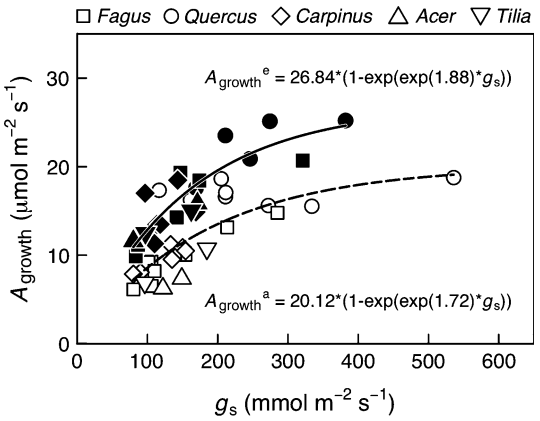

Fig. 2 Net photosynthesis under growth CO_2 concentration (A_{growth}) as a function of stomatal conductance (g_s) in ambient (*white*) and CO_2-enriched foliage (*black*) of five mature deciduous tree species, measured in the peak and late growing season of 2008. Means ± SE, n = 1–5 per species and treatment

unreplicated species was disregarded in the model the interaction term lost its significance. From the early to the late growing season, the average A_{growth} declined significantly by 15–17% under ambient (12.4 vs. 10.7 µmol m^{-2} s^{-1}) and elevated CO_2 (18.3 vs. 15.2 µmol m^{-2} s^{-1}, three-way ANOVA, $P < 0.001$). Stomatal conductance declined towards the end of the growing season by 41 and 33% in ambient and elevated CO_2 (three-way ANOVA, $P < 0.001$). Comparing foliage grown and measured under ambient CO_2 with foliage grown and measured under elevated CO_2 yielded similar stomatal conductance (g_s), but 6 and 9% lower transpiration rates in July and September, respectively. However, these differences in transpiration were statistically not significant (three-way repeated measures ANOVA, $P > 0.2$). Rates of A_{growth} and g_s were tightly coupled, and A^{e}_{growth} was always higher than A^{a}_{growth} at a common g_s (Fig. 2). Since leaf N in CO_2-enriched trees was not reduced (see later), photosynthetic net carbon uptake per unit leaf nitrogen (nitrogen-use efficiency, PNUE) was significantly higher compared to control trees (Table 4).

The carboxylation efficiency of Rubisco (V_{cmax}) and the maximal rate of electron transport leading to RubP regeneration (J_{max}) showed significant inter-specific variation, but CO_2 enrichment as main effect had no significant influence. The significant species × FACE × date interaction for V_{cmax} resulted mainly from increases in V_{cmax} in *Quercus* control trees towards the end of the growing season, rather than from a late-seasonal down-regulation in CO_2-enriched trees. In a number of leaves, A/C_i curves also showed that photosynthesis became limited by triose-phosphate utilisation (5.3–12.6 µmol m^{-2} s^{-1}) when C_i exceeded ~750 ppm.

Across species and treatments, V_{cmax} and J_{max} had significantly declined by 11 and 8% over the growing season

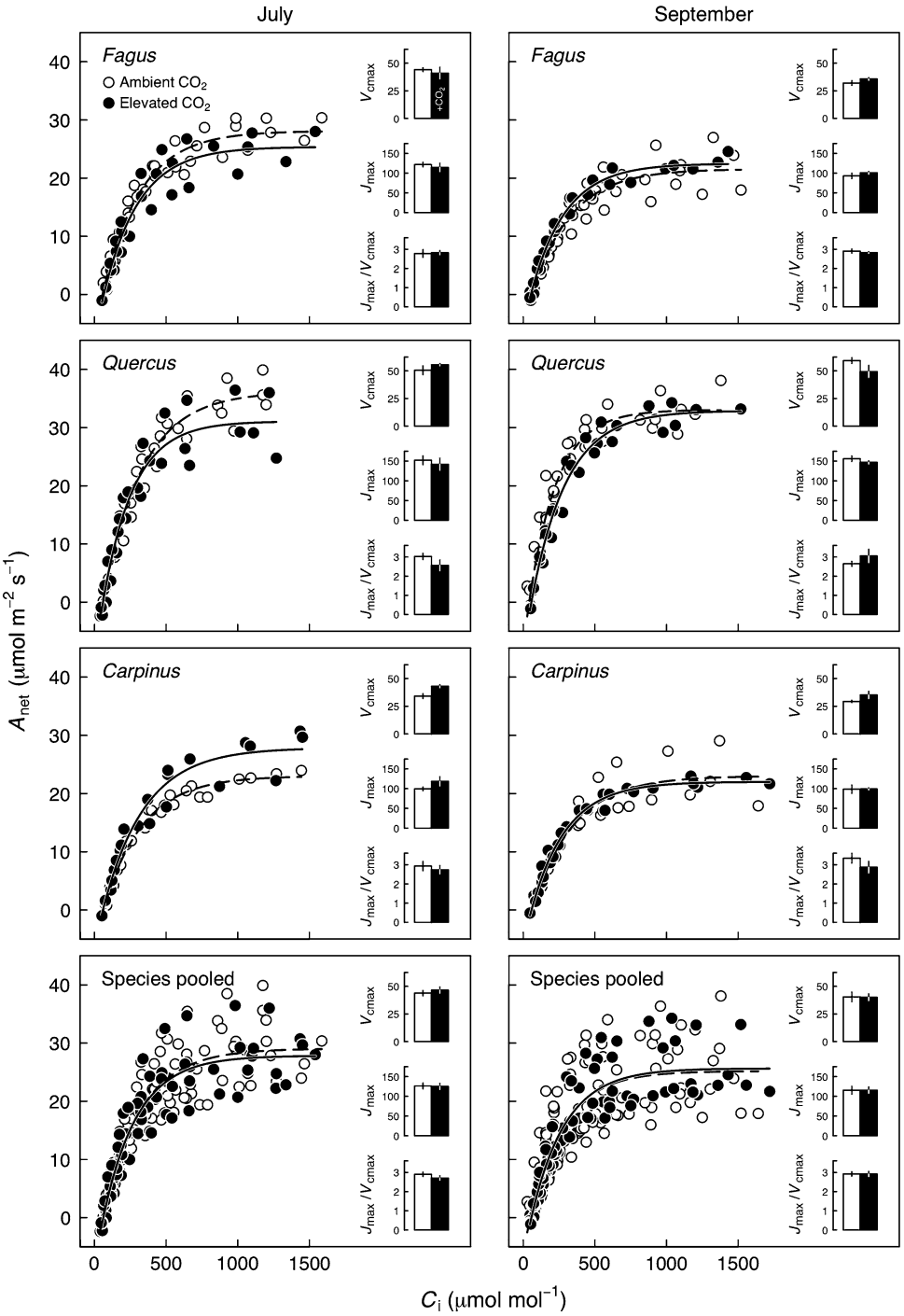

Fig. 3 The seasonal response of light-saturated net photosynthesis (A_{net}) to intercellular CO_2 concentration (C_i) in upper-canopy leaves of mature individuals of the three dominant deciduous tree species growing under ambient (*white*, *dashed lines*) and elevated CO_2 (*black*, *solid lines*) in a near-natural stand at the SCC FACE site, Switzerland. The bar chart inserts give the maximum rate of Rubisco carboxylation (V_{cmax}), the maximum electron transport rate (J_{max}) and the J_{max}/V_{cmax} ratio derived from a Farquhar-type photosynthesis model. The response curves shown in the graph were fitted using nonlinear least squares regression based on the equation used by Herrick and Thomas (2001): $A = A_{max}[1 - (1 - \alpha/A_{max})(1 - C_i/\Gamma)]$, where $A_{max} = A_{net}$ at CO_2 saturation, α = *y*-intercept and $\Gamma = CO_2$ compensation point. Means ± SE, $n = 3$–5 per species and treatment

(Fig. 3; Table 3). We observed a common positive linear relationship between J_{max} and V_{cmax}, which was similar in leaves grown under ambient and elevated CO_2 and did not change with progressing growing season (*F* test, $F = 0.232$, $P = 0.963$, Fig. 4). Neither V_{cmax} nor J_{max} was significantly correlated with leaf N on an area basis (N m^{-2}, data not shown).

Leaf traits

In year 8 of the FACE study, all measured leaf traits showed significant inter-specific variation, but CO_2 enrichment as main effect had no significant influence (Table 4). However, across all trees, CO_2 exposure tended to increase leaf non-structural carbohydrates (NSC,

Table 3 Results of a three-way ANOVA on the effects of species identity, FACE and sampling date (peak and late growing season) on the maximum rate of Rubisco carboxylation (V_{cmax}), maximum electron transport rate (J_{max}) and the ratio J_{max}/V_{cmax} in the three replicated tree species (*Fagus sylvatica*, *Quercus petraea*, *Carpinus betulus*)

Factor	*Df*	*F*	*P*
V_{cmax}			
Species	2, 16	45.976	<0.001***
FACE	1, 16	0.712	0.405
Date	1, 15	7.889	0.009**
Species × FACE	2, 16	2.792	0.078
Species × date	2, 15	4.712	0.016*
FACE × date	1, 15	1.012	0.322
Species × FACE × date	2, 15	3.606	0.039*
J_{max}			
Species	2, 16	37.534	<0.001***
FACE	1, 16	0.003	0.954
Date	1, 15	4.570	0.041*
Species × FACE	2, 16	1.272	0.295
Species × date	2, 15	2.888	0.071
FACE × date	1, 15	0.003	0.961
Species × FACE × date	2, 15	0.977	0.388
J_{max}/V_{cmax}			
Species	2, 16	0.606	0.552
FACE	1, 16	0.948	0.338
Date	1, 15	0.788	0.382
Species × FACE	2, 16	0.693	0.508
Species × date	2, 15	0.464	0.633
FACE × date	1, 15	0.527	0.474
Species × FACE × date	2, 15	2.294	0.118

Df degrees of freedom (numerator, denominator); *F* *F*-value
*** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$

+15%), which was largely driven by *Quercus* that showed 36% increase in leaf NSC under elevated CO_2 (Table 4). There was also a trend towards a decline in specific leaf area (SLA) under elevated CO_2, which disappeared when the two unreplicated species were excluded from the statistical analysis or when SLA was expressed on an NSC-free basis (Table 4). We found species-dependent CO_2 effects on leaf chlorophyll content and leaf N (significant species × treatment interaction). CO_2-enriched *Quercus* trees showed 20% less foliar chlorophyll than conspecific control trees, whilst *Fagus* leaves exhibited ca. 30% higher chlorophyll contents under elevated CO_2. The significant species × treatment interaction with leaf N (on an area basis) resulted solely from the decline seen in *Acer* (−28%) and *Tilia* (−14%). When these two unreplicated species were excluded from the analysis, the interaction term lost significance (Table 4).

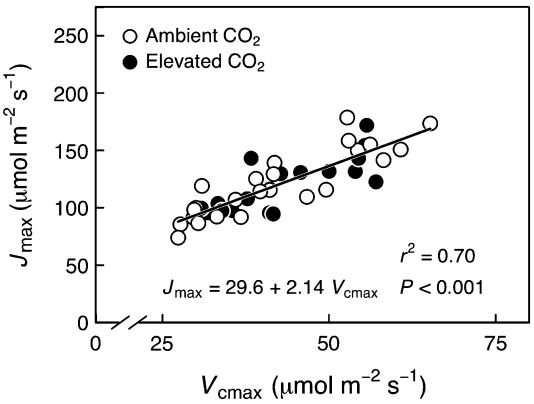

Fig. 4 Correlation between the maximum rate of electron transport (J_{max}) driving RubP regeneration and the maximum rate of Rubisco carboxylation (V_{cmax}) of mature leaves in the outer canopy of the three dominant deciduous tree species at the SCC site [*Fagus sylvatica*, *Quercus petraea*, and *Carpinus betulus*, means ± SE, $n = 9$ (elevated CO_2), $n = 12$ (ambient CO_2)]. Treatment- and date-wise regression was statistically not different from the regression analysis applied to the combined data from ambient and elevated CO_2 in July and September; therefore the pooled data set is shown

Discussion

Leaf gas exchange

The Swiss Canopy Crane gave us the unique possibility to reveal the long-term effects of CO_2 enrichment on photosynthesis in mature, 30–35 m tall deciduous forest trees. In the canopy of the mixed forest stand at the SCC site, net photosynthetic rates of sunlit foliage from mature trees that had been grown under future atmospheric CO_2 for 8 years were consistently higher compared with rates in leaves of control trees ($E = 48\%$ in July and 42% in September, Fig. 1). In the same stand, Zotz et al. (2005) found 36 and 49% photosynthetic enhancement (E) in the mid and late growing season, respectively, after 3 years of CO_2 enrichment (E given here is corrected for the loss of one CO_2-exposed tree in 2006 and was recalculated with the original data using weighted species means). Thus, the magnitude of photosynthetic enhancement at the SCC forest did not change over the study years and agrees well with the numbers reported from other multi-year FACE experiments performed with trees (Herrick and Thomas 2001, *Liquidambar styraciflua* in the understory at Duke-FACE: +63%; Sholtis et al. 2004, *L. styraciflua* at ORNL-FACE: +44%; Liberloo et al. 2007, three *Populus* species at POP-FACE: +49%; Ainsworth and Rogers 2007, review: +46% in trees, Crous et al. 2008, *Pinus taeda* at Duke-FACE: +68% in current year needles, +40% in 1-year-old needles). At the Aspen-FACE site, *Populus tremuloides* clones initially showed somewhat lower stimulation especially under $CO_2 + O_3$ conditions

Table 4 Traits of fully expanded foliage of five forest tree species (*Fagus sylvatica, Quercus petraea, Carpinus betulus, Acer campestre, Tilia platyphyllos*)

Species	*Fagus*		*Quercus*		*Carpinus*		*Acer*		*Tilia*		*P*	FACE	Species × FACE
Treatment	a	e	a	e	a	e	a	e	a	e	Species		
NSC (% d.m.)	14.2 ± 0.8	15.7 ± 1.2	12.9 ± 1.5	17.5 ± 0.4	17.8 ± 1.0	17.3 ± 1.1	12.7	8.7	10.2	11.7	<0.01**	0.10	0.13
											(<0.01**)	(0.11)	(0.10)
Chlorophyll (rel. units)	27.8 ± 2.7	35.9 ± 3.3	33.1 ± 2.4	26.4 ± 1.8	20.3 ± 1.5	22.6 ± 1.3	30.9	28.1	27.5	15.4	<0.05*	0.92	<0.05*
											(<0.01**)	(0.38)	(<0.05*)
Leaf N (% d.m.)	2.5 ± 0.1	2.5 ± 0.0	2.8 ± 0.1	2.6 ± 0.1	2.4 ± 0.1	2.5 ± 0.1	2.5	1.8	3.7	3.2	<0.001***	0.27	<0.05*
											(0.08)	(0.80)	(0.49)
NSC-free leaf N (% d.m.)	2.9 ± 0.2	3.0 ± 0.1	3.2 ± 0.1	3.2 ± 0.2	2.9 ± 0.1	3.0 ± 0.1	2.9	2.0	4.1	3.6	<0.001***	0.65	0.06
											(0.19)	(0.38)	(0.90)
Leaf N ($g\ m^{-2}$)	2.0 ± 0.1	1.8 ± 0.2	2.2 ± 0.1	2.2 ± 0.2	2.3 ± 0.0	2.2 ± 0.1	2.2	1.5	3.5	3.2	<0.001***	<0.05*	0.08
											(<0.01**)	(0.22)	(0.84)
SLA ($m^2\ kg^{-1}$)	10.2 ± 0.5	9.7 ± 0.2	8.8 ± 0.3	8.3 ± 0.1	13.3 ± 0.2	13.9 ± 1.3	11.5	8.2	14.3	14.8	<0.001***	0.09	0.10
											(<0.001***)	(0.76)	(0.75)
NSC-free SLA ($m^2\ kg^{-1}$)	11.9 ± 0.7	11.5 ± 0.4	10.1 ± 0.2	10.1 ± 0.3	16.2 ± 0.0	16.8 ± 1.5	13.2	9.0	16.0	16.8	<0.001***	0.37	0.08
											(<0.001***)	(0.90)	(0.96)
Leaf thickness (μm)	389 ± 14	384 ± 12	337 ± 21	326 ± 5	395 ± 16	403 ± 6	272	302	410	431	<0.001***	0.75	0.71
											(<0.001***)	(0.82)	(0.71)
PNUE ($\mu mol\ mol\ N^{-1}\ s^{-1}$)	86.4 ± 7.1	152.5 ± 12.1	110.6 ± 6.7	140.6 ± 4.0	61.6 ± 6.2	99.3 ± 6.6	47.1	146.0	43.3	66.5	<0.001***	<0.001***	<0.05*
											(<0.001***)	(<0.001***)	(0.09)
Trees (*n*)	5	3	4	3	3	3	1	1	1	1			

Numbers are means ± SE or represent single trees, a = ambient CO_2, e = elevated CO_2. The terminal columns represent the results of two-way ANOVAs for species and treatment effects and the interaction term. For each parameter, the upper row contains the statistical results for all species and the lower row the results for the three replicated species only (see last row for number of replications)

*** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$

(Noormets et al. 2001). However, when LAI had reached steady-state, photosynthetic capacity in CO_2-enriched *Populus* clones and *Betula papyfera* trees was increased by 76 and 115%, respectively, and the addition of O_3 (elevated $CO_2 + O_3$ treatment) did not significantly reduce the CO_2-driven stimulation in photosynthesis (Uddling et al. 2009).

Although previous measurements revealed reduced sapflow in our CO_2-enriched trees (Cech et al. 2003; Leuzinger and Körner 2007), we did not measure reduced g_s under elevated CO_2. However, the relationship between A_{growth} and g_s suggests improved water-use-efficiency (Fig. 2). A similar relationship between A_{growth} and g_s was reported for *Liquidambar styraciflua* trees at the ORNL-FACE site, which, in contrast to our trees, showed a significant 24% decline in g_s under elevated CO_2 (Gunderson et al. 2002).

In their meta-analysis of photosynthetic responses to elevated CO_2 in FACE experiments, Ainsworth and Rogers (2007) reported moderate but significant decreases in V_{cmax} (−6%) in trees growing under high CO_2. At our site, trees receiving CO_2 enrichment did not show reduced V_{cmax} not even towards the end of the growing season when down-regulation due to declining sink strength is most commonly observed. Downward adjustment of J_{max} in response to elevated CO_2 has less often been reported (Long et al. 2004; Ainsworth and Long 2005) and was not apparent in our CO_2-enriched trees.

The lack of photosynthetic down-regulation is consistent with the findings for mature and understory sweetgum trees growing at the ORNL- and Duke-FACE site, respectively, and three poplar species growing at short-rotation coppice at the POP-FACE site as well as for aspen and birch at the ASPEN-FACE stands (Herrick and Thomas 2001; Sholtis et al. 2004; Liberloo et al. 2007; Uddling et al. 2009).

Down-regulation of photosynthesis in response to CO_2 enrichment is often associated with a decline in leaf N (Stitt 1991; Medlyn et al. 1999) and thus occurs more often under limited soil N availability (Oren et al. 2001). Although the steady-state pine forest at the DUKE-FACE facility is such an N-limited system, long-term exposure to elevated CO_2 did not affect needle N irrespective of needle age. Nonetheless, V_{cmax} and J_{max} declined significantly and reduced photosynthetic enhancement by 37% in 1-year-old needles of CO_2-enriched *Pinus taeda* trees (Rogers and Ellsworth 2002; Crous et al. 2008). These declines resulted from NSC accumulation that caused strong selective down-regulation of Rubisco (Rogers and Ellsworth 2002). In current-year needles that showed no down-regulation, the photosynthetic enhancement by elevated CO_2 was therefore substantially higher compared to 1-year-old needles (68 vs. 40%; Crous et al. 2008). Interestingly, N-fertilisation in the last FACE year restored photosynthetic capacity in 1-year-old needles suggesting that CO_2 enrichment reduced the allocation of N to Rubisco and RuBP regeneration and to proteins associated with electron transport in 1-year-old needles. The authors concluded that curtailing the N supply to photosynthesis could make more N available for new foliage growth at low fertility sites (Crous et al. 2008). However, even in well-fertilised sour orange trees, photosynthesis was gradually down-regulated during 14 years of growth in elevated CO_2 (Adam et al. 2004).

The SCC stand grows in an area with ample precipitation and high wet nitrogen deposition (20–25 kg N ha^{-1} a^{-1}) and is thus believed to be free of N-limitation. Consequently, we assumed strong initial growth stimulation by elevated CO_2 that would only start to acclimatise a few years after the FACE initiation (Körner 2006). However, apart from transient growth stimulation in *Fagus* during the early years of the experiment, we did not observe any consistent, significant increases in growth or biomass or the acceleration of turnover processes above or below ground under elevated CO_2 (Körner et al. 2005; Asshoff et al. 2006; Keel et al. 2006; Bader et al. 2009). Although stable isotope data and soil air CO_2 concentration both indicated an increased flux of C into the soil (Keel et al. 2006), we did not detect a corresponding signal in soil respiration that could account for the fate of the extra C assimilated under elevated CO_2 (Bader and Körner 2010). The lack of strong sink capacity for assimilates led us to assume considerable down-regulation of photosynthesis in trees receiving CO_2 enrichment. Instead, we found persistent stimulation of photosynthesis implying a lack of closure in the C budget, clearly pointing to so far unaccounted C fluxes in this CO_2 enrichment experiment. Preliminary findings indicate that parts of the extra C assimilated under elevated CO_2 may leave the system through enhanced leaching of dissolved organic and inorganic C (<20%, F. Hagedorn, personnel communication).

Leaf traits

Similar to previous years, the overall trend towards increased leaf NSC under elevated CO_2 resulted mainly from the strong buildup seen in *Quercus* leaves (Table 4; Körner et al. 2005). As discussed earlier, NSC accumulation in leaves may lead to photosynthetic down-regulation, but photosynthesis (A_{growth} and E') in *Quercus* foliage remained unaffected by the CO_2-induced increase in NSC. Similarly strong accumulation of leaf NSC (+37%) was observed in *Liquidambar styraciflua* (sweetgum) leaves at the ORNL-FACE site and there the sugar signal also failed to down-regulate photosynthesis (Sholtis et al. 2004). Consistent with the lack of photosynthetic down-regulation, leaf N assessed at peak season was not affected by CO_2 enrichment, except for the single *Acer* tree that showed a large decrease in leaf N. This contrasts with the findings from the early years of the

experiment that showed an overall reduction of 10% in leaf N driven by a pronounced decline in *Carpinus* and the dilution by NSC (Körner et al. 2005). A 10% reduction in leaf N (mass based) was also reported for sweetgum trees at the ORNL stands (Sholtis et al. 2004), whilst needle N in *Pinus taeda* growing on low fertile soil at the Duke-FACE forest and leaf N in deciduous trees at the POP- and Aspen-FACE stands remained unaffected by CO_2 exposure (Liberloo et al. 2007; Crous et al. 2008; Uddling et al. 2009).

The changes in area-based chlorophyll content observed in *Fagus* and *Quercus* were not linked to changes in SLA and had no effect on V_{cmax} or J_{max}. Commonly, foliar chlorophyll content is little affected under FACE conditions (Long et al. 2004; Ainsworth and Long 2005), and declines on a mass basis observed in sweetgum trees (ORNL-FACE) were fully explained by reductions in SLA (ca. −10%, Sholtis et al. 2004). During the first 4 years of the SCC experiment, elevated CO_2 diminished SLA by 5–8% in all species but *Fagus* (Körner et al. 2005), whereas in year 8 only *Acer* showed lower SLA under elevated CO_2. Also, *Pinus taeda* growing at the Duke forest showed no SLA response to elevated CO_2 (Rogers and Ellsworth 2002), but SLA declined up to 24% in the upper canopy of a poplar coppice exposed to elevated CO_2 (POP-FACE, Liberloo et al. 2007). In general, leaf traits of our study trees were surprisingly little affected by CO_2 enrichment.

Even if the CO_2-induced decline in SLA observed during the early years of the experiment disappeared over time, it did not preclude changes in leaf production. However, at our site, annual leaf litter production and thus LAI remained unchanged under elevated CO_2 (Körner et al. 2005 and later unpublished data), which was consistent with earlier studies reporting the lack of an LAI response to CO_2 enrichment in closed canopy stands (Hättenschwiler et al. 1997; Gielen et al. 2003; Norby et al. 2003). Steady-state LAI had, however, increased in pure aspen and mixed aspen birch stands growing under elevated CO_2 at the Aspen-FACE site (Uddling et al. 2008).

Conclusions

Photosynthetic enhancement (42–48%) in mature trees of five broad-leaved species was sustained without reductions over 8 years of canopy CO_2 enrichment. Provided that future climatic trends will not strongly affect photosynthesis directly and nutrient availability will remain sufficient, these findings suggest that the enhancement of photosynthesis may persist in these mature deciduous trees under high future atmospheric CO_2 concentrations. The fate of the additional C assimilated by CO_2-exposed trees growing in this closed canopy forest remains uncertain. Above- and below-ground growth responses to elevated CO_2 were inconsistent suggesting that the extra C was not used to build up significantly more biomass in these old trees.

Acknowledgments We thank Erwin Amstutz for crane operations and for his efforts in data collection. We are grateful to the Paul Scherrer Institute (Switzerland) for the provision of equipment. Further, we thank Olivier Bignucolo for various laboratory analyses of leaf parameters. The SCC FACE study was supported by the Swiss National Science Foundation (grant 3100AO-111914/1).

References

Adam NR, Wall GW, Kimball BA, Idso SB, Webber AN (2004) Photosynthetic down-regulation over long-term CO_2 enrichment in leaves of sour orange (*Citrus aurantium*) trees. New Phytol 163:341–347

Ainsworth EA, Long SP (2005) What have we learned from 15 years of free-air CO_2 enrichment (FACE)? A meta-analytic review of the responses of photosynthesis, canopy properties and plant production to rising CO_2. New Phytol 165:351–371

Ainsworth EA, Rogers A (2007) The response of photosynthesis and stomatal conductance to rising CO_2: mechanisms and environmental interactions. Plant Cell Environ 30:258–270

Asshoff R, Zotz G, Körner C (2006) Growth and phenology of mature temperate forest trees in elevated CO_2. Glob Change Biol 12:848–861

Bader M, Körner C (2010) No overall stimulation of soil respiration under mature deciduous forest trees after 7 years of CO_2 enrichment. Glob Change Biol. doi:10.1111/j.1365-2486.2010.02159.x (in press)

Bader M, Hiltbrunner E, Körner C (2009) Fine root responses of mature deciduous forest trees to free air carbon dioxide enrichment (FACE). Funct Ecol 23:913–921

Bonan GB (2008) Forests and climate change: forcings, feedbacks, and the climate benefits of forests. Science 320:1444–1449

Cech PG, Pepin S, Körner C (2003) Elevated CO_2 reduces sap flux in mature deciduous forest trees. Oecologia 137:258–268

Crous KY, Walters MB, Ellsworth DS (2008) Elevated CO_2 concentration affects leaf photosynthesis–nitrogen relationships in *Pinus taeda* over nine years in FACE. Tree Physiol 28:607–614

Curtis PS, Wang XZ (1998) A meta-analysis of elevated CO_2 effects on woody plant mass, form, and physiology. Oecologia 113:299–313

Drake BG, Gonzalez-Meler MA, Long SP (1997) More efficient plants: a consequence of rising atmospheric CO_2? Annu Rev Plant Physiol Plant Mol Biol 48:609–639

Egli P, Maurer S, Spinnler D, Landolt W, Gunthardt-Georg MS, Körner C (2001) Downward adjustment of carbon fluxes at the biochemical, leaf, and ecosystem scale in beech-spruce model communities exposed to long-term atmospheric CO_2 enrichment. Oikos 92:279–290

Ellsworth DS, Reich PB, Naumburg ES, Koch GW, Kubiske ME, Smith SD (2004) Photosynthesis, carboxylation and leaf nitrogen responses of 16 species to elevated pCO_2 across four free-air CO_2 enrichment experiments in forest, grassland and desert. Glob Change Biol 10:2121–2138

Farquhar GD, von Caemmerer S, Berry JA (1980) A biochemical model of photosynthetic CO_2 assimilation in leaves of C_3 species. Planta 149:78–90

Gielen B, Liberloo M, Bogaert J, Calfapietra C, De Angelis P, Miglietta F, Scarascia-Mugnozza G, Ceulemans R (2003) Three years of free-air CO_2 enrichment (POPFACE) only slightly

affect profiles of light and leaf characteristics in closed canopies of *Populus*. Glob Change Biol 9:1022–1037

Gunderson CA, Wullschleger SD (1994) Photosynthetic acclimation in trees to rising atmospheric CO_2—a broader perspective. Photosynth Res 39:369–388

Gunderson CA, Sholtis JD, Wullschleger SD, Tissue DT, Hanson PJ, Norby RJ (2002) Environmental and stomatal control of photosynthetic enhancement in the canopy of a sweetgum (*Liquidambar styraciflua* L.) plantation during 3 years of CO_2 enrichment. Plant Cell Environ 25:379–393

Hättenschwiler S, Miglietta F, Raschi A, Körner C (1997) Thirty years of in situ tree growth under elevated CO_2: a model for future forest responses? Glob Change Biol 3:463–471

Herrick JD, Thomas RB (2001) No photosynthetic down-regulation in sweetgum trees (*Liquidambar styraciflua* L.) after three years of CO_2 enrichment at the Duke forest FACE experiment. Plant Cell Environ 24:53–64

Keel SG, Siegwolf RTW, Körner C (2006) Canopy CO_2 enrichment permits tracing the fate of recently assimilated carbon in a mature deciduous forest. New Phytol 172:319–329

Körner C (2006) Plant CO_2 responses: an issue of definition, time and resource supply. New Phytol 172:393–411

Körner C, Miglietta F (1994) Long-term effects of naturally elevated CO_2 on mediterranean grassland and forest trees. Oecologia 99:343–351

Körner C, Asshoff R, Bignucolo O, Hättenschwiler S, Keel SG, Pelaez-Riedl S, Pepin S, Siegwolf RTW, Zotz G (2005) Carbon flux and growth in mature deciduous forest trees exposed to elevated CO_2. Science 309:1360–1362

Le Quéré C, Raupach MR, Canadell JG, Marland G, Bopp L, Ciais P, Conway TJ, Doney SC, Feely RA, Foster P, Friedlingstein P, Gurney K, Houghton RA, House JI, Huntingford C, Levy PE, Lomas MR, Majkut J, Metzl N, Ometto JP, Peters GP, Prentice IC, Randerson JT, Running SW, Sarmiento JL, Schuster U, Sitch S, Takahashi T, Viovy N, van der Werf GR, Woodward FI (2009) Trends in the sources and sinks of carbon dioxide. Nat Geosci 2:421–436

Leuzinger S, Körner C (2007) Water savings in mature deciduous forest trees under elevated CO_2. Glob Change Biol 13:2498–2508

Liberloo M, Tulva I, Raim O, Kull O, Ceulemans R (2007) Photosynthetic stimulation under long-term CO_2 enrichment and fertilization is sustained across a closed *Populus* canopy profile (EUROFACE). New Phytol 173:537–549

Long SP, Bernacchi CJ (2003) Gas exchange measurements, what can they tell us about the underlying limitations to photosynthesis? Procedures and sources of error. J Exp Bot 54:2393–2401

Long SP, Ainsworth EA, Rogers A, Ort DR (2004) Rising atmospheric carbon dioxide: plants face the future. Annu Rev Plant Biol 55:591–628

Medlyn BE, Badeck FW, De Pury DGG, Barton CVM, Broadmeadow M, Ceulemans R, De Angelis P, Forstreuter M, Jach ME, Kellomaki S, Laitat E, Marek M, Philippot S, Rey A, Strassemeyer J, Laitinen K, Liozon R, Portier B, Roberntz P, Wang K, Jarvis PG (1999) Effects of elevated CO_2 on photosynthesis in European forest species: a meta-analysis of model parameters. Plant Cell Environ 22:1475–1495

Moore BD, Cheng SH, Sims D, Seemann JR (1999) The biochemical and molecular basis for photosynthetic acclimation to elevated atmospheric CO_2. Plant Cell Environ 22:567–582

Noormets A, McDonald EP, Dickson RE, Kruger EL, Sober A, Isebrands JG, Karnosky DF (2001) The effect of elevated carbon dioxide and ozone on leaf- and branch-level photosynthesis and potential plant-level carbon gain in aspen. Trees Struct Funct 15:262–270

Norby RJ, Wullschleger SD, Gunderson CA, Johnson DW, Ceulemans R (1999) Tree responses to rising CO_2 in field experiments: implications for the future forest. Plant Cell Environ 22:683–714

Norby RJ, Sholtis JD, Gunderson CA, Jawdy SS (2003) Leaf dynamics of a deciduous forest canopy: no response to elevated CO_2. Oecologia 136:574–584

Nowak RS, Ellsworth DS, Smith SD (2004) Functional responses of plants to elevated atmospheric CO_2—do photosynthetic and productivity data from FACE experiments support early predictions? New Phytol 162:253–280

Oren R, Ellsworth DS, Johnsen KH, Phillips N, Ewers BE, Maier C, Schäfer KVR, McCarthy H, Hendrey G, Mcnulty SG, Katul GG (2001) Soil fertility limits carbon sequestration by forest ecosystems in a CO_2-enriched atmosphere. Nature 411:469–472

Pepin S, Körner C (2002) Web-FACE: a new canopy free-air CO_2 enrichment system for tall trees in mature forests. Oecologia 133:1–9

Rogers A, Ellsworth DS (2002) Photosynthetic acclimation of *Pinus taeda* (loblolly pine) to long-term growth in elevated pCO_2 (FACE). Plant Cell Environ 25:851–858

Roy J, Saugier B, Mooney HA (2001) Terrestrial global productivity. Academic Press, San Diego

Sabine CL, Heimann M, Artaxo P, Bakker DCE, Chen C-TA, Field CB, Gruber N, Le Quéré C, Prinn RG, Richey JE, Lankao PR, Sathaye JA, Valentini R (2004) Current status and past trends of the global carbon cycle. In: Field CB, Raupach MR (eds) Global carbon cycle—integrating humans climate and the natural world. Island Press, Washington, DC, pp 17–44

Saxe H, Ellsworth DS, Heath J (1998) Tree and forest functioning in an enriched CO_2 atmosphere. New Phytol 139:395–436

Schimel DS (1995) Terrestrial ecosystems and the carbon cycle. Glob Change Biol 1:77–91

Sholtis JD, Gunderson CA, Norby RJ, Tissue DT (2004) Persistent stimulation of photosynthesis by elevated CO_2 in a sweetgum (*Liquidambar styraciflua*) forest stand. New Phytol 162:343–354

Stitt M (1991) Rising CO_2 level and their potential significance for cabon flow in photosynthetic cells. Plant Cell Environ 14:741–762

Stitt M, Krapp A (1999) The interaction between elevated carbon dioxide and nitrogen nutrition: the physiological and molecular background. Plant Cell Environ 22:583–621

Tans P (2008) Trends in atmospheric carbon dioxide. Web page NOAA/ESRL http://www.esrl.noaa.gov/gmd/ccgg/trends/. Accessed 27 July 2009

Uddling J, Teclaw RM, Kubiske ME, Pregitzer KS, Ellsworth DS (2008) Sap flux in pure aspen and mixed aspen—birch forests exposed to elevated concentrations of carbon dioxide and ozone. Tree Physiol 28:1231–1243

Uddling J, Teclaw RM, Pregitzer KS, Ellsworth DS (2009) Leaf and canopy conductance in aspen and aspen—birch forests under free-air enrichment of carbon dioxide and ozone. Tree Physiol 29:1367–1380

Zotz G, Pepin S, Körner C (2005) No down-regulation of leaf photosynthesis in mature forest trees after three years of exposure to elevated CO_2. Plant Biol 7:369–374