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Elevated CO_2 affects photosynthetic responses in canopy pine and subcanopy deciduous trees over 10 years: a synthesis from Duke FACE

DAVID S. ELLSWORTH*, RICHARD THOMAS†, KRISTINE Y. CROUS‡, SARI PALMROTH§, ERIC WARD§, CHRIS MAIER¶, EVAN DELUCIA‖ and RAM OREN§

**Hawkesbury Institute for the Environment, University of Western Sydney, Hawkesbury Campus, Locked Bag 1797, Penrith, NSW 2751, Australia, †Department of Biology, West Virginia University, PO Box 6057, Morgantown, WV 26506, USA, ‡Research School of Biology, The Australian National University, GPO Box 475, Canberra, ACT 0200, Australia, §Nicholas School of the Environment, Duke University, Box 90328, Durham, NC 27708, USA, ¶USDA-Forest Service, Southern Research Station, Forestry Sciences Laboratory, PO Box 12254, Research Triangle Park, NC 27709, USA, ‖Department of Plant Biology, University of Illinois at Urbana-Champaign, 505 South Goodwin Ave., Urbana, IL 61801, USA*

Abstract

Leaf responses to elevated atmospheric CO_2 concentration (C_a) are central to models of forest CO_2 exchange with the atmosphere and constrain the magnitude of the future carbon sink. Estimating the magnitude of primary productivity enhancement of forests in elevated C_a requires an understanding of how photosynthesis is regulated by diffusional and biochemical components and up-scaled to entire canopies. To test the sensitivity of leaf photosynthesis and stomatal conductance to elevated C_a in time and space, we compiled a comprehensive dataset measured over 10 years for a temperate pine forest of *Pinus taeda*, but also including deciduous species, primarily *Liquidambar styraciflua*. We combined over one thousand controlled-response curves of photosynthesis as a function of environmental drivers (light, air C_a and temperature) measured at canopy heights up to 20 m over 11 years (1996–2006) to generate parameterizations for leaf-scale models for the Duke free-air CO_2 enrichment (FACE) experiment. The enhancement of leaf net photosynthesis (A_{net}) in *P. taeda* by elevated C_a of +200 μmol mol^{-1} was 67% for current-year needles in the upper crown in summer conditions over 10 years. Photosynthetic enhancement of *P. taeda* at the leaf-scale increased by two-fold from the driest to wettest growing seasons. Current-year pine foliage A_{net} was sensitive to temporal variation, whereas previous-year foliage A_{net} was less responsive and overall showed less enhancement (+30%). Photosynthetic downregulation in overwintering upper canopy pine needles was small at average leaf N (N_{area}), but statistically significant. In contrast, co-dominant and subcanopy *L. styraciflua* trees showed A_{net} enhancement of 62% and no A_{net}–N_{area} adjustments. Various understory deciduous tree species showed an average A_{net} enhancement of 42%. Differences in photosynthetic responses between overwintering pine needles and subcanopy deciduous leaves suggest that increased C_a has the potential to enhance the mixed-species composition of planted pine stands and, by extension, naturally regenerating pine-dominated stands.

Keywords: drought, elevated atmospheric CO_2 concentration, free-air carbon dioxide enrichment, inter-annual variation, leaf nitrogen, *Liquidambar styraciflua*, net photosynthesis enhancement, *Pinus taeda*

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Introduction

Process-based models are central to estimating the impacts of rising atmospheric CO_2 (C_a) on land surface processes and their feedbacks on climate. Physiological data and scaling in such models are used to predict behaviour of CO_2 and H_2O exchange of forested land surfaces with the atmosphere under current and future conditions (Baldocchi & Meyers, 1998; Sitch *et al*., 2003; Prentice *et al*., 2007). These models vary in their structure and level of detail, but all include functions for estimating photosynthesis according to biochemical limitations (e.g., following Farquhar *et al*., 1980) and subsequent developments in Bernacchi *et al*. (2001) and Sharkey *et al*. (2007), diffusional limitations and regulation by bulk leaf stomatal conductance (Collatz *et al*., 1991; Leuning, 1995), and respiration (Atkin & Tjoelker, 2003) as core processes regulating net carbon and water flux processes and their interactions for forests (Cramer *et al*., 2001; Pitman, 2003). To constrain uncertainties in the emergent processes of forest ecosystems in elevated atmospheric [CO_2] (elevated C_a) anticipated in future decades, these core processes need to be well-

Correspondence: David Ellsworth, tel. +61 (0)2 4570 1365, fax +61 (0)2 4570 1314, e-mail: d.ellsworth@uws.edu.au

represented by key physiological parameterizations of carbon and water fluxes between ecosystems and the atmosphere. A central difficulty to using such models for estimating ecosystem processes in tree-dominated ecosystems is the complexity of measurements required to parameterize them, as these models require considerable detailed information in time and space to be well-parameterized (Hanson *et al.*, 2004). Moreover, canopy access is rarely adequate for model parameterization of physiological processes in forests in particular (Bassow & Bazzaz, 1997; Wilson & Baldocchi, 2001; Ozanne *et al.*, 2003).

Under elevated C_a, a number of leaf physiological attributes may change (Table 1), each of which contributes to, or offsets, a potential stimulation of leaf net photosynthesis (A_{net}) within forest canopies. Estimating changes in light-saturated A_{net} with long-term elevated C_a requires an understanding of regulation by both diffusional and biochemical components. Diffusional constraints to photosynthesis are given in the Fick's Law equation, rearranged as follows:

$$A_{net} = \frac{g_s}{1.6} \times C_a\left(1 - \frac{C_i}{C_a}\right) \quad (1)$$

where g_s is the stomatal conductance to water vapour, C_a is the atmospheric CO_2 mole fraction and C_i is the mole fraction of CO_2 inside the leaf. This equation can provide a useful framework for evaluating the major elements of plant physiological responses to long-term elevated C_a. Following from this equation as well as the instantaneous response of A_{net} to $[CO_2]$ from the leaf-level photosynthesis model of Farquhar *et al.* (1980) that expresses the biochemical constraints to photosynthesis (Eqn 3; see Supporting Information), the principal photosynthetic and stomatal responses to elevated C_a can be anticipated (see Table 1; Long *et al.*, 2004). These short-term responses to C_a include a significant enhancement in leaf-level A_{net} by elevated C_a (Curtis & Wang, 1998; Saxe *et al.*, 1998), reductions in g_s and no change in C_i/C_a with elevated C_a (Sage, 1994; Medlyn *et al.*, 2001)(Table 1). The combined photosynthetic and stomatal responses to elevated C_a based on the photosynthetic response predicted by the model of Farquhar *et al.* (1980) also suggest greater proportional photosynthetic enhancement under drought conditions assuming that stomatal closure dominates the leaf-level response. Under drought conditions, plants operating more on the steep initial linear phase of this photosynthetic response would experience a larger short-term photosynthetic enhancement as the rate of change in A_{net} with increasing C_i is increased in such situations (Jackson *et al.*, 1994; Morgan *et al.*, 2001). Thus, a well-known framework underlies the major elements of the photosynthetic response to rising atmospheric C_a and can readily be incorporated into models if they are supported in data from long-term elevated C_a experiments.

With long-term elevated C_a, there may be departures from the short-term responses stated in Table 1 (Ellsworth *et al.*, 2004; Ainsworth & Rogers, 2007). A major departure from these responses has been detected in reductions in photosynthetic capacity in long-term elevated C_a, termed as photosynthetic downregulation (Sage, 1994). Photosynthetic downregulation in trees in elevated C_a has been observed mostly under conditions such as low nutrient availability (Ellsworth *et al.*, 2004)

Table 1 General hypothesized effects of elevated C_a in free-air CO_2 enrichment experiments on plant physiological processes and leaf traits based on analyses from previous C_a enrichment chamber experiments

Parameter	Description	Expected effect	References
A_{net}	Light-saturated net photosynthesis	↑	Curtis & Wang (1998); Saxe *et al.* (1998)
A_{max} or J_{max}	Net photosynthesis with both CO_2- and light saturation	~	Ainsworth *et al.* (2007)
Carboxylation efficiency or V_{cmax}	Net photosynthesis under carboxylation-limited conditions	~	Ainsworth *et al.* (2007)
C_i/C_a	Ratio of leaf internal CO_2 to external CO_2 concentration	~	Sage (1994); Medlyn *et al.* (2001)
g_s	Leaf stomatal conductance to water vapour	↓	Medlyn *et al.* (2001)
M_a	Leaf mass per unit area	↑	Yin (2002)
Leaf N_m and N_{area}	Leaf nitrogen concentration or leaf nitrogen per unit area	↓	Cotrufo *et al.* (1998), Yin (2002)
ΦCO_2	Apparent quantum efficiency for photosynthesis	↑	Drake *et al.* (1997)

The general expected effects of elevated C_a for trees are indicated either as general increases (↑), decreases (↓) or no change (~), with the expectation that photosynthetic down-regulation has not occurred.

or in older leaves of evergreens (Tissue *et al.*, 2001; Rogers & Ellsworth, 2002; Crous *et al.*, 2008). However, such photosynthetic adjustments have not been observed in most deciduous tree species (DeLucia & Thomas, 2000; Herrick & Thomas, 2001; Zotz *et al.*, 2005), which show important differences from conifers in how N is allocated to the photosynthetic apparatus. We hypothesized that deciduous trees would be less responsive to elevated C_a-induced downregulation than co-occurring pine species, owing to the growth of deciduous trees in more shaded micro-sites than conifers in the region, and hence deciduous trees would be more responsive than conifers in terms of A_{net} enhancement (Kerstiens, 2001). However, even where photosynthetic downregulation has been observed, it is unclear if photosynthetic and stomatal responses to light and atmospheric vapour pressure deficits are similarly affected. Long-term measurements are needed to examine to what extent photosynthetic capacity is coupled to, or regulated independently of, stomatal conductance (Wilson & Baldocchi, 2001).

Meta-analyses have consistently suggested that there are many interactive variables that can modulate downregulation associated with elevated C_a (Medlyn *et al.*, 1999; Poorter & Perez-Soba, 2001; Ainsworth *et al.*, 2003), suggesting that year-to-year variability in photosynthetic enhancement could occur as environmental conditions vary. However, even at a single site, differences in the incidence and magnitude of downregulation have emerged as a result of differences in timing of measurements and methods/approaches as well as how the data have been processed and analysed. For example, although a number of studies (Rogers & Ellsworth, 2002; Crous & Ellsworth, 2004) have found evidence of photosynthetic downregulation in *Pinus taeda* consistent with work on other species of *Pinus* (Tissue *et al.*, 2001), Springer *et al.* (2005) concluded that there was scarce evidence of CO_2-induced loss of stimulation of photosynthesis in elevated C_a. Where photosynthetic down-regulation has been reported, it has not been sufficiently large as to negate the stimulating response of net photosynthesis to elevated CO_2. Thus, we ask to what extent are photosynthetic enhancement and photosynthetic properties of trees diminished by long-term growth in elevated C_a, if at all? Are there differences in this regard between evergreen conifer and deciduous trees? Often model parameterizations of photosynthesis follow those for major plant functional types, two of which are temperate needle-leaved evergreen trees and broadleaved deciduous trees (Sitch *et al.*, 2003). Robust leaf-level physiological modules with explicit expressions for conductance, photosynthesis and intercellular CO_2, are needed by the modelling community to examine higher-order or longer-term ecological processes using a common underlying physiological basis (Dewar *et al.*, 2009; Katul *et al.*, 2010).

Herein, we synthesize information from physiological measurements collected from maturing tree canopies over the course of 10 years effort at the Duke forest free-air CO_2 enrichment (FACE) experiment, a research site that has detailed information for adequately parameterizing fundamental physiological process models for incorporation into larger-scale models. To date, a number of modelling analysis have been conducted with a focus on the Duke FACE experiment (Tissue *et al.*, 1997; Luo *et al.*, 2003; Schäfer *et al.*, 2003; Hickler *et al.*, 2008; Franklin *et al.*, 2009), each with its own derivation or data source for key functional relationships underlying leaf-scale models of CO_2 and H_2O exchange with the atmosphere. These models have employed different levels of detail ranging from hourly ecophysiological controls to daily carbon and water budgets (Luan *et al.*, 1999; Katul *et al.*, 2000; Luo *et al.*, 2003; Hickler *et al.*, 2008; Franklin *et al.*, 2009), but all use some form of the photosynthesis model of Farquhar *et al.* (1980). The model parameter V_{cmax} is central to estimating carboxylation-limited photosynthesis by the Farquhar *et al.* (1980) model, and whole-ecosystem CO_2 flux has been found to be highly sensitive to this parameter (Potter *et al.*, 2001; Schäfer *et al.*, 2003; Siqueira *et al.*, 2006). Similarly, functional relationships between photosynthesis or V_{cmax} and leaf N concentration (mass or area-based) have varied considerably across studies of loblolly pine (Peterson *et al.*, 1999; Springer *et al.*, 2005; Crous *et al.*, 2008) and have been inconsistently applied in models (Luan *et al.*, 1999; Luo *et al.*, 2001, 2006). Our intent here is to provide future modelling efforts with a core set of findings and model parameters based on the considerable data amassed on different tree species over 10 years of intensive experimentation in FACE at Duke. There are still only a handful of papers reporting data measured over 10 years from a single site and even fewer that have done so in a consistent fashion. The 10-year span of physiological data from the Duke FACE experiment permits detection of changes in leaf traits through time and seasonal variation, as well as contributing to analyses of long-term physiological parameters regulating forest canopy photosynthesis, in contrast to the shorter-term responses that are typically studied.

Methods

The Duke FACE site is located in Duke Forest, Orange County, NC, USA (35.977°N, 79.093°W, 170 m a.s.l.), described in Ellsworth *et al.* (1995), Oren *et al.* (1998) with additional detail provided in Hamilton *et al.* (2002). Mean annual precipitation at this location is approximately 1150 mm yr^{-1}. Over the 10-year period from which data were derived, annual rainfall varied from 930 to 1350 mm yr^{-1}, with the warmest and driest year in 2002 and the coolest year on average in 2003. Soils are a low-fertility Ultic Alfisol typical of the southeastern US, characterized as a clay loam in the upper horizon underlain by a dense, clay-rich horizon. Soil moisture was measured by four frequency-domain sensors in each replicate ring, each penetrating to 30 cm depth (CS-615; Campbell Scientific, Logan, UT, USA) (Schäfer *et al.*, 2003).

The forest was planted in 1983 with a temperate pine species (*P. taeda* L.). The stand was subjected to a target elevated atmospheric CO_2 mole fraction of ambient +200 μmol mol^{-1} beginning in May 1994 in a 30-m diameter prototype FACE system (Hendrey *et al.*, 1999) and in three additional replicates in August 1996. There were also three fully instrumented control plots, with an average 24 h CO_2 concentration during the first 6 years of the experiment of 388 μmol mol^{-1}. Elevated CO_2 treatment plots achieved ambient + 196 μmol mol^{-1} for the mean [CO_2] at the top of the canopy in FACE (see http://public.ornl.gov/face/DUKE/duke_data_co2weather.shtml). After 6 years of nearly continuous CO_2 enrichment 24 h a day, daytime-only enrichment was conducted. Data from this study constitutes the first 10 years of CO_2 enrichment of this forest stand. In 1998, the prototype plot and its reference complex was split by trenching, and fertilized (Oren *et al.*, 2001), and in 2005 the rest of the plots were treated similarly.

Data

Leaf-level measurements of photosynthetic net CO_2 assimilation (A_{net}) responses to different environmental factors [CO_2 concentration in air surrounding a leaf, photosynthetic quantum flux density (Q) and leaf temperature] collected during a 10-year period of the Duke FACE experiment (1996–2006) were gathered into a common database for analysis (list of sources in the Supporting Information). Leaf photosynthetic responses to the environment have a key role in current models of CO_2 assimilation due to the utility of inferring biochemical rate constants from such measurements (Sharkey *et al.*, 2007). The Duke FACE physiology database included both published and unpublished photosynthesis-environmental response data from researchers working at the site (see Supporting Information), made with common instrumentation (e.g., portable photosynthesis systems utilizing infra-red gas analyzers). A large majority of measurements (over 90%) were made using the Li-6400 portable photosynthesis system (Li-Cor, Lincoln, NE, USA), but other instruments (Ciras-1; PP-Systems, Hitchin, UK) were used as well. Over 11 000 gas exchange measurements made by these open gas exchange systems comprising more than 1100 controlled-response curves, with 46% of the measurements from *P. taeda*, the dominant species in this ecosystem, and the remainder measured on various broadleaved tree species included in the forest understory (DeLucia & Thomas, 2000; Naumburg & Ellsworth, 2000). Measurement temperatures ranged from 10 to 38 °C, comprising the physiological range over which photosynthesis is observed for species in this ecosystem, as temperatures >38 °C are rare at the site and photosynthesis does not occur below 5 °C (Ellsworth, 2000). Measurements reported here were for unfertilized trees, with fertilization results reported elsewhere (Crous *et al.*, 2008; Maier *et al.*, 2008; Domec *et al.*, 2009). The similarities in equipment design, configuration and operating procedures facilitated a common analysis of all data collected by various researchers. Central variables of interest for this article as well as for many models such as leaf net CO_2 assimilation rate (A_{net}), bulk stomatal conductance (g_s) and leaf internal CO_2 mole fraction (C_i) were computed in a common fashion using raw CO_2 and H_2O mole fraction measurements according to equations from Li-Cor (as in Open v.5.3, Li-6400 manual at http://www.licor.com/env/; Li-Cor). To better compare across needleleaf and broadleaf species, all gas exchange and related parameters are expressed on a projected needle basis (using a total surface to projected leaf area ratio of 2.364), even though some previous publications have presented data for pine, only expressed on a needle surface area basis (Ellsworth, 1999; Crous & Ellsworth, 2004; Domec *et al.*, 2009). This was done in part to facilitate comparisons between the conifer and hardwood tree responses to elevated C_a and leaf N using a common project area basis for physiological parameters. A number of relevant studies could not be included in the analysis (Myers *et al.*, 1999; Singsaas *et al.*, 2004), as raw data were inaccessible or not provided. Several other studies (Ellsworth, 1999; Springer *et al.*, 2005; Springer & Thomas, 2007; Logan *et al.*, 2009) that measured leaf photosynthesis at growth C_a and/or light levels were not used, as additional physiological parameters could not be extracted, and therefore the data were outside the scope of this dataset.

Model-fitting controlled photosynthetic responses to CO_2 concentration and light (Q)

The most commonly measured responses were the responses of A_{net} to [CO_2] (so-called A_{net}–C_i curves) and to the quantum flux of photosynthetically active radiation (here defined as A_{net}–Q curves) at a defined or controlled leaf temperature (T_{leaf}) and leaf-air vapour pressure deficit (D). A_{net}–C_i measurements were all conducted at saturating Q ($Q \geq 1500$ μmol quanta m^{-2} s^{-1}). Both A_{net}–C_i and A_{net}–Q curves allow for interpretations of biochemical or photochemical processes regulating A_{net} (Farquhar *et al.*, 1980; Long, 1991; Niinemets & Tenhunen, 1997) and are critical in models to accurately scale up from leaf processes (see Supporting Information). These response curves were generated by manipulating either the [CO_2] entering the chamber or the incident light level within the leaf chamber in 9–10 steps, as commonly described in the Licor model 6400 operating manual (http://www.licor.com/env/). Both of these responses were fitted to widely used models such as the biochemical model of photosynthesis of Farquhar *et al.* (1980), as described in the Supporting Information, and avoided from common pitfalls associated with model-fitting (see Sharkey *et al.*, 2007; Singsaas *et al.*, 2001). Data from both A_{net}–C_i response curves and A_{net}–Q curves were fitted using custom programs following equations from Farquhar *et al.* (1980) and Thornley (2002) and are summarized in detail for *P. taeda* and *Liquidambar styraciflua* at different canopy heights in a table in the Supporting Information). Modelling of the photosynthetic light response is strictly empirical, but the asymptote of A_{net} at light saturation is highly influenced by g_s and hence C_i. Thus, rather than modelling photosynthetic responses to C_i with a biochemical model, but empirically modelling photosynthetic responses to Q, we adopted an approach analogous to Niinemets & Tenhunen (1997), where the response of electron transport to Q was modelled using the equation for electron transport rate (J) as a function of Q from von Caemmerer & Farquhar (1981) and then based on the response of C_i to Q following Katul *et al.* (2000), the overall response of A_{net} to Q could be recovered. The advantage of this approach was that biochemically based parameters could be extracted from both A_{net}–C_i and A_{net}–Q controlled-response curves, and the A_{net}–Q response can be more readily modelled.

Stomatal responses

In addition to biochemical drivers of photosynthesis, we considered vapour-phase diffusional limitations. Although empirical, the Ball–Berry model often proves the best simple empirical fit to field data according to the improvements suggested in Leuning (1995). However, the dataset was not optimal for deriving the parameters for this model in that the controlled-response curves were not conducted under the full range of conditions experienced diurnally, largely because other factors such as carbohydrate accumulation and stomatal closure at high D_s would have confounded the parameters derived from steady-state response curves. Instead, the proportion of limitations to net CO_2 assimilation attributed to diffusion through stomata was assessed by the approach of Jones (1985) using the quotient of the stomatal resistance to CO_2 by the total resistance (sum of stomatal and mesophyll resistances in a well-mixed cuvette system), and using the 'supply function' for A_{net} and the first-order derivative of A_{net} as a function of C_i. A more elaborate version of this approach was summarized by Wilson *et al.* (2000), but not applied here as the assumptions of their finite differencing approach were found not to be generally applicable in this dataset.

Statistical analyses

Statistical analyses were conducted in JMP v. 5.0.2. (SAS Institute, Cary, NC, USA). Overall, the dataset was unbalanced with regard to major categorical factors such as species, canopy position and leaf age over the years and months during which measurements were made, given that data were contributed by different investigators with different objectives and study timeframes. Canopy position for *P. taeda* was defined as 'upper canopy' for leaves in the upper 10% of the tree crown, and 'lower canopy' was defined as leaves located on the lowest live whorl of branches (Crous & Ellsworth, 2004). For *P. taeda*, not all leaf age classes and/or canopy positions were measured in all years, but the full suite of categorical variables was available in five of the 10 measurement years (1997–2006). The principal measurements of *L. styraciflua* L. were conducted in 1997–1998 and 2003–2004. Thus, two repeated-measures ANOVAs were performed following the precept that true replicates in the FACE experiment were exposure rings, which were included in each seasonal and annual series of measurements. The first ANOVA was an analysis of interanual variation in *P. taeda* across 5 years (1998, 2001, 2002, 2003 and 2005) and between CO_2 treatments, canopy locations and needle age classes. For this repeated-measures ANOVA, differences in A_{net}, $A_{net\text{-}360}$ and $V_{cmax\text{-}25}$ and $J_{max\text{-}25}$ were analysed using a mixed-level, four-way factorial repeated-measures ANOVA (JMP v.9; SAS Institute 2006) with rings across years as repeated factors. The second ANOVA compared overwintering *P. taeda* needles with *L. styraciflua* leaves, both specifically measured in 1998 and 2004 that were the only years of complete overlap in these measurements. Herein, differences in $V_{cmax\text{-}25}$, $J_{max\text{-}25}$ and $A_{net\text{-}360}$ were contrasted between species, CO_2 treatments, canopy location and sampling year using a mixed-level, four-way factorial repeated-measures ANOVA. In all repeated-measures ANOVAs, when main factors and first-order interactions were significant, Tukey's HSD test was performed to test for separate means. Appropriate tests for linearity and equal variances were conducted and variables transformed if necessary. Replicates were $N = 3$ plots per C_a treatment for the repeated-measures ANOVA, though in some years, $N = 4$ plots were used to compute means in the first ANOVA when the additional treatment ring was sampled. Two of the understory species were only measured in $N = 2$ plots per C_a treatment.

In addition to repeated-measures ANOVA, regression techniques were used to explore relationships between key variables of interest, particularly with regard to leaf nitrogen per unit area. Herein, differences in these relationships with regard to ambient and elevated CO_2 were tested using dummy variables and linear contrasts for the same number of true replicates (e.g., FACE arrays) as described above.

Results

The actual enrichment in C_a was +52% with an increase in C_a from 377 to 574 ppm during the growing season, close to the target of +200 μmol mol^{-1} C_a enrichment in FACE. Photosynthetic enhancement by the 52% C_a enrichment in the *P. taeda* forest canopy during the months May–September was highly significant and sustained over the 10 years of the experiment ($P < 0.0001$ for C_a treatment in Table 2). The mean stimulation in light-saturated A_{net} averaged over the growing season of all years and across canopy positions and needle age classes was 53 ± 7% for Ambient-Elevated C_a plot pairs, similar to the enhancement calculated in previous analyses with fewer years (Crous & Ellsworth, 2004). The absolute enhancement of A_{net} was 5.8 ± 0.7 μmol m^{-2} (needle projected area) per second pooled across years, canopy locations and needle ageclasses. However, there were highly significant differences in A_{net} between canopy locations ($P = 0.0017$),

Table 2 Results of a mixed-level four-way factorial ANOVA with repeated measures across years for light-saturated net photosynthesis measured at the long-term growth C_a (A_{net}), stomatal conductance measured at the time of maximum $A_{net\text{-}Ca}$ (g_s) and light-saturated net photosynthesis at a common C_a of 360 ppm ($A_{net\text{-}360}$) in the *Pinus taeda* forest canopy

		Repeated measures ANOVA					
		A_{net}		g_s		$A_{net\text{-}360^*}$	
Source of variation	df*	*F*-value	*P*-value	*F*-value	*P*-value	*F*-value	*P*-value
CO_2	1	94.52	**<0.0001**	2.02	0.206	2.10	0.190
Position	1	28.88	**0.0017**	9.0	**0.024**	32.43	**0.0007**
Ageclass	1	1.38	0.284	0.023	0.883	0.40	0.549
Year	4	27.63	**0.011**	11.12	**0.038**	25.04	**0.0019**
Year × CO_2	4	8.03	0.059	5.47	0.097	9.41	**0.017**
Year × position	4	4.94	0.110	1.90	0.313	1.33	0.364
Year × ageclass	4	40.94	**0.006**	11.74	**0.036**	30.79	**0.0012**
CO_2 × position	1	0.21	0.662	0.28	0.616	0.22	0.654
CO_2 × ageclass	1	14.92	**0.008**	8.15	**0.029**	9.30	**0.019**
Position × ageclass	1	7.59	**0.033**	1.25	0.306	4.01	0.085
CO_2 × position × ageclass	1	0.0006	0.981	0.03	0.866	0.047	0.835
Year × CO_2 × position	4	1.45	0.396	0.67	0.657	0.23	0.872
Year × CO_2 × ageclass	4	2.08	0.286	0.51	0.737	1.82	0.261
Year × position × ageclass	4	2.53	0.236	0.24	0.896	0.64	0.620
Year × CO_2 × position × ageclass	4	2.42	0.247	0.79	0.601	0.59	0.646

In the table, CO_2 refers to the C_a treatment effect and position refers to position within the forest canopy (upper or lower positions). *P*-values for the repeated-measures ANOVA are in bold when significant ($P < 0.05$), and df denotes the degrees of freedom.

[*] $A_{net\text{-}360}$ has missing data for 2005, and therefore only 4 years analysed due to design balance. Thus, for $A_{net\text{-}360}$, df = 3 rather than 4 where year was a factor in the ANOVA for this variable.

with 28% greater A_{net} in the upper canopy compared with the lower canopy in both current year and overwintering needles across C_a treatments (Fig. 1a–d). Thus, upper and lower canopy positions in *P. taeda* are considered separately in Fig. 1.

We found a significant canopy position × ageclass effect in A_{net} ($P = 0.033$), as new needles showed a larger canopy position difference in A_{net} from the lower to upper canopy (+52% across C_a treatments) than did old needles (+9%). Thus, although the upper canopy A_{net} was enhanced by 67% in current-year needles and 25% in overwintering needles compared with the lower canopy, both current and overwintering needles in the lower canopy showed very similar enhancement of A_{net} in elevated C_a, e.g., 65% and 33%, respectively, across the 10 years of the experiment (see Appendix S1). Hence, needle age class was important to consider when evaluating differences with regard to CO_2 treatments as well as canopy positions in *P. taeda* (Fig. 1). There were no overall significant differences in A_{net} between different age classes ($P > 0.10$), though there were significant CO_2 × needle ageclass effects on A_{net} ($P = 0.0083$, Table 2). Overall, the relative enhancement of A_{net} by CO_2 enrichment was similar in magnitude to the overall canopy position/shading effects within the pine forest canopy, but statistically stronger (Table 2), suggesting that it is important to better understand sources of variation in the photosynthetic enhancement by elevated C_a. The amount of N in the upper canopy is critical to photosynthetic performance, and hence we examined upper canopy leaf N for elevated C_a-induced changes over 10 years of FACE. There was no overall effect of elevated C_a on leaf N_{area} in the upper canopy in either foliage ageclass (Fig. 1e and f).

Interannual variability in A_{net} enhancement by elevated C_a

Measurement year strongly affected A_{net} as there was significant year effect on A_{net} across C_a treatments ($P = 0.011$, Table 2). There were particularly low A_{net} values in 2002 and 2005 [e.g., mean light-saturated $A_{net} < 13$ μmol m^{-2} (needle projected area) per second across ages classes and canopy positions; Fig. 1], both of which were dry growing seasons. Cumulative rainfall over April–August in these years was 298 and 348 mm, respectively, compared with a cumulative mean of nearly 500 mm over these 5 months for 1998–2006. Pan evaporation for Chapel Hill, NC, USA for April–August exceeds 800 mm (National Weather Service, unpublished results). There was also a significant year × ageclass effect on A_{net} ($P = 0.006$), with the

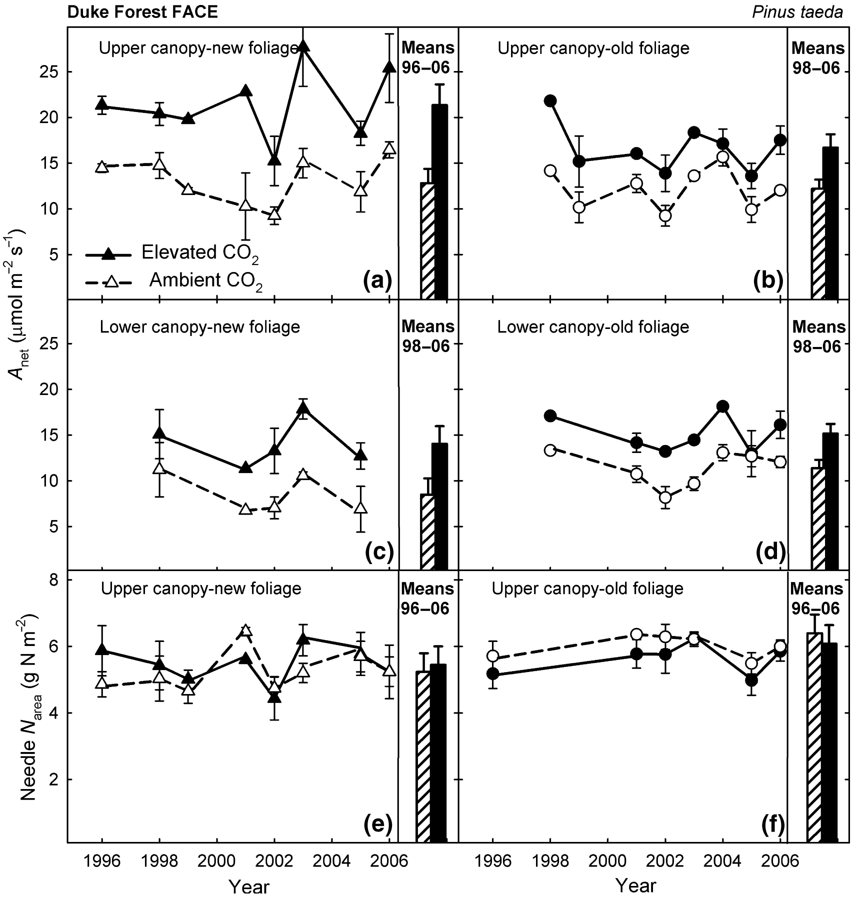

Fig. 1 Mean growing season light-saturated A_{net} per m^2 projected area and N content (N_{area}, projected area basis) of *Pinus taeda* needles across a decade of measurements (1996–2006) as a function of C_a treatment (closed symbols = elevated C_a, open symbols = ambient C_a). Data are shown according to canopy position (upper vs. lower canopy positions) and needle age class (current-year needles in a, c, e are denoted as ▲, △ = new and overwintering needles in b, d, f are indicated as ●, ○ = old), pooled within rings within a given year and averaged across true replicates. Data points are means of $N = 3$ free-air CO_2 enrichment (FACE) rings in each C_a treatment for each year repeatedly sampled over time, with SE among FACE rings within a treatment. Vertical bars at right indicate means for the specified ageclass and canopy position across all years ± standard error of experimental units (plots).

greatest CO_2 enrichment of light-saturated A_{net} in current-year pine needles (+67% across canopy positions, compared with +37% for overwintering needles; Fig. 1). However, the current-year A_{net} enhancement was also the most variable among different years (Range +36% to +95%, Fig. 1). There was a significant year effect ($P = 0.011$) and a marginally significant year × CO_2 treatment effect on A_{net} ($P = 0.059$), but no significant three-way effect ($P > 0.10$ for year × CO_2 treatment × ageclass). This indicates that A_{net} and its enhancement by C_a enrichment were affected by varying annual conditions in this 10-year study.

There are a number of factors that can explain the year–year variability in C_a enrichment effects on A_{net}, but we focussed the analysis on g_s, given the variability in growing season precipitation at the Duke site from 1997 to 2006 and the fact that other proximal sources of variation across measurements and years were minimized. There was no overall CO_2 treatment effect on g_s measured concurrently with light-saturated A_{net} (Table 2), in accord with previous analyses (Ellsworth, 1999; Domec *et al.*, 2009). However, there were both significant year and year × ageclass effects on g_s ($P = 0.038$ and $P = 0.036$, respectively; Table 2). Given that there were similar though statistically stronger effects of these same factors on A_{net}, there is support to the hypothesis that interannual variation in A_{net} could be ascribed in part to interannual variation in tree water status, through feedbacks on g_s. However, variation in photosynthetic enhancement by elevated CO_2 could not be simply explained by average growing season g_s, which was generally similar between ambient and elevated C_a with overall means of 0.15 and 0.16 mol m^{-2} s^{-1} for *P. taeda* under ambient and elevated C_a, pooled across years, canopy locations and needle ageclasses. Given the lack of a strong difference in g_s across C_a treatments, but a significant enhancement of A_{net}, there was a significant increase in instantaneous transpiration efficiency in elevated C_a. Hence, g_s appeared to mediate the interannual variability in A_{net}, but not its enhancement by elevated C_a.

To further explore inter-annual variation in A_{net} enhancement by elevated C_a indicated in the repeated-measures ANOVA (year and year × ageclass effects on A_{net}, *P*-values of 0.01 or better) and weak year × CO_2 treatment effect ($P = 0.059$), A_{net} and its enhancement by elevated C_a were examined as a function of inter-annual precipitation and soil moisture (Fig. 2). Current-year needle A_{net} in elevated C_a was well-correlated with both soil moisture ($r^2 = 0.91$, $P < 0.0001$) and precipitation in recent months ($r^2 = 0.72$, $P < 0.0001$), with $A_{net} > 20$ μmol m^{-2} s^{-1} (projected area basis) in wet growing seasons with cumulative precipitation for the season more than 400 mm, and $A_{net} < 20$ μmol m^{-2} s^{-1} in dry growing seasons (cumulative precipitation for the season of <300 mm). In contrast, A_{net} in ambient C_a showed poorer correlations for each of these variables ($r^2 = 0.29$ and $r^2 = 0.29$ for soil moisture and precipitation, respectively; Fig. 2a and b), with A_{net} always between 10 and 15 μmol m^{-2} s^{-1}. As a result of this stronger dependence of A_{net} on soil moisture in elevated C_a compared with ambient C_a, the absolute enhancement of A_{net} in elevated C_a was itself strongly correlated with both soil moisture ($r^2 = 0.97$, $P < 0.0001$) and precipitation in recent months ($r^2 = 0.72$, $P < 0.0001$). As shown from the trends in A_{net} in ambient and elevated C_a, the A_{net} enhancement ratio varied from +39% to +96% for current-year needles, but was not correlated with either soil moisture or precipitation (Fig. 2e and f). Neither A_{net} in overwintering needles nor its enhancement was significantly related to soil moisture or precipitation (data not shown). Hence, the absolute, but not relative A_{net} enhancement is smaller during drought years than wet years. The sensitivity of current-year needle A_{net} to soil moisture and precipitation, particularly in elevated C_a, contributes to the highly significant CO_2 treatment × ageclass interactions that were observed in Table 2.

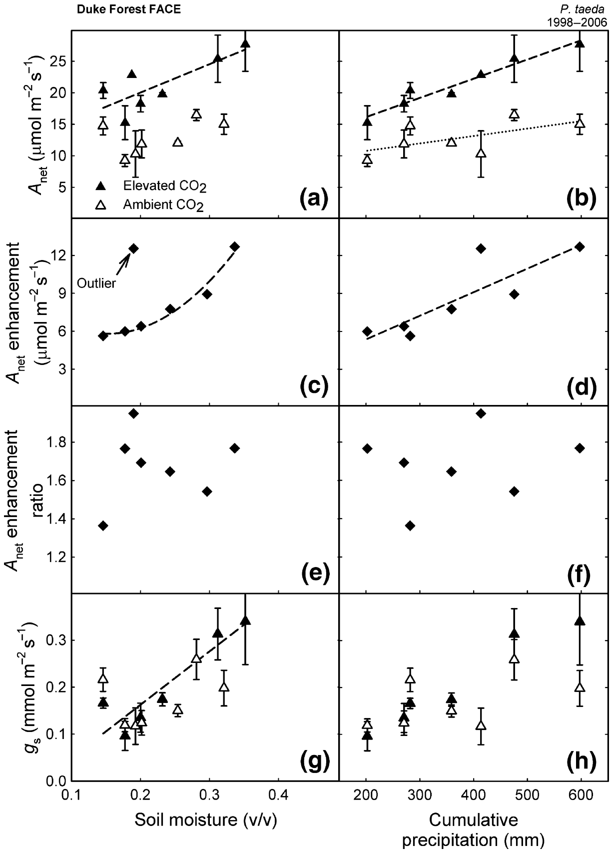

Fig. 2 Annual mean growing season A_{net} (panels a, b), absolute enhancement of A_{net} by elevated C_a (panels c, d) and relative A_{net} enhancement ratio (panels e, f) of current-year *Pinus taeda* needles as a function of mean soil moisture over 30 days preceding A_{net} measurement and cumulative precipitation (April–August) preceding A_{net} measurement. A_{net} was calculated as the difference between A_{net} at elevated C_a and A_{net} at ambient C_a using the ring replicate data for each summer season in each year. The fits shown in c and d are: A_{net} enhancement = 10.30 − 58.77 × *X* + 192.21 × X^2 ($r^2 = 0.97$) and A_{net} enhancement = 1.6283 + 0.0187 × *X* ($r^2 = 0.72$) where *X* is mean soil moisture and cumulative precipitation respectively. The soil moisture fit excludes the identified outlier in c. There was no significant relationship between relative A_{net} enhancement ratio and soil moisture or precipitation as shown in e and f.

To evaluate changes in photosynthetic capacity, photosynthesis at a common measurement C_a of about 360 ppm ($A_{net\text{-}360}$) was evaluated for its dependence on C_a treatment, position and ageclass. Effects for $A_{net\text{-}360}$ were similar to those for A_{net} (e.g., year and year × ageclass effects, and CO_2 treatment × ageclass effects), except for C_a treatment, which in the case of $A_{net\text{-}360}$ was not significant (Table 2). Principal effects on $A_{net\text{-}360}$ were CO_2 treatment × age class ($P = 0.019$) and year × CO_2 treatment ($P = 0.017$), similar to such effects on A_{net} (Table 2). The reductions in $A_{net\text{-}360}$ of pine needles during summer periods was greatest for overwintering needles, concurrent with the lower photosynthetic enhancement in this age class (see Table 2). There was no overall effect of elevated C_a on $A_{net\text{-}360}$ ($P > 0.10$) suggesting that if photosynthetic down-regulation occurred, it was not resolved in this variable.

Seasonal effects on A_{net} and its enhancement by elevated C_a

Given the strength of interactions between CO_2 enrichment and needle aging in previous studies (Tissue *et al.*, 2001; Crous & Ellsworth, 2004) and in this analysis (Table 2), we sought to provide greater insight into leaf aging phenomena in evergreen *P. taeda* by examining seasonal variation in A_{net} and related leaf traits across the lifespan of a needle (Fig. 3). This analysis necessarily lumped data across different years, as data were not available in all months and years. However, with exception of a few instances (e.g., data during wintertime), data from at least two different years were available for the months used in the analysis. The relative increase in C_a was +52% in the FACE experiment (Fig. 3a). C_a enrichment was less during the winter months as the FACE system was not operated when temperatures were below 5 °C, hence actual C_a exposure was less during these months than during summer (Fig. 3a). Pine needles typically emerged in March and reached full elongation to be measured for photosynthesis around mid-May (Rogers & Ellsworth, 2002). Starting with these newly emerged needles, maximum A_{net} on a projected leaf area basis varied between 10 and 15 μmol m^{-2} s^{-1} during the first growing season (May–September), but declined below 10 μmol m^{-2} s^{-1} during winter. Overwintering needles regained photosynthetic rates during the subsequent growing season prior to declines leading up to needle senescence (Fig. 3b). These general patterns in temporal variation in A_{net} were followed in elevated C_a with significant enhancement in A_{net} in all months when measured at or >20 °C.

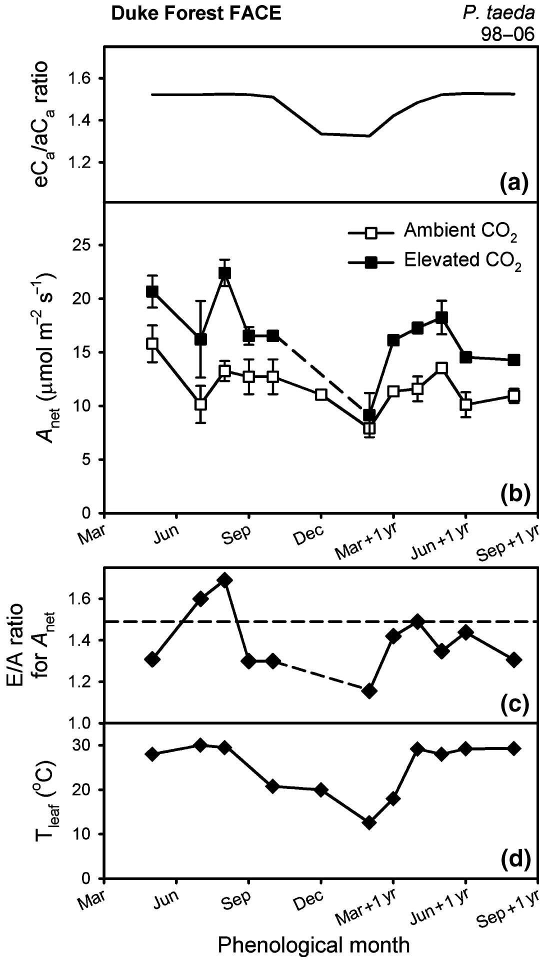

Fig. 3 Seasonal time course through the lifetime of pine foliage for (a) the enhancement ratio for elevated C_a within free-air CO_2 enrichment (FACE) compared with ambient C_a (C_e/C_a), taking into account the operating time for FACE, (b) light-saturated A_{net} in both elevated CO_2 and ambient CO_2-grown leaves measured under atmospheric C_a treatments, (c) the enhancement ratio given as the ratio of A_{net} in elevated CO_2 to A_{net} in ambient CO_2-grown leaves and (d) leaf temperature (T_{leaf}) for all measurements of different age classes within a month. Data collected in the same month was averaged by month and then a composite mean across all years was calculated (1996–2006).

Theoretically, an A_{net} enhancement of 48% computed following Nowak *et al.* (2004) could be expected with enriched C_a (dashed line in Fig. 3c). Across summer months and different needle ages, a mean 62% enhancement of A_{net} was observed (Fig. 3c). Photosynthetic enhancement ratios greater than 48% would be possible either due to higher temperatures that would steepen the initial slope of the A_{net}–C_i curve (Medlyn *et al.*, 2002) or lower C_i that would put the C_a enrichment range in the more linear portion of the A_{net}–C_i curve.

During each growing season in the lifespan of pine needles, photosynthetic enhancement varied in a hump-shaped fashion (May–September data; Fig. 3c). Young, newly emerged pine needles showed A_{net} enhancement that was considerably <48%, as did overwintering needles during cooler months (October–February). The greatest enhancement of A_{net} over the needle life-span occurred in the first summer as needles emerged, as did the greatest variability in A_{net}, in part for reasons discussed above (see Fig. 2a and b). In the summer and autumn after overwintering, A_{net} declined as did the relative enhancement of A_{net} by elevated C_a. Variable relative enhancement of A_{net} in elevated C_a during the growing season could be attributed to variation in C_i/C_a, reflecting variation in stomatal limitations to photosynthesis (Fig. 4a). During the growing season, overwintering needles showed higher C_i/C_a than current-year needles (0.64 vs. 0.61 across C_a treatments; Fig. 4), and there was no significant difference in C_i/C_a between C_a treatments. The C_i/C_a ratio was less variable over time than g_s, which also did not vary between C_a treatments (Table 2) except for newly

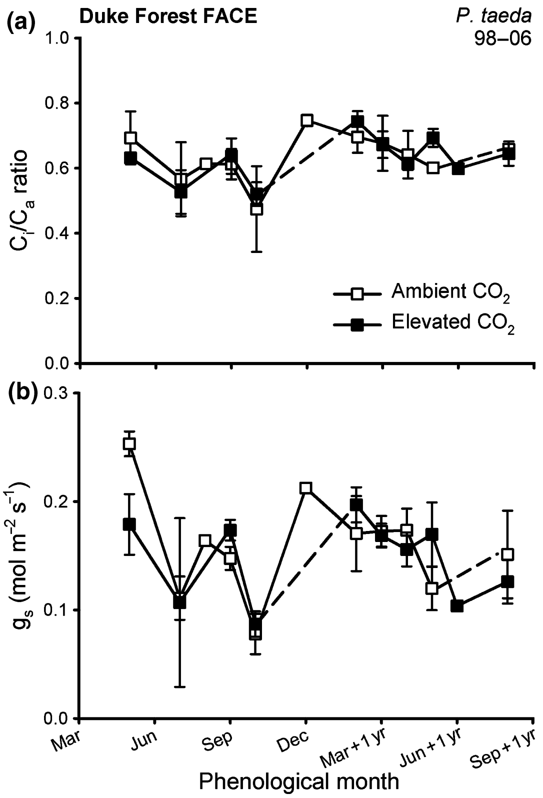

Fig. 4 (a) Timecourse of needle internal CO_2 concentration (C_i) to external air CO_2 concentration (C_a) of pine through the foliage lifetime as a composite of measurements averaged across all years in the experiment. (b) Seasonal comparison of leaf stomatal conductance of pine through the foliage lifetime as a composite of measurements averaged across all years in the experiment.

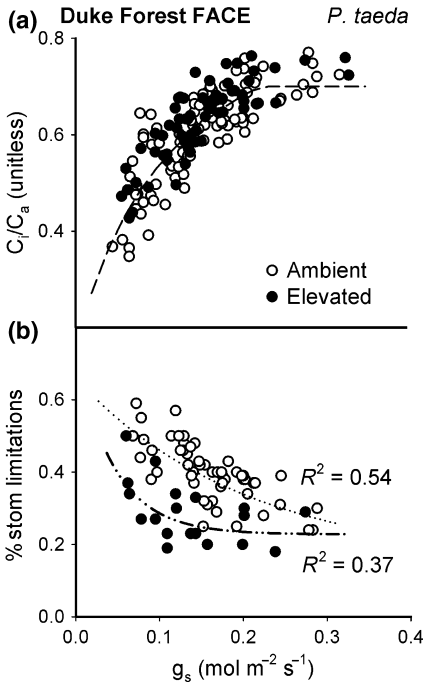

Fig. 5 (a) Dependence of C_i/C_a ratio from light-saturated gas exchange measurements on stomatal conductance for *Pinus taeda* needles in elevated CO_2 across different exposure durations. The dashed line in panel a is from the hybrid model of Katul *et al.* (2000) based on independent data from the Duke free-air CO_2 enrichment experiment. Similar hyberbolic dependence is also predicted by an optimization model (Katul *et al.*, 2009). The parameters of the hybrid morel are: g_{crit} of 0.14 mol m^{-2} s^{-1}, R_c of 0.7 and a of 0.076 mol m^{-2} s^{-1}. (b) Calculated relative stomatal limitations for *P. taeda* needles in ambient and elevated C_a (see Methods). Dashed lines represent the best-fit negative exponential function to the data.

emerged needles (Fig. 4b). The large variance in g_s in current-year needles in summer (Fig. 4b) can be ascribed to interannual variability as discussed in the previous section. Generally, after overwintering, g_s was relatively stable during summer months compared with current-year needles, lending evidence to the needle age class effects on g_s noted earlier (Table 2).

As demonstrated in Eqn (1), C_i/C_a is strongly related to both A_{net} and g_s, but *P. taeda* at the Duke FACE site rarely ever showed a difference in g_s with elevated C_a treatment (Fig. 4, but see Domec *et al.*, 2009) though A_{net} is significantly enhanced (Table 2, Figs 1 and 3). Thus, following Eqn (1), as a consequence of the same diffusional flux inside stomata in ambient and elevated C_a treatments, but a higher C_a, the photosynthetic flux A_{net} is significantly enhanced (Fig. 5a). However, a consequence of increased C_a with C_a enrichment and increased C_i, but no change in C_i/C_a, is a reduction in relative stomatal limitations in elevated C_a (Fig. 5b), as leaves in elevated C_a, operate closer to the CO_2 saturation point for A_{net}. The relationship in Fig. 5b suggests that the difference in stomatal limitations to A_{net} in elevated C_a vs. ambient C_a should decrease as g_s becomes larger, a logical outcome of leaves with high g_s operating at progressively higher C_i relative to the saturation C_i for photosynthesis.

In addition to changes in C_i/C_a, biochemical changes during leaf ageing may be important in regulating A_{net}. The underlying biochemical regulation of photosynthesis was described by the parameters of the photosynthesis model of Farquhar *et al.* (1980), V_{cmax} and J_{max} (see Supporting Information). Both parameters varied considerably between the growing season and cooler months due to their inherent temperature dependence and activation energies (Fig. 6a and c). However, when normalized to a common, standard temperature of 25 °C using activation energies derived from instantaneous temperature responses (Medlyn *et al.*, 2002), the corresponding parameter $V_{cmax\text{-}25}$ was remarkably consistent over the lifetime of needles (Fig. 6b). By

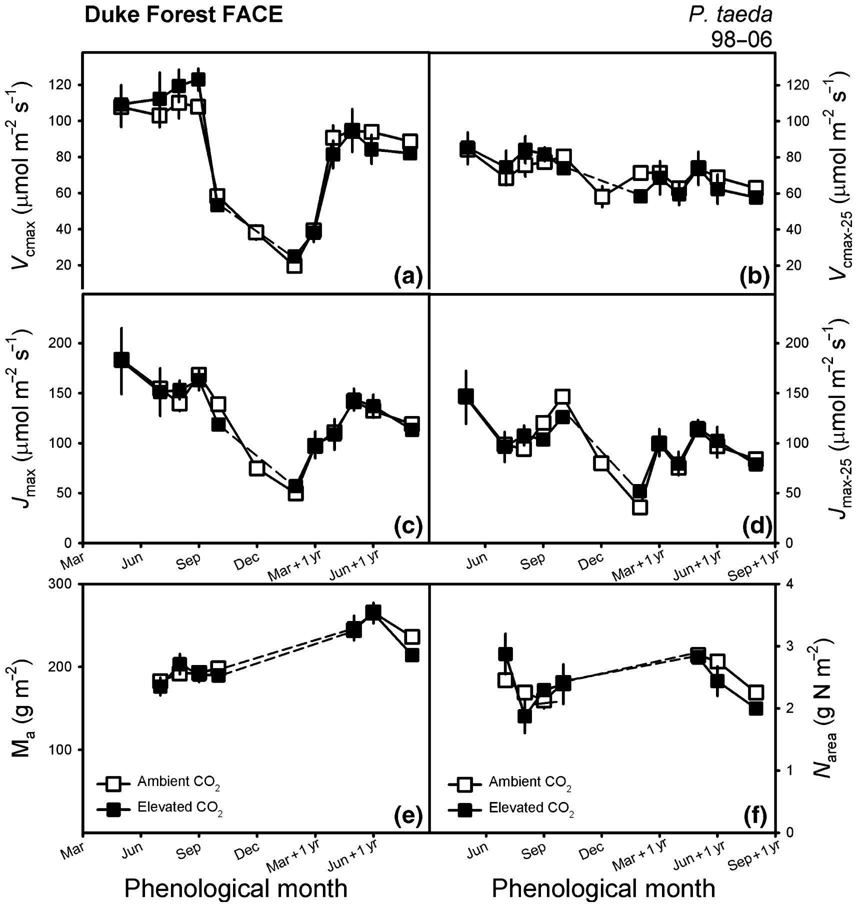

Fig. 6 Timecourse of upper canopy pine needle physiological parameters representing *in situ* maximum rates of carboxylation (V_{cmax}; panel a) and electron transport in photosynthesis (J_{max}; panel c) through the foliage lifetime in the upper canopy, computed from averages across all years of the experiment. V_{cmax} and J_{max} normalized to a standard leaf temperature (25 °C; $V_{cmax-25}$ and J_{max-25}) are shown in panels b and d respectively. Panel e shows leaf mass per unit area (M_a) as an index of leaf structure, and leaf nitrogen (N_{area}) across leaf lifetime is shown in panel f.

removing variability due to temperature, a declining trend for both $V_{cmax-25}$ and J_{max-25} can be seen with respect to leaf aging. On average, the decline in $V_{cmax-25}$ due to leaf ageing in elevated C$_a$ was about 15% larger than the age-related decline in ambient C$_a$ by the time needles were 16 months old in August, with a similar elevated C$_a$ difference for J_{max-25} (Fig. 6b and d). Despite age-related declines in $V_{cmax-25}$ and J_{max-25}, there was a trend towards increasing needle N_{area} from young to overwintering needles. A decline in N_{area} occurred only in the last few months of needle age. There was no apparent difference in either M_a or N_{area} with regard to C$_a$ treatment (Fig. 6e and f).

Role of N in photosynthetic capacity

In accordance with previous work at this site, V_{cmax} and J_{max} were significantly related to leaf N_{area} in *P. taeda* with slopes similar to those reported in (Crous *et al.*, 2008) (Table 3). Needle ageclass significantly affected these relationships ($P = 0.001$; Fig. 7, Table 3), with diminished slopes for $V_{cmax-25}$ as a function of N_{area} as well as for $J_{max–25}$–N_{area} for overwintering needles compared with current-year needles. There was an age-class × CO$_2$ treatment effect on the $V_{cmax–25}$ relationship with N_{area}, as there was no significant difference between ambient and elevated C$_a$ in this relationship for current-year needles, but there was such a difference for overwintering needles (Table 3, Fig. 7). There were also significant relationships between $V_{cmax-25}$ and N_{area} as well as $J_{max–25}$ and N_{area} in *L. styraciflua* (Fig. 8) with important differences in this regard from pine. First, there was no significant difference with regard to treatment C$_a$ on the slopes of these relationships for *L. styraciflua*, whereas there were treatment C$_a$ effects on V_{cmax}–N_{area} for overwintering needles of *P. taeda* (Table 3). Second, the range of N_{area} was smaller for *L. styraciflua* than for *P. taeda*, but the slopes of

Table 3 Statistical relationships and regression statistics for V_{cmax} and J_{max} (at measurement temperature, or standardized to 25 °C) as a function of N_{area} throughout the canopy in both *Pinus taeda* across two age classes (current needles and old, overwintering needles; Fig. 7) and in *Liquidambar styraciflua* (Fig. 8)

Relationship	Species	CO_2 treatment	Age class	N	Dependent variable	Intercept	Slope	R^2
$V_{cmax\text{-}25}$–N_{area}	*P. taeda*	Ambient and elevated	Current	183	$V_{cmax\text{-}25}$	53.3 ± 3.7	10.6 ± 2.3	0.12^{***}
	P. taeda	Ambient	Old	62	$V_{cmax\text{-}25}$	35.1 ± 7.9	13.6 ± 3.2	0.23^{***}
	P. taeda	Elevated	Old	57	$V_{cmax\text{-}25}$	43.7 ± 6.9	7.3 ± 2.9	0.10^{*}
	L. styraciflua	Ambient and elevated	Current	107	$V_{cmax\text{-}25}$	11.0 ± 2.1	34.9 ± 2.6	0.64^{***}
V_{cmax}–N_{area}	*P. taeda*	Ambient and Elevated	Current	183	V_{cmax}	10.5 ± 4.5	41.2 ± 2.7	0.59^{***}
	P. taeda	Ambient	Old	62	V_{cmax}	51.1 ± 11.0	16.4 ± 4.5	0.18^{***}
	P. taeda	Elevated	Old	57	V_{cmax}	79.1 ± 2.7	–	0.01^{NS}
	L. styraciflua	Ambient and elevated	Current	107	V_{cmax}	9.3 ± 4.3	55.1 ± 5.1	0.53^{***}
$J_{max\text{-}25}$–N_{area}	*P. taeda*	Ambient and elevated	Current	183	$J_{max\text{-}25}$	108.9 ± 2.8	–	0.01^{NS}
	P. taeda	Ambient	Old	62	$J_{max\text{-}25}$	29.0 ± 12.4	28.5 ± 5.1	0.34^{***}
	P. taeda	Elevated	Old	57	$J_{max\text{-}25}$	35.7 ± 11.8	23.2 ± 4.9	0.29^{***}
	L. styraciflua	Ambient and elevated	Current	107	$J_{max\text{-}25}$	5.5 ± 3.1	65.0 ± 3.7	0.75^{***}
J_{max}–N_{area}	*P. taeda*	Ambient and elevated	Current	183	J_{max}	71.1 ± 5.6	34.6 ± 3.4	0.39^{***}
	P. taeda	Ambient	Old	62	J_{max}	47.5 ± 14.0	34.0 ± 5.7	0.37^{***}
	P. taeda	Elevated	Old	57	J_{max}	75.8 ± 12.7	18.8 ± 5.3	0.19^{***}
	L. styraciflua	Ambient and elevated	Current	107	J_{max}	3.3 ± 4.4	89.6 ± 5.2	0.74^{***}

Linear regression parameters ± their standard errors are shown. The relationships shown are significant at
$^{***}P < 0.005$ unless denoted
$^{*}P < 0.05$ or
^{NS} indicated not significant ($P > 0.05$). The 'Old' age class refers to overwintering needles. All variables are expressed on a projected area basis, and V_{cmax} and J_{max} were fit using procedures described in the Supporting Information.

these relationships were generally higher than for *P. taeda* (Table 3). Taken together, the results suggest that there is a reduction in the relationships of biochemical parameters with leaf N_{area} in overwintering needles in *P. taeda* due to elevated C_a, but no such effect for deciduous *L. styraciflua.*

Both *P. taeda* and *L. styraciflua* were sampled in the upper and lower canopy or understory early in the FACE experiment in 1998 as well as later in the experiment (2004, after 8 years of continuous elevated C_a treatment) (Fig. 9). Herein, overwintering needles of *P. taeda* are used for comparison with *L. styraciflua* leaves given that projected area-based V_{cmax} was relatively similar between these species when this age class was used, and these leaves for the different species were sampled in common years. In a four-way repeated-measures ANOVA (C_a treatment × canopy position × year with a species contrast), there were very highly significant species differences ($P = 0.0004$) for $V_{cmax\text{-}25}$ with 55% higher $V_{cmax\text{-}25}$ and 39% higher $A_{net\text{–}360}$ for *P. taeda* overall (on a projected area basis). There were also highly significant species × canopy position differences for V_{cmax} and $A_{net\text{–}360}$ ($P = 0.0008$ and $P = 0.0004$, respectively; Fig. 9). There was no overall effect of elevated C_a on $V_{cmax\text{-}25}$ or $A_{net\text{–}360}$ across species and canopy positions ($P > 0.10$). However, there was a significant species × C_a treatment effect for $A_{net\text{–}360}$ ($P = 0.031$). For the upper canopy, there was a non-significant trend towards reductions in $V_{cmax\text{-}25}$ in elevated C_a in *P. taeda* in 2004 vs. in 1998, whereas $V_{cmax\text{-}25}$ tended to stay the same across this time period in ambient C_a (Fig. 9). Thus, there was evidence of reduced photosynthetic capacity in elevated C_a in *P. taeda* as opposed to *L. styraciflua* after 8 years of the FACE experiment.

Photosynthetic parameters from the response to quantum flux density

We compared photosynthetic performance of different species using the response of A_{net} to Q (see (Singsaas *et al.*, 2001). The overstory trees *P. taeda* and *L. styraciflua* showed strong differences in photosynthetic characteristics (Table 4) as noted above (Fig. 9). Though not necessarily measured in the same years as analysed in Fig. 9, A_{net} and V_{cmax} derived from responses of A_{net} to Q were similar to those determined from A_{net}-C_i curves. Understory species were *Acer rubrum*, *Carya glabra*, *Cercis canadensis*, *Cornus florida*, *L. styraciflua and Liriodendron tulipifera*. These understory species were

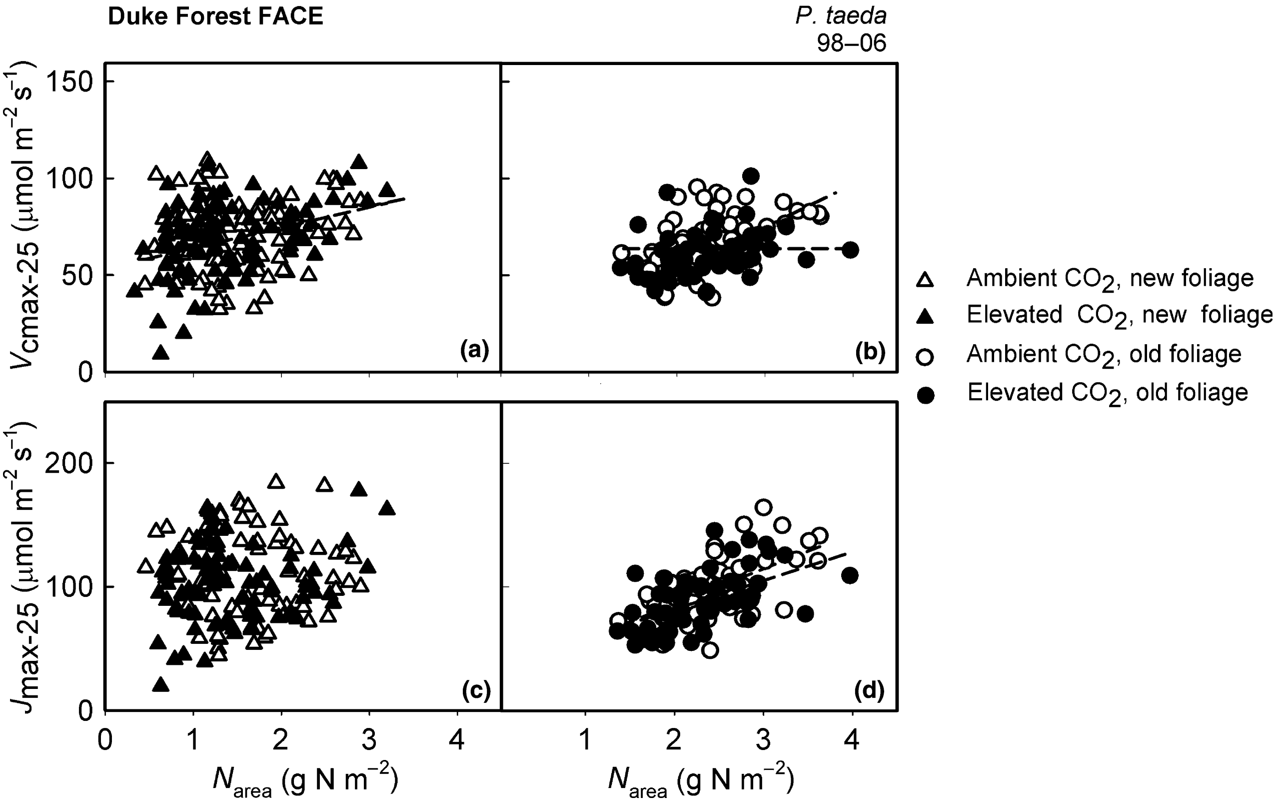

Fig. 7 Carboxylation rate at 25 °C ($V_{\mathrm{cmax}\text{-}25}$) or maximum photosynthetic electron transport rate at 25 °C ($J_{\mathrm{max}\text{-}25}$) as functions of leaf nitrogen on a projected area basis (N_{area}) in *Pinus taeda* grown in ambient and elevated C_a in free-air CO_2 enrichment for current year ('new', left panels a, c) and overwintering needles ('old' age class) (right panels, b and d). Regression statistics for the relationships shown are given in Table 3, and symbols are as in Fig. 1.

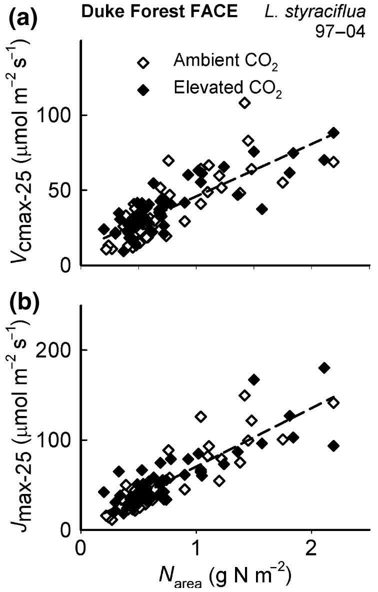

Fig. 8 $V_{\mathrm{cmax}\text{-}25}$ or $J_{\mathrm{max}\text{-}25}$ as functions of N_{area} for *Liquidambar styraciflua* leaves grown in ambient and elevated C_a in free-air CO_2 enrichment in summer months. Regression statistics for the relationships shown are given in Table 3.

stimulated by elevated C_a similar to the overstory canopy, with an overall significant enhancement of A_{net} across species of 42% ($P = 0.0083$), though light-saturated photosynthetic capacity was lower overall in the understory than the lower canopy (compare Figs 9d and 10). Understory species differed significantly in light-saturated A_{net} ($P = 0.0159$), initial slope of the photosynthetic light response ($P = 0.0085$), but not in R_{dark} ($P > 0.10$). Elevated C_a also had no apparent effect on R_{dark} analysed from A_{net}-Q response curves ($P > 0.10$; Fig. 10c). The obligate understory species *C. florida* had significantly higher A_{net} and initial slope of the photosynthetic light response than all the other species (multiple comparison using Student's *t*-test, $P < 0.05$), and the largest enhancement of A_{net} by elevated C_a (+90%) as well as the largest increase in initial slope of the photosynthetic light response in elevated C_a (+46%). For the remaining five species studied, all able to grow as overstory trees at various successional stages, A_{net} enhancement by elevated C_a was smaller than for *Cornus* and marginally not significant ($P = 0.105$).

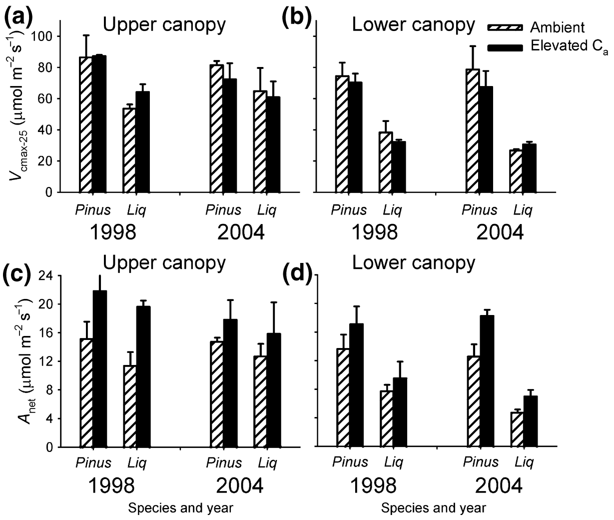

Fig. 9 Comparison of growing season mean $V_{\mathrm{cmax\text{-}25}}$ (top panels a, b) and $A_{\mathrm{net\text{-}360}}$ (bottom panels c, d) in *Pinus taeda* overwintering needles (*Pinus*) and *Liquidambar styraciflua* leaves (*Liq*) and across years (1998 vs. 2004) as a function of C_a treatment for upper canopy (a, c) and lower-canopy foliage (b, d). Data points are means in each C_a treatment for each year repeatedly sampled over time, with SE among free-air CO_2 enrichment rings within a treatment. For $V_{\mathrm{cmax\text{-}25}}$, there was a highly significant species × canopy position effect ($P = 0.0008$), and for $A_{\mathrm{net\text{-}360}}$, there was a significant species × C_a treatment effect ($P = 0.031$) and a highly significant species × canopy position effect ($P = 0.0004$).

Discussion

The magnitude of photosynthetic enhancement by elevated C_a drives carbon available to the non-photosynthetic parts of the ecosystem. Predicting carbon and water fluxes in forests at a variety of scales is improved by considering the ability of plants to adjust photosynthetic capacity when exposed to long-term elevated atmospheric C_a (Schäfer *et al.*, 2003; El Maayar *et al.*, 2006), which along with hydrological and nutrient feedbacks drive leaf area production (McCarthy *et al.*, 2007) and gross and net primary productivity (NPP) on an annual basis (McCarthy *et al.*, 2006, 2010) The magnitude and extent of photosynthetic adjustments vary considerably among species (Ellsworth *et al.*, 2004), with duration of elevated C_a exposure (Ainsworth & Rogers, 2007) and site nutrient status (Ellsworth *et al.*, 2004; Crous *et al.*, 2010). We observed significant enhancement of A_{net} in *P. taeda* and deciduous trees that was sustained over a wide range of conditions over 10 years in this ecosystem, including during canopy closure (Ellsworth, 1999), following storm damage (McCarthy *et al.*, 2007) and in summer drought. Given the comprehensive data assembled for these species at the FACE experiment, the photosynthetic results are potentially stronger than can be stated in individual studies or meta-analyses, particularly because the latter approach is unable to resolve absolute changes nor the substantial seasonal variation that typified variation in A_{net} and V_{cmax} with leaf aging in pines (Figs 3 and 6).

Seasonal variation of photosynthetic enhancement in wet and dry years

There was substantial seasonal variation in photosynthetic parameters in *P. taeda* owing not only to seasonal temperatures but also to variable summer rainfall (Table 2; Figs 3 and 4). Given the nature of the intrinsic photosynthetic CO_2 response of C_3 plants, greater photosynthetic enhancement by elevated C_a is expected

Table 4 Light response curve parameters for current-year needles of *Pinus taeda* and leaves of *Liquidambar styraciflua* in ambient and elevated C_a from fits to the non-rectangular function of Prioul & Chartier (1977) shown in Eqn (6) (see Supporting Information for equations)

Species	Canopy position	C_a treatment	Φ_{CO_2}	Apparent quantum efficiency	Asymptotic A_{net}	Observed light-saturated A_{net}	θ	R_{dark}
P. taeda	Upper	Ambient	0.068	0.044	15.2	12.2	0.15	−1.3
		Elevated	0.062	0.045	23.5	18.0	0.15	−1.0
	Lower	Ambient	0.049	0.033	11.8	9.9	0.30	−0.5
		Elevated	0.052	0.034	17.7	13.8	0.25	−0.6
L. styraciflua	Upper	Ambient	0.067	0.045	13.7	10.9	0.27	−1.4
		Elevated	0.077	0.055	23.2	18.4	0.47	−1.9
	Lower	Ambient	0.063	0.040	7.9	6.6	0.45	−0.8
		Elevated	0.088	0.056	12.2	10.5	0.47	−0.9

A_{net} is given as the asymptotic fit to the non-rectangular function as well as the observed light-saturated A_{net}. Φ_{CO2} is the theoretical maximum quantum efficiency based on the non-rectangular fits, whereas the apparent quantum efficiency is the linear regression fit to gas exchange data for $Q < 150$ μmol m^{-2} s^{-1} as an operational quantum efficiency.

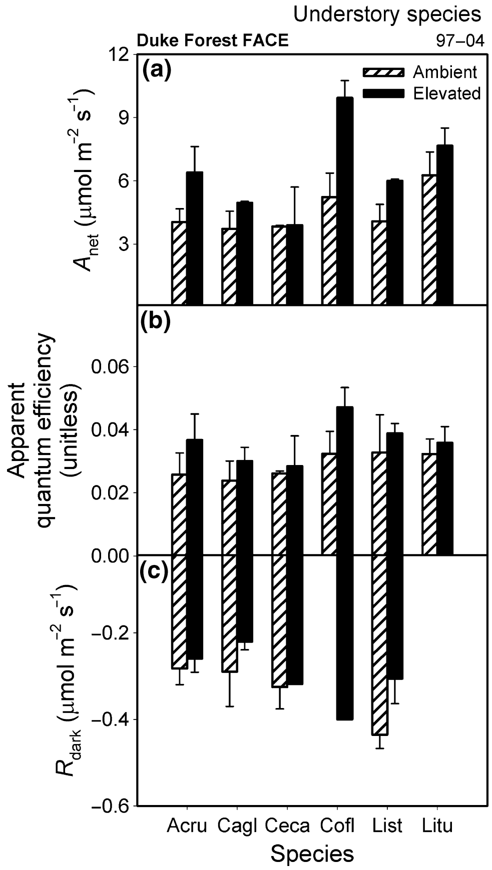

Fig. 10 Photosynthetic characteristics of understory species in the *Pinus taeda*-dominated forest at the Duke free-air CO_2 enrichment site derived from controlled A_{net}–Q responses across years in each species. (a) Light-saturated A_{net} (b) apparent quantum yield at $Q < 150$ µmol m^{-2} s^{-1}, and (c) dark respiration (R_{dark}) as a function of $\mathrm{C_a}$ treatment for *Acer rubrum*, *Carya glabra*, *Cercis canadensis*, *Cornus florida*, *Liquidambar styraciflua* and *Liriodendron tulipifera*, with each species designated by the first two letters of each part of the latin binomial.

under dry conditions than under wet conditions. As drier conditions prevail, the operational set-point for plant gas exchange tends to favour the more responsive part of the photosynthetic CO_2 response curve (e.g., the initial slope region) when all other conditions are assumed to be similar (Nowak *et al.*, 2004). This CO_2-drought enhancement hypothesis is a central paradigm of elevated $\mathrm{C_a}$ effects (Strain & Bazzaz, 1983). Indeed, in herbaceous grasslands, the biomass response to elevated $\mathrm{C_a}$ is more pronounced in dry years than in wet years (Morgan *et al.*, 2004). Our results for mature evergreen *P. taeda* trees are counter to these expectations. The observed stimulation of photosynthesis was highest in *P. taeda* in wet years such as 2003 (Fig. 1), and particularly large in current-year needles during summer months with ample soil moisture (Fig. 2b). Precipitation and soil moisture were major sources of year–year variation in A_{net} and its CO_2 enhancement response to elevated $\mathrm{C_a}$, as has been demonstrated for net primary production at the site (McCarthy *et al.*, 2010).

How was it that photosynthetic enhancement by elevated $\mathrm{C_a}$ was larger in wet compared with dry years in this ecosystem? Based on first principles, the opposite response may be expected (Nowak *et al.*, 2004). Even though current-year needle g_s was nearly half in the drought of August 2002 than in the subsequent wet summer of 2003, g_s in elevated $\mathrm{C_a}$ was also more improved in ‘wet’ conditions in the 2003 summer (by three-fold) than it was in ambient $\mathrm{C_a}$ (by less than two-fold; Fig. 2h). This is consistent with findings in Domec *et al.* (2009), where elevated $\mathrm{C_a}$ was associated with higher maximum leaf hydraulic conductance and higher sensitivity of this conductance to leaf water potential. V_{cmax} as well as leaf N were depressed more in the dry year of 2002 compared with other years, and recovered after drought (increase in V_{cmax} by 25% after drought). The combination of up-regulation in V_{cmax} and recovery of g_s in elevated $\mathrm{C_a}$ after a strong drought year meant greater photosynthetic enhancement under wet conditions, which was more important than the instantaneous effect of an elevated $\mathrm{C_a}$-drought enhancement hypothesis in *P. taeda* (counter to Morgan *et al.*, 2004). This was the case following two strong drought events (2002 and 2005; Fig. 1). The short-term photosynthetic enhancement by a sudden step change in $\mathrm{C_a}$ measured in the leaf cuvette was indeed larger in 2002 (60% enhancement in A_{net} by +200 ppm $\mathrm{C_a}$) than in 2003 (42%; data not shown) in agreement with the general expectation in Nowak *et al.* (2004) and other work, but only on an instantaneous basis, and not considering the strong biochemical adjustments to the photosynthetic apparatus that may accompany progressive drought (Galmés *et al.*, 2007). An improvement in soil moisture through indirect effects of elevated $\mathrm{C_a}$ in moist conditions observed by Schäfer *et al.* (2002) could also have contributed to the increased recovery in elevated $\mathrm{C_a}$ leaves after drought. The gas exchange data used here may represent some bias in that trees with very low $\mathrm{g_s}$ (e.g., approximately $g_s < 0.09$ mol m^{-2} projected area s^{-1}) were excluded from the analysis given that $\mathrm{C_i}$ could not be reliably determined under conditions where patchy stomatal closure and/or Rubisco deactivation may be prevalent (Crous & Ellsworth, 2004). Despite the strong sensitivity of current-year pine needles to drought, A_{net} was less variable across different years in overwintering needles (Fig. 1).

The photosynthetic enhancement responses to elevated $\mathrm{C_a}$ are mirrored in part by the pine biomass accumulation responses to elevated $\mathrm{C_a}$ across different years. The biomass response to elevated C_a is less variable than the A_{net} response except in the very dry year of 2002 (McCarthy *et al.*, 2010), which may partially be explained by compensatory photosynthesis that might have occurred outside the main dry summer period in other years, particularly in years where late winter and/or early spring were normal or better in terms of precipitation. In contrast, winter 2001–2002 and spring in 2002 were dry, thus precluding any early-season photosynthesis, reflected in the biomass response of 2002 (McCarthy *et al.*, 2010). Evergreen conifers like *P. taeda* persist in photosynthesis through a wide range of conditions (Ellsworth, 2000) and hence seasonal adjustments of biochemistry and stomatal regulation serve to maintain photosynthetic activity during unfavourable climate conditions to a greater extent than for sympatric deciduous tree species.

Were there photosynthetic adjustments in elevated C_a?

Photosynthetic downregulation has frequently been related to plant N status, as N-containing amines are needed for synthesizing and maintaining photosynthetic proteins (Ellsworth *et al.*, 2004; Bloom *et al.*, 2010). There have long been concerns that sub-optimal N supply from the soil coupled with increased demands for N with a step change to elevated C_a could lead to reductions in photosynthetic proteins and/or activation state, and thus diminished photosynthetic enhancement by elevated C_a over long time frames (Zak *et al.*, 1993; Finzi *et al.*, 2002, 2006; Yin, 2002; Luo *et al.*, 2004; Reich *et al.*, 2006b). We investigated whether elevated C_a affected leaf N and photosynthetic capacity or its biochemical component processes for *P. taeda* over 10 years of elevated C_a exposure using the largest dataset available for such an analysis at a single, long-term experiment.

Decreases in leaf N are frequently observed in elevated C_a experiments (Curtis & Wang, 1998; Yin, 2002), but our 10-year dataset including pre-treatment data suggests that leaf N_{area} in the *P. taeda* canopy has been relatively stable over 10 years in FACE. This suggests that these pines have been able to maintain their canopy N pool by foraging for N (Finzi *et al.*, 2007; Drake *et al.*, 2011). However, assimilation of N into organic compounds was significantly reduced in overwintering needles of *P. taeda* in elevated C_a (Natali *et al.*, 2009), which could potentially lead to a reduction in photosynthetic capacity in that treatment (see Fig. 7). It is unclear how long enhanced N requirements in elevated C_a can be satisfied thus maintaining canopy photosynthetic capacity, but the degree to which leaf N has been maintained over a decade of C_a enrichment is surprising (Drake *et al.*, 2011) and counter to nitrogen limitation hypotheses (Luo *et al.*, 2004; Reich *et al.*, 2006a), suggesting a strategy of deeper or intensified root foraging in elevated C_a than in ambient C_a.

Despite the maintenance of leaf N and elevated C_a-induced photosynthetic enhancement in the pine canopy over a decade, there was evidence indicating that the biochemical parameters underlying photosynthetic capacity of *P. taeda* were not necessarily maintained constant over time in elevated C_a. For instance, an accelerated decline in V_{cmax} across the leaf aging sequence (Fig. 5) and a weakened relationship and smaller slope between V_{cmax} and leaf N_{area} for overwintering needles, evident at higher N_{area} (Fig. 7), suggest downregulation in elevated C_a in these overwintering pine needles. The downregulation effect on the V_{cmax}–N_{area} relationship in overwintering needles is physiologically meaningful at high N_{area} only, because the relative difference diminishes very quickly with decreasing N_{area} due to the high intercepts of photosynthetic-N relationships (Table 3). Lower mean leaf N status and variation among FACE array sites (Finzi *et al.*, 2002; McCarthy *et al.*, 2007) would potentially mask an average statistical effect across replicate FACE arrays. The observed downregulation effect in overwintering needles was potentially small relative to the increase in C_a and year–year variation in g_s (Figs 1 and 2). Thus, overall enhancement of A_{net} was sustained in *P. taeda* over a decade served to sustain the observed increased NPP under elevated C_a (McCarthy *et al.*, 2010). The results here broadly support previous analyses of elevated C_a effects on N nutrition of pine needles from this ecosystem with regard to leaf age and canopy position (Rogers & Ellsworth, 2002; Crous *et al.*, 2008; Maier *et al.*, 2008).

Needle aging had a significant effect on aspects of photosynthetic performance in *P. taeda* that were observed in key functional relationships. For aging pine needles in the upper canopy, there were reductions in J_{max} that were consistent with declines in photosynthetic capacity and tended to de-couple this capacity from leaf N_{area} (e.g., slope reductions for J_{max} as a function of N_{area}, Fig. 7 and Table 3). Such age-related phenomena have been observed in the past in temperate evergreen pines growing under elevated C_a on several continents (Jach & Ceulemans, 2000; Tissue *et al.*, 2001; Luomala *et al.*, 2003). There is a substantial role for aging pine needles to serve as stores for N, later reallocated to younger, actively growing needles. It remains unclear if the observed photosynthetic downregulation here was part of a pattern of accelerated needle senescence in elevated C_a, yet no effect was detected in direct observation of needle expansion and fall (McCarthy *et al.*, 2007). However, this idea is bolstered by observations that photosynthetic downregulation of aging overwintering pine needles in elevated C_a was ameliorated by the addition of N fertiliser (Crous *et al.*, 2008; Maier *et al.*, 2008). In addition, Natali *et al.* (2009) found diminished leaf nitrate reductase activity of overwintering pine needles in elevated C_a, in one of the years in which photosynthetic downregulation was noted (Crous *et al.*, 2008). N nutrition as well as water status from year-to-year probably contributed to variable photosynthetic enhancement by enhanced C_a as well as interannual variation in the C_a-induced stimulation of pine productivity (McCarthy *et al.*, 2010).

There is recent evidence suggesting that the source of N may be linked to plant N status and N assimilation capacity in elevated C_a in a manner that explains the feedback regulation of leaf photosynthetic responses to elevated C_a (Bloom *et al.*, 2010). In such NH_4^+-dominated systems as this research site (Finzi *et al.*, 2002; Hofmockel *et al.*, 2007), little downregulation response to elevated C_a is expected even after a decade of treatment (Erickson *et al.*, 2007; Bloom *et al.*, 2010), as opposed to nitrate-dominated ecosystems. Consistent with this hypothesis, overwintering needles demonstrated lower NO_3^- assimilation in elevated C_a than in ambient C_a, in concert with photosynthetic downregulation in the former (Crous *et al.*, 2008; Natali *et al.*, 2009). Moreover, there were no detectable effects of elevated C_a on NO_3^- assimilation in hardwood species at the site, in systems where such hardwood species tend to prefer NH_4^+ sources for leaf organic N (Constable *et al.*, 2001), no downregulation would be expected (Bloom *et al.*, 2010). Hence, the available evidence is broadly consistent with differential species photosynthetic performance associated with the ability to acquire and assimilate N into leaves (Bloom *et al.*, 2010) affecting the elevated C_a responses of forest trees.

Species differences

Given the differential photosynthetic-N requirements and leaf longevity between pines and their deciduous neighbours (Reich *et al.*, 1995), it might be expected that photosynthetic downregulation would be manifested in deciduous trees earlier in time than in pines upon elevated C_a exposure or to a greater extent. The Duke FACE experiment has provided a unique opportunity to directly test for differences in photosynthetic responses of these co-occurring major species groups and plant functional types with different photosynthetic-N relationships (Table 3). *Pinus taeda* had greater photosynthetic rates per unit projected area than *L. styraciflua* on a projected area basis (compare V_{cmax} and J_{max}; Fig. 9), but was less sensitive to N (lower slopes; Table 3). Consistent with earlier analyses, there was some evidence of downregulation of photosynthetic capacity in *P. taeda* in the upper canopy, but not in *L. styraciflua*, though only for $A_{net-360}$ and not V_{cmax} in *P. taeda* (Fig. 9). Thus, in contrast to the initial hypothesis, *L. styraciflua* showed somewhat greater photosynthetic enhancement than *P. taeda* in the overstory, considering both age classes of *P. taeda* and year–year variation. These differences in photosynthetic responses are associated with different strategies for acquiring and allocating N to photosynthesis in these different species (Constable *et al.*, 2001; Natali *et al.*, 2009), and may or may not represent broader functional group differences associated with leaf habit.

There were strong reductions in photosynthetic capacity from the upper crown to the base of the live crown for both *P. taeda* and *L. styraciflua*, though these adjustments to shade were larger for *L. styraciflua* (Fig. 9). The comparison of these species is somewhat biased considering that the pines were planted and hence dominated the overstory, whereas deciduous *L. styraciflua* had colonized 'gaps' in the growing plantation and there were few trees occupying the 'sun' portion of the canopy. The magnitude of shade acclimation of photosynthetic capacity indicates that caution should be applied to categorical comparisons between 'sun' and 'shade' foliage or upper- and lower-canopy foliage if such comparisons do not specify the light environment in which foliage has developed, especially as the shade-induced forcing of photosynthetic capacity was larger and statistically better resolved than that by long-term elevated C_a in this forest (Tables 2 and 4 and Fig. 9).

After a decade of study, it is clear that understory species differentially respond to elevated C_a in terms of A_{net} enhancement at light saturation (DeLucia & Thomas, 2000; Naumburg & Ellsworth, 2000). The photosynthetic responses of shaded, understory leaves suggests a capacity to increase photosynthetic carbon capture in elevated C_a in shade-grown plants when measured in sunflecks (DeLucia & Thomas, 2000). Among six intensively studied understory tree species, the most shade-tolerant *C. florida* was most responsive to elevated C_a for A_{net} at light saturation as well as apparent quantum efficiency, whereas the least shade-tolerant species (*Cercis*, *Carya*, and *Liriodendron* were least responsive to elevated C_a with small enhancements to A_{net} (Fig. 10a and b). These physiological results suggest a competitive advantage to shade-tolerant species adapted for carbon capture in high sunlight or sunflecks in the understory over less shade-tolerant species. If sustained over time, we speculate that such an advantage may drive altered understory tree species dynamics in elevated C_a. However, given the ability of shaded trees to persist for long periods in the understory, these dynamics may only be testable for very small seedlings or over longer time periods than a decade.

Conclusions

A significant stimulation of light-saturated photosynthesis was sustained throughout a full decade of growth in elevated C_a in FACE in both *P. taeda* and *L. styraciflua* in the upper and lower canopy, as has been previously hypothesized (Table 1). The stimulation of stand NPP also was sustained over this interval, and was probably driven by the increase in photosynthesis. This open-air field experiment largely supports the null hypothesis that stimulation of photosynthesis under elevated C_a in an ammonium-dominated ecosystem is not a transient phenomenon, and extends to multiple canopy positions and species. The information generated from the study can thus inform process-based models and shows how leaf-level data can provide an understanding of canopy physiological behaviour under long-term elevated C_a. Differences in photosynthetic responses between the overstory pines and deciduous tree subcanopy suggest that increased C_a may have the potential to enhance the mixed-species composition of planted pine stands.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. List of parameter values derived from the Duke FACE experiment for data 1996–2006.
Data S1. Data sources for the studies included in the Duke FACE experiment database.
Data S2. Modelling analysis of field A_{net}–C_i curves for Farquhar *et al*. model parameters.